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Seed Germination as a Metric of Invasive Potential in Winter-Hardy *Prunus*

Sarah A. Kostick[1], Emily E. Hoover[2], Neil O. Anderson[3], John Tillman[4], and Emily Tepe[4]

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Abstract

Invasive species threaten the survival of native flora through the alteration of the structure and processes of natural communities. After species are introduced to a new location, seed germination is vital for the formation of diverse, self-sustaining populations. In this study we measured seed germination of a selection of winter-hardy *Prunus* fruit types of apricot, tart cherry, and plum genotypes. This experiment examined seed germination requirements parsed by fruit type, genotype within fruit type, environment, and scarification. Higher germination percentages were observed in the greenhouse compared to the field. Scarification was dependent on genotype within a fruit type and germination environment. From this study we concluded that most genotypes examined will not become invasive due to low and/or inconsistent germination. Apricots had high overall germination whereas tart cherries were lower. The plums had variable germination percentages but progeny from the plum genotypes 'Hazel', 'Whittaker', 'South Dakota', and 'Hennepin' had high germination, indicating the potential to become invasive.

Prunus, a large and economically important genus in the Rosaceae, includes many species with lengthy and rich histories of human cultivation (Das et al., 2011; Griffiths, 1994; Potter, 2012; Wen et al., 2008). Although fruit production is the most prominent use of many of the cultivated species in this genus, others serve functions as landscape plants, for timber production, and medicinal use (Potter, 2012). However, few of these species can be successfully cultivated in USDA zones 3 and 4 because of low midwinter temperatures and flower damage during spring frosts (Andersen and Weir, 1967; Taylor, 1965). Even winter-hardy species are often short lived and fail to produce consistent fruit crops (Andersen and Weir, 1967). In northern climates, breeding programs in the 1900s focused on releasing winter-hardy genotypes that had relatively good fruit quality and produced viable pollen to ensure fruit set (Andersen and Weir, 1967). These goals were accomplished through the hybridization of high quality fruiting species (e.g. *P. domestica* L.) with native, winter-hardy species like *P. americana* Marsh., which often had poor quality and astringent fruit (Andersen and Weir, 1967). Although a number of winter hardy genotypes have been released, little is known about their invasive potential.

Baskin and Baskin (1998) theorized that mechanical dormancy might not be separate from physiological dormancy as some species overcome dormancy through a period of cold stratification without scarification. However, *Prunus* seeds overcome mechanical and deep physiological dormancy to germinate through scarification (Baskin and Baskin, 1998; Hartmann et al. 1997). Scarification leads to variable effects on germina-

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tion in *Prunus*. For *P. americana*, *P. cerasus* L., and *P. persica* Batsch., scarification was shown by Chen et al. (2007), Grisez et al. (2008) and Kristiansen and Jenson (2009) to increase both the percent and rate of germination. In *P. domestica* L. and *P. angustifolia* Marsh., scarification did not alter germination percentage or rate (Grisez et al. 2008; McMahon et al. 2015).

Physiological dormancy is overcome through a long period of moist, cold stratification (Baskin and Baskin, 1998; Westwood, 1993). However, in some *Prunus* species, moist and warm stratification increased seed germination (Baskin and Baskin, 1998; Chen et al. 2007; Grisez et al. 2008; Westwood, 1993). *Prunus armeniaca* L. requires 50 days of cold stratification whereas other species such as *P. domestica* and *P. cerasus* require 90 or 90-150 days, respectively (Jauron, 2000; Grisez et al. 2008; Seeley and Damavandy, 1985). As stratification period lengthens, germination is often higher. For example, germination in *P. persica* begins after 56 days of cold stratification and continues to until 84 days at an increasing rate (Martınez-Gómez and Dicenta, 2001).

The spread of invasive species is often the result of human activities including agriculture, horticulture, and forestry (Reichard and White, 2001; Vanhellemont et al. 2009). Many winter-hardy *Prunus* genotypes have been cultivated since the early 1900s (Andersen and Weir, 1967; Brooks and Olmo, 1997). Some *Prunus* species have escaped cultivation and become invasive. For example, *P. serotina* Ehrh., a species native to North America, has escaped cultivation in parts of Europe and become invasive (Deckers et al. 2005). Phartyal et al. (2009) estimated that 44% of mature seed of the invasive species *P. serotina* germinated *in situ*. *Prunus americana* has also demonstrated high invasive potential as it is adapted to a variety of habitats and is spread across a wide geographic range (Francis, 2004). Whether other *Prunus* species and genotypes will become invasive is not known.

These examples from *Prunus* provide a basis to study whether winter-hardy *Prunus* have invasive potential. Kolar and Lodge (2001) define the first stage in invasiveness as the transport of the species into a new environment. Once present in the new environment, a viable population establishes itself and becomes reproductive (Kolar and Lodge, 2001). Thus, seed germination and seedling establishment are important to understand invasiveness. The objective of our study was to determine winter-hardy *Prunus* seed germination as it relates to invasive potential.

Materials and Methods

Genotypes and Seed Collection. We examined three fruit types of *Prunus* for germination of open pollinated seed including 28 *Prunus* winter-hardy genotypes (Table 1). Fruit type was defined as apricot, tart cherry, or plum. Although there are two types of tart cherries, amarelle and morello genotypes (Brown et al. 1989), all tart cherries were classified under one category for the purposes of this experiment. In 2012, all apricot, tart cherry, and plum fruits were collected from trees at the University of Minnesota research plots in Excelsior, MN (44°52'06.4" N lat., -93°38'00.5" W long.) during weeks 25-26 and 31-34. Week number is defined as the number of weeks from the first week of the year beginning 1 Jan.

Experimental Design. For each genotype, 48 seeds were randomly chosen and divided into two groups of 24 each. One group was mechanically scarified with a hammer hard enough to crack the stony endocarp (pit); the endocarps were left in place when the seeds were sown. Three seeds per pot (11.43 x 11.43 cm Jumbo Junior pots, Belden Plastics, St. Paul, MN) were planted in BM2 germination mix (Berger, Quebec Canada) for the greenhouse or pasteurized field soil (Waukegin silt loam) collected from the University of Minnesota St. Paul campus (44°59'17.8" N lat., -93°10'51.6" W long.) for the field. The pots, rather than individual seeds, were considered experimental units.

Table 1. Fruit type, species, and genotype names for *Prunus* germplasm tested in the germination experiment. All seed was collected at the University of Minnesota research plots in Excelsior, MN in 2012.

Fruit Type	Species	Genotype
Apricot	*P. armeniaca* L.	'Moongold'
		'Sungold'
		'Westcot'
Tart Cherry	*P. cerasus* L.	'Bali'
		'Mesabi'
		'Meteor'
		'N81755'
		'Suda'
Plum	*P. americana* L.	'Hazel'
	P. besseyi x *P. hortulana* L.	'Compass'
	P. domestica L.	'Mount Royal'
		'Opal'
		'Stanley'
		'Todd'
	P. munsoniana Wright and Hedrick	'Whittaker'
	P. nigra Aiton	'Bounty'
	Prunus spp. L.	'Alderman'
		'Gracious'
		'Hennepin'
		'La Crescent'
		MN598
		'Monitor'
		'Pipestone'
		'Redcoat'
		'South Dakota'
		'Superior'
		'Tecumseh'
		'Toka'
		'Underwood'
		'Winona'

After planting, a warm stratification treatment was applied to all pots at 20-25°C (day/night) in darkness for two weeks beginning week 41 in 2012. Pots were monitored and watered as necessary for the duration of warm stratification. After warm stratification, 4 pots of each treatment were divided for the greenhouse or field environments. Pots for the greenhouse environment were placed in a cooler (5°C; complete darkness) for a 112-day period of cold stratification, week 43, 2012 – week 7, 2013. During the cold stratification period, pots were monitored for seed germination and hand-watered as necessary. Pots for the field were covered with fine netting to prevent rodents and other herbivores from destroying the seeds. These pots were planted in a randomized complete block design into the field at the University of Minnesota Saint Paul, MN (44°59'18.4"N,

-93°10’21.5”W) in week 43, 2012. Pots in the field were buried with the soil level of the pots equal to the field soil level. As a result, about 2.5 cm of the rim for each pot was above the soil line. Pots in the field were overwintered. Average monthly soil temperature (10.2 cm depth) and the number of days with average temperatures above and below 0°C per month during this experiment were calculated from average soil temperatures at the University of Minnesota St. Paul Climatological Observatory (44°59’25.1” N long., -93°10’35.2” W lat.; Minnesota DNR, 2016; Table 2).

When the cold stratification period in the cooler was completed, pots were placed in a randomized complete block design in the greenhouse. The average day/night temperature for the greenhouse environment was 17.8°C. Germination was monitored for a seven-week period. A seed was considered germinated once the plumule was observed above the soil surface (Huntzinger, 1971). The week each seed germinated was denoted using different colored toothpicks placed next the seedling for each week of germination assessment. The average number of weeks for germination for each pot was calculated by: summing the number of weeks to germination for all germinated seedlings and then dividing by the number of seedlings that germinated in the pot. If a seed did not germinate, it was not used to calculate average number of weeks for germination.

In the spring of 2013, the pots in the field were monitored for germination *in situ*. Starting when the first seedling’s pumule became visible, germination for all pots was monitored for seven weeks. Nongerminated seeds were evaluated for decay at the germination period. Average number of weeks to germination for individual seedlings was recorded with the same methodology as in the greenhouse.

Data Analyses. The statistical package R, version 3.3.3 (2017-03-06), was used for statistical analyses. Data within a fruit type (i.e. apricot, tart cherry, and plum) were analyzed using univariate, linear model type III analysis of variance (ANOVA). Block was considered a fixed effect nested within germination environment. Germination percentage data was transformed using arcsine square root transformation and all analyses, except for correlations, used the transformed data. To correct for non-constant variance (heteroscedasticity), White’s correction for heteroscedasticity was used. If the genotype x germination environment x scarification interaction was significant, genotype means within a given environment and scarification treatment were compared using Tukey’s Honest Significant Difference test (HSD) at a significance $\alpha \leq 0.05$. If genotype x scarifica-

Table 2. Average monthly soil temperature (°C) from Oct. 2012 to May 2013 at 10.2 cm depth and number of days with average soil temperatures below and above 0°C. Temperature data were recorded at the University of Minnesota Saint Paul campus (Minnesota DNR, 2016).

Month	Year	Avg. Temp.	Days below 0°C	Days above 0°C
Oct.	2012	10.5	0	31
Nov.	2012	3.3	6	24
Dec.	2012	0.4	3	28
Jan.	2013	-1.9	27	4
Feb.	2013	-1.9	28	0
March	2013	-0.3	29	1
April	2013	3.6	5	15[z]
May	2013	13.8	0	31

[z] Temperature probe failed to record ten days in April.

tion, or the genotype x germination environment x scarification treatment interactions were significant, single degree of freedom linear contrasts were used to compare non-scarified and scarified seed germination within a genotype. Germination percentage data within a fruit type were compared using Spearman correlations ($\alpha \leq 0.05$) between field and greenhouse environments.

Results

Apricots. The main effects of germination environment ($p<0.001$) and cultivar ($p<0.05$) significantly affected % germination in the apricot fruit type. Scarification did not have a significant effect ($p=0.096$). The environment x cultivar interaction ($p<0.05$) was significant. All other interactions were not significant: environment x block ($p=0.71$), environment x scarification ($p=0.29$), cultivar x scarification ($p=0.42$), and environment x cultivar x scarification ($p=0.98$). Since the environment x cultivar interaction was significant, cultivar means were calculated and compared within a germination environment across scarification treatments. Average % germination was higher in the greenhouse environment (70.8%) than in the field (37.5%, Table 3); nongerminated seeds had decayed. Average germination in the greenhouse ranged from 91.7% to 45.8% with 'Moongold' and 'Sungold' differing significantly from 'Westcot' (Table 3). In the field environment, mean germination rates ranged from 66.7% to 20.8% with 'Sungold' differing significantly from 'Moongold' and 'Westcot' (Table 3). 'Sungold' had the highest germination in both environments. Regardless of the environment, most apricot seed germinated by the end of week 2 (data not shown).

Tart cherries. Within the tart cherry fruit type, main effects of the greenhouse and field environments ($p=0.45$), cultivar ($p=0.36$), and scarification (0.06) did not significantly affect germination. The interactions environment x block ($p=0.89$), environment x cultivar ($p=0.51$), environment x scarification ($p=0.46$), cultivar x scarification ($p=0.30$), and environment x cultivar x scarification ($p=0.14$) were also not significant. In both environments, germination of tart cherry genotypes was ≤ 33.3% with no significant variation among genotypes (data not shown). Average % germination across environments, tart cherry cultivars, and scarification treatments was 4.3% (data was pooled for all main effects and, thus, is not shown). All nongerminated seeds had decayed. On average, all tart cherry seeds germinated by week 2, 2013 (data not shown), similar to apricots.

Plums. Within the plum fruit type, main effects of cultivar ($p<0.001$) and scarification treatment ($p<0.001$) had significant effects on % germination whereas environment ($p=0.14$) did not. The interactions environment x block ($p=0.55$) and environment x scarification ($p=0.80$) were not significant whereas environment x cultivar ($p<0.001$) and environment x cultivar x scarification ($p<0.05$) were significant. Since the environment x cultivar x scarification interaction was significant, average % germination among genotypes were examined within an environment x scarification treatment combination. Averages for non-scarified seed of

Table 3. Average % seed germination after cold stratification for apricot seeds (pooled across non-scarified and scarified treatments) in the greenhouse and field environments.[z]

Cultivar	Greenhouse	Field
'Moongold'	91.7 a	20.8 b
'Sungold'	75.0 a	66.7 a
'Westcot'	45.8 b	25.0 b
Mean	70.8	37.5

[z] Means within columns followed by common letters do not differ at the 5% level by Tukey's HSD.

Table 4. Average percent seed germination after cold stratification for non-scarified and scarified plum seeds in the greenhouse and field environments.

	Greenhouse		Field	
Cultivar	Non-scarified[z]	Scarified[z]	Non-scarified[z]	Scarified[z]
'Hazel'	25.0 cdef	50.0 ab	75.0 a*[y]	16.7 ab*[y]
'Compass'	33.3 bcdef*[y]	83.3 ab*[y]	50.0 abc*	8.3 ab*
'Mount Royal'	41.7 abcdef	25.0 ab	0.0 d	0.0 b
'Opal'	100.0 a	75.0 ab	0.0 d	8.3 ab
'Stanley'	33.3 bcdef	25.0 ab	0.0 d	0.0 b
'Todd'	41.7 abcdef	58.3 ab	16.7 bcd	8.3 ab
'Whittaker'	58.3 abcdef	91.7 a	41.7 abcd	41.7 ab
'Bounty'	41.7 abcdef	75.0 ab	66.7 a*	33.3 ab*
'Alderman'	16.7 def*	58.3 ab*	0.0 d	16.7 ab
'Gracious'	16.7 def*	58.3 ab*	16.7 bcd	33.3 ab
'Hennepin'	83.3 abc	50.0 ab	58.3 ab	66.7 a
'La Crescent'	91.7 ab	91.7 a	16.7 bcd	16.7 ab
'MN 598'	25.0 cdef	50.0 ab	0.0 d	0.0 b
'Monitor'	25.0 cdef	33.3 ab	0.0 d*	33.3 ab*
'Pipestone'	41.7 abcdef	50.0 ab	0.0 d	16.7 ab
'Red Coat'	8.3 ef	41.7 ab	0.0 d*	33.3 ab*
'South Dakota'	75.0 abcd	75.0 ab	75.0 a*	33.3 ab*
'Superior'	8.3 ef*	75.0 ab*	0.0 d	16.7 ab
'Tecumseh'	8.3 ef	16.7 b	0.0 d	0.0 b
'Toka'	66.7 abcde	66.7 ab	58.3 ab*	25.0 ab*
'Underwood'	25.0 cdef	50.0 ab	0.0 d	8.3 ab
'Winona'	0.0 f*	75.0 ab*	8.3 cd	8.3 ab
Mean	39.4	58.0	22.0	19.3

[z] Means within columns followed by common letters donot differ at the 5% level.
[y] An asterisk refers to a significant difference ($p<0.05$) within a genotype and germination environment across scarification treatments.

plum genotypes ranged from 0.0% for 'Winona' to 100.0% for 'Opal' with a pooled average of 39.4% (Table 4). The range in mean germination of scarified plum seeds in the greenhouse was 16.7% for 'Tecumseh' to 91.7% for 'La Crescent' and 'Whittaker' (Table 4). The main effect means for scarified seed was 55.7% and 39.4% for non-scarified seed (Table 4). There were significant differences for % germination between non-scarified and scarified seed for 'Alderman', 'Compass', 'Gracious', 'Superior', and 'Winona' ($p<0.05$; Table 4). All nongerminated seeds had decayed.

In the field environment, average germination percentages for non-scarified seed ranged from 0.0% for 'Alderman', MN598, 'Monitor', 'Mount Royal', 'Opal', 'Pipestone', 'Red Coat', 'Stanley', 'Superior', 'Tecumseh', and 'Underwood' to 75% for 'Hazel' and 'South Dakota' (Table 4). Average % germination for scarified seed ranged from 0.0% for MN598 and 'Tecumseh' to 66.7% for 'Hennepin' (Table 4). Main effect means for non-scarified and scarified plum seed were 22.0% and 19.3%, respectively (Table 4). There were significant differences for % germination between non-scarified and scarified seed for 'Bounty', 'Compass', 'Hazel', 'Monitor', 'Red Coat', 'South Dakota', and 'Toka' (Table 4).

Correlations. The only significant correlation between % germination in the greenhouse and field was for plums (r=0.19, p<0.05, data not shown). The remaining Spearman correlation coefficients were not significant (p>0.05; data not shown).

Discussion

Successful germination is the first step towards establishing a self-sustaining population and, as a result, species with higher % germination compared to native species may be more likely to become invasive (Hock et al. 2015). In our experiment, seed germination across environments for apricots was high whereas tart cherries were low. The plum genotypes we studied had variable germination, which is perhaps due to the diverse genetic background (Table 1). Some plum genotypes like *P. americana* 'Hazel', *P. munsoniana* 'Whittaker', and Japanese-American hybrids 'South Dakota', and 'Hennepin' had high seed germination across both environments and scarification treatments. In contrast, *P. domestica* 'Mount Royal' and *P. spp.* 'Monitor' had variable germination percentages across environments and scarification treatments. In comparison to native species, genotypes with higher % germination across environments could potentially become invasive compared to genotypes with low germination (Hock et al. 2015).

Inbreeding depression could potentially provide an explanation for why low % germination among tart cherry genotypes was observed. Most tart cherry genotypes are self-compatible but naturally outcrossing and thus, inbreeding depression is possible in tart cherry progeny (Lansari and Iezzoni, 1990; Krahl et al. 1991). According to Baskin and Baskin (2015), inbreeding has a variable effect on germination; in some cases, inbreeding depression has a negative relationship with germination. Lansari et al. (1994) states that inbreeding depression in almond (*P. dulcis* Miller) can result in reduced seed germination. Inbreeding depression in the tart cherry genotypes tested could have played a role in the lower germination observed. Even though most tart cherry genotypes had low % germination, germination still occurred, thus not eliminating the potential to become invasive. Other factors that may affect a genotype's invasive potential include crop load, seed dispersal mechanism, and seedling establishment (Bullock et al. 2002; Deckers et al. 2008). According to Deckers et al. (2008) the invasive *P. serotina* has inconsistent crop loads but its avian dispersal system makes it highly effective at spreading throughout the landscape. Tart cherries are often consumed completely or damaged by birds (Lindell et al. 2012). The potential for seed dispersal via birds coupled with good stand establishment may result in higher invasive potential.

Germination can be impeded at many steps in the process. The uptake of water initiates germination (Chong et al. 1994). Hard seed coats or stony endocarps can prevent or reduce water uptake (Chong et al. 1994; Hartmann et al. 1997). The endocarp of stone fruits prevents the expansion of the embryo so no radical emergence can occur (Hartmann et al. 1997). These seed types often need to be cracked or softened through scarification to initiate water uptake and thus, germination (Chong et al. 1994; Hartmann et al. 1997). In our experiment, endocarps of seeds were mechanically scarified prior to planting. Scarification had a significant effect on germination of plum seed in both the greenhouse and field environments. However, scarification significantly increased % germination of some plum genotypes in the greenhouse but decreased germination in some plum genotypes in the field. In most cases, germination of non-scarified seed and scarified seed was similar in the field. A potential reason for this is the freeze-thaw cycle. According to Chong et al. (1994), scarification of the seed can result through the freeze-thaw action of the soil. During the overwintering period in our field experiment, the soil at a 10.2 cm depth oscillated above and below 0°C (Table 2). Scarification via freezing and thawing of the soil in the field could have been sufficient to crack the endocarp of non-scarified seeds and resulted in similar germination between non-scarified and scarified seed of most plum genotypes.

Kristiansen and Jenson (2009) observed greater germination for *P. cerasus* seeds with the endocarp removed whereas Grisez et al. (2008) reported that after 90 days of cold stratification, *P. armeniaca* seeds achieved 95% germination with an intact endocarp. McMahon et al. (2015) observed no significant difference for germination between non-scarified and scarified *P. angustifolia* seed and reasoned that the lower percentages of seeds germinating could have been caused by inadequate endocarp removal. For example, when Kristiansen and Jenson (2009) removed the entire endocarp from *P. cerasus* seed, there was a significant positive effect on germination. However, scarification did not have a significant effect on germination in both the apricot and tart cherry fruit types. In greenhouse and field environments of our study, scarification significantly affected plum germination. However, within most plum genotypes germination was not significantly affected by scarification in both environments. For most genotypes in our study, the combination of warm and cold stratification may have sufficiently overcome dormancy and eliminated the need for scarification. Higher germination was observed for scarified seed in most plum genotypes in the greenhouse whereas lower germination was observed for scarified seed in the field environment. Scarification of some plum genotypes' seed prior to planting in the field could have resulted in lower germination because scarification may have resulted in higher susceptibility of seeds to disease and other environmental pressures (i.e. temperature fluctuations) not present in the greenhouse. For most genotypes, there was not a significant difference for average number of weeks for germination between non-scarified and scarified seed. Germination percentages were similar and most seeds germinated within three weeks, thus indicating that some genotypes do not require scarification for successful germination.

Chong et al. (1994) states that moisture is the most important factor for initiation of seed germination and lack of consistent moisture during germination can result in drying of the seed leading to failed germination and potentially seed death. Across fruit types, we observed higher percent seed germination in the greenhouse than the field. In the greenhouse, pots were consistently monitored and watered whereas in the field watering ceased once the field soil froze and did not begin again until the soil thawed. Inconsistent moisture in our field soil could have resulted in lower germination across fruit types.

