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CONTENTS

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Effects of Ethephon as a Blossom and Fruitlet Thinner on Yield and Fruit Quality of 'Jubileum' European Plum in a Nordic Climate

Mekjell Meland[1] and Clive Kaiser[2]

Additional index words: abscission, fruit set, return bloom, *Prunus domestica* L.

Abstract

European plum cultivar Jubileum (*Prunus domestica* L.) blooms abundantly most years and too many fruit can be set if flowers and/or fruitlets are not properly thinned. In 2007, 2008 and 2009, mature 'Jubileum/St. Julien A' trees were treated with ethephon either at full bloom, at concentrations of 250, 375 and 500 mg/l or when fruitlets averaged ~12 mm in diameter at concentrations of 125, 250 and 375 mg/l. In general, flower-thinning treatments reduced fruit set significantly. Fruit set decreased with increasing ethephon concentrations, and the highest rate of ethephon applied either at full bloom (500 mg/l) or post bloom (375 mg/l) resulted in excessive over-thinning. Up to 375 mg/l of ethephon was required at full bloom whereas only 125 mg/l of ethephon was required post bloom for marked fruitlet thinning. Yields confirmed the fruit set response and yield reductions were significant. In most years, all thinning treatments resulted in fruit larger than 38 mm in diameter compared to fruit from unthinned control trees. Fruit quality, characterized by blue surface color and soluble solids content was generally higher and increased significantly with the reduction in crop load. Fruit firmness of fruit from all ethephon applications was lower than that of the fruit from unthinned control trees. In contrast, titratable acidity did not show a clear response to ethephon thinning. Return bloom the following year was mostly unaffected by all ethephon applications compared to the control. In conclusion, an ethephon application at a rate of up to 375 mg/l applied at full bloom will result in adequate thinning of 'Jubileum' plums and achieved a target of about 10-15 % reduction in fruit set. When weather conditions are not conducive during flowering, a post bloom ethephon application at 125 mg/l may be applied however, this should only be considered in years of excessive flowering and as a last resort.

The European plum cultivar 'Jubileum', which is widely grown in Norway, frequently produces too many flowers and sets too many fruit. Consequently, unless flowers and/or fruitlets are thinned, regular yields of marketable fruit of acceptable quality and size cannot be achieved. Unlike other European countries, the Norwegian market requires European plums of at least 36-38 mm in diameter. In addition, branches may break under the heavy crop load and flowering may be reduced in the subsequent season. Hand thinning of flowers and/or fruitlets is both tedious and costly. Consistent annual yields of high quality fruit may be achieved in commercial orchards when this cultivar is thinned at full bloom using mechanical thinning (Seehuber et al., 2011; Weber, 2013) or a chemical agent such as ammonium thiosulfate (ATS) (Seehuber et al., 2011; Meland, 2004). Further crop load adjustments are usually made by hand following "June drop". Should it be possible to avoid hand thinning, this will reduce labor costs and improve fruit quality, thereby significantly increasing the value of the crop.

Exogenously applied ethephon stimulates ethylene production, which in turn causes fruit abscission (Wertheim, 2000). Previous evaluations of ethephon on stone fruit at

[1] Norwegian Institute of BioEconomy Research, NIBIO, Ullensvang, N-5781, Lofthus, Norway
Corresponding author: mekjell.meland@nibio.no
[2] Associate Professor and Umatilla County Extension Horticulturist, Department of Horticulture, Oregon State University, 418 N Main St, Milton-Freewater, OR, 97862, USA.

full bloom or two weeks after full bloom with warm weather conditions demonstrated that ethephon is a successful thinning agent (Meland, 2004) however, results were not always predictable nor consistent (Webster and Spencer, 2000). Usually ethephon performs better as a fruitlet thinner. This may be attributable to the higher temperatures later in the season and/or increased sensitivity of the fruit to ethephon at the later 'pit hardening' stage (Webster and Spencer, 2000). Chemical thinning of blossoms permits reduction of the potential overset at the earliest possible stage, thus reducing the impact on photoassimilate reserves, but fruit can abscise under Nordic conditions due to a post bloom late frost. In Scandinavia, fruit thinning with ethephon at the early bloom stage or lime sulphur at full bloom have been recommended (Kvåle, 1978). A single dilute application of 250 mg/l ethephon at full bloom reduced fruit set and crop load, and increased fruit quality and return bloom of the cultivar 'Victoria' (Meland 2007). However, these chemicals occasionally produce inconsistent results on a commercial scale. Fruit thinning following bloom permits a more exact evaluation of fruit set before any application of a thinning agent. Jakob (1998) found that the mixture ethephon-NAA applied to plums 30–40 days after bloom had a significant thinning effect. Using ethephon alone at post bloom was too unpredictable and caused over-thinning.

Martin et al. (1975) found that 'French Prune' could be effectively thinned using ethephon spays if applied when the seeds were approximately 8-9 mm long. However, the main problem with these sprays was the inconsistent response from site to site and from season to season. Consequently, warm weather (>15 ºC) at the time of spraying and ethephon concentrations of between 200-250 mg/l appear most appropriate for thinning European plum cultivars and in general this coincides with the fruitlet stage reported by Webster and Spencer (2000). Basak et al. (1993) found that 'Opal' and 'Common Prune' were thinned effectively using 200 mg/l ethephon applied two weeks after flowering and Seehuber et al. (2011) and Weber (2013) using ATS and/or ethephon four weeks after flowering.

The aim of the present investigation was to evaluate the effect of ethephon at different concentrations as a thinning agent for 'Jubileum' plum when applied at full bloom or post bloom.

Materials and Methods

In 2007, a field trial was initiated on six-year-old European plum 'Jubileum', grafted on 'St. Julien A' rootstock in a commercial orchard near the shore of the Hardangerfjord near Nibio, Ullensvang (60.2 ºN). Productive, uniform slender spindle trees, spaced at 2 x 4 m and pruned to a maximum height of 2.5 m with an optimum yield of 15-18 kg/tree. Trees were grown in a loamy sand with ~4% organic matter and sprayed in 2007, 2008 and 2009 either at full bloom at concentrations of 250, 375 or 500 mg/l ethephon or post bloom at concentrations of 125, 250 or 375 mg/l ethephon when fruitlets averaged ~12 mm in diameter. Optimum yield was set at ≥10 kg/tree. The experiment was a two by three factorial (2 timings and 3 ethephon concentrations) plus an untreated control. Before budbreak each year, trunk circumference (cm) was measured at 0.25 m above the soil level. Experimental trees were blocked using cm^2 trunk cross sectional area (TCSA) measured before bloom the first year. Subsequently, each tree received the same treatment each year. Orchard floor management consisted of frequent mowing of the interrows and a 1 m wide herbicide strip was maintained in the intrarow. Trees were irrigated by drip irrigation when water deficits occurred. All trees received the same amount of fertilizers based on soil and leaf analysis.

The ethephon source was 'Cerone' (48 % a.i. ethephon w/v) (Bayer Crop Science, Monheim am Rhein, Germany). In all three years, treatments were applied to whole trees as dilute sprays with a handgun to the

point of run-off with approximately 2 l/tree. To prevent spray drift during application a portable plastic shield was placed between each tree. No surfactants or other additives were included with the sprays. The date of application for each year, maximum daily temperature on the day of application and the highest maximum daily temperature on the 3 days following application; maximum solar radiation on the day of application and the highest solar radiation on the 3 days following application, and relative humidity on the day of application are presented in Table 1.

Fruit set was calculated each year by counting the number of flowers on three branches per tree prior to ethephon application. Subsequently, fruit counts were measured each year shortly before harvest on the same branches. At harvest, fruit were selectively picked on two occasions one week apart. Fruit were harvested according to commercial fruit standards and the first selective picked dates were 08/31/2007, 09/4/2008 and 09/08/2009.

Total yields were recorded for each tree at harvest and graded according to current standards (Standardization Organization of Norway, 1999). A sample of 10 randomly selected fruit from each experimental tree was used to determine fruit quality. Fruit firmness was measured on two sides of each fruit, using a fruit texture digital table penetrometer (Durofel®, Copa-Technology CTIFL, Vandoeuvre-lès-Nancy, France). Surface color was rated on a scale from 0 to 100%, where 0 % = no blue color and 100% blue color, covering the entire fruit surface area. From each sample total soluble solids concentration was evaluated using a handheld digital refractometer (Atago® ,Tokyo, Japan). Titratable acidity (TA) was measured using an auto-titrator (model TIM865 Titration Manager, Radiometer Analytical SAS, Lyon, France) with 0.1 mol/l NaOH to endpoint pH 8.2 and expressed as percentage of malic acid (%). The following spring, return bloom was recorded as the total number of flowers per branch from the same three sample branches. Data were evaluated using Genstat® 17 statistical software (VSN International, Rothamsted, UK) testing for differences between all crop load parameters and effects on fruit quality. Unless noted otherwise, only results significant at $P \leq 0.05$ are discussed.

Results and Discussion

2007. Both TCSA and the number of flowers per branch were uniform at the start of the experiment (Table 2). All thinning treatments reduced crop load compared to the unthinned control. Fruit set was reduced curvilinearly with increasing concentration of ethephon. The two highest rates of ethephon, 500 mg/l at full bloom and 375 mg/l post bloom, resulted in insufficient yields for commercial

Table. 1: Climate data in Ullensvang, Norway on the day of application of ethephon at full bloom or post bloom and the 3 days following application between 2007 and 2009.

Year	Application time	Date	Max. temp. (°C)	Highest max. temp subsequent 3 days (°C)	Daily solar radiation (W/m^2)	Max daily solar radiation subsequent 3 days (W/m^2)	Relative humidity (%)
2007	Full bloom	13 May	13.7	10	498	513	40
	Post bloom	17 June	17.2	23.5	402	825	50
2008	Full bloom	5 June	17.9	20.3	642	758	40
	Post bloom	16 June	18.4	16.6	888	512	46
2009	Full bloom	1 May	19.8	14.4	658	706	65
	Post bloom	15 June	17.7	18.9	725	834	29.5

Table 2: Effects of different ethephon concentrations applied in 2007 at full bloom or post bloom on trunk cross sectional area (TCSA), fruit set, yield, yield efficiency (YE) and return bloom of 'Jubileum' plum in Ullensvang, Norway.

Ethephon concentration (mg/l)	TCSA (cm^2)	Harvested fruit/100 flowers	Yield (kg/tree)	YE (kg/cm^2)	flowers/branch in 2008
0 control	29.0	21.4	21.5	0.105	149
250 full bloom	27.0	19.8	20.8	0.130	141
375 full bloom	27.9	14.2	14.2	0.148	147
500 full bloom	29.5	6.8	7.4	0.086	153
125 post bloom	28.2	16.3	13.0	0.224	130
250 post bloom	30.2	14.6	12.3	0.215	145
375 post bloom	30.9	2.4	1.5	0.054	123
Significance	NS	***	***	**	NS
LSD (P = 0.05)	4.06	6.9	5.0	0.109	-

production (7.4 and 1.5 kg/tree respectively). Furthermore, fruit were more sensitive to ethephon at the later treatment date. All ethephon treatments resulted in a significantly higher percentage of fruit larger than 38 mm in diameter at harvest (data not shown). Fruit weight increased when ethephon was applied at 375 or 500 $mg \cdot L^{-1}$ (Table 3) and as expected, the largest fruit were on trees with the lowest fruit set. However, linear regression of fruit size versus yield combined for all treatments was poorly correlated (R^2=0.124). At harvest, only those fruit from trees sprayed with 375 mg/l ethephon post bloom had significantly higher average soluble solids (17.6%) but the lowest concentration of ethephon at bloom reduced soluble solids (9.8%) relative to the untreated control trees (11.7%). None of the ethephon treatments had a marked effect on fruit firmness compared to fruit from the untreated control trees. Fruit surface color was improved for all treatments applied after bloom. Fruit acidity and return bloom were similar for all treatments.

Table 3: Effects of different ethephon concentrations applied in 2007 at full bloom or post bloom on fruit weight and fruit quality at harvest of 'Jubileum' plum in Ullensvang, Norway.

Ethephon concentration (mg/l)	Fruit weight (g)	Fruit firmness[z] (units)	Fruit surface color[y] (%)	Soluble solids (%)	Acidity (%)
0 control	40.0	75	73.3	11.7	3.1
250 full bloom	43.5	72	60.0	9.8	3.2
375 full bloom	50.9	75	72.5	10.8	3.2
500 full bloom	54.2	77	78.7	11.9	3.1
125 post bloom	40.1	75	81.7	12.1	3.1
250 post bloom	42.1	75	80.8	12.6	3.0
375 post bloom	46.6	78	95.0	17.6	2.9
Significance	***	*	***	***	**
LSD (P = 0.05)	7.3	3.2	8.5	1.6	0.2

[z] Fruit firmness measured with Durofel, Copa-Technology, CTIFL, Vandoeuvre-lès-Nancy, France
[y] Fruit surface color rated 0-100%, where 0 = no blue color and 100% = blue color covering entire fruit surface

Table 4: Effects of different ethephon concentrations applied in 2008 at full bloom or post bloom on trunk cross sectional area (TCSA), fruit set, yield, yield efficiency (YE) and return bloom of 'Jubileum' plum in Ullensvang, Norway.

Ethephon concentration (mg^1)	TCSA (cm2)	Harvested fruit/100 flowers	Yield (kg/tree)	YE (kg/cm^2 TCSA)	flowers/branch in 2008
0 control	35.0	17.6	11.1	0.316	65
250 full bloom	34.4	12.9	6.2	0.184	70
375 full bloom	32.3	11.6	8.0	0.263	73
500 full bloom	33.9	4.5	4.4	0.136	42
125 post bloom	32.4	9.6	9.1	0.280	74
250 post bloom	34.6	6.3	5.2	0.155	47
375 post bloom	37.4	3.9	2.5	0.074	37
Significance	NS	**	**	**	*
LSD (P = 0.05)	4.06	7	5.0	0.109	2.97

2008. Flowers per branch was less than half the previous year for all treatments (Table 4) and it is likely that this was due to inclement weather earlier that spring. Effects of ethephon thinning with respect to fruit set and yield were similar to those in 2007 and both were significantly lower than the untreated control. The highest concentration of ethephon applied at full bloom or post bloom resulted in over-thinning with 4.5 and 3.9 fruit at harvest/100 flowers, respectively. This was reflected in the unacceptably low yields for these same treatments of 4.4 and 2.5 kg per tree at harvest, respectively when compared to the untreated control trees (11.1 $kg.tree^{-1}$). However, linear regression of fruit size versus yield pooled over all treatments was again poorly correlated (R^2=0.031). As in 2008, there were no significant effects of these high ethephon concentrations on return bloom in 2009 (Table 4). Both the lowest and the intermediate concentrations of ethephon applied at bloom (250 and 375 mg/l) as well as the lowest concentration applied post bloom (125 mg/l) resulted in satisfactory fruit set (12.9, 11.6 and 9.6 fruit/100 flowers at harvest), respectively (Table 4). These thinning effects were also resulted in acceptable yields (6.2, 8.0 and 9.1 kg/tree at harvest respectively) when compared to the control (11.1 kg/tree). All other treatments resulted in significantly reduced yields (≤5.2 kg/tree).

In 2008 ethephon applied at bloom did not affect fruit weight, fruit firmness, soluble solids concentration or acidity, but the lowest concentration enhanced surface color (Table 5). Post bloom applications of 250 mg/l reduced fruit weight, and 375 mg/l reduced flesh firmness. Yields and fruit acidity in 2008 were in general almost half that of the previous year, but soluble solids were almost double, which infers that fruit maturity is markedly affected by crop load.

2009. TCSA and return bloom in 2010 were unaffected by any of the ethephon treatments (Table 6). Ethephon applications of 250 mg/l at full bloom or at concentrations of 250 or 375 mg/l post bloom did not affect fruit set (69.1, 85.6 and 78.7 fruit/100 flowers) compared to the untreated control (57.7 fruit/100 flowers) (Table 6). In addition, only the 250 mg/l ethephon application at full bloom resulted in fruit that was numerically greater than fruit on control trees (92.7 g vs. 88.5g, respectively). In contrast, all post bloom ethephon treatments resulted in significantly smaller fruit than the untreated control (all ≤ 78.7 g) (Table 7). Furthermore, all post bloom applications at 250 or 375

Table 5: Effects of different ethephon concentrations applied in 2008 at full bloom or post bloom on fruit weight and fruit quality at harvest of 'Jubileum' plum in Ullensvang, Norway.