Lockley (1980) recorded a significant positive correlation between greenhouse and field for germination and seedling emergence of *P. virginiana* L., leading to the conclusion that germination in the greenhouse was indicative of germination in the field. If the environments in our germination experiment were correlated, germinated seed in the greenhouse could be predictive of germination under field conditions. This would be a useful tool for quickly screening multiple genotypes. However, we found that within most species there was no significant correlation for % germination between the two environments. There was a significant positive correlation between environments for the plums. However, this correlation coefficient was low ($r<0.20$) and, thus, germination in the greenhouse environment may not be an accurate predictor of field response. Further investigation is required.

Conclusions

Although successful germination is an important step in the invasion process, many factors contribute to the invasive potential of a species including vigor of seedlings, tendency to vegetatively propagate, herbivore pressure, crop load, and seed dispersal mechanisms (Deckers et al. 2008; Kolar and Lodge, 2001; Siemann and Rogers, 2001). As a result, high % germination does not necessarily mean that a genotype will become invasive. Many of the *Prunus* genotypes examined in this study will probably not become invasive due to poor and/or inconsistent germination. According to Brooks and Olmo (1997) tart cherry genotypes like 'Meteor' tended to be productive and bear regularly. On average, a 10 to 20-year-old tart cherry tree ('Montmorency') produces 36 kg to 45 kg of fruit (Me-Nsope, 2009). Seed production differences between years could greatly influence invasive potential, particularly since apricots do not set a fruit crop consistently across years due to early spring frosts during the bloom period (Hoover and Zins, 1998; Hoover et al., 2015). Even with relatively low germination, high fruit yields could result in large numbers of propagule units and thus, could potentially result in a moderate number of seedlings. Progeny from the plum genotypes *P. americana* 'Hazel', *P. munsoniana* 'Whittaker', and the hybrids 'South Dakota' and 'Hennepin' exhibited high germination across environments and years, indicating the potential to become invasive. Further research would be necessary to determine seedling stand establishment of these plums as well as the effects of enhanced fruit yield and/or germination differences across years in all tested genotypes.

Even though some genotypes examined in this experiment exhibit characteristics indicative of the potential to become invasive, escapes from cultivation by these genotypes have not yet been documented. Horticultural practices like mowing, tilling, hand pulling, and the application of herbicides can control the spread invasive species (Beasley and Pijut, 2010; Culley and Hardiman, 2007). As a result of these practices, horticulturalists may inadvertently be preventing the escape of *Prunus* genotypes into surrounding environments. However, winter-hardy *Prunus* genotypes may become invasive if present in an abandoned field or in a circumstance where horticultural control practices are not applied, as has occurred with the invasive, ornamental Pyrus *calleryana* Decne in parts of the United States (Culley and Hardiman, 2007; Taylor et al. 1996). Another potential reason that these genotypes have not escaped cultivation is that these genotypes are not extensively cultivated in the landscape. This lack of cultivation results in a low number of propagules that could potentially develop self-sustaining populations.

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Diurnal Patterns of Photosynthesis and Water Relations for Four Orchard-Grown Pomegranate (*Prunica granatum* L.) Cultivars

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Additional index words: berries, cultivars, germplasm, physiology, USDA

Abstract

Long-term drought, coupled with tighter regulations on limited water resources have caused growers to seek drought tolerant cultivars of common tree crops in California. Yet information on pomegranate physiology is lacking, even though it is grown throughout the world in various climates. The purpose of this research was to determine the effect of time of day and cultivar on pomegranate photosynthesis and water relations, and calculate values for water-use efficiency, defined as photosynthetic carbon gain divided by water lost during transpiration. The study utilized four field-grown cultivars in their fourth year of growth ('Eversweet,' 'Haku Botan,' 'Parfianka,' and 'Wonderful'), in Riverside, California. Variables analyzed included photosynthesis, stomatal conductance, transpiration, instantaneous water-use efficiency, intrinsic water-use efficiency, and pre-dawn and midday water potential. Differences were detected for time of day, with higher rates of assimilation, transpiration, and stomatal conductance in morning. Intrinsic water-use efficiency was higher in the afternoon compared to the morning. There were also differences among cultivars for stomatal conductance and transpiration during the morning but not during the afternoon, with 'Eversweet' having significantly lower rates of stomatal conductance and transpiration than 'Parfianka': other cultivars were intermediate. These results further our understanding of how pomegranate cultivars function on a physiological level during different times of the day, and suggest that efficiency of production can be improved through cultivar selection.

Increasing global temperatures coupled with unpredictable changes in climate threaten food security globally (Altieri and Nicholls, 2017). California has experienced extreme drought conditions for several years, causing fruit growers to face water limitations affecting production and leading to hundreds of millions of dollars in crop revenue losses in 2016 alone (Medellín-Azuara et al., 2016). To lessen the impacts of climate change and increasing temperatures on food security, it is important to utilize diversified cropping systems to reduce vulnerability to extreme climatic events as experienced in California and other regions of the United States (Altieri and Nicholls, 2017). Long term drought in California and other regions of commercial tree fruit production in the United States has caused growers to abandon fruit crops and seek alternatives with less water demand in the short term. Options for mitigating long term drought in California have included crop abandonment, stress irrigation, switching to alternative crops with new plantings (Medellín-Azuara et al., 2016) and utilization of lower quality secondary water sources.

It has been proposed that physiologists and breeders focus on increasing the efficiency of water use in agriculture (Wallace, 2000).

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Improving production efficiency and drought tolerance through cultivar or variety selection has been proposed in tree crops, such as citrus (Savé et al., 1995) *Prunus* species (Rieger and Duemmel, 1992), dates (Djibril et al., 2005), and coffee (DaMatta, 2004). Because tree crops can have a considerable amount of variability in terms of physiological traits, it is useful to study diversity in crop species to determine if there are cultivars that use water more efficiently or are able to be productive in stressful conditions. Because pomegranate (*Punica granatum* L.) is a drought tolerant crop, especially once established (Stover and Mercure, 2007), it is a candidate crop for growers wishing to switch from more water-intensive species, such as avocado, citrus or almond.

Pomegranate is a drought tolerant crop that has been grown in California since the Spanish missionaries arrived from Spain and planted mongrel seeds at missions up and down the coast (Day and Wilkins, 2009; Stover and Mercure, 2007). The pomegranate variety collection located at the United States Department of Agriculture - Agricultural Research Service (USDA-ARS) National Clonal Germplasm Repository, Davis, CA (NCGR) conserves about 200 genotypes of pomegranate sourced from all over the world, many of which have unique phenotypic traits (Stover and Mercure, 2007). Experiments have demonstrated differences in morphology and vegetative growth traits, including differences in relative chlorophyll content, plant vigor, and branching habit, which can be observed during propagation and in the field (Chater et al., 2017). Although available literature on pomegranate physiology is scarce, research has shown that there can be differences among cultivars for many physiological traits of pomegranate in other collections, including transpiration rate, stomatal conductance, water use efficiency, photosynthetic rate and chlorophyll content (Drogoudi et al., 2012). The objectives of this study were 1) to evaluate four unique pomegranate cultivars for physiological field performance in a semi-arid agroecosystem during morning and afternoon hours; and 2) to determine if there are differences among cultivars for physiological traits that would be conducive to commercial crop production in drought conditions.

Materials and Methods

Site conditions. The site was located at the Department of Agricultural Operations in field 5E (33° 58’ 9.39” N, 117° 20’ 46.93” W) at University of California, Riverside. Riverside is a semi-arid climate with hot, dry summers and cool winters. The mean annual precipitation of the area is 262 mm and mean maximum temperatures are 28.1 and 35.6° C for June and Aug., respectively. Mean minimum temperatures are 12.9 and 18.1° C for June and Aug., respectively. The soil is a sandy loam with good drainage and was previously an established lemon grove. All trees were growing under natural light, outside in field conditions and were irrigated three times per week. All experimental trees were in their third and fourth years of growth and were located on the inside of the grove, with at least one border tree acting as a buffer to reduce the edge effect.

Plant material. An established pomegranate cultivar trial was utilized for this study during years three and four of tree development. The cultivars in the study were ‘Eversweet,’ ‘Haku Botan,’ ‘Parfianka,’ and ‘Wonderful’ (Table 1). All plants were propagated as dormant hardwood cuttings at the same time in winter of 2012 and sourced from the National Clonal Germplasm Repository, Davis, CA, USA. All trees included were mature and had fruit set typical of trees in commercial production. Trees were grown under conventional commercial management practices and fertilized in spring with urea and sulfate of potash, totaling 31.75 kg N and 34 kg K per year, respectively, over approximately 0.81 ha. The healthiest tree in each of three blocks was selected (among 15 trees total per cultivar in the trial). The trial was planted in a randomized complete block design.

Table 1. Descriptions of the four pomegranate cultivars sourced from the USDA-ARS National Clonal Germplasm Repository used in this study. Variables known include county of origin, countries of commercial production, acidity, flavor, peel color, aril color, and seed hardness.

Cultivar	Country of origin	Countries of commercial production	Acidity	Flavor	Peel color	Aril color	Seed hardness
Eversweet	USA	USA	Very Low	Sweet and yellow	Pink	Pink	Soft
Haku Botan	Japan	Japan, USA	Very High	Sour yellow	White/	White	Hard
Parfianka	Turkmenistan	USA, Australia	High	Sweet-tart	Red	Red	Soft
Wonderful	USA	USA, Chile, Peru, Israel, Mexico, Argentina, South Africa, Uruguay, Turkey, Italy, Spain, Greece	Medium-high	Sweet-tart	Red	Red	Medium hard

'Wonderful' is the industry standard in many countries and was chosen as a control in the experimental cultivar field trial. The other cultivars were selected for their unique phenotypes. 'Eversweet' is a dwarf-like cultivar bred for coastal climates, with pink fruit peel and aril color, and soft seeds. 'Haku Botan' is an ornamental Japanese cultivar that has an upright growth habit with double white flowers and darker green foliage than most other pomegranate cultivars and lacks visible anthocyanin pigments in stem, leaves and fruit. The fruit is very acidic and very light yellow in color. 'Parfianka' is an internationally-renowned cultivar that has a bright red peel and arils with soft seeds and a balanced sweet-tart flavor. The tree is extremely thorny and has a bushy, highly branched growth habit with smaller leaves than other pomegranate cultivars. 'Wonderful' is commercially widely-grown, and in the USA it accounts for approximately 90-95% of production. It is a highly vigorous, thorny tree that has high yield with red fruit and red seeds with moderate seed hardness and a sweet-tart flavor. The growth habit is willowy, with a tendency to sucker at the base of the tree.

Photosynthesis measurements. During fruit development (late June through Aug.), an infrared gas analyzer (6400, Li-Cor, Lincoln, NE, USA) was used to measure maximum rates of net CO_2 assimilation (A), stomatal conductance (g_s), and transpiration (E) during the morning (9:00 - 12:00 hr) and afternoon (15:30 – 17:30 hr). Morning photosynthetically active radiation (PAR) ranged from 1500-1600 μmol $m^{-2}\cdot s^{-1}$ photosynthetic photon flux density (PFD), while afternoon PAR was 1990 μmol $m^{-2}\cdot s^{-1}$ PFD. Morning measurements were pooled for the four cultivars, which occurred on 22, 23 Aug. 2015 and 26 June 2016. Afternoon measurements were taken on 30 June 2016, which was representative of a typical summer afternoon in Riverside (30-34 °C; 32-37% Relative Humidity). Gas exchange characteristics were measured on two leaves per tree and a minimum of three trees per cultivar. All leaves were collected for leaf area, which was quantified on a leaf area meter to normalize photosynthesis data (Li-Cor, Lincoln, NE, USA).

Only the most recently fully-formed, sun-exposed leaves were selected for this study. Cuvette temperatures were allowed to vary with field conditions. Leaves were measured in a chamber that provided 1500 µmol $m^{-2}\cdot s^{-1}$ (PFD). Instantaneous water-use efficiency was calculated as $A\cdot E^{-1}$ and intrinsic water-use efficiency was calculated as $A\cdot g_s^{-1}$.

Stem water potential measurements. Predawn and midday stem water potential measurements were recorded for each data tree. For predawn water potential, non-actively growing shoots were covered with a plastic bag for 10 min before being pruned, placed in a sealed plastic bag and kept in a cooler bag until transferred to an indoor environment for plant moisture stress measurements with a pressure chamber (Model 1000 Pressure Chamber, PMS Instrument Company, Albany, USA). For afternoon stem water potential measurements, canopy-shaded non-actively growing shoots were covered with a plastic bag for 10 min before being pruned, placed in a sealed plastic bag and kept in a cooler until immediately transferred to a cool lit, indoor environment. Stem water potential was immediately measured after being removed from the cooler bag. One stem was measured from three individual trees per cultivar, for a total of three trees, for predawn and midday stem water potential.

Statistical analysis. All variables were analyzed with Analysis of Variance (ANOVA). When ANOVA indicated significant differences, post-hoc comparisons were performed utilizing Tukey's honestly significant difference (HSD) with an experiment-wise type 1 error rate of α = 0.05. Relationships between all variables were analyzed using linear regression (α = 0.05), with relationships among parameters determined using general regression with Minitab Software, version 16 (Cov-

Table 2. Mean values of maximum rates of net CO_2 assimilation (µmol CO_2 $m^{-2}\cdot s^{-1}$, *A*), stomatal conductance (mol H_2O $m^{-2}\cdot s^{-1}$, g_s), transpiration (mmol H_2O $m^{-2}\cdot s^{-1}$, *E*), intrinsic water use efficiency ($A\cdot g_s^{-1}$), and instantaneous water-use efficiency ($A\cdot E^{-1}$) for four pomegranate cultivars grown in Riverside, CA USA. All measurements were made in the morning and afternoon hours during fruit development in summers of 2015 and 2016.

Cultivar	*A*	g_s	*E*	$A\cdot g^{-1}$	$A\cdot E^{-1}$
Morning					
Eversweet	13.27 ± 1.18[z]	0.10 ± 0.02b[y]	1.73 ± 0.38b	147.4 ± 24.8	8.25 ± 1.27
Haku Botan	16.49 ± 1.02	0.15 ± 0.02ab	2.41 ± 0.15ab	112.8 ± 4.7	6.85 ± 0.20
Parfianka	19.80 ± 1.57	0.18 ± 0.03a	2.66 ± 0.26a	117.4 ± 13.3	7.52 ± 0.33
Wonderful	19.38 ± 3.71	0.15 ± 0.02ab	2.04 ± 0.17ab	124.1 ± 9.5	9.44 ± 1.66
P-Value	0.289	0.049	0.037	0.540	0.238
Afternoon					
Eversweet	11.98 ± 1.29	0.07 ± 0.02	1.85 ± 0.40	170.0 ± 22.8	6.87 ± 1.03
Haku Botan	10.24 ± 0.78	0.05 ± 0.02	1.29 ± 0.11	208.5 ± 32.4	8.10 ± 1.01
Parfianka	11.78 ± 1.32	0.07 ± 0.03	1.63 ± 0.41	193.4 ± 26.2	7.79 ± 1.15
Wonderful	9.24 ± 1.10	0.04 ± 0.01	1.10 ± 0.17	235.4 ± 6.4	8.34 ± 0.59
P-Value	0.439	0.334	0.446	0.762	0.442
Time of day					
Morning	17.74 ± 1.24a	0.15 ± 0.01a	2.26 ± 0.15a	124.3 ± 7.1b	8.05 ± 0.53
Afternoon	10.81 ± 0.60b	0.06 ± 0.01b	1.47 ± 0.15b	201.8 ± 12.5a	7.78 ± 0.45
P-Value	< 0.001	< 0.001	0.001	< 0.001	0.708

[z] Values expressed as means ± standard error (Morning n = 15, Afternoon n = 12).
[y] Values within columns and variables followed by common letters do not differ significantly by Tukey's HSD test ($P < 0.05$).

entry, UK). Block was coded as a random effect and interaction terms were included in the models. For the purposes of this work, the R^2 value is the proportion of variation in one variable that is explained by the variation in the regressor variable. Regression models were fit to determine differences in slope coefficients and constants (y-intercept) among variables.

Results and Discussion

The pomegranate cultivars were actively photosynthesizing and transpiring during morning and afternoon hours during all days of data collection. There were significant differences among cultivars for morning measurements only (Table 2). 'Eversweet' had significantly lower rates of g_s ($P = 0.049$) and E ($P = 0.037$) than 'Parfianka' during the morning. There were no other differences detected for gas exchange variables among cultivars.

Time of day significantly affected pomegranate leaf physiology (Table 2). Morning rates of A were 64% higher on average than during the afternoon ($P < 0.001$). Similarly, rates of g_s during the morning were 250% higher on average than rates of g_s during the afternoon ($P < 0.001$). Rates of E were 54% higher on average in morning than in afternoon ($P = 0.001$). In contrast, intrinsic water use efficiency was 62% higher in afternoon than in morning ($P < 0.001$). Instantaneous water-use efficiency was similar in morning and afternoon. According to our findings, net CO_2 assimilation and intrinsic water use efficiency were more variable in the afternoon.

Stem water potential was significantly different among cultivars ($P = 0.012$). 'Haku Botan' had higher stem water potential than 'Eversweet' and 'Wonderful.' 'Parfianka' had a higher stem water potential than 'Wonderful' (Fig. 1). There were no differences in stem water potential among cultivars for midday measurements. Although the difference among means in pre-dawn and afternoon were of similar magnitude, variability was much higher in afternoon than during pre-dawn, leading to no significant differences. There was a large difference between time of day for stem water potential ($P < 0.001$). Stem water potential was much less negative in morning than in midday, with average readings of -0.825 and -2.420 MPa, respectively.

There were positive and negative correlations between physiological variables for morning, afternoon and for data pooled for the two times of day. The relationship between A and g_s was positive and linear and significant for morning measurements ($P < 0.001$, $R^2 = 0.7275$), afternoon measurements

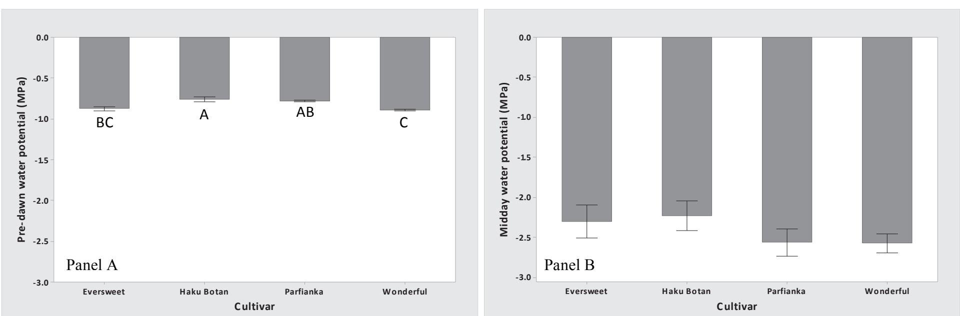

Figure 1. Mean stem water potential (MPa) of four pomegranate cultivars grown in Riverside, CA USA (n = 3 for each cultivar). All stem water potential measurements were made in the pre-dawn (Panel A) or afternoon (Panel B) hours during fruit development in summer of 2015 and 2016. Values followed by common letters do not differ significantly ($P < 0.05$).

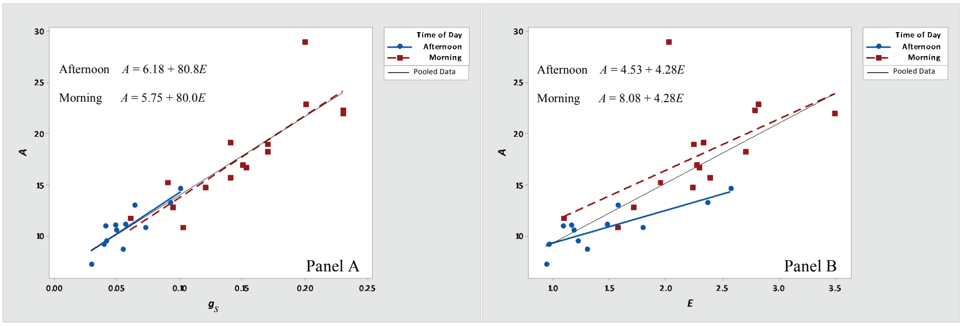

Figure 2. Relationships between maximum rates of net CO_2 assimilation (μmol CO_2 $m^{-2}\cdot s^{-1}$, *A*), stomatal conductance (mol H_2O $m^{-2}\cdot s^{-1}$, g_s) (Panel A), and net CO2 assimilation (μmol CO_2 $m^{-2}\cdot s^{-1}$, *A*) and transpiration (mmol H_2O $m^{-2}\cdot s^{-1}$, *E*) (Panel B). Data represents four pomegranate cultivars grown in Riverside, CA USA (n = 27). All leaf photosynthesis measurements were made in the morning or afternoon hours during fruit development in summer of 2015 and 2016.

($P = 0.001$, $R^2 = 0.7178$) and for pooled data ($P < 0.001$, $R^2 = 0.8533$) (Fig. 2). For *A* and g_s slopes and intercepts did not differ significantly for time of day. There was also a weak, positive correlation between *A* and *E* in the morning ($P = 0.019$, $R^2 = 0.3560$), a stronger relationship in the afternoon ($P = 0.001$, $R^2 = 0.6809$) and a moderately strong relationship for pooled data ($P < 0.001$, $R^2 = 0.5893$), each of which had a weaker relationship than between *A* and g_s. Intercepts ($P = 0.025$), but not slopes, for the relationship between *A* and *E* differed between morning and afternoon. There was a significant interaction between time of day and cultivar for g_s ($P = 0.05$). Eversweet' had similar g_s regardless of time of day, whereas g_s was much higher during the morning for the other cultivars (Fig. 3).

The objectives of this study were to evaluate four pomegranate cultivars for field performance and to determine differences among them for leaf physiological traits. Our findings suggest that all cultivars evaluated in this field study function satisfactorily on an eco-physiological scale for commercial production purposes if the industry standard, 'Wonderful,' is used as the standard. Physiological trait values obtained for 'Wonderful' were much different than those reported for purportedly the same cultivar grown in Greece (Noitsakis et al., 2016). Values were typically of the same order of magnitude, which suggests differences in climate or cultural practices between the two sites may have influenced results because the instrumentation in the two studies are normally well-calibrated against a standard. We found evidence that there are differences among cultivars for physiological traits including stomatal conductance, transpiration and pre-dawn water potential. Values for physiological traits were generally similar in other studies (Hepaksoy et al., 2000; Rodríguez et al., 2012). Strong differences were also detected for time of day, with higher rates of assimilation, transpiration, and stomatal conductance in the morning than afternoon. Intrinsic water-use efficiency was higher in afternoon compared to morning. There were also differences among cultivars for stomatal conductance and transpiration during the morning but not during the afternoon, with 'Eversweet' having significantly lower rates of stomatal conductance and transpiration than 'Parfianka,' and other cultivars were intermediate. Because the larger differences occurred in the afternoon, primarily for g_s and *E* which describe water loss, afternoon water loss characteristics offer a promising direction for improving water-use efficiency during cultivar selection.