Ethephon concentration (mg/1)	Fruit weight (g)	Fruit firmness (z) (units)	Fruit surface color (y) (%)	Soluble solids (%)	Acidity (%)
0 control	76.0	73.2	70	21.3	1.4
250 full bloom	80.0	73.8	78	20.3	1.4
375 full bloom	76.7	73.5	67	17.1	1.2
500 full bloom	72.0	70.7	72	21.7	1.7
125 post bloom	68.7	68.7	73	19.4	1.3
250 post bloom	62.1	70.3	73	21.2	1.2
375 post bloom	71.6	65.1	82	22.0	0.9
Significance	**	**	**	NS	NS
LSD (P = 0.05)	8.4	4.4	7.2	-	-

(z) Fruit firmness measured with Durofel, Copa-Technology, CTIFL, Vandoeuvre-lès-Nancy, France
(y) Fruit surface color rated 0-100%, where 0 = no blue color and 100% = blue color covering entire fruit surface

mg/l ethephon resulted in significantly lower yields (12.8 and 7.8 kg/tree respectively) than the untreated control trees (16.8 kg/tree) (Table 6). The relationship of fruit size versus yield pooled over all treatments was once again very poorly correlated (R^2=0.145). All ethephon treatments in 2009 had little effect on fruit quality (Table 6). Compared to control fruit, the lowest concentration of ethephon significantly reduced surface color, but color was acceptable for the market. The highest post bloom ethephon concentration increased soluble solids concentration from 16.0 to 17.8%.

Many factors must be taken into account for consistent thinning with ethephon, including site, cultivar, spray volume, timing of application and temperature (Marini, 2004). However, in the present study many of these factors were constant. We believe spray volume was adequate in the present study, since all treatments were applied to run-off with a

Table 6: Effects of different ethephon concentrations applied in 2009 at full bloom or post bloom on trunk cross sectional area (TCSA), fruit set, yield, yield effiiciency (YE) and return bloom of 'Jubileum' plum in Ullensvang, Norway.

Ethephon concentration (mg/1)	TCSA (cm^2)	Harvested fruit/100 flowers	Yield (kg/tree)	YE (kg/cm^2 TCSA)	flowers/branch in 2008
0 control	41.5	57.7	16.8	0.406	141
250 full bloom	40.7	69.1	16.1	0.407	151
375 full bloom	41.1	55.3	17.5	0.435	150
500 full bloom	41.3	63.8	19.5	0.488	123
125 post bloom	40.5	53.3	17.1	0.425	110
250 post bloom	41.7	85.6	12.8	0.318	98
375 post bloom	43.4	78.7	7.8	0.185	91
Significance	NS	**	***	**	NS
LSD (P = 0.05)	4.06	8.93	5.0	0.109	-

Table 7: Effects of different ethephon concentrations applied in 2009 at full bloom or post bloom on fruit weight and fruit quality at harvest of 'Jubileum' plum in Ullensvang, Norway.

Ethephon concentration (mg/l)	Fruit weight (g)	Fruit firmness[z] (units)	Fruit surface color[y]	Soluble solids (%)
0 control	88.5	66.8	77	16
250 full bloom	92.7	67.1	68	15.2
375 full bloom	86.4	65.1	73	15.1
500 full bloom	85.8	63.5	74	15.3
125 post bloom	77.3	65.7	79	16
250 post bloom	78.7	67.7	80	16.8
375 post bloom	70.7	66.2	78	17.8
Significance	***	n.s.	**	**
LSD (P = 0.05)	8.13	-	6.09	1.40

[z] Fruit firmness measured with Durofel, Copa-Technology, CTIFL, Vandoeuvre-lès-Nancy, France
[y] Fruit surface color rated 0-100%, where 0 = no blue color and 100% = blue color covering entire fruit surface

handgun. In our experiment the nearest day to the indicated phenological stage with temperature above 15 °C was selected in order to achieve optimum thinning (Table 1). In a cooler climate like in Norway, optimum weather conditions may not be adequate during bloom to thin plum trees successfully. For this reason it is important for the growers to have a second thinning window at fruitlet stage. Under the conditions reported, ethephon proved to be an effective fruit thinner at different concentrations and could be applied at either bloom or post bloom.

Ethephon reduced fruit set significantly with increasing rate of the thinner. A higher dosage of ethephon at bloom is needed compared to the fruitlet stage in order to achieve the same fruit set. These results are contrary to those with apple. 'Golden Delicious' apple trees were most sensitive to thinning at pink bud stage. After bloom higher rates were necessary in order to get the same reduction in fruit set (Koen and Jones, 1985). In both 2007 and 2008, concentrations of up to 375 mg/l ethephon applied at bloom and 125 mg/l post bloom reduced crop load to the target of about 10-15 fruit/100 flowers, which is required in order to fulfill the market requirements for fruit quality. This is in accordance with previous reports (Kvåle, 1978; Meland, 2007; Webster & Spencer, 2000).

Reducing plum crop load usually increases fruit weight due to less competition for carbohydrates among the remaining fruit on the tree during fruit growth. In this study yield was high and fruit were smaller on average in 2007, but fruit weight was higher following bloom thinning than post bloom thinning. Soluble solid concentration is usually negatively related to crop load but in our study, timing of thinning had little if any effect on soluble solid concentration. Embree et al. (2001) found that ethephon applied to small fruitlets resulted in advanced fruit maturity at harvest, increasing fruit color and blossom density the following year. Similarly, Seehuber et al (2011) and Weber (2013) found that post bloom applications of ethephon resulted in advanced maturity with cv. 'Ortenauer' plums and softer fruit postharvest. In the current study, in fruit firmness was little affected by ethephon treatment.

A general response to heavy thinning is increased return bloom the following season. Kvåle (1978) found that return bloom was positively affected for 'Opal' and 'Victoria' plums when thinned with ethephon at bloom

the subsequent year. Treating apple trees with ethephon at or shortly after bloom in a biennial bearing "on year", promoted return bloom (Bukovac et al., 2006; Meland and Gjerde, 1993; McArtney et al., 2007). This observation was not confirmed in the present study and there were no improvements in the amount of bloom the year after ethephon application either at full bloom or post bloom.

Conclusions

Ethephon applications at either 250 or 375 mg/l applied at full bloom resulted in adequate thinning of 'Jubileum' plums and successfully resulted in a target of about 10 -15 % fruit set in most years. Furthermore, these concentrations had negligible impact on fruit size, internal fruit quality or external fruit color and in some years they were actually improved. Post bloom ethephon applications to 'Jubileum' did not result in optimal fruit set, yield or fruit size and should not be considered as an alternative to blossom thinning. Indeed, high concentrations of post bloom ethephon (250 and 375 mg/l) applied to 'Jubileum' plum trees often resulted in unacceptably low yields and small fruit. The higher concentrations should be avoided because the negative effects appeared cumulative over three years. However, it is not always possible to apply foliar sprays during flowering in Norway, due to inclement weather and under these circumstances a 125 $mg \cdot L^{-1}$ ethephon applied post bloom to the fruitlets is recommended. In conclusion, if weather conditions during bloom are not conducive to applying ethephon at concentrations of 250 or 375 $mg \cdot L^{-1}$, a window of opportunity still exists for spraying 'Jubileum' plum trees with 125 $mg \cdot L^{-1}$ when average fruitlet diameter is ~12 mm, but this should only be considered an option in years of excessive bloom or consider mechanical blossom thinning.

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Trends in Public and Private Peach Breeding in the Republic of Korea

Ji Hae Jun, Jung Hyun Kwon[1], Eun Young Nam, Kyeong Ho Chung, Ik Koo Yoon, Seok Kyu Yun, and Sung Jong Kim

Additional index words: *Prunus persica*, cultivar, fruit quality

Abstract

Peaches (including nectarines) are the fifth most important deciduous fruit in the Republic of Korea after apples, grapes, persimmons, and pears. Usually consumed as fresh fruit, 217,000 metric tons of peaches were produced in the country in 2015, and the total cultivated area was 16,704 ha. Peaches and nectarines account for 82% and 18%, respectively, of cultivated area in the Republic of Korea. The Republic of Korea's National Institute of Horticultural and Herbal Science (NIHHS), a division of the Rural Development Administration, initiated a public peach breeding program in 1961. The main purpose of this program has been to breed new peach cultivars to satisfy consumers and producers through the development of high-quality fruit and improved shelf life. 'Yumyeong', a white peach, was the first cultivar bred by the NIHHS, and was released in 1977. This peach has a good shelf life and firm flesh. To date, the NIHHS has released 10 peach and 4 nectarine cultivars through the national peach breeding program. 'Yumyeong' has been used as a main cross parent to improve fruit size, sweetness, and shelf life. It was a cross parent for 4 of the 10 peach cultivars bred by the NIHHS. The passage and implementation of the Seed Industry Law in December 1997 and subsequent membership in the International Union for the Protection of New Varieties of Plants has encouraged private breeders to release new cultivars. As a result, the number of such cultivars has increased annually. As of 2015, 108 applications for new cultivars have been submitted under the Plant Variety Protection legislation, and private breeders have released 87 of these cultivars. Most of these cultivars originated from bud sports of other key cultivars, such as 'Yumyeong', and chance seedlings.

Peaches (including nectarines) have a short shelf life compared with other fruit crops, and cultivars that can be harvested continuously are required for fresh produce markets. More than 60 cultivars are shipped to fresh markets from June to September in the Republic of Korea (Korea Statistical Information Service, 2016). Most peach farmers in the country cultivate more than 10 cultivars in their orchards and have expressed interest in planting new cultivars to generate more income. Moreover, with the growth of cultivation areas, demand for new and marketable cultivars is growing (Park et al., 2016).

The passage and implementation of the Seed Industry Law in December 1997 strengthened Plant Variety Protection rights. The enforcement of these rights has, in turn, promoted private breeding activity. Private breeding programs for peaches are the most active and dynamic among fruit crops, and breeders have continuously released new cultivars since 1998 (Korea Seed and Variety Service, 2016). Private breeders were responsible for 87 of the 108 peach and nectarine cultivars bred between 1998 and 2015 (Figure 1). Although breeding programs are active and productive in the Republic of Korea, few comprehensive reviews and reports on the subject are available. Thus, this review aims to provide an overview of the trends in public and private peach breeding in the Republic of Korea.

[1] To whom reprint requests should be addressed; e-mail kwon1101@korea.kr
Fruit Research Division, National Institute of Horticultural and Herbal Science, Wanju 55365, Republic of Korea

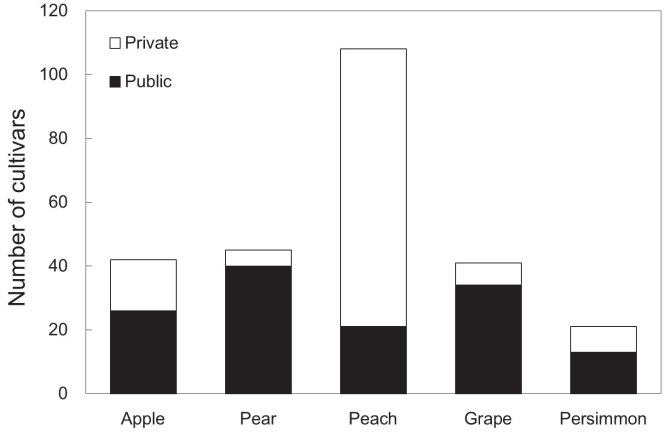

Fig. 1: The number of fruit cultivars submitted for Plant Variety Protection by public and private breeders between 1998 and 2015 in the Republic of Korea.

Peach and nectarine production

Peaches (including nectarines) are the fifth most important deciduous fruit in the Republic of Korea after apples, grapes, persimmons, and pears (Korea Statistical Information Service, 2016). Most peaches produced in the Republic of Korea are consumed as fresh fruit. About 2% of the total production volume is processed into products such as jams, beverages, and canned fruit (Park et al., 2016). The total planted area for peach trees has increased by 63.7% between 1995 and 2015 (Figure 2). This area is expected to grow another 13.8% between 2016 and 2025 (Park et al., 2016). Fresh fruit markets throughout the Republic of Korea are now open to imports from several countries and regions, including Chile, the United States, and the European Union, due to free trade agreements (FTAs). After the conclusion of a FTA between the Republic of Korea and Chile in 2004, the area used for peach cultivation in the Republic of Korea decreased between 2005 and 2009 (Figure 2). At that time, most farmers were concerned about fresh fruit imports from Chile. However, to date, no fresh peaches have been imported from any other country because of the Plant Protection Act. Compared with other fruit crops, peaches have a short juvenile period and maintain relatively high prices (Korea Statistical Information Service, 2016). These two features have led to an increase in the peach cultivation area, and thus increased peach fruit production. However, frost and freezing damage in 2010 and 2013 resulted in production declines of 30% and 17% compared with the respective previous years (Figure 2).

Peaches and nectarines account for 82% and 18%, respectively, of the cultivated area in the Republic of Korea. More than 70% of peach cultivars are white, non-acidic flesh, whereas most nectarines are yellow, moderately acidic flesh. The main peach cultivars are ‘Kawanakajima Hakuto’, ‘Changhowonhwangdo’, ‘Yumyeong’, and ‘Mibackdo’, and the primary nectarine cultivars are ‘Cheonhong’, ‘Redgold’, ‘Fantasia’, and ‘Sunfre’ (Korea Statistical Information Service, 2016).

Results of public peach breeding

The Republic of Korea’s National Institute of Horticultural and Herbal Science (NIHHS), a division of the Rural Development Administration, initiated a public peach breeding program in 1961 (Kim et al., 1978).

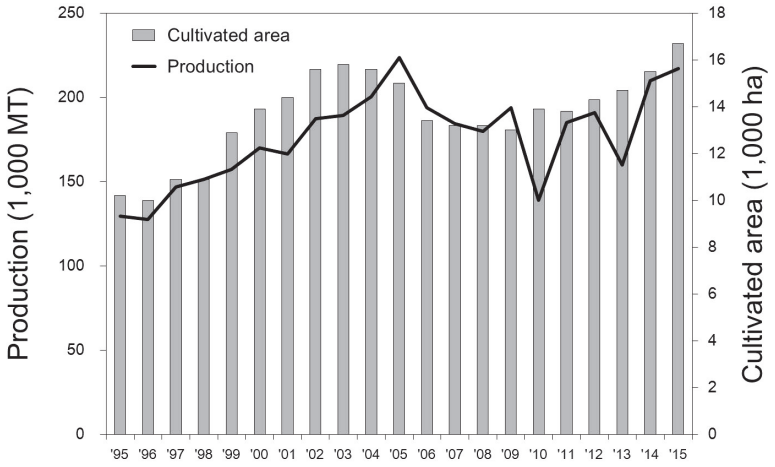

Fig. 2: Peach and nectarine production volume and total cultivated area in the Republic of Korea from 1995 to 2015.

The main purpose of this program has been to breed new peach cultivars to satisfy consumers and producers through the development of high-quality fruit and improved shelf life.

'Yumyeong' was the first cultivar bred by the NIHHS, in 1977 (Kim et al., 1978). As it has firm flesh, it is easy to handle during harvest and transportation. The amount of cultivation area devoted to 'Yumyeong' increased dramatically until the early 1990s. 'Yumyeong' occupied 23% of the total peach cultivation area in 1992. However, as consumers' preferences changed to sweet, juicy, and more soft-fleshed fruit, the cultivation area for 'Yumyeong' has decreased steadily (Jun et al., 2007a).

Although the popularity of 'Yumyeong' is decreasing, it has been a good resource for the improvement of fruit quality. 'Yumyeong' has been used as a main cross parent to improve fruit size, sweetness, and shelf life; it was used as a cross parent in 4 of the 10 peach cultivars bred by the NIHHS (Table 1). Besides, 'Yumyeong' has been used as a parent in other countries. 'Ghiaccio' peach series obtained by open pollination of 'Yumyeong' were released in Italy (Nicotra et al., 2002) and 'Coconut Ice' and 'Scarlet O'Hara' derived from 'Yumyeong' were released in New Zealand (Okie et al., 2008).

Before enforcement of the Seed Industry Law in 1997, new cultivars bred by public breeding programs were not protected. These cultivars were distributed to nurseries and farmers free of charge. At the time, the supply of new cultivars to domestic producers without charging them was popular, as taxes were used to fund public breeding programs. This approach was considered to be an efficient way to expand the number of new cultivars over a short period of time. That 'Yumyeong' and 'Chenhong' became the leading peach and nectarine cultivars, respectively, over a very short period of time may be a natural consequence of this practice (Jun et al., 2007a). These cultivars were distributed to nurseries and farmers free of charge from 1978 to 1993.