The most interesting finding of this field study is that there are differences among cultivars for important leaf physiological traits, such as E, g_s and water potential. This finding suggests there may be other cultivars in the national germplasm or in other germplasm collections that have even greater production efficiencies than those represented in this study. This finding is important, not only for growers looking for crops and cultivars that use water more efficiently and sustainably, but also for breeders who can use this information for genotype selection. Hepaksoy et al. (2000), studying cultivars 'Lefon,' 'Kadi,' 'Keyiz,' 'Seedless,' 'Siyah,' and 'Koycegiz,' reported that transpiration rate and water use efficiency of pomegranate are correlated with fruit cracking, which means that these cultivars demonstrating differences among these physiological traits should be followed in the field to determine their effects on pomegranate's most destructive physiological disorder, fruit cracking. The next step in this discovery of differences in leaf physiological traits is to investigate why on a genomic or physiological scale some cultivars are more water efficient.

Although literature regarding pomegranate leaf physiology is limited, the results of this study support previous pomegranate cultivar field studies with other germplasm collections, identifying differences among cultivars (Drogoudi et al., 2012). Another interesting finding in this study is that we were able to demonstrate that pomegranates, like other tree fruit crops, fix most carbon in the morning to take advantage of the mild, high light conditions. During the warmer afternoons carbon fixation significantly decreases, which is attributed to stomata closing to reduce water loss in the dry heat of inland Southern California.

Stem water potential values reported in this present study agree with other mid-day

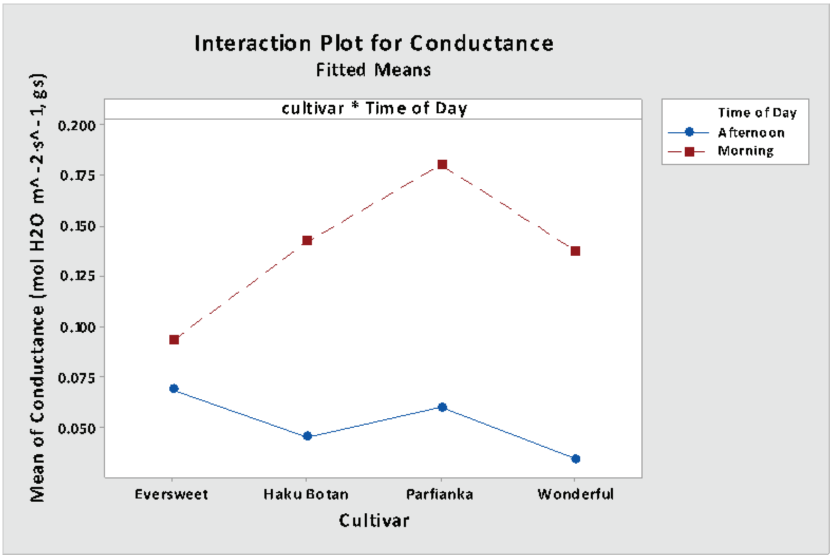

Figure 3. Factorial plot with stomatal conductance (mol H_2O $m^{-2}\cdot s^{-1}$, g_s) as the response variable, visualizing the interaction between time of day and cultivar. The interaction between cultivar and time of day was significant (P-value = 0.05). Data represents four pomegranate cultivars grown in Riverside, CA USA (n = 27) at different times of day (afternoon and morning). All leaf photosynthesis measurements were made in the morning or afternoon hours during fruit development in summer of 2015 and 2016.

values in the literature for pomegranate (Hepaksoy et al., 2000; Rodríguez et al., 2012). These may be the first values for water potential in pre-dawn hours for pomegranate, however there are reports for afternoon water potential. There were significant differences among cultivars for morning, but not afternoon, water potential. These findings indicate there may be differences in water uptake at night as well as differences in water loss at night. This could be a result of differences in root uptake or structure or stomatal number and/or size of stomatal aperture at night compared to other cultivars. Because vapor pressure deficit (VPD) can remain high (ranging from 1.56 to 2.49 kPa) at night in Riverside, CA, it is understandable that there could be differences in these stomata-based factors at night as well (Dawson et al., 2007).

We studied four unique pomegranate cultivars displaying very different phenotypes and found some interesting differences among them in terms of physiological traits and water relations. Despite these differences, the cultivars investigated performed similar to 'Wonderful' in a semi-arid climate, which means they may have potential in commercial orchards. Because we found differences among cultivars for various leaf physiological traits during certain times of day, the next steps would be to investigate these traits in additional cultivars, but to also carry out these physiological and water relations measurements during different times of year as well as different times of day. It would also be important to investigate these cultivars on molecular, morphological or anatomical scales to determine the underlying causes of these differences among cultivars for breeding purposes, specifically for marker assisted selection (MAS).

This investigation was the first of its kind to evaluate diurnal patterns in photosynthesis and water relations in California-grown 'Wonderful' and other pomegranate cultivars available on the market and sold by American nurseries. These results further our understanding of how pomegranate trees function on a physiological level among unique cultivars and during different times of the day in a semi-arid climate, and suggest that efficiency of production can be improved through cultivar selection. We emphasize that the strongest differences among cultivars in leaf gas exchange occurred in the morning, and largely involve water loss traits. More cultivars should be evaluated for their production efficiency using experimental cultivar trials to identify those that are productive under high temperature conditions with less applied irrigation water.

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About The Cover:

'Emma K' black walnut (*Juglans nigra*) is a commonly-grown cultivar for commercial nut production in the Midwestern region of the United States. This cultivar was selected for its large kernel size (> 35% by weight), relatively thin nut shell, and productivity. Grafted trees may start bearing a few nuts in the second year after planting, whereas seedlings do not commonly produce a nut crop until seven years after planting. Black walnuts are mechanically harvested with a tree shaker, collected, hulled, and then dried before cracking. About 13.7 million kg of black walnuts are harvested annually from 15 states, resulting in 1.3 t of marketable kernels. Photo by Michele Warmund, University of Missouri.

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Characterization of Southern Highbush Blueberry Floral Bud Cold Hardiness through Dormancy in a Sub-Tropical Climate

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Abstract

Southern highbush blueberry (SHB; *Vaccinium corymbosum* L. interspecific hybrids) is highly susceptible to freeze damage in sub-tropical climates, but the process is poorly understood. To address the issue, freeze tolerance experiments were conducted on two common cultivars of SHB, Emerald and Farthing, during the winters of 2015-16 and 2016-17. Floral buds (attached and excised from stems) were preconditioned overnight at 4.0 °C or -2.0 °C and exposed to temperatures of -3.0 to -21.0° C. The samples were then stored at 4.0 °C for a week and afterwards examined for bud damage. A lethal temperature threshold of 50% (LT_{50}) was calculated on visual ratings for damage. Cold hardiness varied with preconditioning, bud type (attached or excised), and sampling date, and ranged from -6.8 °C to -20.2 °C in both cultivars. On average, LT_{50} was lower in the attached than in the excised buds and when buds were preconditioned at -2.0 °C than at 4 °C. Attached buds displayed increasing hardiness as the chill hours accumulated, until the buds began to swell, at which time the hardiness decreased. Cold hardiness did not change in the excised floral bud in either cultivar or year. Preconditioning increased the hardiness of the attached buds at -2 °C, which may be artificially inflated considering sub-tropical climate. This work shows that attached SHB floral buds are sensitive to chill hour accumulation and deacclimation. Whereas, excised buds did not respond with similar sensitivity. For SHB, the best estimation for hardiness can be obtained through attached buds preconditioned at 4 °C.

Southern highbush blueberries were bred from northern highbush blueberry (NHB; *V. corymbosum* L.) and low chill blueberries (e.g. *V. darrowii* Camp) for cultivation in sub-tropical climates (Lyrene and Sherman, 2000; Lyrene, 2008a). In the southeastern U.S., the mean temperature for the coldest three months of winter may be as high as 15 °C, whereas, NHB may not be productive unless a mean temperature of 10 °C is attained due to insufficient chilling (Lyrene and Sherman, 2000; Retamales and Hancock, 2012).

Northern Florida and southern Georgia producers grow SHB cultivars that require an average winter chill of ≤ 300 h. Chill is commonly calculated as an accumulation of hours between 0 °C to 7 °C (Chandler et al., 1937), which is the easiest and widely accepted method used by most producers to estimate chill hour accumulation. However, in sub-tropical climates there may be periods during winter months where temperatures are within the range for plant growth. For example, SHB cultivars with chill hour requirements of 300 h to 600 h were grown in a non-dormant production system in which only 69 and 118 chilling hours (<7 °C) were accumulated over two seasons, respectively (Reeder et al., 1998). All of the cultivars tested had sufficient vegetative and reproductive growth suggesting the SHB cultivars tested were not regulated by endodormancy and may flower when conditions for physiological and morphological development are met

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(Reeder et al., 1998). 'Emerald' will flower with above normal temperatures (> 15 °C) in late winter and early spring (Lyrene, 2008a) and in Georgia, 'Emerald' has been observed to flower and set fruit in the fall (E. Smith, personal communication). Concomitantly, *V. darrowii* and SHB are photoperiod sensitive and flowering was shown to be promoted by short day photoperiods (8 h) (Spann et al., 2003). These data show that SHB can flower without prolonged dormancy and with low to no accumulated chilling.

In Michigan, NHB harvest dates were observed to be a function of chill hour accumulation, low temperature threshold, and starting date for heat-unit accumulation (Carlson and Hancock, 1991). Their modeling suggested that blueberries tend to advance their harvest date with warm late winter temperatures. In sub-tropical climate, winter temperatures can be sufficiently warm to break dormancy of SHB, and without prolong periods of cold, may cause tight floral buds to have greater sensitivity to cold than blueberry grown in temperate climates.

Two important concerns in blueberry production are winter hardiness and susceptibility to spring frosts (Moore, 1993). Blueberry cold hardiness throughout dormancy has been reported on NHB and SHB cultivars grown in northern climes (Ehlenfeldt et al., 2009; Rowland et al., 2005; Rowland et al., 2008). However, low chill hour (< 300 h) SHB cultivars sensitivity to freezing temperatures, while dormant, is not well understood under sub-tropical field conditions. In sub-tropical growing regions the chill is not steadily accumulated and periods of warmth between cold can trigger plant growth. The objective of this study was to investigate two SHB cultivars, Emerald and Farthing, sensitivity to cold using freeze tolerance tests over two winter seasons. Our expectation was that lethal freezing temperatures, where 50% of the florets are damaged (LT_{50}), would be higher than what has been reported for SHB grown in northern climes (Ehlenfeldt et al., 2009).

Materials and Methods

Plant material. 'Emerald' and 'Farthing' SHB terminal fruit bud bearing shoots were harvested periodically from contiguous rows at a commercial blueberry farm in Lakeland, GA (31°06'28.00" N, 83°06'42.76" W) during the seasons 2015-16 (Nov. to Feb.) and 2016-17 (Nov. to Jan.). At the initiation of the experiment, the planting was in its fifth year of production under conventional farming practices for the southeastern U.S. At each sampling, shoots of 25 cm to 31 cm were collected from a minimum of 30 plants. In the field, stems were placed in sealable plastic bags wrapped in a moist paper towel and transported to the laboratory. At the lab, samples were prepared for shipment by removing leaves near the pruning cut and wrapping the pruned end of the stems in moist paper towels. Samples were shipped overnight to the University Georgia's Cold Hardiness Laboratory at the Griffin Campus, Griffin, GA. Samples were transported and shipped at ambient temperature to maintain field conditions. From previous blueberry floral bud hardiness studies, Ehlenfeldt et al. (2009) and Rowland et al. (2005) described transport using ice from samples collected on fields where temperatures ranged from 13.4 °C to 3.7 °C or above the snowline. Whereas, in south Georgia, winter season daily high temperatures regularly reach 21 °C (GAEMN, 2017) and placing samples on ice would not reflect field conditions. Upon arrival in Griffin, samples were held at 4 °C until preparation (within 48 h). The samples were prepared by removing remaining leaves; then stems with intact attached buds were trimmed to 5 cm segments with a minimum of 1.5 cm of stem remaining below the proximal bud. Excised buds were carefully cut to exclude stem tissue (Flinn and Ashworth, 1994).

Freeze hardiness determinations. For both 'Emerald' and 'Farthing' at each sampling date, eight sets of six randomly selected samples of attached (5 cm stems) or excised buds were placed into a moistened paper towel (Kimwipe, Irving, TX) and enclosed in a sealable freezer bag. In season 2015-16, sample sets were treated to an overnight temperature of 4 °C (from 16 Nov. to 12 Jan.) and -2 °C (from 25 Jan. to 15 Feb.). During the 2016-17 season, a set of samples were each pre-treated at either 4 °C or -2 °C overnight before each freeze test. The bags were organized and placed on a rack in the ESPEC EY-101 (Tabai Espec Corp., Osaka, Japan) freeze chamber and set to a freezing rate of 4 $°C·h^{-1}$. Removal occurred every 3 °C, from -3 °C to -21 °C. A control bag of each tissue type (attached vs. excised) and cultivar was held at 4 °C. The freeze treated and control floral buds were stored in a refrigerator at 4 °C for a week. Samples were then brought to room temperature, dissected, and inspected for discolored floral tissue (e.g. browning) under a light microscope (Olympus BX51, Tokyo, Japan). The lethal temperature where 50% of the floral tissue was damaged, LT_{50}, was rated as the percentage of injured florets compared to total florets within a floral bud (Arora et al., 2000; Flinn and Ashworth, 1994).

Floral bud mass and size. Water relations within the bud and tissue type can significantly alter the cold hardiness of a floral bud (Kader and Proebsting, 1992; Flinn and Ashworth, 1994). Floral buds of *Prunus* spp. that were dehydrated expressed lower hardiness temperatures than hydrated samples (Kader and Proebsting, 1992); while, blueberry with excised floral bud were less tolerant to freezing than attached buds (Flinn and Ashworth, 1994). However, both the *Prunus* spp. and blueberries were grown in temperate climates with longer duration of freeze through winter period, where blueberry grown in the subtropics may have interrupted periods of chill (> 7.2 °C). To identify subtropical cultivation on blueberry water relationship to freezing, fresh bud weight and length were measured using a scale (Model ML203E, Mettler Toledo, Columbus, OH) and a micrometer (Traceable, VWR International, Radnor, PA). Ten labeled excised buds from each cultivar were measured on each sampling date. After fresh weight measurement, samples were dried in an oven (Thermo Electron Corporation, Beverly, MA) at 60 °C to a constant weight for about of 48 h and recorded.

Weather Data. All weather data was collected from the University of Georgia Automated Environmental Monitoring Network (GAEMN, 2017) accessing data from the Homerville, GA station (31°00'58" N, 82°39'02"W), which is 5° S and 44 km from the sample collection site. Chill hours were calculated as chill hours accumulated between 0 °C to 7 °C using the calculator provided by GAEMN. Daily minimum and maximum temperatures were downloaded from 14 Nov. 2015 to 15 Feb. 2016 and 27 Nov. 2016 to 31 Jan. 2017, which covers the sampling period for each respective season.

Statistical Analysis. The LT_{50} curves were calculated as a nominal logistic model in JMP Pro (SAS, Cary, NC) version 13. All other analyses were evaluated using Proc GLM with SAS 9.4 (SAS Institute Inc., Cary, NC, U.S.) and means were separated at $P<0.05$ level using Tukey's honest significant difference (HSD) test.

Results and Discussion

Mean daily maximum and minimum temperatures were erratic during the study and averaged 20.3 and 7.1 °C, respectively, in 2015-2016 and 21.3 and 7.7 °C, respectively, in 2016-2017 (Fig. 1). Both cultivars received sufficient chill hours for bloom, which is 100-400 h for 'Emerald' and 300 h for 'Farthing' (Lyrene, 2008 a, b) (Table 1, 2, 3, and 4).

Excised floral buds were generally less hardy than attached floral buds, regardless of cultivar or the preconditioning temperature (Table 1 and 2). Flinn and Ashworth (1994) also observed lower hardiness in excised buds in NHB and concluded that ice nucleators associated with woody tissue are removed when the stem is excised yielding more conservative results. In the present study, LT_{50} of attached floral buds reached a

Table 1. Lethal temperature at which 50% of attached or excised buds were damaged by freezing (LT_{50}) in 'Emerald' and 'Farthing' southern highbush blueberry. The buds were sampled during the winter months of 2015-2016.

		LT_{50} (deg. C)							
		Emerald				Farthing			
Sample Date[z]	Chill Hours[y]	Attached Buds		Excised Buds		Attached Buds		Excised Buds	
16 Nov.	18					-9.8 a	A	-6.8 a	A
7 Dec.	44	-11.7 ab	A[x]	-10.9 bc	A	-11.6 ab	A	-10.8 c	A
21 Dec.	96	-16.1 cd	B	-8.2 ab	A	-14.6 bc	B	-7.5 ab	A
12 Jan.	164	-16.4 cd	B	-13.0 c	A	-12.3 abc	A	-11.9 c	A
25 Jan.	266	-17.7 d	B	-10.9 bc	A	-17.7 d	B	-10.9 c	A
1 Feb.	314	-14.0 bc	B	-7.5 a	A	-16.7 d	B	-9.6 bc	A
8 Feb.	371	-16.7 d	B	-10.3 abc	A	-18.6 d	B	-11.7 c	A
15 Feb.	433	-10.4 a	A	-9.4 ab	A	-16.4 cd	B	-12.1 c	A

[z] Prior to freezing, bud samples were preconditioned overnight at 4 °C from 16 Nov. to 12 Jan. and at -2 °C from 25 Jan. to 15 Feb.
[y] Total hours at 0-7 °C.
[x] Means followed by the same lower-case letter within a column, or by the same upper-case letter within a row and cultivar, are not significantly different at $P \leq 0.05$ according to Tukey HSD.

minimum of -17.7 and -18.6 °C in 'Emerald' and 'Farthing', respectively, in 2015-2016 and -20.2 and -20.2 °C, respectively, in 2016-2017. In each case, the minimum occurred in January or early February, and LT_{50} increased to -10.0 to -16.0 °C in the weeks afterwards. Excised floral buds, on the other hand, had only -7.0 to -10.0 °C over the entire sampling period, regardless of year, cultivar, or preconditioning. Clearly, excision reduced hardiness of the buds and is a poor method for determining freeze tolerance in SHB. Rowland et al. (2013) observed that when testing the freeze tolerance of blueberry flowers using stem tissue with buds attached responded similarly to whole plant freeze tolerance comparisons. This work suggests that attached floral buds reflect natural freezing in the field.

Preconditioning at or below freezing temperatures is often used in freeze tolerance tests to minimize ice nucleation and to allow intercellular water to supercool and better tolerate subfreezing conditions (Ashworth, 1991; Quamme, 1983). In our case, attached buds preconditioned at -2 °C were typically hardier than those preconditioned at 4 °C (Table 2). Bittenbender and Howell (1975) also found that preconditioning increases hardiness in 'Jersey' NHB in Michigan. When attached buds were preconditioned at -2 °C, 'Emerald' reached a maximum LT_{50} of -10.4 and -13.6 °C on the last sampling date of each year, respectively (Table 1 and 2), while 'Farthing' reached a maximum LT_{50} of -16.4 and 13.4 °C on last sample date of each year, respectively (Table 1 and 2). In Georgia, a majority of the commercial SHB production occurs within USDA hardiness zone 8B, where the 30-year-average temperature is -6.7 to -9.4 °C (USDA, 2017). The lowest temperatures recorded at the Homerville weather station during the present study were -3.6 °C during the winter of 2015-2016 and -4.4 °C during the following winter. At no point did the samples of either cultivar show any browning florets in the control samples, which indicate critical temperatures had not been surpassed in the field. Because temperatures rarely fall below -10 °C or remain below 0 °C for 12 continuous hours (Fig. 1), preconditioning floral bud samples of SHB grown in the subtropics could be artificially increasing hardiness of field conditions.

Table 2. Lethal temperature at which 50% of attached or excised buds were damaged by freezing (LT_{50}) in 'Emerald' and 'Farthing' southern highbush blueberry. The buds were sampled during the winter months of 2016-2017. Prior to freezing, bud samples were preconditioned overnight at 4 or -2 °C.

		Emerald				Farthing			
Sample	Chill	Attached buds		Excised buds		Attached buds		Excised buds	
Date	Hours[z]	4 °C	-2 °C	4 °C	-2 °C	4 °C	-2 °C	4 °C	-2 °C
29 Nov.	118	-13.3 bc B[y]	-17.3 bc C	-10.5 ab A	-10.5 b A	-10.2 a A	-15.6 a B	-8.1 a A	-8.1 a A
13 Dec.	170	-14.0 c B	-18.1 bc C	-9.9 a A	-13.0 c B	-14.4 c B	-18.4 b C	-11 c A	-10.5 c A
5 Jan.	235	-14.5 c B	-19.0 c C	-10.3 ab A	-8.8 a A	-17.0 d B	-20.2 b C	-11 c A	-10.8 c A
17 Jan.	287	-13.8 bc B	-15.5 ab B	-10.5 ab A	-10.5 b A	-16.8 d B	-18.8 b B	-9.7 b A	-10.5 c A
24 Jan.	287	-9.8 a A	-13.7 a B	-11.2 b AB	-10.5 b AB	-11.5 ab A	-14.7 a B	-11 c A	-10.6 c A
31 Jan.	333	-11.5 ab B	-13.6 a C	-11.0 b AB	-9.9 b A	-12.6 bc B	-13.4 a B	-11 c AB	-9.3 b A

[y] Means followed by the same lower-case letter within a column, or by the same upper-case letter within a row and cultivar, are not significantly different at $P \leq 0.05$ according to Tukey HSD.

Floral bud moisture content (%) in blueberry has been shown to decrease as water content increases during de-acclimation (Biermann et al., 1979; Bittenbender and Howell, 1975). At each sampling for both years and cultivars, bud water content, dry weights, and lengths are reported (Table 3 and 4). For 'Emerald', dry weights, water content and length increased from initial to final sampling in both years. In 2016, 8 Feb. to 15 Feb. showed a significant increase in fresh and dry weight (84% and 54%, respectively) (Table 3). For 2017, increase in fresh and dry weight was observed from 24 Jan. to 31 Jan. at 46% and 53%, respectively, though not significant (Table 4). The lack of significance suggests that 'Emerald' had deacclimated and as was progressing towards bloom by 24 Jan. Similar trends were observed in bud length (Table 3 and 4) where increasing length of tight floral buds was associated with deacclimation. 'Farthing' demonstrated a similar pattern for both years for weight and length measurements of the last two sample dates (Table 3 and 4). Comparing years, 'Farthing' had 38% less weight at the end of sampling in 2016 than in 2017 (Table 1 and 2) and this could be attributed to higher temperatures in 2017. In 2016, the last 14 days of sampling had min/max temperatures of 18.1/4.2 °C with 3 days

Table 3. Dry weight, length, and water content of the floral buds collected from 'Emerald' and 'Farthing' southern highbush blueberry. The buds were sampled during the winter months of 2015-2016.

		Emerald			Farthing		
		Dry Bud	Bud Water	Bud Length	Dry Bud	Bud Water	Bud Length
Date	Chill Hours[z]	wt (mg)	content (%)	(mm)	wt (mg)	content (%)	(mm)
16 Nov.	18	10.8 b[y]	63.7 cd	4.6 b	10.0 e	62.2 a	5.7 c
7 Dec.	44	13.5 b	60.8 d	4.8 b	10.5 de	62.3 a	5.7 c
21 Dec.	96	15.8 b	63.6 cd	5.2 b	12.6 cde	65.0 a	6.2 bc
12 Jan.	164	14.2 b	64.4 bc	4.9 b	16.1 abc	64.8 a	6.8 ab
25 Jan.	266	13.2 b	66.0 bc	4.9 b	14.5 b-e	64.8 a	6.5 abc
1 Feb.	314	15.2 b	67.5 b	5.1 b	17.5 ab	64.4 a	6.8 ab
8 Feb.	371	14.4 b	66.2 bc	5.0 b	15.3 bcd	61.9 b	6.5 bc
15 Feb.	433	22.2 a	72.0 a	6.7 a	20.3 a	65.8 a	7.6 a

[y] Total hours at 0-7 °C.
[x] Means followed by the same letter within a column are not significantly different at $P \leq 0.05$ according to Tukey HSD.

Table 4. Dry weight, length, and water content of the floral buds collected from 'Emerald' and 'Farthing' southern highbush blueberry. The buds were sampled during the winter months of 2016-2017.

		Emerald			Farthing		
Date	Chill Hours[z]	Dry Bud wt (mg)	Bud Water content (%)	Bud Length (mm)	Dry Bud wt (mg)	Bud Water content (%)	Bud Length (mm)
29 Nov.	118	8.4 b[y]	56.1 c	4.2 b	5.4 d	59.0 bc	4.5 d
13 Dec.	170	12.3 b	57.5 c	4.5 b	8.6 cd	56.3 c	5.0 cd
5 Jan.	235	11.5 b	57.8 c	4.4 b	12.5 bc	59.5 abc	5.5 cd
17 Jan.	287	13.4 b	61.0 bc	4.8 b	12.6 bc	61.4 abc	5.9 bc
24 Jan.	287	18.0 ab	71.0 a	5.4 ab	18.0 b	66.2 ab	6.9 ab
31 Jan.	333	27.5 a	68.6 ab	7.0 a	26.2 a	68.3 a	7.9 a

[y] Total hours at 0-7 °C.
[x] Means followed by the same letter within a column are not significantly different at $P \leq 0.05$ according to Tukey HSD.

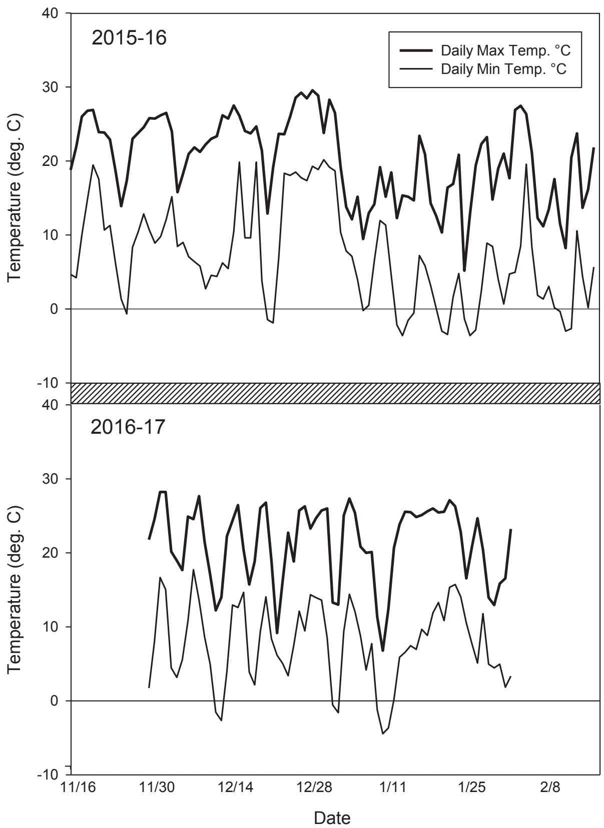

Figure 1. Daily minimum and maximum temperature measured over floral bud sampling period for 2015-16 (16 Nov. to 15 Feb.) and 2016-17 (29 Nov. to 31 Jan.). Data gathered from Georgia Automated Environmental Monitoring Network (http://www.georgiaweather.net/), Homerville, GA station.

below 0 °C (9, 10, 11 Feb. at -0.3 °C, -3.0 °C, and -2.7 °C, respectively); whereas, the last 14 days of sampling in 2017 had min/max of 21.2/9.1 °C with no freezing temperatures recorded (Fig 1). Water content in floral buds can fluctuate during dormancy (Quamme, 1983) and sensitivity is affected by the level of moisture within the bud (Bittenbender and Howell, 1975; Hewett et al., 1979). Our work suggests that increasing water content and length of tight floral buds are indicators of a loss of hardiness for subtropical field grown 'Emerald' and 'Farthing'.

Conclusions

The findings indicate that experimentally freezing excised SHB ('Emerald' and 'Farthing') floral buds tended to be more sensitive to freeze than attached floral buds. Preconditioned floral buds below freezing can significantly increase hardiness of the buds, which may be inflating hardiness of subtropical SHB. Attached floral buds for both cultivars with 4.0 °C preconditioning in both years were not > -9.8 °C, which is hardier then the 30 average for hardiness zone 8b. The least hardy buds were at the beginning of chill accumulation and when buds beginning to swell, which indicated deaccimation. This work should give subtropical SHB growers clear indication that tight SHB floral buds are not being damaged during average winters.

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Walnut Cultivars Through Cross-Breeding: 'DİRİLİŞ' and '15 TEMMUZ'

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Additional index words: *Juglans regia* L., cross-breeding, laterally fruitful, fruit set, phenological, pomological

Abstract

This study considered two new walnut cultivars ('Diriliş' and '15 Temmuz') and aimed to evaluate their performance, phenological and pomological traits. The cultivars were obtained through cross-breeding in Turkey. The phenological and pomological traits of mature trees of the cultivars 'Diriliş' and '15 Temmuz' were compared with those of 'Pedro', 'Chandler' and 'Maraş 18' cultivars. Late leafing and a high level of lateral fruitfulness were the most prominent plant traits of the new cultivars 'Diriliş' and '15 Temmuz'. The nut weight, kernel weight and kernel percentage measured 13.70g, 7.23g and 52.83% in the 'Diriliş' cultivar, and measured 14.32g, 7.72g and 53.92% in '15 Temmuz' respectively. Results indicated that these new cultivars have good performance, with phenological and pomological traits that are better or comparable to common walnut cultivars.

Walnut (*Juglans regia* L.) is a commercially important species because of its high quality wood, nutritious nuts and leaves that have significant pharmacological values (Avanzato et al., 2014). Walnut species are found throughout the world including Southern Asia, South Eastern Europe and the Americas (McGranahan and Leslie, 1991; Vahdati et al., 2015). Walnuts are one of several fruit species indigenous to Anatolia, and their long history of fruit cultivation is commonly known (Şen, 1986). The amount of walnuts produced in the world each year is about 2 million tons. Turkey nets an annual production of 200,000 metric tons, and is ranked fourth among the walnut producing countries of the world (FAO, 2016).

Widely used in various fields around the world, walnuts (*Juglans regia* L.) have been studied from many perspectives. Frequently studies have focused on the improvement of productivity and fruit quality, propagation and recently releasing new rootstocks (Dehghan et al., 2009; McGranahan et al., 2009; Vahdati et al. 2004). Meanwhile, spring late frost is one of the most serious problems that cause loss of production in walnuts, and therefore late leafing is a desirable attribute for walnuts grown in regions that are susceptible to spring late frosts (Aslani Aslamarz et al., 2010).

Some genotypes with high variation are important in breeding programs. Walnut breeding programs are characterized by efforts to achieve earlier fruiting, higher yield, lateral fruitfulness, late leafing, good adaptability to different ecological conditions, good fruit quality and tolerance to pests and diseases (Aslantas, 2006; Germain, 1998; Sutyemez, 2016).

Formerly, most walnut cultivars were obtained through selection rather than cross-breeding. Such cultivars include 'Franquette', 'Parisienne', 'Corne', 'Marbot', 'Sorrento', 'Sibisel', 'Payne', 'Maraş 18', 'Sütyemez 1' and 'Kaman 1' (Painter and Rawlings, 1961; Ölez, 1971; Çelebioğlu, 1978; Şen, 1986; Ramos, 1998; Sutyemez, 2016). Nonetheless, walnuts have also been cross-bred over the past two to three decades, and a few cultivars

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with superior traits have been commercialized as a result, e.g. 'Fernor' and 'Fernette' from France (Germain, 1998) and 'Chandler' from USA (Ramos, 1998).

The walnut cultivar improvement program conducted by Forde and Serr at the University of California, Davis from 1948 to 1978 was the first cross-breeding improvement project in the world. This program produced many new cultivars with improved productivity and quality attributes including high lateral bud fruitfulness, large nut size, kernel percentage, light kernel color, easy removal of kernels from the shell and relatively thin shells (Mc Granahan and Forde, 1985). 'Serr', 'Chandler', 'Sundland', 'Chico' and 'Tulare' were produced by this program (Tulecke and McGranahan, 1994). The ongoing demands for walnut production and export have encouraged scientists to breed new walnut cultivars (Germain, 1998; Ramos, 1998; Sütyemez and Kaşka, 2002; Özcan et al., 2017).

Our breeding program was started in 2004, in order to generate promising cultivars characterized by significant production and late leafing, early bearing, higher nut quality and resistance to bacterial blight. The aim of the present study was to evaluate the performance, phenological and pomological traits of two new walnut cultivars produced by the walnut breeding program at the University of Kahramanmaras Sutcu Imam (KSU). The breeding program spanned 13 years, from 2004 to 2016, and the cultivars were registered and patented as 'Diriliş' and '15 Temmuz'. The objective of this study is to evaluate the performance, phenological and pomological traits of 'Diriliş' and '15 Temmuz' in comparison with 'Chandler'.

Materials and Methods

'Diriliş' and '15 Temmuz' were the progeny of 'Pedro' and 'Maraş 18'. The 'Chandler' cultivar was also evaluated and contrasted with the two new cultivars. The study was located in the Nut Application and Research Center (SEKAMER), Kahramanmaras, Turkey. SEKAMER is located at 37° 35' 27" N latitude, 37° 03' 28" E longitute and 930 m above sea level. The region has

Table 1. Descriptors of the phenological traits used to compare five walnut cultivars [z].

Traits	Description
Leafing date	Date when 50% of terminal buds had enlarged and the bud scales had split exposing the green leaves
First male bloom date	When first pollen shedding occurred
Last male bloom date	When last pollen shedding occurred
First female bloom date	Date of initial pistillate flower receptivity
Last female bloom date	Date of last pistillate flower receptivity
Harvest date	When nuts are harvestable
Defoliation date	Date of defoliation
Male flowering times	Catkins receptive duration
Female flowering times	Female flower receptive duration
Female abundance	Female flower abundance: 3 low; 5 intermediate; 7 high
Catkin abundance	Male flower abundance: 3 low; 5 intermediate; 7 high
Lateral bud flowering (lateral fruitfulness)	Percent of lateral buds with female flowers
Dichogamy	Female flowers and catkins receptive duration overlap status: 1 Protandrous; 2 Protogynous; 3 Unknown
Estimated yield	Rate in relation to age and volume of tree: 3 Low; 5 Intermediate; 7 High

[z] Source: IPGRI, 1994

Table 2. Descriptors of the pomological traits used to compare five walnut cultivars [z].

Traits		Description
Shell texture		1: Very smooth; 3: Smooth; 5: Medium; 7: Rough; 9: Very rough
Shell color		1: Very light; 3: Light; 5: Medium; 7: Dark; 9: Very dark
Shell seal		1: Open or very weak; 3: Weak; 5: Intermediate; 7: Strong; 9: Very strong
Shell strength		1: Paper; 3: Weak; 5: Intermediate; 7: Strong
Shell thickness (mm)		Near center of half shell was measured with digital caliper.
Shell integrity		1: Incomplete shell; 2: Intermediate; 3: Complete shell, no holes
Nut weight (g)		Average of total 400 nuts
Kernel weight (g)		Average of total 400 nuts
Nut diameter (mm)		Average of total 400 nuts
Nut height (mm)		Average of total 400 nuts
Kernel percentage (%)		Kernel weight/nut weight × 100
Packing tissue thickness		1: Very thin and sparse; 3: Thin; 5: Medium; 7: Thick; 9: Very thick
Kernel veins (%)		Percent of kernels with conspicuous veins
Kernel fill		3: Poor; 5: Moderate; 7: Good
Kernel plumpness		3: Thin; 5: Moderate; 7: Plump
Ease of removal of kernel halves		1: Very easy; 3: Easy; 5: Moderate; 7: Difficult; 9: Very difficult
Kernel color	Extra light (%)	Percent of kernels in extra light category
	Light (%)	Percent of kernels in light category

[z] Source: IPGRI, 1994

a mild climate - an average between Mediterranean and Continental climates with 727 mm yearly precipitation and 16.9°C average yearly temperature. The soil structure in general is suitable for walnut cultivation, and the plants in this study were irrigated regularly. The trees of each cultivar, were propagated on 'Maraş 18' seedling rootstocks. Ten plants with similar growing qualities were used for each genotype. The research started in 2004, and the phenological and pomological data were collected since 2012, when the plants reached maturity and the orchard became productive. Data collection continued until 2016, and IPGRI procedures and criteria were used (IPGRI, 1994; Anonymous, 1999; Hendricks et al., 1985).

Phenological traits. Phenological traits were assessed according to the walnut descriptor (IPGRI, 1994) (Table 1). Phenological observations were recorded for 10 different trees per cultivar. In this study, 14 phenological traits were evaluated to assess the range of variation among the cultivars, and data pertaining to 2014-2016 are reported.

Pomological traits. Pomological measurements were made on 20 healthy nuts taken from each of 10 trees per cultivar during 2014-2016. Mean values were calculated after measuring several traits with laboratory equipment. Nut and kernel weights were measured using an electronic balance with 0.01 g precision. Nut diameter, length and shell thickness were measured using a digital caliper. Additional traits of the nuts and kernels were determined according to IPGRI, 1994 (Table 2).

Data analysis

In this study, the results were analysed statistically by One Way Analysis of Variance (ANOVA) and means were compared with Tukey's HSD Post Hoc Test using SPSS version 20.0 package program. In the analysis of phenological data, the number of days between 1 Jan. and the date of data collection were considered as the time span for analyses.

Table 3. Five walnut cultivars grown at the Nut Application and Research Center (SEKAMER), Kahramanmaras, Turkey.

Cultivar	Geographic origin	Genetic Origin
Diriliş	University of Kahramanmaras Sutcu Imam, Turkey	Maraş 18 × Pedro
15 Temmuz	University of Kahramanmaras Sutcu Imam, Turkey	Pedro × Maraş 18
Chandler	University of California, USA	Pedro × UC 56-224
Pedro	University of California, USA	Conway-Mayatte × Payne
Maraş 18	University of Kahramanmaras Sutcu Imam, Turkey	Selection from local populations from Kahramanmaras province

Results and Discussion

Phenological traits. The phenological traits of the cultivars were monitored annually throughout the growing season (Table 4). The harvest period of 'Diriliş' was 10-17 Sept. (average, 14 Sept.) and the harvest period of '15 Temmuz' was 21-25 Sept. (average, 23 Sept.). Accordingly, the new cultivar 'Diriliş' was harvested 9 days before 'Chandler', 15 days before 'Pedro' and 1 day before 'Maraş 18'. Meanwhile, '15 Temmuz' was harvested 21 days before 'Chandler', 6 days before 'Pedro' and 8 days after 'Maraş 18'.

Leaves emerged during 28 April - 6 May for 'Diriliş' and during 5-13 May for '15 Temmuz', compared to 25 April - 3 May for 'Chandler', 26 April - 5 May for 'Pedro' and 14-24 April for 'Maraş 18' cultivars. On average, the leafing date between 2014 and 2016 was 2 May for 'Diriliş' and 9 May for '15 Temmuz'. Late leafing is a desirable trait for walnuts grown in regions that experience late spring frosts. This study indicated that the first leafing date of 'Diriliş' and '15 Temmuz' occurred after the leafing for 'Chandler', 'Pedro' and 'Maraş 18' (Table 4; Fig. 1). Defoliation date is another important trait in fruit breeding to avoid early autumn frosts. In this respect, the defoliation dates for 'Diriliş' and '15 Temmuz' were 13 and 17 Nov., respectively (Fig. 1).

Fruit set on lateral branches is a very important trait that influentially defines the productivity of a walnut cultivar and substantially determines the process of selecting a walnut genotype and introducing it as a new cultivar (Ramos, 1998). Furthermore, female flower abundance is also an important indicator of phenological productivity. Today, 'Chandler' is acknowledged as a quite productive walnut cultivar in terms of phenological productivity because its fruit set occurs prominently on the lateral sides of branches (85-90%) (Ramos, 1998). The 'Diriliş' cultivar was found to be similar to 'Chandler' in terms of productivity and the amount of fruit set on lateral sides of branches. However, the '15 Temmuz' cultivar had higher yield compared to 'Diriliş' and 'Chandler' (Table 5).

Table 4. Some important phenological traits of 'Diriliş' and '15 Temmuz' compared to their parents and 'Chandler'.

Cultivars	Leafing date	Leafing date range	Harvest date	Harvest date range	Defoliation date	Defoliation date range
Diriliş	2 May b [z]	28 April-6 May	14 Sept. d	10-17 Sept.	13 Nov. c	8-18 Nov.
15 Temmuz	9 May a	5-13 May	23 Sept. c	21-25 Sept.	17 Nov. c	6-26 Nov.
Chandler	29 April c	25 April-3 May	5 Oct. a	1-10 Oct.	22 Nov. b	16-28 Nov.
Pedro	30 April bc	26 April-5 May	29 Sept. b	26 Sept.-2 Oct.	28 Nov. a	21 Nov.-3 Dec.
Maraş 18	20 April d	14-24 April	15 Sept. d	12-19 Sept	30 Oct. d	21 Oct.-5 Nov.

[z] Values within columns followed by common letters do not differ at the 5% level, by Tukey HSD.

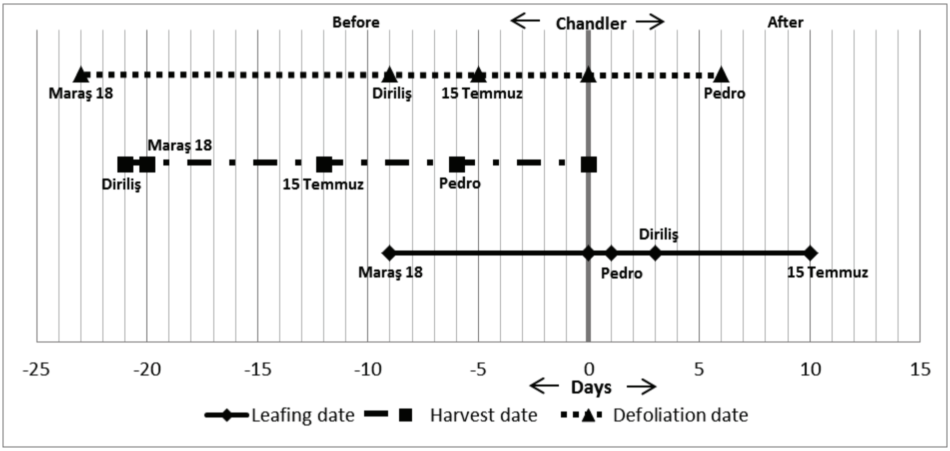

Figure 1. Comparison of some important phenological traits of five walnut cultivars.

Pomological traits. Pomological data were collected during 2014 and 2016, and average values were calculated for each cultivar (Tables 6 and 7). In this study, 'Diriliş' had an average nut weight of 13.70 grams. Its kernel weight measured 7.23 grams and the kernel percentage was 52.83%. On the other hand, '15 Temmuz' had an average nut weight of 14.32 grams, with a kernel weight of 7.72 grams. Its kernel percentage was 53.92%. 'Diriliş' and '15 Temmuz' had heavier nuts and kernels compared to 'Chandler' and 'Pedro'. The kernel percentage was also higher for the two new cultivars, but the values were lower when compared to 'Maraş 18'. Regarding both 'Diriliş' and '15 Temmuz', the

Table 5. Phenological traits of five walnut cultivars grown at the Nut Application and Research Center (SEKAMER), Kahramanmaras, Turkey.

Traits	Diriliş	15 Temmuz	Chandler*	Pedro*	Maraş 18*
Male bloom date range	22 April-2 May	29 April-9 May	18 April-1 May	22 April-1 May	12-19 April
Female bloom date range	3-12 May	8-17 May	29 April-8 May	28 April-8 May	17-28 April
Male flowering times	10	10	13	9	7
Female flowering times	9	9	9	10	11
Catkin abundance	Heavy	Heavy	Heavy	Heavy	Heavy
Female flower abundance	Heavy	Heavy	Heavy	Heavy	Intermediate
Lateral bud flowering range (%)	80-90	85-95	80-90	90-95	50-60
Dichogamy	Protandrous	Protandrous	Protandrous	Protandrous	Protandrous
Estimated yield	High	High	High	High	Intermediate

* Reference Cultivars: Parents ('Pedro' and 'Maraş 18') and 'Chandler'.

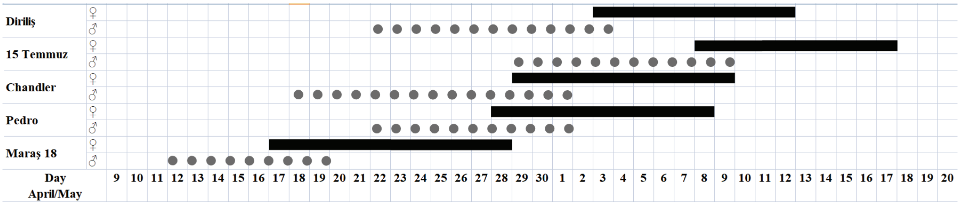

Figure 2. Pollen-shedding period in relation to the time of pistillate blooming of five walnut cultivars.

DİRİLİŞ **15 TEMMUZ**

Figure 3. Fruits of 'Diriliş' and '15 Temmuz' walnut cultivars.

nut shell's texture was 'very smooth' and the shell color was 'very light'. The kernel could be removed easily from the shell, and the color of kernels were 'extra light' or 'light' in both cultivars (Tables 6 and 7).

The nut weights and kernel percentage of some cultivars which are commonly grown in Europe, the US and other regions are as follows, according to existing reports: 'Franquette' (10.75g, 44.50%); 'Mayette' (10.50g, 43.50%); 'Parisianne' (10.75g, 46.50%); 'Corne' (10g, 37.50%); 'Tulare' (13.30g, 53.30%); 'Midland' (12.00g, 44.00%); 'Payne' (11.40g, 50.00%); 'Serr' (13.68g, 57.00%); 'Chandler' (13.26g, 49.00%) and 'Cisco' (12.39g, 46.00%) (Germain, 1988; Glagolev, 1969; Jelenkovic, 1975; Kornienko, 1974; Liu et al., 1991; Ramos, 1998; Schonberg, 1984; Zhadan and Strukov, 1977). By comparing previous reports with data obtained from this study, it is likely that 'Diriliş' and '15 Temmuz' possess higher quality fruit.