Another public peach breeding program has been developed by the Cheongdo Peach Experimental Station (CPES) and implemented by municipal governments. The CPES was established in 1994 in Cheongdo, North Gyeongsang Province, which is the main production region for peaches in

Table 1. Major characteristics of peach and nectarine cultivars released by public breeding programs in the Republic of Korea.

Cultivar	Release year	Parentage	Breeding program	Fruit type	Days to ripen from blooming	Flesh color	Flesh firmess	Reference
Yumyeong	1977	Yamatowase × Sunagowase	NIHHS	Peach	120	White	Stony hard	Kim et al., 1978
Baekmijosaeng	1983	Mishima Hakuto × Sunagowase	NIHHS	Peach	60	White	Soft melting	Kang et al., 1986
Cheonhong	1992	Open pollination of Garden State	NIHHS	Nectarine	100	Yellow	Soft melting	Kang et al., 1999a
Baekhyang	1994	Open pollination of Garden State	NIHHS	Peach	130	White	Soft melting	Kang et al., 1999b
Jinmi	1999	Hakuto × Nunomewase	NIHHS	Peach	125	White	Soft melting	Kang et al., 1999c
Daemyeong	2002	Bud sport of Yumyeong	CPES	Peach	110	White	Stony hard	Kwon et al., 2002
Suhong	2004	SunGlo × Cheonhong	NIHHS	Nectarine	115	Yellow	Soft melting	Jun et al., 2007c
Soomee	2005	Yumyeong × Chiyomaru	NIHHS	Peach	138	White	Soft melting	Jun et al., 2007d
Mihwang	2005	Kawanakajima Hakuto × Chiyomaru	CPES	Peach	78	Yellow	Soft melting	Choi et al., 2007
Mihong	2006	Yumyeong × Chiyomaru	NIHHS	Peach	77	White	Soft melting	Jun et al., 2007b
Chowhang	2007	Kawanakajima Hakuto × Chiyomaru	CPES	Peach	85	Yellow	Soft melting	Choi et al., 2008
Misshong	2008	Yumyeong × Chiyomaru	NIHHS	Peach	109	White	Soft melting	Jun et al., 2013a
Yumi	2009	Yumyeong × Chiyomaru	NIHHS	Peach	82	White	Soft melting	Jun et al., 2013b
Hahong	2009	SunGlo × Cheonhong	NIHHS	Nectarine	118	Yellow	Soft melting	Jun et al., 2014
Osubaekdo	2010	Unknown (chance seedling)	CPES	Peach	88	White	Soft melting	Park et al., 2014
Soohwang	2010	Nishio Gold × Chiyomaru	CPES	Peach	97	Yellow	Soft melting	Kim et al., 2010
Seonmi	2012	Hakuto × Baekhyang	NIHHS	Peach	114	White	Soft melting	Nam et al., 2012
Geumhwang	2012	NishioGold × Chiyomaru	CPES	Peach	93	Yellow	Soft melting	KSVS, 2016
Hwanghoo	2014	Open pollination of Changhowon Hwangdo	NIHHS	Peach	111	Yellow	Soft melting	Jun et al., 2014
Soobaek	2014	Okubo × Chiyomaru	CPES	Peach	80	White	Soft melting	Park et al., 2015
Seolhong	2015	Self-pollination of Baekhyang	NIHHS	Nectarine	134	White	Soft melting	NIHHS, 2015
Juwolhwangdo	2015	Bud sport of Hikawa Hakuho	CPES	Peach	80	Yellow	Soft melting	KSVS, 2016
Hongbaek	2015	Odoroki × Hikawa Hakuho	CPES	Peach	110	White	Soft melting	KSVS, 2016

NOTE: NIHHS, National Institute of Horticultural and Herbal Science; CPES, Cheongdo Peach Experiment Station; KSVS, Korea Seed and Variety Service.

the Republic of Korea. The CPES focused initially on developing of peach cultivation systems to achieve more stable and efficient production in North Gyeongsang Province. However, after public breeding programs began to raise money by patenting their releases, the CPES also focused on the release of new cultivars that were marketable and well adapted to the North Gyeongsang area. It released nine peach cultivars between 2002 and 2015 (Table 1). Although the history of the CPES' breeding program is short, the program has produced good results because of the advantage of its location in the district with the most production. Whereas most peach cultivars bred by the NIHHS are white fleshed, five of the nine cultivars bred by the CPES are yellow fleshed. Two cultivars released by the CPES originated from bud sports of 'Yumyeong' and 'Hikawa Hakuto'.

Of the 19 peach cultivars bred by the NIHHS and CPES, nine originated from the same cultivar, 'Chiyomaru', which was used as a male cross parent (Table 1). 'Chiyomaru' was bred in Japan and produces delicious, yellow-fleshed, early-ripening fruit (Yamaguchi et al., 1989).

Development of private peach breeding

Enforcement of the Seed Industry Law in 1997 and the Republic of Korea's entry into the International Union for the Protection of New Varieties of Plants encouraged private breeders to release new cultivars. Since that time, the number of such cultivars has increased annually.

As fruit breeding programs are long-term proejcts and require large fields and much capital, to be effective, the development of new cultivars is challenging for private breeders. However, peaches have a short juvenile period of 2–3 years, compared with the 5–10 years required for the maturation of most other fruit tree species. In addition, mutations commonly called bud sports are found frequently in peaches (Scorza and Sherman, 1996). As a result, private peach breeding is quite active compared with breeding of other fruit crops. More than 80% of the registered peach cultivars in the Republic of Korea

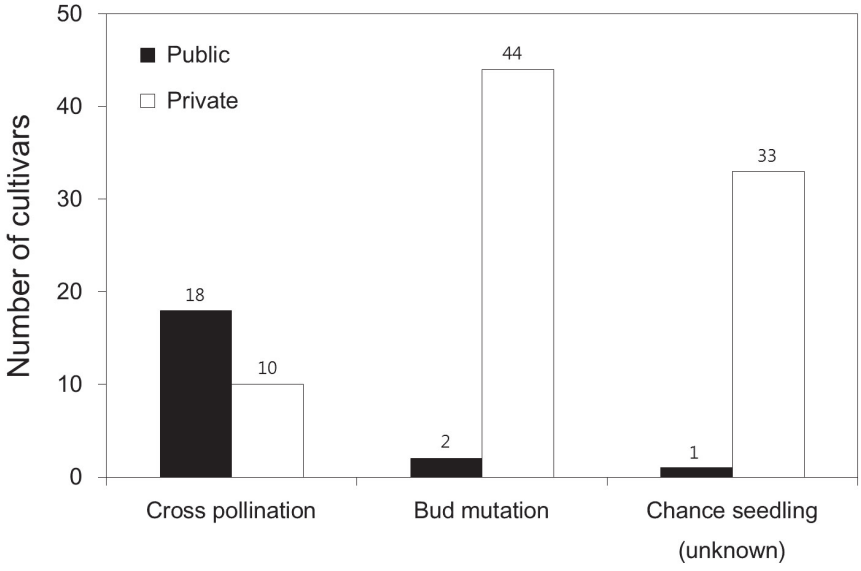

Fig. 3: Genetic origin and breeding programs of peach and nectarine cultivars released in the Republic of Korea between 1998 and 2015.

were released by private breeders (Figure 1). Of these 87 cultivars, 32 are yellow-fleshed peaches (Korea Seed and Variety Service, 2016).

Most cultivars released by private breeders originated from bud sports of the main cultivars, such as 'Yumyeong', or from chance seedlings with unknown parents. However, active private breeders currently try to select new cultivars from open-pollinated seedlings with known seed parents, or from cross pollination between two known cultivars (Figure 3).

Although private breeders have released 87 cultivars, few have become main cultivars (Korea Statistical Information Service, 2016). The development of main cultivars is not easy because most cultivars bred by private breeders have not been tested sufficiently to determine their qualities and adaptability to various regions throughout the country. For example, in 2010 and 2013, many new cultivars that came from Japan and private breeding programs experienced damage from freezing and frost in the northern regions of the Republic of Korea (National Institute of Horticultural and Herbal Science, 2015). Exaggerated promotion of new cultivars released by private breeders often results in the disappointment of farmers, who expect the new cultivars to be of high quality.