This study also reveals that the cross-bred cultivars ('Diriliş' and '15 Temmuz') had favorable values for nut weight and kernel percentage; their qualities are remarkably higher than several domestic and foreign cultivars that are currently planted commercially worldwide. Both new cultivars had high kernel yields and late season leafing dates, i.e. 3-11 days later than the leafing date of 'Chandler'. Both cultivars were harvested earlier than 'Chandler'.

Conclusion

'Diriliş' and '15 Temmuz' walnut cultivars were registered and patented within the framework of walnut breeding studies at the University of Kahramanmaras Sutcu Imam (KSU). Both cultivars possess higher fruit yields, superior fruit quality, later leafing dates and earlier harvesting periods compared to other commercially grown cultivars in Turkey. In the future, researchers can consider planting these two cultivars in other countries and in different climates to evaluate their adaptability and fruit yields.

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Table 6. Some important nut traits of 'Diriliş' and '15 Temmuz' compared to their parents and 'Chandler'.

Cultivars	Nut weight (g)	Kernel weight (g)	Kernel percentage (%)	Shell thickness (mm)	Nut diameter (mm)	Nut height (mm)
Diriliş	13.70 ± 0.89 c[z]	7.23 ± 0.46 c	52.83 ± 2.10 b	1.43 ± 0.16 b	33.22 ± 1.00 c	42.23 ± 1.66 a
15 Temmuz	14.32 ± 0.93 b	7.72 ± 0.51 b	53.92 ± 1.89 a	1.44 ± 0.15 b	34.60 ± 1.74 b	37.91 ± 2.13 d
Chandler	12.64 ± 1.01 d	6.21 ± 0.56 d	49.14 ± 2.38 c	1.46 ± 0.16 b	34.54 ± 1.53 b	38.90 ± 2.64 c
Pedro	11.41 ± 0.84 e	5.43 ± 0.38 e	47.63 ± 1.99 d	1.44 ± 0.19 b	35.47 ± 1.61 a	39.73 ± 1.85 b
Maraş 18	15.61 ± 0.84 a	8.34 ± 0.63 a	53.44 ± 2.80 ab	1.53 ± 0.14 a	34.66 ± 1.28 b	41.72 ± 1.73 a

[z] Means ± standard deviations within columns followed by common letters do not differ at the 5% level of significance, by Tukey's HSD.

Table 7. Some nut traits of five walnut cultivars.

Traits	Diriliş	15 Temmuz	Chandler*	Pedro*	Maraş 18*
Shell texture	Very smooth	Very smooth	Smooth	Smooth	Very smooth
Shell color	Very light	Very light	Light	Light	Light
Shell strength	Weak	Weak	Weak	Weak	Intermediate
Shell integrity	Complete shell	Complete shell	Complete shell	Complete shell	Complete shell
Packing tissue thickness	Medium	Medium	Medium	Medium	Medium
Kernel veins %	0	0	0	3	4
Kernel fill	Good	Good	Moderate	Moderate	Good
Kernel plumpness	Plump	Plump	Plump	Plump	Plump
Ease of removal of kernel halves	Very easy	Very easy	Very easy	Very easy	Very easy
Extra light % (kernel colour)	49	63	64	52	35
Light % (kernel colour)	51	37	36	48	65

*Reference Cultivars: Parents ('Pedro' and 'Maraş 18') and 'Chandler'.

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Seasonal Variation in Mineral Nutrient Concentration of Primocane and Floricane Leaves in Trailing Blackberry Cultivars Produced in an Organic System

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Abstract

The impact of floricane-fruiting trailing blackberry (*Rubus* L. subgenus *Rubus,* Watson) cultivar ('Black Diamond', 'Marion', 'Obsidian', and 'Onyx') and leaf sampling time during the growing season were studied for 2 years in an organic production system to evaluate impacts on leaf nutrient concentration in primocane and floricane leaves. Primocane leaves were sampled every 2 weeks from late May through early October, whereas leaves on fruiting laterals (floricane) were sampled every 2 weeks from bloom (early May) through fruit harvest (late July) and were analyzed to determine concentration of macro- and micronutrients. Sampling date through the season, cultivar, and year had an effect on the concentration of all nutrients in the primocane leaves, though patterns of change were similar between years and cultivars. Primocane leaf N, S, and Cu concentration generally declined over the season while P, K, and Zn generally increased. Primocane leaf Mg, Ca, B, Fe, Mn, and Al concentrations peaked during the harvest season. The concentration of nutrients in floricane leaves generally decreased (N, P, K), remained steady (Mg, S, Cu), or increased (Ca, B, Fe, Mn, Zn, Al) from bloom through fruit harvest. 'Black Diamond' tended to have lower primocane but higher floricane leaf nutrient concentrations than the other cultivars. 'Obsidian' tended to have among the highest concentrations in both primocane and floricane leaves for many nutrients. Our results confirm the need to sample cultivars separately. The primocane leaf nutrient concentrations measured in this study were below the published recommended sufficiency levels for N (in 'Black Diamond'), Mg (in 2014), K (in 'Onyx'), Ca, and B, indicating the sufficiency levels for these nutrients and cultivars may need to be revised for this region.

Oregon is the leading producer of trailing blackberry (*Rubus* L. subgenus *Rubus*, Watson) in the USA, with about 2500 ha harvested mainly for processed markets in 2016 (Oregon Department of Agriculture, 2017). The primocanes of these floricane-fruiting cultivars are vegetative in their first year of growth. In their second year, when they are called floricanes, they flower, fruit, and then senesce. The primocanes of trailing types are not self-supporting and they are kept on the ground, under the floricane canopy, until trained to the trellis after fruit harvest and floricane pruning (typically done in late August) (Strik and Finn, 2012).

The nutrient status in trailing blackberry plants and fields is monitored by commercial growers using soil nutrient analysis, observations of plant growth, and annual primocane leaf tissue analysis. Fertilizer programs are developed based on recommended starting rates of nitrogen (N), which depend on planting age, and are adjusted for N and other macro- and micronutrients based on field observation and plant tissue nutrient testing (Bolda et al., 2012; Bushway et al., 2008; Fernandez and Ballington, 1999; Hart et al., 2006; Krewer et al., 1999). A review of plant nutrient uptake and plant assessment of nutrient status is provided in Strik and Bryla (2015). In floricane-fruiting blackberry and raspberry, leaf sampling of primocanes in mid- to late-season informs growers of plant nutrient requirements for fruit production the following season.

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Primocane leaf nutrient concentrations varied over the growing season in erect (Clark et al., 1988; Strik and Vance, 2017) and trailing (Mohadjer et al., 2001; Strik and Vance, 2017) floricane-fruiting blackberry, primocane-fruiting blackberry (Strik, 2015), and floricane-fruiting raspberry (Hughes et al., 1979; John and Daubeny, 1972; John et al., 1976; Kowalenko, 1981, 1994; Wright and Waister, 1980). While nutrients in floricane leaves in blackberry changed over the fruiting season (Pereira et al., 2015; Strik and Vance, 2017) standards for this leaf tissue type have only recently been developed in Brazil (Pereira et al., 2015).

In Oregon, leaf sampling for tissue analysis is recommended for primocanes from late July to early August with values compared to published sufficiency levels (Hart et al., 2006), though Strik and Vance (2017) found that mid- to late-August provided a more consistent sampling time when most nutrients were not changing rapidly. Sufficiency levels in Oregon were developed based on 15 years of tissue samples submitted by growers of 'Marion' blackberry (Hart et al., 2006). However, there are many more cultivars currently being grown. Cultivars of blackberry (Fernandez-Salvador et al., 2015a, b, c; Dixon et al., 2016; Harkins et al., 2014; Strik, 2015; Strik and Vance, 2017) and raspberry (John and Daubeny, 1972; John et al., 1976) differed in primocane leaf nutrient levels when sampled in mid-season. In addition, nutrient sufficiency levels may need to differ between conventional and organic production systems, as was suggested in blueberry by Strik and Vance (2015). Organic sources of N and other nutrients are often more slowly released to the plants as they may come from sources such as compost, cover crops, and animal-based manures and fertilizers (Archer et al., 2016). This requires careful management to ensure adequate nutrients are available at the correct times. Organic fertilizer sources often contain nutrients other than N. For example, the high level of potassium (K) in yard-debris compost and fish emulsion fertilizer has led to high rates of K application when these fertilizer sources were used (Fernandez-Salvador et al., 2015a, Harkins et al., 2014; Strik et al., 2017), potentially impacting plant nutrient uptake. Other practices such as the use of permeable polyethylene ground cover as a weed barrier in organic systems rather than maintaining bare soil with herbicides has been suggested to impact nutrient uptake and plant nutrient status in blackberry (Dixon et al., 2016). There are no existing standards for leaf aluminum (Al), but it can be useful as an indicator of low soil pH, in which Al uptake increases and inhibits the uptake of other cations, and can eventually cause toxicity resulting in improper root growth and insufficient nutrient and water uptake (Foy et al., 1978; Ryan and Kochian, 1993; Vitorello et al., 2005).

The objectives of this study were to evaluate the changes in primocane leaf nutrient concentrations in trailing, floricane-fruiting blackberry cultivars grown in an organic production system over two seasons, with a goal of comparing actual nutrient levels in each cultivar to published sufficiency levels for this crop. In addition, we evaluated the nutrient concentration of fruiting lateral leaves to better understand changes in nutrient allocation within the floricane during fruiting and whether this may provide another method of assessing nutrient needs in this type of blackberry.

Materials and Methods

Study sites. The study was conducted in 2013 and 2014, in a mature field planting at Oregon State University's North Willamette Research and Extension Center, Aurora, OR [lat. 45°16'47"N, long. 122°45'23"W; USDA hardiness zone 8b (U. S. Department of Agriculture, Agricultural Research Service, 2014); the weather records for this site can be viewed at U.S. Department of Interior (2014)]. The soil is mapped as a Willamette silt loam, classified as a fine-silty, mixed, superactive mesic Pachic Ultic Argixeroll.

The site was planted in spring 2010 and was certified organic by a USDA accredited agency (Oregon Tilth, Certified Organic, Corvallis, OR). Plants were spaced 1.5 m in the row with 3 m between rows (2222 plants/ha). The in-row area was covered with a 1.4-m-wide strip of black, woven polyethylene ground cover (TenCate Protective Fabrics; OBC Northwest Inc., Canby, OR) centered on the row and secured using 0.1-m-long nails. According to the manufacturer, the weed mat had a density of 0.11 $kg \cdot m^{-2}$ and a water flow rate of 6.8 $L \cdot h^{-1} \cdot m^{-2}$. The ground cover (referred to hereafter as weed mat) was placed on top of the row just prior to planting, and openings were cut for each plant (planting hole). Weeds were manually removed from the planting hole area and seams in the weed mat, as required throughout the study. The between row area was planted to a grass cover crop ('Aurora Gold' hard fescue, *Festuca brevipila* Tracey) that was mowed as needed.

Plants were irrigated with a single line of drip tubing (UNIRAM; Netafim USA, Fresno, CA) containing pressure-compensating emitters (1.9 $L \times h^{-1}$ in-line) spaced every 0.6 m. The drip tubing was placed along the ground at the base of the plants under the weed mat. More information on planting establishment is available from Fernandez-Salvador et al. (2015b).

Fertilization in both years was done through the drip irrigation system (fertigation). In 2013, a total target rate of 90 $kg \cdot ha^{-1}$ of N was applied, half using a soluble grain fermentation and nitrate of soda blend (4N–0.9P–0.8K; Converted Organics of California, LLC, Gonzales, CA) and half as a fish hydrolysate and fish emulsion blend combined with molasses (TRUE 512; 5N–0.4P–1.7K; True Organic Products, Inc., Spreckels, CA). In 2014, the same rate of N was applied using only TRUE 512 as a source. The fertilizers were applied in eight equal portions from early April to early July of each year. Additionally, 2.2 $kg \cdot ha^{-1}$ of boron (B; Solubor, 20 Mule Team Borax, Englewood, CO), 560 $kg \cdot ha^{-1}$ of pelletized dolomitic lime [62 $kg \cdot ha^{-1}$ magnesium (Mg) and 112 $kg \cdot ha^{-1}$ of calcium (Ca; Pro-Pell_it! Pelletized Dolomite; Marion Ag Service Inc., St. Paul, OR)], and 2242 $kg \cdot ha^{-1}$ of pelletized lime [717 $kg \cdot ha^{-1}$ of Ca (Pro-Pell-it! Pelletized Lime; Marion Ag Service Inc., St. Paul, OR)] were broadcast applied to the plots and aisles on 8 Mar. 2013. An additional 2.2 $kg \cdot ha^{-1}$ of B was applied in early Mar. 2014. Ground covers, as used in our study, have been shown to be permeable to fertilizers applied on top (Zibilske, 2010). A copper (Cu) fungicide (Nu-Cop; Albaugh Inc., Ankeny, IA) was applied to all plants (1.1 $kg \cdot ha^{-1}$ of Cu) on 24 Mar. 2014 to control for the cane diseases purple blotch [*Septocyta ruborum* (Lib.) Petr.] and cane rust [*Kuehneola uredines* (Link) Arthur].

Plants were grown in an every-year production system (Strik and Finn, 2012). New primocanes were bundled and tied to a 0.3-m-high trellis wire, below the floricane canopy, until Aug. each year. Primocanes were then trained to the upper trellis wires in mid- to late-Aug. by dividing the primocanes produced by each plant into two bundles and looping half in one direction from the upper to middle trellis wire and bringing it back towards the plant with one or two twists; the other half was looped in the opposite direction. Plants were trained on a two-wire vertical trellis system in each row with the wires attached to steel posts at 1.0 m and 1.6 m above the ground.

Cultivars. The cultivars studied were 'Black Diamond', 'Marion', 'Obsidian' and 'Onyx' trailing blackberry. More information on the fruiting season, growth and yield of the cultivars grown at this site was reported by Fernandez-Salvador et al. (2015b).

Leaf sampling. Tissue samples for nutrient testing were collected approx. every 2 weeks by choosing the most recent fully expanded leaves of primocanes (19 May to 6 Oct. 2013 and 2014) and fruiting laterals on floricanes (6 May to 29 July 2013 and 2014) for a total of 11 and 7 samples in each year for primocanes and floricanes, respectively. Stage of plant development and fruiting season was recorded for each cultivar. Yield data were not recorded, but the field had a good, typical commercial yield for this production region (Fernandez-Salvador et al., 2015b).

Approximately 6 to 12 of the most recent, fully expanded primocane and fruiting lateral leaves, respectively, including petioles, were sampled per plot on each date. Leaves were not washed prior to tissue analysis (Hart et al., 2006). Sampled leaves were priority shipped to Brookside Laboratories, Inc. in New Bremen, OH for analysis. Leaf N was determined using a combustion analyzer with an induction furnace and a thermal conductivity detector (Gavlak et al., 1994). Other nutrients, including phosphorus (P), K, Ca, Mg, Al, B, Cu, manganese (Mn), iron (Fe) and zinc (Zn) were determined using an inductively coupled plasma (ICP) spectrophotometer after wet ashing the samples in nitric/perchloric acid (Gavlak et al., 1994).

Soil testing. Soil samples were collected on 12 Nov. 2013 and 21 Oct. 2014 using a 2.4-cm-diam., 0.5-m-long, slotted, open-side, chrome-plated steel soil probe (Soil Sampler Model Hoffer, JBK Manufacturing, Dayton, OH). Soil was sampled to a depth of 0.2 m at the center of the row, approx. 0.3 m from the crown between plants and within the water emitter drip zone or fertilization area. Only one, combined sample (unreplicated) was sent for analysis of macro- and micronutrient content and pH to Brookside Laboratories each year.

Data analysis. Cultivars were arranged as a randomized complete block design with four replicates for each of the four cultivars. Each experimental unit consisted of a four-plant plot. Data were analyzed by tissue type (primocane or floricane) separately, as our goal was to observe nutrient changes throughout the season rather than to compare canes. Research has suggested that floricanes and primocanes act independently in blackberry as well (Bryla and Strik, 2008). Leaf nutrient data were analyzed by sample date for the effect of year using PROC MIXED (SAS version 9.3) with year as the main effect (n=2), and cultivar as the subplot effect (n=4) using a Satterthwaite approximation, as needed, for main effect comparisons. Mean comparison was performed using a protected LSMEANS.

PROC UNIVARIATE and the Shapiro-Wilk procedure were used to assess normality of the data for all the aforementioned analyses. As the tissue nutrient concentration of many nutrients was not normal, a log transformation was used to improve homogeneity of variance and to assess proportional effects. Data were back transformed for presentation.

Results and Discussion

Phenological development. Key phenological stages and harvest season for the four cultivars studied are shown in Table 1. Fruiting season and plant development were quite similar for the two years of the study (data not shown). 'Black Diamond' and 'Obsidian' had similar harvest dates, which were earlier than 'Marion' and 'Onyx'. By the end of July, all fruit harvest was complete and floricanes were removed ("caned-out") in mid-August.

Soil properties. Soil pH and all nutrients were within recommended levels in both years (Table 2), other than soil B (0.5 to 1.0 ppm; Hart et al., 2006); there are no published recommended levels for soil Fe, Cu, Zn, and Al for blackberries in this region.

Year effect. Year had a significant main effect on primocane and floricane leaf nutrient concentration on several sample dates throughout the season and for many nutrients on at least one sample date. In primocane leaves, the concentrations of most nutrients (N, Mg, Ca, S, Fe, Zn, and Al) were similar, higher, or lower in 2014 than 2013 depending on the time of the season. Leaf B and Mn concentrations were generally lower in 2014 than 2013, while the concentrations of P, K, and Cu were higher in 2014 for most

Table 1. Development stages for primocanes and floricanes through the season (2013 and 2014 averaged) for floricane-fruiting trailing blackberry cultivars grown in an organic production system at Oregon State University's North Willamette Research and Extension Center. Show in approximate order of fruit ripening.

Cultivar	Approximate stage of development on each date[z]										
	15 May	1 June	15 June	1 July	15 July	1 Aug.	15 Aug.	1 Sept.	15 Sept.	1 Oct.	15 Oct.
'Black Diamond'	late fruit set	mid green fruit	begin harvest	harvest	end of harvest	P[y] growing	P growing	P growing	P growing	P growing	P growing
'Marion'	mid bloom	early green fruit	red fruit	begin harvest	late harvest	P growing	P growing	P growing	P growing	P growing	P growing
'Obsidian'	late fruit set	mid green fruit	begin harvest	harvest	end of harvest	P growing	P growing	P growing	P growing	P growing	P growing
'Onyx'	early bloom	early green fruit	red fruit	begin harvest	harvest	P growing	P growing	P growing	P growing	P growing	P growing

[z] Approximate stage of development is provided. The beginning and end of fruit harvest for a particular cultivar may have occurred between the dates provided. Primocane leaves were sampled for tissue analysis on 6 May, 19-20 May, 2-3 June, 16-17 June, 30 June-1 July, 14-15 July, 28-29 July, 12 Aug., 25-27 Aug., 8-10 Sept., 22-23 Sept., and 6-7 Oct. (depending on year). Leaves on fruiting laterals were sampled for tissue analysis on 6 May, 19-20, May, 2-3 June, 16-17 June, 30 June-1 July, 14-15 July, and 28-29 July.

[y] "P"=primocanes. Primocane growth would have slowed toward the end of the season (e.g. 1 Oct.) as temperatures declined in autumn.

of the season (Figures 1 and 2). Leaf Cu was likely higher in 2014 as a result of the Cu-based fungicide applied in late winter of that year.

In floricane leaves, N, S, Fe, Mn, and Zn were lower in 2014 than in 2013 on at least one date while leaf B and Cu were higher in 2014 on at least one date (data not shown). Floricane leaf Cu concentrations spiked in the early part of 2014 due to the application of a copper-based fungicide used for disease control and remained higher for the duration of the season. Concentrations of K and Ca were either higher or lower in 2014 depending on the time of the season.

The patterns of change in leaf nutrients for both primocanes and floricanes were relatively similar through the season between years (primocanes: Figures 1 and 2; floricanes: data not shown), despite the above-noted differences between years. There was a year x cultivar interaction on several dates for multiple nutrients. An example of these interactions can be seen for one sample date within the recommended sampling time suggested by Strik and Vance (2017), in Table 3. The data presented herein support their recommendation of mid- to late-August as a good time to conduct primocane leaf tissue nutrient sampling based on the relative stability of many nutrients during that period (Figures 3 and 4). We have chosen to present only 2013 data here in order to more clearly show seasonal changes for these four cultivars.

Primocane leaf nutrient concentration. Primocane leaf N fluctuated throughout the season for all cultivars, but decreased overall in 'Black Diamond' whereas in 'Marion', 'Obsidian', and 'Onyx', leaf N was similar at the end of the season as in the spring (Figure 3). Primocane leaf N was significantly affected by cultivar. 'Obsidian' had higher leaf N than the other cultivars on several sampling dates, particularly early and late season, while 'Black Diamond' had lower leaf N than the other cultivars for most of July through the end of the season. Fewer differences among trailing cultivars were reported by us (Strik and Vance, 2017) in primocane leaf N in conventional production.

Leaf P concentration was either steady ('Marion') or declined slightly ('Black Diamond', 'Obsidian', 'Onyx') until the end of the fruiting season (late July, Figure 3), after which it increased in all cultivars through early September. Leaf P declined in all cultivars from September into October.

Table 2. Soil pH, organic matter and nutrient content in the organic production system at Oregon State University's North Willamette Research and Extension Center, 12 Nov. 2013 and 21 Oct. 2014.

		Organic	ppm												
Year	pH	matter (%)	NH_4-N	NO_3-N	P[z]	K	Ca	Mg	S	B	Fe	Mn	Cu	Zn	Al
2013	6.4	3.1	10.3	0.9	234	228	1127	132	12	0.23	337	51	0.8	1.9	1201
2014	6.1	3.0	3.8	1.4	218	289	1306	176	21	0.41	327	29	1.6	2.3	1297

[z] P as determined by Bray I.

During the fruiting season, leaf K concentration declined in 'Marion' and 'Obsidian', while it increased in 'Black Diamond' or remained relatively steady in 'Onyx' (Figure 3). However, leaf K increased in all cultivars from mid-Aug. until early Sept.. Blackberry fruit are high in K (Harkins et al., 2014), which may be why leaf K was lower during the portion of the season when fruit were present. Similar patterns in leaf K concentration during the fruiting season were reported for conventional production (Strik and Vance, 2017), except 'Marion' had considerably higher leaf K in late season than 'Black Diamond' in the present study.

Leaf Ca and Mg showed opposite patterns to leaf K, as concentrations were highest during the harvest season for all cultivars (Fig. 3). After fruiting, leaf Ca and Mg declined to levels similar to those measured in the spring, except for leaf Mg in 'Obsidian', which was high at the end of the season. The decline in

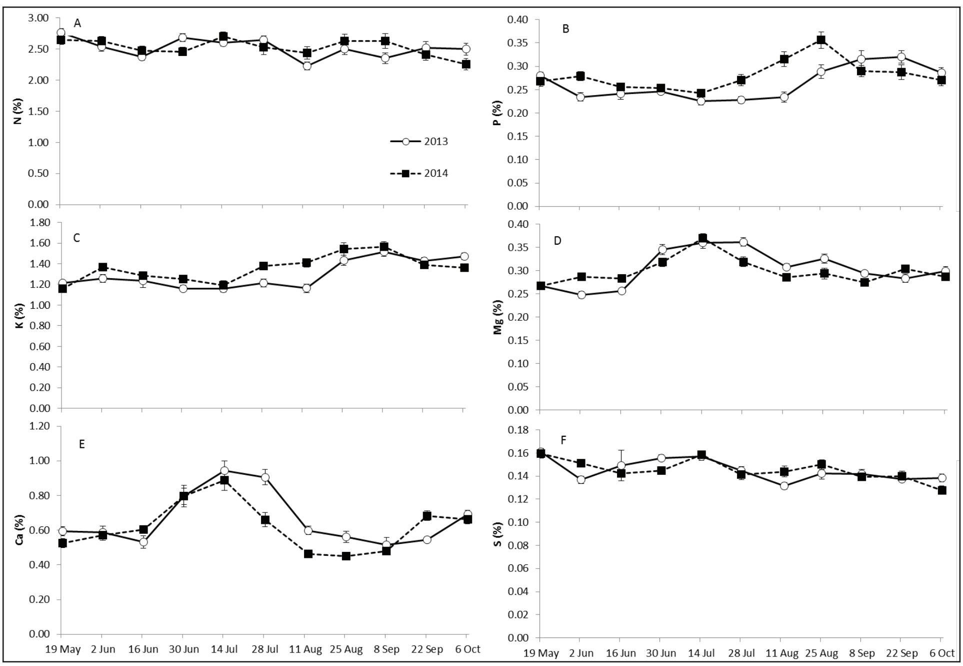

Figure 1. Effects of year and sample date on the concentration of macronutrients in primocane leaves of floricane-fruiting, trailing blackberry when sampled over the growing season in 2013 and 2014. Values are the means of four cultivars grown in an organic production system at Oregon State University's North Willamette Research and Extension Center, Aurora, Ore. **A** = nitrogen; **B** = phosphorus; **C** = potassium; **D** = magnesium; **E** = calcium; **F** = sulfur. Bars indicate standard error for cultivar (n = 16).

Table 3. Effect of year and cultivar on primocane leaf nutrient concentration of floricane-fruiting, trailing blackberry cultivars when grown in an organic production system at Oregon State University's North Willamette Research and Extension Center, Aurora, Ore. and sampled on 27 Aug. 2013 and 25 Aug. 2014.

	%								ppm								
	N[z]	P		Mg		K	Ca	S	B	Fe		Mn	Cu		Zn	Al	
Tissue Standards[y]	2.3–3	0.19–0.45		0.3–0.6		1.3–2.0	0.6–2.0	0.1	30–70	60–250		50–300	6–20		15–50	n/a	
Year																	
2013	2.50	0.29 b[x]		0.33		1.43 b	0.56 a	0.14	23.1	156 a		163 a	8.0 b		34.9	115 a	
2014	2.62	0.36 a		0.29		1.54 a	0.45 b	0.15	22.0	115 b		123 b	11.7 a		35.6	89 b	
Cultivar		2013	2014	2013	2014					2013	2014		2013	2014		2013	2014
Black Diamond	2.11 d	0.27 e	0.28 de	0.29 bc	0.26 c	1.33 b	0.46 b	0.13 c	21.9 b	144 ab	157 a	137 ab	8.9 c	11.3 b	31.3 ab	103 bc	140 a
Marion	2.76 b	0.33 c	0.39 b	0.36 a	0.29 bc	1.64 a	0.60 a	0.15 b	24.1 b	158 a	91 c	131 b	7.3 d	11.5 b	37.2 bc	124 ab	68 e
Obsidian	3.01 a	0.35 c	0.45 a	0.35 a	0.36 a	1.72 a	0.52 ab	0.17 a	27.1 a	150 ab	124 b	165 a	8.9 c	13.3 a	43.0 a	97 bcd	80 cde
Onyx	2.37 c	0.21 f	0.31 cd	0.30 b	0.26 c	1.27 b	0.46 b	0.13 c	17.1 c	172 a	90 c	139 ab	6.9 d	10.8 b	29.4 c	135 a	69 de
Significance[w]																	
Year	NS	0.0031		NS		0.0467	0.007	NS	NS	0.0225		0.0435	<0.0001		NS	0.0176	
Cultivar	<0.0001	<0.0001		<0.0001		<0.0001	0.025	<0.0001	<0.0001	NS		NS	<0.0001		<0.0001	0.0119	
Year*Cultivar	NS	0.0161		0.0086		NS	NS	NS	NS	0.0006		NS	0.0059		NS	0.0001	

[z] N=nitrogen; P=phosphorus; Mg=magnesium; K=potassium; Ca=calcium; S=sulfur; B=boron; Fe=iron; Mn-manganese; Cu=copper; Zn=zinc; Al=aluminum.
[y] Recommended sufficiency range for blackberry when sampled in late July to early August (Hart et al., 2006); no sufficiency levels are available for aluminum (n/a).
[x] Means followed by the same letter within treatment or the interaction are not significantly different (LSMeans) ($P > 0.05$).
[w] Non-significant ("NS") or actual P value provided when significant by analysis of variance.

leaf Ca after fruiting may have been related to the relatively high rate of primocane growth that occurs during the latter part of the season in trailing blackberry (Cortell and Strik, 1997). Similar patterns and levels were reported in conventional production (Strik and Vance, 2017). Primocane leaf S fluctuated throughout the season, but all cultivars ended the season with slightly lower leaf S than in spring.

Primocane leaf B, Fe, Mn, and Al concentrations increased or reached their highest levels during fruiting and declined into Aug. and Sept., ending the season at levels that were similar (B) or higher (Fe, Mn, Al) than those in the spring (Figure 4). Leaf Zn increased during fruiting and ended the season with higher concentrations than in spring (except 'Onyx', which had similar leaf Zn at the beginning and end of the season). 'Black Diamond' and 'Obsidian' had significantly higher leaf Cu than 'Marion' and 'Onyx' for the majority of the season. Leaf Cu concentration fluctuated in all cultivars and was lower at the end of the season than in spring. We found less variability in primocane leaf micronutrient concentrations over the season in this study as compared to conventionally-grown trailing blackberry where canes were on bare soil through much of the season (Strik and Vance, 2017).

Comparison to standards. Many of the primocane leaf nutrient concentrations measured were within the recommended sufficiency range during the mid- to late-August sampling period

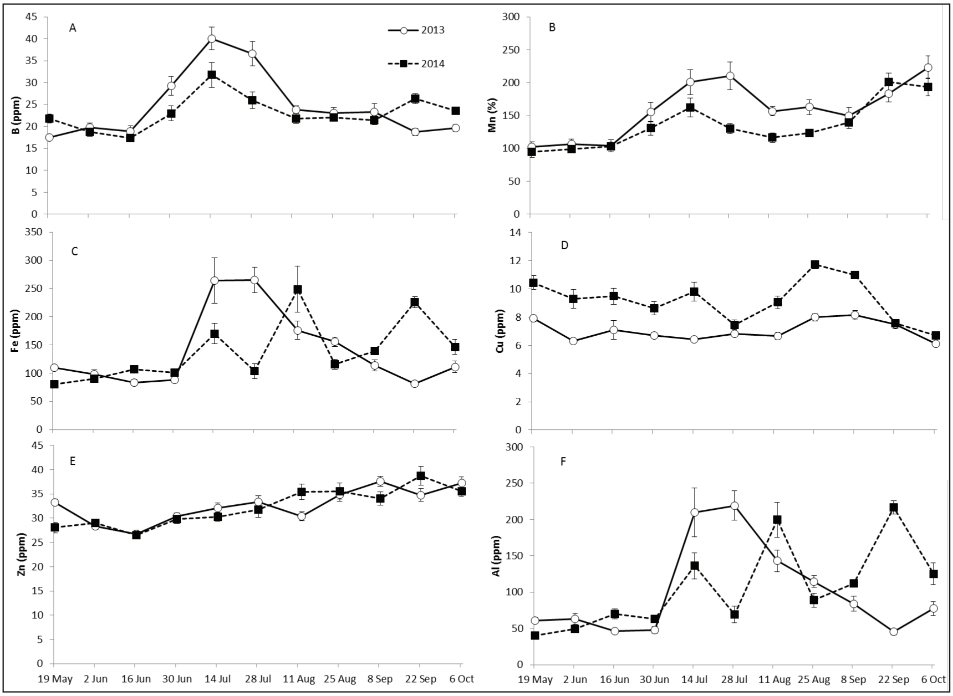

Figure 2. Effect of year and sample date, averaged over cultivar, on the concentration of macronutrients in primocane leaves of floricane-fruiting, trailing blackberry when sampled over the growing season in 2013 and 2014. Values are the means of four cultivars grown in an organic production system at Oregon State University's North Willamette Research and Extension Center, Aurora, Ore. **A** = boron; **B** = manganese; **C** = iron; **D** = copper; **E** = zinc; **F** = aluminum. Bars indicate standard error for cultivar (n = 16).

recommended by Strik and Vance (2017), including leaf P, S, Fe, Mn, Cu, and Zn. 'Black Diamond' had low leaf N compared to the other cultivars and was below the recommended range, as was found previously in both organic (Dixon et al., 2016, Harkins et al., 2014) and conventional (Strik and Vance, 2017) production systems. Leaf Mg was low in all cultivars except 'Obsidian' in 2014, while all cultivars except 'Black Diamond' were within (but at the low end) of the range in 2013. 'Onyx' had particularly low leaf K, while 'Black Diamond' was just within the recommended range in late Aug. and below it in mid-Aug.. All cultivars were below the existing tissue standard for leaf Ca and B, though it is typical in this region for leaf B to be low unless supplemental foliar B is applied. Soil B was also below recommended levels at this site (Table 2). Clearly, cultivars vary in leaf nutrient levels, even when fertilized the same, indicating differences in nutrient requirements or uptake. We thus confirm that cultivars should be sampled separately for leaf tissue analysis. The proposed new sufficiency standards proposed by Strik and Vance (2016) for blackberries in Oregon would encompass the ranges of nutrients observed in this study.

Floricane leaf nutrient concentration. While floricane leaf nutrient concentrations changed similarly from spring through the fruiting season in all cultivars (Figures 5 and 6), there was a significant cultivar effect on many sampling dates. Whereas 'Black Diamond' had the lowest primocane leaf N

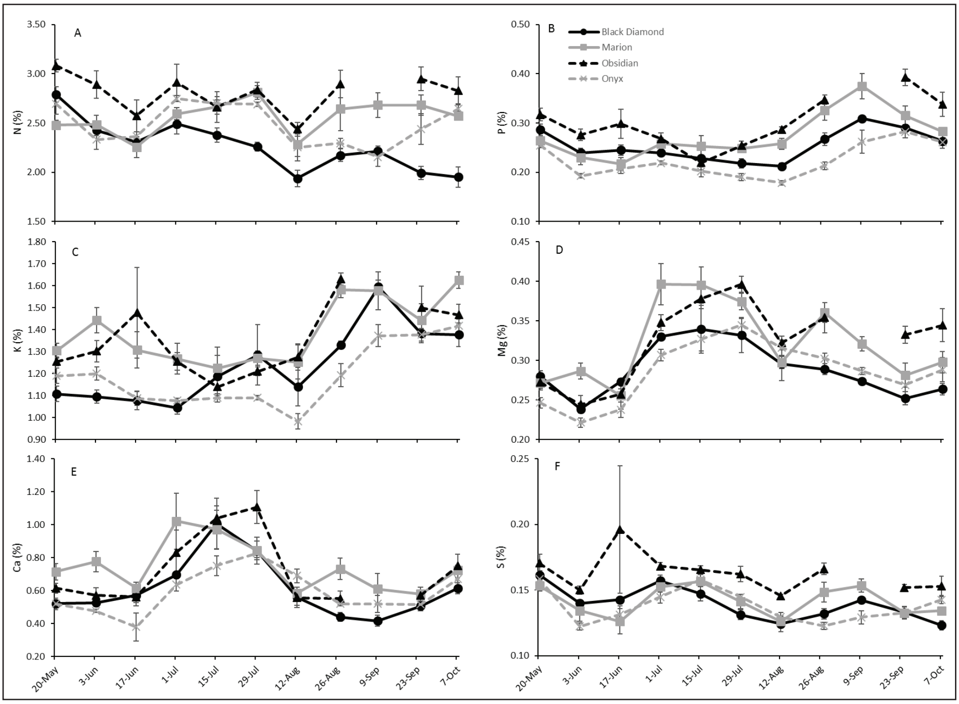

Figure 3. Effect of sample date and cultivar on the concentration of macronutrients in primocane leaves of floricane-fruiting, trailing blackberry when sampled over the growing season in 2013 in an organic production system at Oregon State University's North Willamette Research and Extension Center, Aurora, Ore. **A** = nitrogen; **B** = phosphorus; **C** = potassium; **D** = magnesium; **E** = calcium; **F** = sulfur. Bars indicate standard error for cultivar (n = 4). No data were collected for 'Obsidian' on Sept 9 due to insufficient leaf tissue after primocane training.

on many dates, it had the highest floricane leaf N concentration for most of the season. 'Marion' had much lower floricane leaf N than the other three cultivars. 'Obsidian' and 'Onyx' had similar floricane leaf N concentrations, but 'Obsidian' had higher levels on several dates. Differences in floricane leaf N concentration may be related to vigor of the fruiting lateral or yield. For example, 'Black Diamond' has very short fruiting laterals with dark green leaves, as compared to relatively long laterals with lighter green leaves in 'Marion' (Finn et al., 1997, 2005). In addition, the concentration of N in the floricane leaves may have declined as N was mobilized to the developing fruit which, in these cultivars, ranges from 0.9 to 1.4% of dry weight (Dixon et al., 2016; Harkins et al., 2014; Strik and Vance, 2017). Leaf S followed much the same pattern by cultivar as leaf N.

Leaf P concentration declined more rapidly in the early season compared to leaf N, but mostly leveled out through the fruiting season. 'Black Diamond' and 'Marion' had the highest and lowest leaf P, respectively, relative to the other cultivars for most of the season. Floricane leaf K, Mg, and Ca were similar between 'Black Diamond' and 'Marion', while concentrations in 'Obsidian' were either lower (K) or higher (Mg and Ca) than the other cultivars for much of the season. Floricane leaf K remained at a more consistent, higher concentration in our study

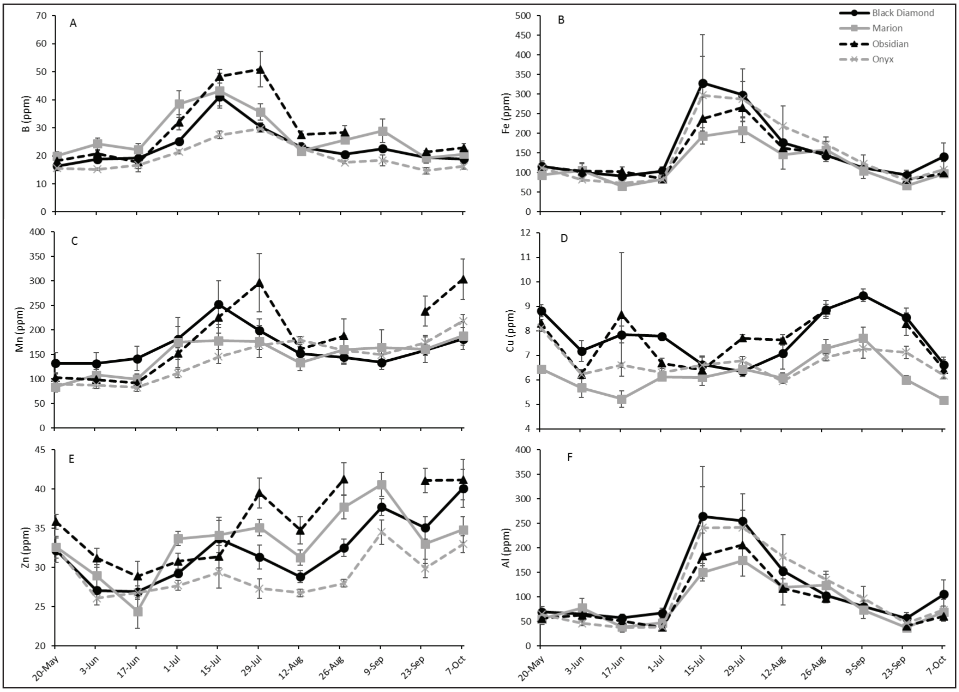

Figure 4. Effect of sample date and cultivar on the concentration of macronutrients in primocane leaves of floricane-fruiting, trailing blackberry when sampled over the growing season in 2013 in an organic production system at Oregon State University's North Willamette Research and Extension Center, Aurora, Ore. **A** = boron; **B** = iron; **C** = maganese; **D** = copper; **E** = zinc; **F** = aluminum. Bars indicate standard error for cultivar (n = 4). No data were collected for 'Obsidian' on Sept 9 due to insufficient leaf tissue after primocane training.

than many of the same cultivars grown in a conventional production system with bare soil in the row (Strik and Vance, 2017). The differences observed between these two studies may be because plants in this study were being fertigated, including applications later in the season (as opposed to granular fertilizer applications used by Strik and Vance, 2017), or were due to differences in nutrient availability related to production method (weed mat in the row as compared to bare soil). Dixon et al. (2016) showed that trailing blackberries grown with weed mat (which is commonly used in organic blackberry production in this region) had higher K in floricanes than plants grown with bare soil.

Floricane leaf Mg concentration peaked just prior to fruit harvest in 'Obsidian' and 'Black Diamond' and again at the end of the floricane season, but remained relatively stable all season with a slight increase toward the end of harvest in 'Marion' and 'Onyx' (Figure 5). Leaf Ca increased for the majority of the floricane season in all cultivars, with the highest levels in 'Obsidian' (Figure 5). Leaf Ca and Mg concentrations likely increased because these fruiting lateral leaves were aging, as has been reported in older primocane leaves of raspberry (Hughes et al., 1979), and there are relatively low concentrations of these nutrients in trailing blackberry fruit (Dixon et al., 2016; Harkins et al., 2014).

In general, floricane leaf B, Fe, Mn, and

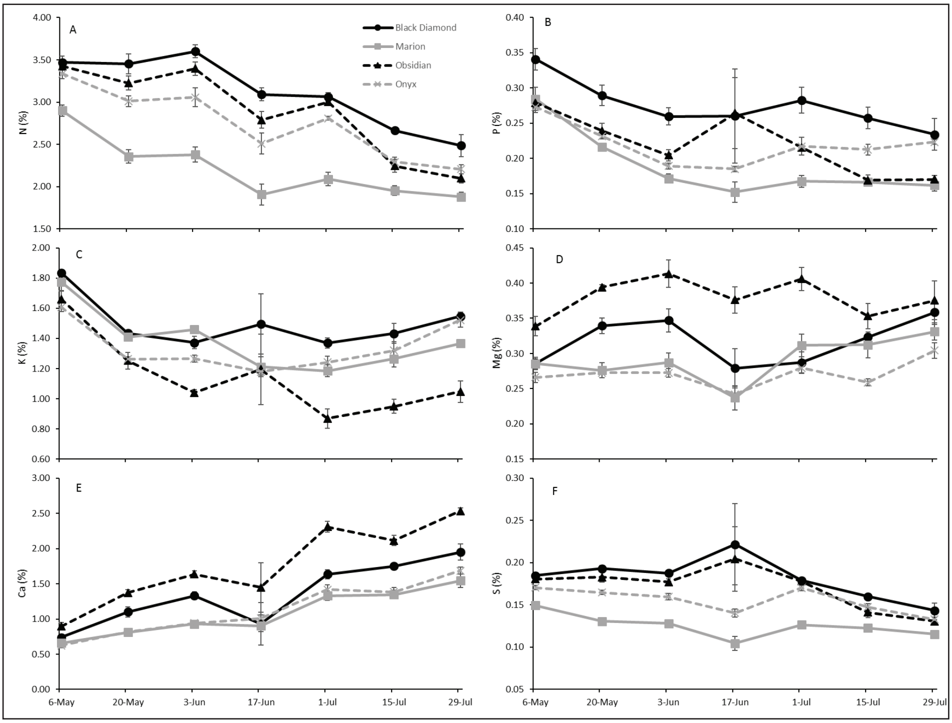

Figure 5. Effect of sample date and cultivar on the concentration of macronutrients in floricane leaves of floricane-fruiting, trailing blackberry when sampled over the growing season in 2013 in an organic production system at Oregon State University's North Willamette Research and Extension Center, Aurora, Ore. **A** = nitrogen; **B** = phosphorus; **C** = potassium; **D** = magnesium; **E** = calcium; **F** = sulfur. Bars indicate standard error for cultivar (n = 4).

Zn, and Al concentrations increased during the floricane season, with the exception of lower values of each nutrient for 'Black Diamond' just prior to fruit harvest (Figure 6). Leaf Cu concentration was relatively stable in the fruiting lateral leaves of most cultivars, except for an increase before fruit harvest in 'Obsidian' and a slight decrease at this time in 'Marion' and 'Onyx'. 'Black Diamond' had higher floricane leaf Fe and Al (all season except just prior to fruit harvest), leaf Mn (to mid-June), and Cu in spring than the other cultivars, as has been reported in other studies (Dixon et al., 2016; Harkins et al., 2014; Strik and Vance, 2017). 'Marion' had the lowest concentrations for all or the majority of the season for all micronutrients except B.

Summary

Primocane leaf nutrient concentrations changed through the growing season and were often affected by year and cultivar. Sampling cultivars separately, as currently recommended (Hart et al., 2006; Strik and Vance, 2016), is thus important in addition to keeping records to monitor variability among years. The primocane leaf nutrient concentrations measured in this study were below the published recommended sufficiency levels for N (in 'Black Diamond'), Mg (in 2014), K (in 'Onyx'), Ca, and B, indicating the sufficiency levels for these nutrients and cultivars may need to be revised for this region (Hart et al., 2006; Table 3), as we have suggested for conventional production (Strik and Vance, 2016, 2017).