Conclusion

Much progress in peach breeding in the Republic of Korea has been achieved in the last 20 years. Active work continues in public and private breeding programs to meet the demands of fresh produce markets. Thus far, public and private peach breeding have been concentrated on the fresh domestic market. The extension of the harvest period and improvement of fruit size and sweetness are the main breeding targets in public and private programs.

However, trends in fruit consumption, production system practices, and orchard locations have been changing. Because consumers have become more aware of the health benefits of fruit, the potential exists to create a new market for cultivars developed specifically for health benefits, perhaps by incorporating "ingredients" such as carotenoids, anthocyanins, and polyphenols. The consumption of flat peaches is expected to increase because consumers are curious about this novel fruit and because these peaches can be eaten more easily than conventional round peaches. As trends in fruit consumption change, peach breeding programs should focus on high fruit quality, variety of fruit types, and possible health benefits. The high labor cost and the aging population of orchard workers are factors leading to the demand for easily cultivated new cultivars. Cultivars that exhibit dwarfing in rootstock or scion cultivars, good fruit firmness, and better post-harvest fruit characteristics are more important future breeding targets. As global climate change progresses, production areas are also changing. Cold-hardy and frost-hardy cultivars will be in great demand in the future. In the near future, low-chill cultivars may also be in demand. Most importantly, fruit breeders should be aware of the broad trends and focus on the development of reliable new cultivars to meet these needs.

Acknowledgements

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Pomegranate: The Grainy Apple

John E. Preece and Jeff Moersfelder

Additional index words: *Punica granatum*, fruit quality, germplasm

The pomegranate (*Punica granatum* L.) is an ancient fruit crop that offers a wide variety of choices for the consumer. Fruit range in color from light yellow to deep maroon to black, from very sweet to lemon-like tartness, and seed hardness can be along the spectrum from very soft (called seedless) to very hard. Hard seeds can be either crunchy or chewy and difficult to bite through. Pomegranates are consumed fresh as arils; processed as juice, candy, confections, or nutraceuticals; fermented into a sweet to semi-dry wine; used as grenadine to flavor cocktails; and dried seeds with attached pulp (anardana) are utilized as a souring agent in Indian cooking, and roasted seeds add aroma and flavor to Middle Eastern dishes. Currently the market in the USA is dominated by 'Wonderful' pomegranate; however, there is a variety of alternative cultivars that may have greater consumer acceptance as a fresh product, such as 'Parfianka' pictured on the cover of this issue and described below.

Pomegranate originates from Iran and Afghanistan (Levin, 2006) and the surrounding areas of the near east, including Turkmenistan and northern India (Holland et al., 2009). Cultivation began in Iran (Kahramanoglu and Usanmaz, 2016), or the Transcaucasia-Caspian region (Still, 2006) sometime in the Neolithic era (9000 BCE to 3000 BCE, Levin, 2006, Holland et al., 2009). It was 3,000 to 7,000 years from the beginning of the Neolithic transition to agriculture when pomegranate was introduced to new regions (Levin, 2006). For example, more than 5,000 years ago, pomegranates had been moved to and were being grown as far away as the Middle East, demonstrating widespread adoption.

Domestication was likely from fruit similar to wild pomegranates, which are generally sour and small. However, wild pomegranates differ depending on where each population evolved. For example, large pomegranates grow wild in the Kandahar region of Afghanistan and soft-seeded fruit are found in the wild in the Tagab Valley, Afghanistan (Levin, 2006). During domestication, pomegranates were selected for larger fruits and seeds, for their color, their resistance to splitting (Still 2006), their sweetness, seed hardness (or lack of), and flavor. This has resulted in more than 500 cultivars throughout the world, but only 50 that are in common use (Still, 2006).

Pomegranate is heterozygous and does not come true from seeds, so it is interesting to note that pomegranates have been propagated by rooting suckers for about 5,000 years in Jericho, Cyprus, Greece, and Mesopotamia (Hummer et al., 2015). The selection of pomegranates for clonal propagation demonstrates that it was known that the desirable phenotypes could only be reliably reproduced vegetatively. This also advanced domestication and widespread adoption of the crop because pomegranate hardwood cuttings root relatively easily, facilitating the movement of the most desirable clones. It is also likely that when selections were made for fruit characteristics, inadvertent selection was also made for rootability because those selections that rooted readily multiplied more quickly and therefore must have become the dominate cultivars in production.

National Clonal Germplasm Repository, USDA-ARS, One Shields Avenue, University of California, Davis, CA 95616-8607 email: John.Preece@ars.usda.gov

Pomegranate seeds, and stamen, anther, and skin fragments were recovered from a 14th century BCE ship wreck near Turkey (Ward, 2003). The other items on the ship included ivory, precious metals, amber, and ostrich eggs. Therefore the pomegranates were being shipped with exclusive and luxury items, perhaps indicating that pomegranates were considered an "elite" fruit that was desired by the rich.

Pomegranates became sufficiently important to have some religious significance. They are mentioned in the Hebrew Bible 23 times, three times in the Qur'an, but not at all in the Christian Bible (Janick, 2007). They have also been used on both ancient and modern Jewish coins and in Christian Renaissance artwork.

Pomegranates were brought to the New World (Central and South America) by the Spanish in the 1500s and 1600s (Stover and Mercure, 2007) and in the 1700s, they were planted in Florida and Georgia. By 1770, Jesuit missionaries had introduced them to California (Holland et al. 2009). According to Father Eusebio Francisco Kino, Dolores 1695, "This mission has his church adequately furnished with … Castilian fruit trees, grapes, peaches, quinces, figs, pomegranates ..." (Garcia-Yanez and Emanuel, 2016). When writing about pomegranates and other fruits at the American missions, Ignaz Pfefferkorn, 1725 stated: "These fruits are superior in size, juiciness, sweetness, and flavor to those which are grown in Europe…" (Garcia-Yanez and Emanuel, 2016). The increased quality of the fruit could be related to better growing conditions in the new world, or perhaps the clonal selections that were sufficiently valuable to make it to the new world were among the most desirable.

Remnants of these old pomegranates remain in the New World. For example, PomNatural, LLC, from Steinhatchee, FL has been scouting and finding pomegranate trees that are at least 50-100 years old in Florida and Georgia (Bonsteel and Bice, 2015). Old pomegranates that apparently have greater cold hardiness than most have been found at old estates in Georgia, and several of those discovered in Florida seem to have adapted well to the humidity and rainfall of the southeastern USA because their fruit have few blemishes. It is unclear if these trees are of seedling or clonal origin. If they are seedlings, this could indicate some selection for the humid climate and genetics that might be exploited to breed better adapted fruit. Therefore, there may be gems in some of these old heirloom cultivars or selections that can be exploited for production, lack of splitting, and possible disease resistance.

By 1916, there were five USDA Plant Introduction Gardens or Field Stations receiving new germplasm that entered the USA (Dorsett, 1916). At these Stations, plants were grown, evaluated for economic importance, and the best were propagated for distribution based on orders received in Washington, DC at the home location of the Office of Foreign Seed and Plant Introduction. The recipients of the plants released by the Plant Introduction Gardens were state experiment stations, private researchers, special cooperators, and plant breeders throughout the USA. Specifically, pomegranates were received by the Chico Plant Introduction Field Station, and the pomegranate with the lowest Plant Inventory number, PI 179 was received in March, 1898 from Turkestan by N.E. Hansen (USDA, 1898). Under the description of the accession, they state in quotation marks: "Seeds saved from large, fine fruits picked in the garden of the Emir of Bokhara's summer palace in Old Amu Daria." (The Emirate of Bokhara is now part of Uzbekistan; USDA, 1898, p.22). The first clonal cultivar , PI 731, had large red fruit and was received from Tiflis, Transcaucasia, Russia through N.E. Hansen in 1897 (USDA, 1898).

By 1922 (Anon, 1922), the Chico Station was offering plants of 6 pomegranate cultivars (Table 1). Interestingly, they offered accessions (presumably identical) with the same name under different plant introduction numbers, indicating different origins and

Table 1. Pomegranate cultivars available for researchers from the Plant Introduction Garden, Chico, CA in 1922 (Anon, 1922).