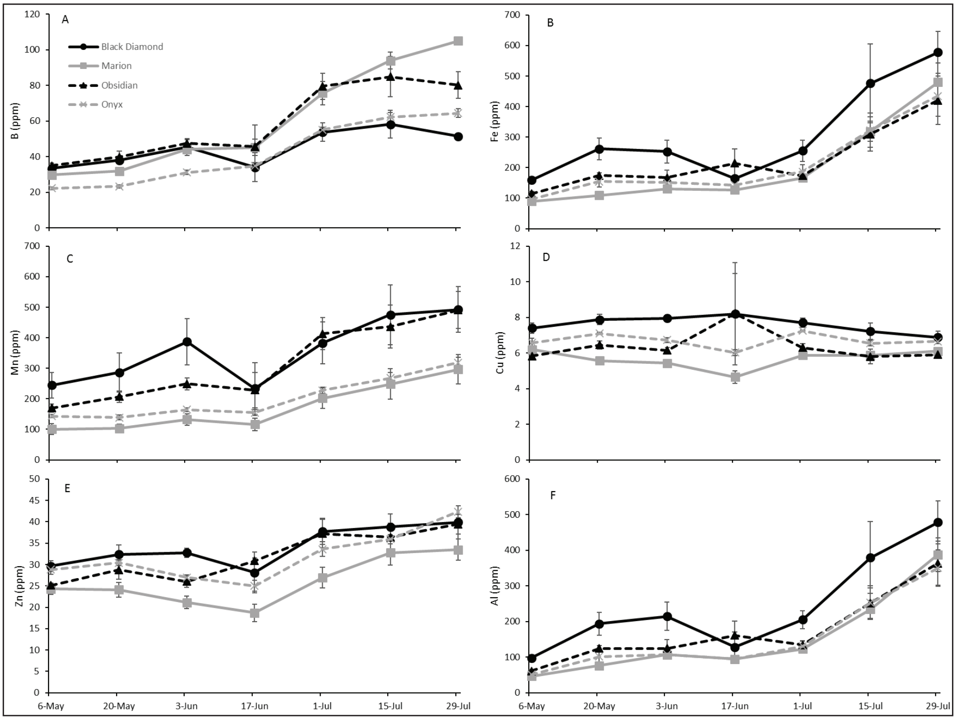

Figure 6. Effect of sample date and cultivar on the concentration of macronutrients in floricane leaves of floricane-fruiting, trailing blackberry when sampled over the growing season in 2013 in an organic production system at Oregon State University's North Willamette Research and Extension Center, Aurora, Ore. **A** = boron; **B** = iron; **C** = maganese; **D** = copper; **E** = zinc; **F** = aluminum. Bars indicate standard error for cultivar (n = 4).

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High Tunnel Performance of Seven Primocane Red Raspberry Cultivars in Western NY

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Additional index words: *Rubus idaeus,* yield

Abstract

Seven primocane fruiting red raspberry (*Rubus idaeus* L.) cultivars ('Autumn Britten', 'Caroline', 'Heritage', 'Himbo Top', 'Jaclyn', 'Joan J' and 'Polka') were cultivated under high tunnels to assess their relative performance in a protected agriculture system in western New York. 'Joan J' had the highest yield over three seasons averaging over 14 $t \cdot ha^{-1}$ per year while 'Autumn Britten' and 'Jaclyn' were the lowest yielding with mean annual yields of less than 7.5 $t \cdot ha^{-1}$ per year. 'Caroline', 'Himbo Top', 'Polka' and 'Heritage' produced intermediate yields similar to each other. 'Autumn Britten' had the greatest mean annual berry weight but was very similar to 'Jaclyn', 'Himbo Top', 'Joan J', and 'Polka'. 'Heritage' consistently had the lowest mean berry weight in all years. The beginning of harvest varied widely from season to season. It started as early as 23 July and as late as 11 Aug. in the earliest cultivar, 'Autumn Britten', with a similar range among the remaining cultivars. Harvest lasted for 6 to 9 weeks for individual cultivars depending on cultivar and approximately 10 weeks across all cultivars in a given season. The cultivars 'Joan J', 'Himbo Top', 'Polka' and 'Heritage' showed the best potential to produce high quality fruit over extended period using high tunnels in New York and regions of similar climate.

Red raspberry (*Rubus idaeus* L.) production in the eastern United States has a long history and was once concentrated in New York state with over 4,200 ha under cultivation in 1919 (Hedrick, 1925). That is greater than the area that was cultivated in California in 2016 (USDA-NASS, 2017) although the historical industry was primarily for processing berries using floricane cultivars, and productivity was considerably lower. Over the last 100 years, market conditions and production problems have reduced the eastern industry to hundreds of hectares across the region. The vast majority of U.S. production today is centered in California for fresh market sales and in Washington for frozen berries used whole or in processing (USDA-NASS, 2017). The increased availability of fresh raspberries in supermarkets made possible through improvements in production practices in combination with the adoption of primocane fruiting cultivars in warm climate production regions (Pritts, 2008) has also driven interest in local sources of fresh raspberries for farm-direct retail outlets and farmers' markets as well as regional wholesale outlets in the Northeast. Increased demand for locally grown fruit for use in local processing for the tourist trade has also provided more opportunities for growers in the temperate regions in the Midwest and Northeastern U.S. to market fruit directly.

The introduction of high tunnels for raspberry production has been instrumental in the expansion of the fresh market raspberry industry in the U.S. and around the world (Gaskell, 2004). Fruit quality improvements due to post-harvest handling advances combined with new cultivars enabled the widespread shipment of fresh raspberries from production areas in the west to the entirety of the U.S. This technology has also made widespread production in temperate regions more feasible and possibly competitive to

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California production of fresh raspberries, when all cost and productivity factors are considered. Multiple reports on performance of open field (Goulart and Demchak, 1999; Hanson et al., 2005; Weber et al. 2005) and high tunnel (Demchak, 2009; Hanson et al., 2011; Yao and Rosen, 2011) trials with primocane cultivars in temperate climate conditions have been published. However, many of the cultivars that were evaluated are not currently grown widely and information on the performance of some newer cultivars is unavailable to growers.

The goal of this project was to compare the performance of seven currently available commercial primocane fruiting raspberry cultivars in a high tunnel production system to aid in evaluating their suitability for the system and to demonstrate the potential for fresh red raspberry production in New York. Yield components and fruit quality observations were made to evaluate the potential of the cultivars for NY production and their utility for use in the Cornell berry breeding program for the development of improved cultivars for protected production in temperate climate regions.

Materials and Methods

A trial of seven primocane fruiting red raspberry cultivars was established in a randomized complete block design at Cornell University's New York State Agricultural Experiment Station (NYSAES) in Geneva, NY (lat. 42°8'N, long. 77°0'W). The cultivars included 'Autumn Britten', 'Caroline', 'Heritage', 'Himbo Top' (cv. 'Rafzaqu'), 'Jaclyn', 'Joan J' and 'Polka', which represent standard and newly introduced cultivars in the region from multiple breeding programs around the world (Weber, 2013). Bare root canes of each cultivar were sourced from commercial nurseries and planted in 30.5 cm high raised beds in a Honeoye loam soil with less than 3% slope in a 3-bay (7.32 m width) high tunnel structure (Haygrove Ltd., Ledbury, UK). Each bay was treated as a block in a randomized complete block design with one 6-plant plot (5.49 m per plot) of each cultivar randomly located in each block (bay). Initial in-row spacing was 0.9 m within row and 2.44 m between row centers with 3 rows in each bay.

A three-level V-trellis with a width of 46 cm at the base and 60 cm at 1.5 m height was installed after planting and drip irrigation was provided to deliver approximately 25 mm of water per week after the tunnels were covered prior to bloom and approximately 51 mm of water per week during the fruit development period through harvest after which the tunnel covers were removed for the winter. Fertilization was based on recommendations for high tunnel (Heidenreich et al., 2012) and field production practices (Bushway et al., 2008) and was applied through the drip irrigation. Weed barrier fabric (GreenhouseMegastore, International Greenhouse Co., Danville, IL) was applied between the rows and supplemental hand weeding was utilized within the rows. Predator mites (*Phytoseiulus persimilis*) (Biobest USA, Inc., McFarland, CA) were released prophylactically three times each summer to suppress two-spotted spider mite (*Tetranychus urticae* Koch) populations. To ensure good pollination, a quad-hive of bumble bees (*Bombus impatiens* Cresson) (Biobest USA, Inc., McFarland, CA) was placed at the end of the tunnel at the beginning of bloom each year.

Fruit was harvested for the same 2 m of row within each block for three seasons after the establishment year for annual and cumulative yield calculations. The first harvest began approximately 14 months after planting. Yield was converted to $t \cdot ha^{-1}$ based 4099 m of $row \cdot ha^{-1}$ at the spacing stated above. Fruit were harvested on Mondays, Wednesdays and Fridays, for each plot throughout the harvest period. For mean fruit weight calculations, a random 10-fruit sample was taken at each harvest date per block per cultivar being harvested. Mean fruit weight values over the whole season were calculated for each year, and total mean fruit weight values across all three years were calculated. All mean yield and fruit weight values for each cultivar were subjected to one-way analysis of variance (ANOVA) and mean separation by Duncan's multiple range test ($P \leq 0.05$) using Microsoft Excel software (Microsoft Corp., Redmond, WA) following the procedures of Gomez and Gomez (1984). Harvest began when any plot had ripe fruit and ended when the last plot had fruit. The date of first harvest, peak harvest, and last harvest were recorded each year for each cultivar with peak harvest being the date with the greatest 3-plot cumulative daily yield. Air temperature and rainfall measurements were recorded at the New York State Agricultural Experiment Station Research North Farm weather station approximately 1.5 km from the trial site to identify any gross differences in annual weather conditions between years that may have affected the trial results.

Results and Discussion

The performance of the cultivars in the trial under high tunnels compared very favorably to similar trials in open field conditions (Goulart and Demchak, 1999; Hanson et al., 2005; Weber et al., 2005; Yao and Rosen, 2011). Mean yield across the cultivars in the first season was over 89% higher compared to an open field trial previously conducted in a nearby field at the NYSAES (Weber et al. 2005) and 48% higher than first season yields in Michigan (Hanson et al., 2005). Yields in subsequent seasons were similarly higher. While these studies cannot be compared directly because they were completed in different years and with a different cultivar mix, they were completed under similar conditions with several of the same cultivars. These same cultivars showed similar yield differences between trials as the overall mean comparisons, suggesting that the comparison between high tunnel and open field systems provides a good estimate of predicted performance.

Mean yields across the cultivars in this trial were highest in harvest seasons one and two, at over 10 $t \cdot ha^{-1}$ but with wide differences among the cultivars (Table 1). This is less than that achieved by Demchak (2009) in Pennsylvania and by Yao and Rosen (2011) in Minnesota but still double or more

Table 1. Yield of seven primocane red raspberry cultivars in a high tunnel field trial at Geneva, NY over three harvest seasons. Field spacing was equivalent to 4099 m of $row \cdot ha^{-1}$ at 3.44 m center to center row spacing.

Cultivar	Mean yield[z] ($t \cdot ha^{-1}$)[y]			Total cumulative Yield[x] ($t \cdot ha^{-1}$)[y]
	Year 1	Year 2	Year 3	
Joan J	14.9 a	15.6 a	12.5 a	43.0 a
Caroline	11.6 b	12.3 ab	8.9 ab	32.8 b
Himbo Top	9.8 bc	10.7 ab	9.4 ab	29.9 bc
Polka	11.6 b	9.4 bc	8.1 b	29.2 bcd
Heritage	8.4 c	10.8 ab	8.6 b	27.8 bcd
Jaclyn	11.7 b	4.8 c	6.0 b	22.5 cd
Autumn Britten	7.6 c	7.2 bc	5.8 b	20.6 d
Mean[w]	10.8	10.1	8.5	

[z] Means (n=3) within columns followed by common letters are not significantly different by Duncan's multiple range test at $P \leq 0.05$.

[y] Multiply $t \cdot ha^{-1}$ by 890 for equivalent $lb \cdot ac^{-1}$.

[x] Mean for all cultivars.

Table 2. Mean fruit weights of seven primocane red raspberry cultivars over three harvest seasons at Geneva, NY.

Cultivar	Mean fruit weight [z] (g)			Three-year mean fruit weight [z,y] (g)	Maximum 10-fruit mean weight (g)			Minimum 10-fruit mean weight (g)		
	Yr1	Yr2	Yr3		Yr1	Yr2	Yr	Yr1	Yr2	Yr3
Autumn Britten	3.09 a	3.03 a	3.03 a	3.05 a	3.8	3.9	3.6	2.3	2.0	2.2
Jaclyn	3.09 a	2.87 a	2.87 ab	2.95 a	3.9	3.8	3.3	2.3	2.4	2.6
Himbo Top	3.01 a	2.99 a	2.81 ab	2.93 a	4.5	3.9	3.4	2.1	2.2	2.3
Joan J	2.90 ab	3.05 a	2.72 ab	2.89 a	5.0	4.0	3.7	1.9	2.2	2.0
Polka	2.63 bc	2.96 a	2.95 ab	2.84 a	3.4	4.0	3.4	1.9	2.2	2.2
Caroline	2.53 c	2.63 a	2.50 bc	2.55 b	3.4	3.8	3.5	1.7	1.7	1.8
Heritage	2.10 d	1.82 b	2.28 c	2.07 c	3.0	2.8	2.0	1.6	1.0	1.2
Mean[x]	2.76	2.76	2.74							

[z] Means (n=3) within columns followed by common letters are not significantly different by Duncan's multiple range test at $P \leq 0.05$.
[y] Mean across all three harvest seasons.
[x] Seasonal mean across all cultivars.

than some field trials (Goulart and Demchak, 1999; Yao and Rosen, 2011). Overall yield in harvest season three was lower than in the first two seasons and this was consistent among all cultivars except 'Jaclyn' where the lowest yield was in season two (Table 1). Over the three harvest seasons, 'Joan J' was consistently the highest yielding cultivar with 'Caroline' and 'Himbo Top' having similar yield in seasons two and three but significantly less cumulative yield over the three season period. Overall there was a 2-fold difference in cumulative yield among the cultivars over three harvest seasons (Table 1).

Additionally, mean fruit weight was higher in this trial compared to some of the same cultivars in the open field trials, with the overall average of 2.8g per fruit in this trial compared to 1.7g in the first season of the open field trial in NY (Weber et al., 2005). This trend of larger/heavier fruit was consistent over subsequent seasons. The mean fruit weight of the cultivars was also consistent over the three harvest seasons (Table 2). 'Autumn Britten' consistently produced the largest fruit (though not significantly larger than most other cultivars in the trial) and 'Heritage' the smallest (Table 2). 'Jaclyn', 'Himbo Top', 'Joan J' and 'Polka' were all very similar to 'Autumn Britten' with 'Caroline' being intermediate. In the Michigan (Hanson et. al., 2005) and Minnesota trials (Yao and Rosen, 2011) the size difference between tunnel production and open field production was not as pronounced but the general trend was the same.

The fruit in this trial was largest at the beginning of the season and dropped off in size as the season progressed, which also occurred in the Minnesota trial (Yao and Rosen, 2011). However, the lowest fruit weights in most plots were recorded at or just following the peak harvest date before rebounding towards the end of the season when the crop load was reduced. The rebound often lasted until the final harvests in the last week to 10 days. The decline in fruit size was as much as 64% in 'Heritage' in year 2, but the mean decline for 'Heritage' over all 3 seasons was only 36%. The greatest mean seasonal fruit weight decline over the 3 seasons was observed in 'Caroline' at 51%, followed closely by 'Joan J' (47%) and 'Polka' (44%). Growers will need to determine their own threshold for when fruit weight and crop load make harvest uneconomical. Further research may be useful in determining if the observed fruit size reduction near peak crop load can be mitigated through more precise water management leading up to this period. The lowest mean fruit weight declines were recorded for 'Himbo Top', 'Autumn Britten' and 'Jaclyn' at 26%, 30% and 31%, respectively. This wide difference in fruit weight uniformity among the cultivars suggests a strong genetic effect on this character. Therefore, improving this uniformity is likely to be possible through breeding. Future trials with newer cultivars will determine if progress has been made and the potential for future improvements.

The length of the harvest season extended to several weeks for all cultivars, starting as early as 23 Jul. in year 1 and as late as 11 Aug. in year 2 for the early cultivars (Table 3) and lasted 10 weeks cumulatively each year for all the cultivars (Table 3). The late cultivars in this trial stretched the season into Oct. in each year (Table 3). The harvest ended each year not due to cold weather as is often observed in open field production but due to the fruit all being harvested. With the recent development of later producing cultivars, production using high tunnels could stretch well into Nov. in most years in central NY, possibly replacing 'Heritage' as the standard late season cultivar with a cultivar with larger fruit.

Overall, the performance of all seven cultivars under high tunnels was better than that observed in outdoor trials. Pest control requirements were also significantly reduced in the tunnels. No fungicides were used at

Table 3. Harvest dates for 7 primocane raspberry varieties grown under high tunnels over three harvest seasons in Geneva, NY.

Cultivar	Harvest Season	1st Harvest Date[z]	Last Harvest Date[y]	Peak Harvest Date[x]	Season Length (days)
Autumn Britten	1	Aug 11	Sept 28	Sept 1	49
	2	Jul 23	Sept 1	Aug 9	41
	3	Aug 3	Sept 30	Aug 24	59
Caroline	1	Aug 25	Oct 16	Sept 17	53
	2	Aug 9	Sept 24	Aug 20	47
	3	Aug 15	Sept 23	Aug 24	40
Heritage	1	Aug 28	Oct 16	Sept 18	50
	2	Aug 16	Oct 2	Aug 27	48
	3	Aug 17	Oct 11	Sept 12	56
Himbo Top	1	Aug 18	Oct 16	Sept 4	60
	2	Jul 30	Sept 17	Aug 25	53
	3	Aug 3	Sept 26	Aug 30	55
Jaclyn	1	Aug 17	Oct 12	Sept 9	57
	2	Jul 30	Sept 20	Aug 13	53
	3	Aug 8	Sept 23	Sept 6	47
Joan J	1	Aug 17	Oct 16	Sept 9	61
	2	Jul 26	Sept 20	Aug 18	56
	3	Aug 8	Sept 26	Aug 29	50
Polka	1	Aug 18	Oct 8	Sept 16	52
	2	Jul 23	Sept 20	Aug 18	60
	3	Aug8	Sept 30	Aug 22	54
Whole	1	Aug 11	Oct 16		67
Planting	2	Jul 23	Oct 2		72
	3	Aug 3	Oct 11		70

[z] Date when any plot had ripe fruit.
[y] Date when the last fruit from any plot was harvested.
[x] Date with the highest total yield.

any time in the trial and only minimal hand weeding was needed for weed control within the rows. Even without fungicide treatments, no appreciable fruit rots were observed. However, spotted winged drosophila (*Drosophila suzukii*) has become a serious pest in raspberry production in most if not all production regions in the U.S. including NY. A diligent insecticide spray program rotating recommended chemical classes (Pritts et al., 2015) or complete exclusion with netting (http://blogs.cornell.edu/swd1/2016/04/19/exclusion-netting-against-swd/) is currently required to control this pest in order to have marketable fruit.

Additionally, in the first year of production, there were symptoms associated with feeding by potato leafhoppers (*Empoasca fabae*), especially in the cultivars 'Polka' and 'Jaclyn'. These insects moved into the tunnel after the first mowing around the outside of the tunnels. Damage observed included stunted canes, twisted leaves and yellowing of the leaves similar to a nutritional deficiency or viral infection. Considerable damage was done to developing fruit at the time of infestation but, if yield was affected, the effects were marginal as new growth developed normally and overall yield was similar to unaffected cultivars (Table 1). The extent that the tunnel system exacerbated the leafhopper infestation or symptoms is not known but little leafhopper damage has been observed in open production trials at this location. In subsequent seasons, more careful management of the vegetation surrounding the tunnels kept leaf hopper damage to a minimum and yield for 'Polka' and 'Jaclyn' were in line with most of the other cultivars though tending to be in the lowest grouping (Table 1).

It is important to note that while all the cultivars tested performed very well compared to open field conditions, many displayed characteristics that may limit their suitability for some markets. Dark red fruit color, especially after storage was observed in many cultivars, especially 'Joan J', 'Polka' and 'Jaclyn', making them less than ideal for wholesale markets and some retail outlets. Fruit from 'Autumn Britten' and 'Caroline' could also be dark when overripe or after a few days of storage but were not as problematic as the former varieties. Dark red fruit was most problematic in 'Jaclyn' because this cultivar is very difficult to detach until it is completely ripe (when the fruit is darkest). The receptacle is elongated and thin and adheres tightly in the fruit cavity. This can cause damage when extra force is needed for picking. These fruit characteristics severely limit the usefulness of 'Jaclyn' even though it has superior flavor. The darker fruit color observed in many of these cultivars is often perceived by consumers as being overripe and having poor shelf life. This is especially problematic for red raspberries because of their relatively high cost and short shelf life compared to other fresh fruits. This is less problematic in local markets in which fruit is marketed soon after harvest and consumers can often obtain information from the grower concerning variation in cultivars and when the fruit was harvested.

'Himbo Top' had some of the best fruit quality with bright, shiny red, firm fruit with good flavor. However, the fruit was noticeably softer when temperatures at harvest were high, requiring immediate chilling to maintain fruit quality. Additionally, 'Himbo Top' canes are taller than the other cultivars tested. This, in combination with long fruiting laterals and a crop concentrated on the top third of the plant, makes them top-heavy and prone to weeping. Considerably more trellising is required compared to the other cultivars in order to keep the canes upright and easy to harvest. This greater cane height, however, does present the potential of double cropping the floricanes of 'Himbo Top' in the summer since considerable cane length for fruiting remains at the end of the fall (primocane) season. Managing this system can be done but was outside the scope of this study.

'Caroline' performed very well but was very vigorous and the thick foliage often obscured the fruit and slowed harvest. This led to unpicked ripe fruit, which then became overripe fruit with poor shelf life in the next harvest and a target for spotted wing drosophila infestation. Careful and thorough harvest is required to best manage this cultivar. It may also benefit from primocane thinning and precise trellising to mitigate the problem. Conversely, 'Autumn Britten' was the least vigorous cultivar based on sucker production. It was the lowest yielding cultivar, mainly due to fewer canes for harvest. It had very good fruit quality in firmness and flavor, though it too could be dark. Higher initial plant density and careful attention to nutrition may be useful in increasing cane density to increase overall yield.

'Heritage', the standard primocane cultivar for the region, performed as expected with very good cane development and fruit numbers. The round shaped fruit was typically smaller than other cultivars, which reduced harvest efficiency and made it less desirable in the marketplace. However, fruit quality was consistent and local consumers did not object to the size. Overall, even with some shortcomings in the cultivars, none of the fruit was rejected by local wholesale buyers who reported that the fruit was acceptable for local markets. The use of high tunnels for raspberry production continues to be adopted by local producers as the benefits become recognized. Current cultivars can meet the immediate demand for local fruit, but improvements in size, color and yield are required for the industry to expand and take advantage of the expanding local market. Additional trials on more recently developed cultivars would also be useful to local growers.

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Organic Blackberry Cultivar Trials at High Elevation and in High pH Soil in the Southwestern United States

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Abstract

Two semi–erect, three erect floricane-fruiting and one primocane-fruiting blackberry (*Rubus* L. subgenus *Rubus* Watson) cultivars were evaluated in high tunnels and in the field at Alcalde, NM. Semi–erect cultivars Triple Crown and Chester Thornless were planted at 1.5 × 2.5 m in a 5.0 × 12.2 m high tunnel with an identical field planting. Erect cultivars Ouachita, Natchez and Navaho, and primocane-fruiting cultivar Prime-Ark® 45 were planted at 0.6 × 1.7 m in another 5.0 × 12.2 m high tunnel with an identical field planting. Comparing all cultivars, yield of floricane-fruiting cultivars was reduced by winter damage while the primocane-fruiting cultivar Prime-Ark® 45 had reliable fall crops in the high tunnel each year in northern New Mexico. Based on four winters' weather, canes of semi–erect and erect blackberry cultivars overwintered well when mid-winter temperatures dropped to -15 °C, but canes were damaged when temperatures reached -20 °C. 'Chester Thornless' was more cold hardy than 'Triple Crown', and 'Ouachita' was more cold hardy than 'Navaho' and 'Natchez' in the field. 'Triple Crown' produced 1-1.5 times more yield than 'Chester Thornless' in the high tunnel and in the field, while 'Ouachita' had the highest yield in 2015 and the highest cumulative yield from 2012–2015 among the three erect cultivars tested in the high tunnel, while in the field 'Natchez' had the highest cumulative yield. 'Prime-Ark® 45' produced well in the fall in the high tunnel but not in the field. 'Navaho' had small plants and low yields both in the high tunnel and in the field. In northern New Mexico or similar areas with a short growing season, 'Triple Crown', 'Ouachita', 'Natchez' and 'Prime-Ark® 45' are recommended for high tunnels while 'Triple Crown', 'Natchez', and 'Ouachita' are recommended for field planting.

Blackberry (*Rubus* L. subgenus *Rubus* Watson) and raspberry (red raspberry- *Rubus ideaus*) are closely related and together they are called brambles or caneberries. Generally, blackberry is more heat tolerant than raspberry. Raspberry production is prevalent in regions with cool summers like the West Coast. Washington, Oregon and California account for over 80% of raspberry production in the United States, whereas blackberry production is more widely distributed, including southern states with hot summers like Florida, Georgia and Texas (USDA NASS, 2014; Strik et al. 2007). Blackberries have trailing, semi–erect and erect growing habits. In general, erect cultivars and semi–erect cultivars are more cold hardy than trailing types and trailing cultivars are not suitable for cold areas (Black and Lindstrom, 2014; Weber, 2013; Westwood, 1993). Clark (1992) conducted a survey of 13 Southern states and the dominant cultivars were erect cultivars (78%) and the rest were semi–erect. Trailing blackberries are more adapted to the mild climate of the Pacific Northwest and California in the U.S. (Finn et al., 2005a, 2005b, 2005c and 2005d).

Brambles have performed well in high tunnels and yields can be more than double that of those planted in the field (Thompson et al., 2009; Demchak, 2009; Domoto et al., 2008; Rom et al., 2010). By using high tunnels, the harvest season of floricane-fruiting blackberries can be advanced and the harvest season of primocane-fruiting blackberries can be extended (Demchak, 2009; Lamont

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et al., 2003; Rom et al., 2010). There is very limited blackberry research in the Southwest (Walser and Guldan, 2006). The objectives of this study were to evaluate semi–erect, erect and primocane-fruiting blackberry cultivars, compare high tunnel and field planting, and recommend suitable cultivars for growers in northern New Mexico or similar regions with short growing seasons and high pH soils.

Materials and Methods

This study was conducted at New Mexico State University's Sustainable Agriculture Science Center at Alcalde, NM (Lat. 36°05'27.94" N, long. 106°03'24.56" W, and 1735 m elevation). Two semi–erect cultivars, Triple Crown and Chester Thornless, three erect cultivars, Natchez (Clark and Moore, 2008), Navaho, and Ouachita (Clark and Moore, 2005), and one primocane-fruiting cultivar Prime-Ark® 45 (Clark and Perkin-Veazie, 2011) were used in this experiment. All plants were tissue cultured plugs from North American Plants (Lafayette, OR). The two existing high tunnels used in this study were built at the Center with dimensions of 5.0 × 12.2 m. The main structure consisted of vertical metal posts 1.2 m apart and 1.2 m above ground, with two inch plastic pipe (polyvinyl chloride) arched for the upper part with highest point of 2.9 m. The high tunnel had one door at each end and roll-up sides. The cover used was 8 μm Solarig 172 woven plastic (J&M Industries, Inc., Ponchatoula, LA). The two semi–erect cultivars were in one high tunnel while the erect and primocane-fruiting cultivars were in another identical high tunnel. For the high tunnel with semi–erect cultivars, there were two rows in the high tunnel with a randomized complete block design with three replications at a planting density of 1.5 × 2.5 m. For the high tunnel with primocane-fruiting and floricane-fruiting erect cultivars, there were three rows using a randomized complete block design at 0.6 × 1.7 m planting density. There were identical plantings in the nearby field for each high tunnel. All plants were planted in May 2011 and the plantings were in a USDA certified organic field and managed organically. Weeds were managed manually both in the high tunnels and in the open field and pests were scouted weekly during the growing season and managed organically as needed (Bushway et al., 2008).

The soil type was a Fruitland sandy loam (coarse-loamy, mixed, superactive, calcareous, mesic Typic Torriorthents) with 1.6-1.7% soil organic matter in the top 15 cm of soil and the soil pH was 7.9-8.0 (1:1 water extraction) (Yao et al., 2015). About 56 kg/ha nitrogen (N) as cotton seed meal (7.0N-0.9P-0.8K) was applied before planting for all plantings. Two 1.27 cm diameter polyethylene drip irrigation tubing lines with emitters 30 cm apart delivering 3.78 L per h were installed along each row after planting and plants were watered once per week for 4 h or as needed during the growing season each year. Organic fish fertilizer (Neptune's Harvest fish fertilizer 2.0N-1.7P-0.8K, Gloucester, MA) was applied through fertigation at two week intervals at a rate of 1.9 L each time per high tunnel or field planting, two to three times for 2011-2012 and four to five times in June and July for 2013-2015. Semi–erect cultivars were in a single 1.5 m high trellis system in each row, while two 1.2 m high single wire trellises at 0.6 m apart were used for each row to hold the plants of erect cultivars in the row. Semi–erect cultivars were pinched when the canes reached the top of trellis while the canes of 'Prime-Ark® 45' were also pinched when the canes were 90-100 cm in height (Strik et al., 2012). 'Prime-Ark® 45' was grown only for a primocane crop with all canes pruned to the ground while dead floricanes were removed for other cultivars in March each year. The sides of both high tunnels were kept open year round except in Oct. when sides were lowered at night for frost protection.

Cane winter damage was assessed visually in May of each year. If no bud break (lateral emergence) occurred, they were judged as dead. Fruit from all plants in each plot was harvested twice per week or as necessary from 2012-2015. Total fruit weight and weight of 30 fruit from each plot were recorded for each harvest. Fruit weight and fruit yield data of erect and primocane fruiting cultivars were analyzed with ANOVA by Statistix (Tallahassee, FL). Since there was only one high tunnel and one field planting for each blackberry type, they were analyzed separately and could not be compared statistically. For semi-erect cultivars, due to limited degrees of freedom, standard deviation was calculated for yield data instead of ANOVA. Standard deviation was also calculated for cane winter damage data.

Results and Discussion

Weather data and plant winter damage. Fig. 1 presents winter and spring daily minimum temperature data from 2011 to 2015. Blackberry plant performance was closely related to weather conditions in northern New Mexico. The winters of 2011/12 and 2012/13 both had minimum temperatures of -20°C or lower in Dec 2011 and Jan 2013. The winter of 2013/14 had fewer extremes in mid–winter than the winters of 2011/12 and 2012/13, but there were more late frosts (0 °C or lower) in April and May 2014 (Fig. 1). Regardless of growing habit or cultivar, field plantings had more winter cold injury than the high tunnel plantings (Table 1). Plants suffered more winter cane damage in 2012 and 2013 than in 2014 and 2015 (data not shown for 2014 and 2015). Even though the canes in high tunnels were green in spring of 2012 and 2013, the buds on the floricanes of some plants never broke and laterals only emerged on the lower 30-50 cm of floricanes. Clark et al. (2012) mentioned that winter injury of floricane-fruiting blackberry cultivars is a big concern in mid-western and northern U.S. It is also true in the Southwestern U.S. at high elevation areas such as in northern New Mexico. The floricane-fruiting blackberry crop is not reliable in these areas because of winter minimum temperatures.

For the two semi–erect cultivars tested, 'Chester Thornless' was more cold hardy than 'Triple Crown' in the field in 2012 (Table 1). In New York, 'Chester Thornless' was also reported more cold hardy than others (Weber, 2013). For erect cultivars, Ouachita was more cold hardy than 'Natchez' and 'Navaho' in both 2012 and 2013 (Table 1) in the field. In 2014 and 2015, all floricanes over-

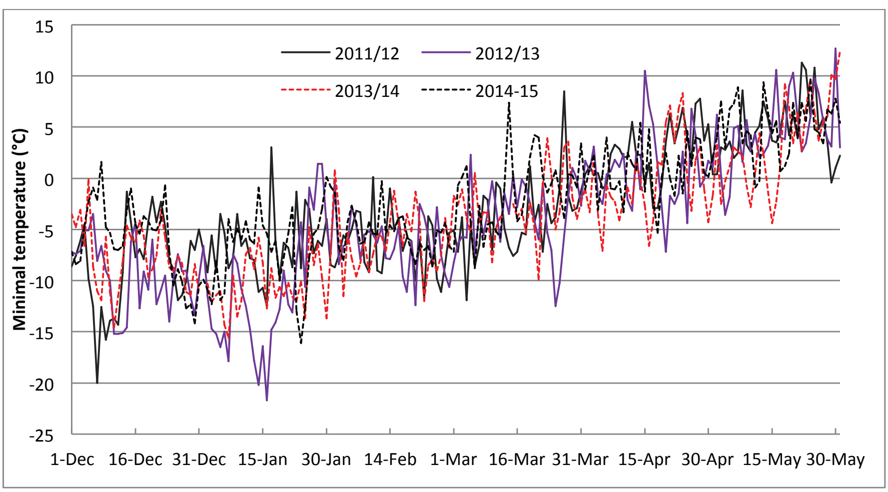

Figure 1. Daily air minimum temperatures for Dec. to May from 2011-2015 at Alcalde, NM.

Table 1. Blackberry cane winter damage in 2012 and 2013 at Alcalde, NM.

Type	Location	Cultivar	Winter damage 2012 [z]	Winter damage 2013
Semi-erect	Field	Chester Thornless	2.6 ± 0.18 [y]	3.1 ± 0.25
		Triple Crown	4.7 ± 0.18	3.2 ± 0.25
	High Tunnel	Chester Thornless	1.0 ± 0.09	1.0 ± 0.00
		Triple Crown	1.2 ± 0.09	1.0 ± 0.00
Erect	Field	Natchez	2.7 ± 0.19	4.4 ± 0.24
		Navaho	3.4 ± 0.19	4.1 ± 0.23
		Ouachita	2.1 ± 0.19	3.3 ± 0.23
	High Tunnel	Natchez	1.1 ± 0.04	1.1 ± 0.10
		Navaho	1.0 ± 0.04	1.1 ± 0.09
		Ouachita	1.0 ± 0.04	1.0 ± 0.09

[z] Damage Rating Scale: 1. No damage; 2. One year old cane tip damaged; 3. Up to half length of cane damaged; 4. More than half of cane length damaged; 5. All canes dead.
[y] Mean ± SE

wintered well in high tunnels for semi–erect and erect cultivars. For the field plantings, there was minor floricane tip damage for both semi–erect and erect cultivars.

Based on weather data from 2011-2015 and blackberry cane winter damage data, floricanes overwintered well at -15 °C but they were damaged at -20 °C. Strong winds and huge temperature fluctuations in the spring in northern New Mexico can also contribute to floricane injury. Even though the sides of high tunnels were open in winter, they still provided protection with warmer daytime temperatures and reduced wind speed. Others also reported that blackberry winter damage was reduced in high tunnels (Demchak, 2009; Strik et al., 2007). However, high tunnels did not provide enough protection for floricanes in mid–winter when temperatures dropped to -20 °C or lower in this study.

Frost damage occurred occasionally during the trial period. The frost on 29 May 2012 killed new primocanes and forced the plants to produce new primocanes and primocane branching. We noticed damage to early blooming flowers for 'Natchez' and 'Ouachita' due to the mid–May frosts both in 2014 and 2015.

Yield. In 2012, the first harvest year after planting, all erect cultivars had a very light crop while there was no crop for semi–erect cultivars (Table 2, Table 3). 'Prime-Ark® 45' had a reliable fall crop each year despite the severe winter weather conditions, while the yields of floricane-fruiting cultivars were related to plant age and weather conditions (Table 2). In the high tunnel, there were no significant differences among 'Prime-Ark® 45', 'Ouachita' and 'Natchez' both in 2013 and 2014, only 'Prime-Ark® 45' had a significantly higher yield than 'Navaho' in 2013, and both 'Prime-Ark® 45' and 'Ouachita' had a greater yield than 'Navaho' in 2014 (Table 2; Fig. 2A). In 2015, the fifth year after planting, 'Ouachita' yielded an average of 5.6 kg/plant, higher than 'Navaho', but not significantly different from 'Natchez' and 'Prime-Ark® 45'. The high yield of 'Ouachita' in a mature stand was also documented in other locations (Clark and Moore, 2005, 2008). 'Navaho' had the smallest plants and lower accumulated yield than 'Ouachita' in the high tunnel (Table 2).

For the field planting, 'Natchez' had higher cumulative yield than 'Navaho' and 'Prime-Ark® 45' (Table 2). 'Natchez' appeared to produce similar yield in the high tunnel and in the field from 2013-2015 while 'Ouachita' and 'Prime-Ark® 45' yielded higher in the high tunnel. 'Navaho' had the smallest plants among the four cultivars in the field planting. Perhaps because of high elevation and a shorter growing season in northern New Mexico, 'Prime-Ark® 45 grew better in the high tunnel than in the field as described in other areas with shorter growing seasons (Clark, 2008). A high tunnel extended the growing season of the primocane-fruiting cultivar Prime-Ark® 45 and ensured that fruit reached maturity for harvest; however, 'Prime-Ark® 45' is not a reliable cropper for planting in the open field in northern New Mexico as in New York and Minnesota (Clark, 2008). 'Prime-Ark® 45' avoided the winter damage issue for floricane-fruiting cultivars in cold areas (Clark, 2008), but early maturing primocane-fruiting cultivars with good fruit quality, especially thornless ones, are needed for short growing season areas (Clark, 2008; Clark et al., 2012).

For the yields of semi–erect cultivars, the improved cold hardiness of 'Chester Thornless' was reflected in the yield in the high tunnel in 2013. While in years with mild winters, 'Triple Crown' produced nearly two- to three-fold the yield of 'Chester Thornless' in the high tunnel in 2014 and 2015, respectively (Table 3 and Fig. 2B). In the field, 'Triple Crown' also produced higher yields than 'Chester Thornless' in both 2014 and 2015. The yield of 'Triple Crown' in the high tunnel in this study is close to that reported

Table 2. Yield of erect and primocane blackberry cultivars in high tunnel (HT) and field from 2012 to 2015 at Alcalde, NM. The yields from high tunnel or field each year were analyzed separately.

Location		Yield (kg/plant)					kg/ha	kg/ha
	Cultivar	2012	2013	2014	2015	2012-15	2015	2012-15
HT	Prime-Ark 45	0.21	1.10 a[z]	1.31 a	2.16 ab	4.78 ab	21,088	46,693
	Ouachita	0.27	0.54 ab	1.33 a	5.55 a	7.68 a	54,279	75,075
	Natchez	0.39	0.42 ab	1.03 ab	2.57 ab	4.41 ab	25,106	43,065
	Navaho	0.02	0.14 b	0.50 b	2.01 b	2.67 b	19,622	26,093
Field	Prime-Ark 45	0.23	0.12 a	0.16 b	0.78 b	1.30 b	7,664	12,670
	Ouachita	0.03	0.05 b	0.26 b	1.63 a	1.96 ab	15,897	19,201
	Natchez	0.04	0.04 bc	0.98 a	2.00 a	3.06 a	19,592	29,877
	Navaho	0.04	0.01c	0.55 ab	0.50 b	1.10 b	4,927	10,764

[z] Means within each column and location not followed by common letters are significantly different at $P \leq 0.05$, by Fisher's protected LSD.

Table 3. Yield of semi-erect blackberry cultivars in high tunnel (HT) and field from 2013 to 2015 at Alcalde, NM. (n=3, mean ± SE)

	Yield (kg/plant)				kg/ha
	2013	2014	2015	2013-15	2013-15
HT-Triple Crown	0.35±0.13	9.45±2.04	10.16±2.63	19.96±4.76	53,219
HT-Chester Thornless	0.82±0.57	4.91±0.54	3.73±1.10	9.46±1.14	25,227
Field-Triple Crown	0.02±0.02	2.41±0.39	7.96±1.42	10.39±1.80	27,696
Field-Chester Thornless	0.06±0.07	1.59±0.19	2.78±0.92	4.42±1.08	11,782

in high tunnels in Pennsylvania (Demchak, 2009).

High tunnels advanced the harvest season for semi–erect cultivars and erect cultivars by one to three weeks and extended the harvest season of the primocane-fruiting cultivar for two weeks in the fall (Fig. 2A and 2B). Similar results have been reported in other states (Demchak, 2009; Rom et al., 2010; Thompson et al., 2009). The harvest season of 'Natchez' in the field planting was similar to that in the high tunnel in 2014 which could be related to the mid-May frost that killed the early blooms and reset the blooming process.

Fruit size. 'Triple Crown' fruit matured earlier and was larger than 'Chester Thornless', while 'Chester Thornless' was firmer than 'Triple Crown' (Table 4, Fig. 2B). 'Prime-Ark® 45' and 'Natchez' had bigger fruit while 'Navaho' had the smallest fruit overall (Table 4). Fruit sizes in high tunnels appeared bigger than those in the field for all cultivars except 'Chester Thornless' which had similar size in the field and in the high tunnel in 2014 (Table 4). Larger fruit in high tunnels was also reported elsewhere (Thompson et al., 2009) and for raspberries (Yao and Rosen, 2011). Clark et al. (2012) mentioned a substantial genotype × environment interaction for three primocane-fruiting cultivars -- smaller fruit for 'Prime-Jan®', 'Prime-Jim®' and 'Prime-Ark® 45' in Arkansas and bigger fruit in cool areas like Oregon. High temperatures (>32°C) during bloom and fruit development stage negatively impact fruit size and quality (Clark et al., 2012). In northern New Mexico, it can be hot (>32°C) during the day but always cool at night and the fruit size of 'Prime-Ark® 45' was similar to or larger than erect or semi–erect cultivars tested. It seems not only the maximum temperature, but also the diurnal temperature difference plays a role in primocane-fruiting blackberry fruit development and fruit quality.

In summary, semi–erect, erect and primocane-fruiting blackberries all can be planted in northern New Mexico, but if minimum temperature drops to -20 °C or lower, floricane damage can be expected. Even high

Table 4. Blackberry fruit weight of different cultivars grown in high tunnels (HT) and field in 2014 at Alcalde, NM.

Cultivar	HT (g)	Field (g)
Prime Ark® 45	7.0 a[z]	6.3 a
Natchez	6.8 ab	5.0 ab
Ouachita	6.6 b	4.8 ab
Navaho	4.0 c	3.0 b
Triple Crown	7.4 a	6.0 a
Chester Thornless	3.1 b	3.3 b

[z] Means within each column and fruiting type (erect or semi-erect) not followed by common letters are significantly different at $P \leq 0.05$, by Fisher's protected LSD.

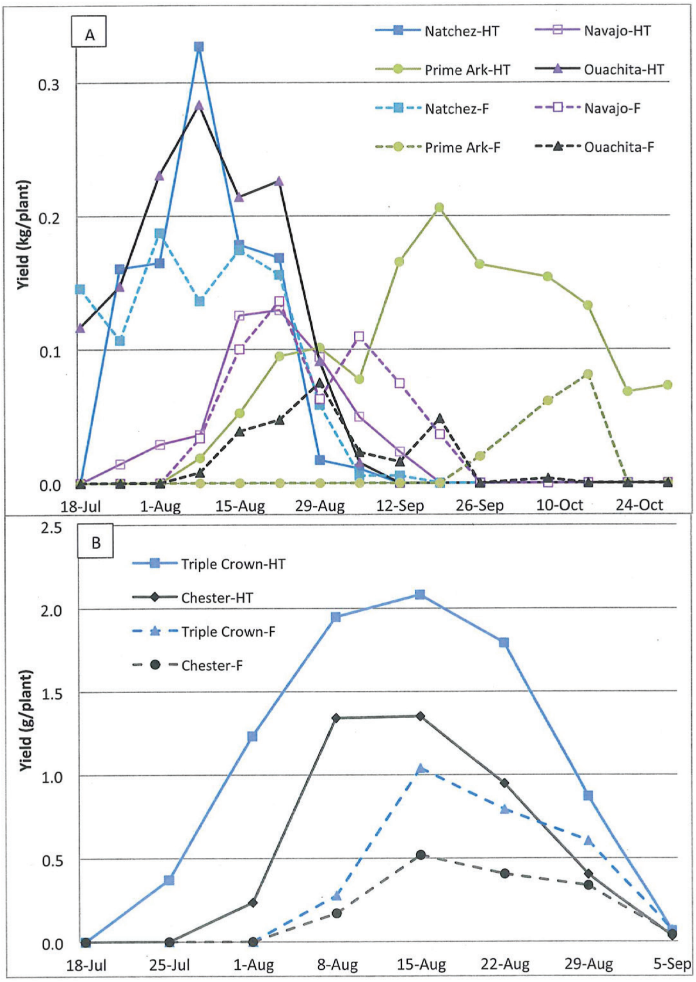

Figure 2. Seasonal yields of erect and primocane-fruiting blackberry cultivars (A), and semi–erect cultivars (B) grown in high tunnels (HT) and the field in 2014 at Alcalde, NM.

tunnels could not protect floricanes from temperatures lower than -20 °C. For cultivar Prime-Ark® 45, if it is considered as primocane-fruiting only as in this trial, winter extreme temperatures do not affect its yields in northern New Mexico.

Blackberries produced higher yields in high tunnels. Semi–erect 'Triple Crown', erect 'Ouachita' and 'Natchez' and primocane-fruiting 'Prime-Ark® 45' performed well in high tunnels. Semi–erect and erect cultivars together with 'Prime-Ark® 45' would greatly extend the fruit supply season which is critical for those selling their produce at local farmers markets. For field planting, 'Triple Crown', 'Natchez', and 'Ouachita' are good choices. 'Prime-Ark® 45' does not perform well in short growing season areas in open field plantings. Blackberry still is a risky fruit in northern New Mexico and can be severely damaged in winters when minimal temperatures drop to -20 °C or lower.

Selecting a protected area or planting them in high tunnels would reduce this risk considerably.

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