Cultivar	Inventory Number	Passport Information
Granado de Rogises	33229	From: Granada, Spain, purchased by P. Giraud at the request of W.T. Swingle, received March 23, 1912. One of the 3 principal cultivars grown in Granada, Spain (Galloway, 1913).
Krylezy-Kabuk	27049	From: near Sukhum-Kale, Caucasus, Russia from a collection of named cultivars via F.N. Meyer, March 10, 1910. (Galloway, 1911a).
Krymisi Kabugh	27966	From: Geok-Tepe, Caucasus, Russia from A. Shelkovnikoff, via F.N. Meyer, April 12, 1910. Large, bright red, sour-sweet fruit. F.N. Meyer thought it to be the same as Inventory No. 27773, 'Cumzi gabuch', which was received from Tiflis, Caucus, Russia on March 22, 1910 (Galloway, 1911b).
Krymisi Kabugh	30615	From: R.H. Kearney, April 26, 1911, who received the cuttings from I. Munro, Putnam, GA. Red, sweet fruit (Galloway, 1912).
Legrellei	24825	From: La Tour-de-Peilz, Vaud, Switzerland.Purchased by J. Brunner at the request of O.F. Sillig (USDA), Received March, 9, 1909. Double flowered cultivar with salmon-red petals with white variegation. Vigorous and hardy and can ripen fruit in the climate of central France (Galloway, 1909).
Negro Monstruoso	33227	From: Granada, Spain, purchased by P. Giraud at the request of W.T. Swingle, received March 23, 1912. One of the 3 principal cultivars grown in Granada, Spain (Galloway, 1913).
Nejidi	8646	From: Bassorah, Arabia (now Iraq) through Mr. Lathrop and David Fairchild, No. 849, Feb. 26, 1902. Large fruit with thin skin, very soft seeded, red-arils (Galloway, 1905).
Nejidi	13298	From: the Georgetown custom-house on March 29, 1905. It had arrived in New York on the steamship Umbria. (Galloway, 1907).

dates from which the material was sourced by the Plant Introduction Station. It is common for genebanks to give different accession numbers when plants are acquired at different times, even if two plants have the same name. It is possible that they are indeed the same, but in some cases, cultivars with the same name will be different because of misidentification, or because of a name being used repeatedly over the centuries to name different genotypes. Therefore, having different PI numbers for the same named cultivar is logical.

In the early 20th century, pomegranate accessions were arriving at the Chico Station from various locations, including Russia, Spain, Switzerland, what is now Iraq, and the USA state of Georgia (Table 1). Spellings of cultivar names can change as they move around, different people handle them, or during translation to English. There are some cultivars at the USDA-ARS, National Clonal Germplasm Repository in Davis, CA (NCGR) with similar or rearranged spellings of two of the cultivars offered in 1922. These may be a result of utilization of the Chico

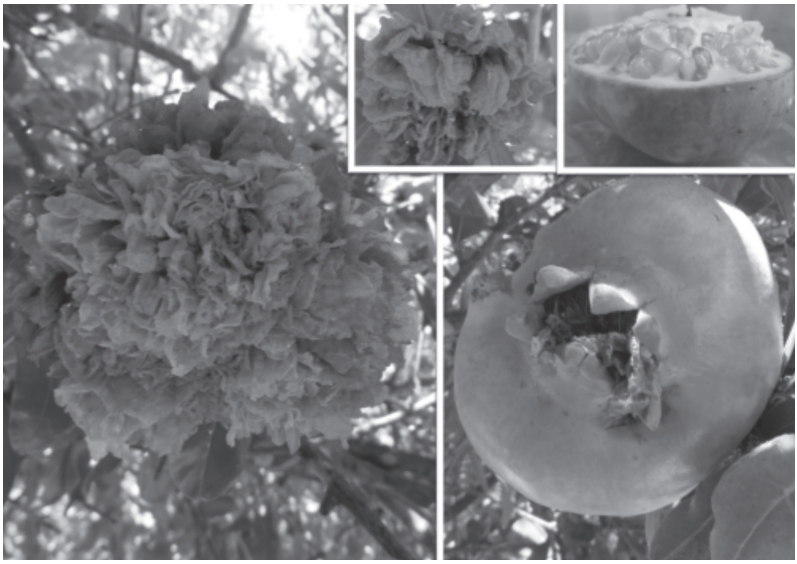

Fig. 1: WEO42, an unnamed pomegranate that was brought to the University of California, Davis from the Chico Plant Introduction Station, between 1954 and 1960 that has similarities with 'Legrellei' that was offered by the USDA Chico Plant Introduction Station in 1922. It is now part of the NCGR collection and has salmon-red double flowers with white variegation. The chimera is not stable (inset), and flowers can be red or have red sectors. WEO42 only had this one mature fruit in 2016. The skin is pink with white arils and crunchy seeds. Remnants of the double flower parts remain attached to the fruit.

Plant Introduction Station material in breeding. One cultivar is 'Dotch Legrelley' from Turkmenistan (similar spelling to 'Legrellei'; however, 'Dotch Legrelley' has double variegated red-white flowers and 'Legrellei' is described with double variegated salmon-red and white petals. Additionally, in the NCGR collection is an accession with the number WEO42 (Fig. 1), and is unnamed and was introduced to Wolfskill Experimental Orchard between 1954 and 1960 from the Chico Plant Introduction Station. It has similar salmon-red and white double variegated petals to "Legrellei." WEO42 has white arils and crunchy seeds. Using 16 SSR markers (unpublished, Aradhya and Preece, 2016), 'Dotch Legrelley' and WEO42 clustered together with other double-flowered cultivars in the NCGR National collection. This is considered evidence that 'Dotch Legrelley' is a seedling of the genotype represented by WEO42, which has a likelihood of being 'Legrellei.'

Another accession in the NCGR collection with a link to a cultivar offered in Table 1 is Hyrdanar x 'Kirmizy-Akbuh,' which was introduced into the collection in September, 1995 as cuttings from Turkmenistan. Interestingly, the pedigree is a cross between a mutant of American dwarf Chico x Kirmizy-kabuh (similar spelling to and most likely the same as 'Krymisi Kabugh' from Table 1, USDA, 2016).

The NCGR maintains and curates the national pomegranate collection that currently consists of approximately 280 accessions that are available for distribution to the research and educational communities. The oldest trees in the collection were collected at the Chico Plant Introduction Station, received by the University of California Davis, and established at Wolfskill Experimental Orchard, Winters, CA between 1954 and 1960 (Kennedy, 2010). These trees include the variegated double-flowered WEO42

listed above. The original names and identifications of most of the remaining 47 trees are missing. Dr. John Lovell was a Professor of Experimental Psychology at Cal. State Hayward who gave some of these trees new cultivar names, including: 'Cloud,' 'Crab,' 'Cranberry,' 'Gold,' and 'Elf.' These names are listed in the Germplasm Resources Information Network (GRIN-Global); whereas their original names are lost.

With the exception of the trees that arrived at the NCGR in the 1950s, the next new pomegranate accessions were received in the late 1980s and are ornamental cultivars, including double flowered cultivars from Japan, some of which are fruitful and others sterile. In 1995, the first accessions from the Turkmenistan Experimental Station of Plant Genetic Resources (TESPGR), Garrygala arrived, and in 1997, 17 accessions from TESPGR and other locations in the Caucus region came into the collection via Byron, GA. These were presumably among most cold hardy accessions in the collection at TESPGR. In 1999, an additional 65 Turkmenistani accessions were received from G. Levin, TESPGR. In 1996, T. Kennedy donated 19 accessions of various backgrounds and both that year and the next, J. LaRocca and J. Chater donated accessions, several of these were from the Chater breeding program. In the 2000s, accessions were received from Albania, Armenia, Azerbaijan, India, and The Republic of Georgia. The collection has been sourced from at least 11 countries.

Some of the variation among fruit characteristics is presented in Table 2 as an example of the diversity in the NCGR pomegranate collection. Pomegranate juice was compared for soluble solids (°Brix), color parameters, and titratable acidity. Juice from 'Girkanets' was the sweetest with soluble solids concentration (SSC) of 16.8 %, and those with the lowest soluble solids were 'Ariana' and 'Nikitski ranni' at 14.6%. The reliably sour 'Haku-Botan' the most acidic with a titratable acidity of 2.10, however, with 15.7% SSC, it had higher soluble solids than the industry standard, 'Wonderful.' 'Wonderful' is intermediate for both soluble solids and titratable acidity, demonstrating that there are sweeter and more sour fruited cultivars, which could offer much more culinary diversity to consumers. For example, 'Parfianka' with its moderate soluble solid level of 15.2% and titratable acidity of 1.04% offers a nice sugar/acid balance and the soft seeds make eating the arils a pleasure. They are a nice addition sprinkled on top of a tossed lettuce-based salad.

Pomegranates are typically propagated clonally by rooting cuttings. Adding about 3,000 ppm (mg/L) auxin, such as indolebutyric acid (IBA), naphthaleneacetic acid (NAA) or a combination will enhance rooting. Pomegranate plants tend to grow more as bushes than trees because they freely produce suckers from the base of the plant. *Punica granatum* grows naturally in areas where fires are an ecological feature. Suckering is an adaptation to fire and the plants are quickly able to recover following fire events by resprouting via these suckers. However, suckers mean work and expense for growers who typically prune them off and train the plants as trees for ease of management. This pruning is an annual event and therefore a recurring orchard expense. There is now a non-suckering *P. granatum* rootstock named 'Pjered One' that was selected in Italy in 2007 (Preka et al., 2016). This rootstock roots readily from hardwood cuttings and grafts well using cleft grafting. The result is a non-suckering pomegranate tree. It would appear that the extra expense of grafting, compared to rooting cuttings, would pay for itself quickly with the great reduction in pruning costs.

Pomegranate production practices are described in Kahramanoglu and Usanmaz (2016), which is reviewed in this issue and are therefore not detailed here. The authors also include pest, disease, and weed management, harvest and postharvest considerations, as well as the health benefits of this crop in their book.

Table 2. Pomegranate juice soluble solids concentration (SSC), color, and titratable acidity on selected accessions harvested on 25 November from the USDA-ARS National Clonal Germplasm Repository, Davis, CA.

Cultivar	Accession Number	SSC (%)	Juice Color L	C	h	Titratable Acidity (%)
Al-sirin-nar	DPUN0060	14.8	53	34	26	0.63
Andalib	DPUN0137	15.4	42	53	30	1.85
Myagkosemyannyi						
Rozovyi	DPUN0139	14.9	68	15	18	1.11
Ariana	DPUN0125	14.6	44	53	32	1.16
Desertnyi	DPUN0108	15.1	44	55	33	1.24
Fleishman's	DPUN0028	15.4	70	10	45	0.18
Girkanets	DPUN0126	16.8	40	54	31	0.97
Haku-Botan	DPUN0007	15.7	73	07	97	2.10
Ink	DPUN0167	16.3	40	52	28	0.85
Kara Gul	DPUN0155	15.4	36	54	31	1.83
Kara-Kalinskii	DPUN0118	16.2	37	53	31	1.43
Khoramabad	DPUN0078	15.4	42	53	30	1.04
Medovyi Vahsha	DPUN0109	14.9	49	43	23	0.21
15/4 Pamyati						
Rozanova	DPUN0113	15.4	43	53	28	1.11
Molla-Nepes	DPUN0128	15.8	39	58	33	1.79
Nikitski ranni	DPUN0067	14.6	54	38	22	0.88
Dorosht 5 hahanshahi						
Palermo	DPUN0093	16.2	36	54	30	1.22
Parfianka	DPUN0015	15.2	44	52	31	1.04
Purple Heart	DPUN0056	15.8	40	54	31	0.92
Sakerdze	DPUN0059	16.0	43	52	29	1.10
Sirenevyi	DPUN0151	15.2	53	38	23	0.22
Sogdiana	DPUN0143	15.6	48	45	23	0.20
Vina	DPUN0035	14.8	71	09	43	0.25
Wonderful	DPUN0037	15.6	43	52	17	0.90
Average Deviation		0.6	10	14	8	0.45

Currently, Iran is the largest producer with 63,733 ha cultivated, followed by India, the USA, Turkey and Spain (54,755, 14,000, 8,500, and 3,000 ha, respectively, Iran Fruit Center, 2016).

The fruit are berries and the seeds are borne in arils, which are juicy, specialized outgrowths of the seeds, making what resembles a small juice and seed-filled sac. Linneaus gave it the name *Punica granatum* which was a change from its original name, *Malum punicum*, which meant the apple of Carthage because Punica is a Roman name for Carthage; Linneaus chose to retain the reference to Carthage (Punica) in the genus (Stover and Mercure 2007). The specific epithet "*granatum*" means that the fruit is grainy or seedy. Apples are pome fruits, therefore the word "pomegranate" actually means seedy or grainy apple and "*Punica granatum*" references the grainy apple from Carthage.

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Changes in Morphological, Biochemical and Physiological Traits in Strawberry in the Northeastern United States During One Hundred Years of Breeding

Rebecca M. Harbut, Marvin P. Pritts[1] and Lailiang Cheng

Additional index words: anthocyanins, chlorophyll, carotenoids, photosynthesis, fruit quality

Abstract

Two of the more popular northeastern strawberry cultivars from each decade spanning 1891 – 2003 were obtained from various sources and grown in a common environment. Morphological, physiological and biochemical traits were measured in each cultivar to determine if directional changes have occurred through selective breeding over time. Fruit firmness, size, and fruit set increased over time, whereas soluble solids and leaf area ratio (LAR) decreased. Photosynthesis tended to become less efficient over time, while plant pigments showed no consistent change. Yields peaked in the 1980s and have remained somewhat constant for the past 30 years. For most traits, cultivars exhibited values midway between those of the progenitor species, suggesting that traits are partially heritable. *F. chiloensis* appears to have a more efficient photosynthetic apparatus than *F. virginiana*, so might be a good candidate for recurrent breeding. We suggest several approaches for productivity improvement including increasing fruit number per plant, modifying plant architecture and carbon allocation, improving carbon assimilation and increasing photosynthetic efficiency. Incorporating day neutrality into adapted cultivars also could have a significant impact on yield.

Evidence of strawberry cultivation can be found in literature dating back to the sixth century. The first systematic breeding of strawberries began in England in 1817 by Thomas A. Knight. Early American strawberry cultivars were selections of the small fruited species *F. virginiana*, known as the Scarlet strawberry, and at least 30 cultivars were available by 1820 (Jones, 1976). However, when the much larger fruited *F.* x *ananassa* cultivars from Europe were introduced into the United States, they quickly became the dominant strawberry grown and formed the basis of new American breeding programs (Hancock, 1999). Cultivars continued to be released in the early part of the 20th century such as 'Dunlap', 'Klondike', 'Howard 17' and 'Aberdeen,' all of which played an important role in the growing American strawberry industry (Darrow, 1966). During the early stages of cultivar development improvement in fruit size was a priority, but increasingly, characteristics such as disease resistance and fruit quality (i.e. flavor, firmness, color) were also considered. By the early 1900s, breeders were focused on developing cultivars for a particular region; this regional focus on cultivar development continues today.

We were interested in the changes that occurred in strawberry cultivars over the past 100 years of breeding in the northeastern United States (US). One might assume that yield, fruit size and quality have improved as older cultivars have been replaced with newer, but little is known about the basis for this improvement, especially changes in the plant's biochemistry and physiology that might be related to improved performance. The objective of this study was to evaluate

[1] Corresponding author
Horticulture Section, School of Integrative Plant Science, Cornell University, Ithaca, NY 14853
Special thanks to the National Germplasm Repository in Corvallis, OR for assistance in providing plant material.

the more popular cultivars that have been released in the northeastern U.S. over the past century, identify morphological, physiological and/or biochemical changes over time, and determine if any traits might be related to changes in performance. By identifying possible factors associated with growth and yield, new strategies might be identified to improve cultivar performance.

Materials and Methods

Greenhouse trial. Twenty strawberry (*F.* x *ananassa*) cultivars (Table 1) and one representative genotype of each of the progenitor species, *F. chiloensis* (PI551455) and *F. virginiana* (PI612495), were obtained from various commercial nurseries or from the National Germplasm Repository (Corvallis, OR) during spring 2005. Plants were set into a greenhouse mist bed where they were deflowered and derunnered. Over the next several months stolons were transplanted into 4-L pots filled with 1:2:1 (perlite:peat:vermiculite). Transplants were placed in a greenhouse under supplemental high pressure sodium lights to facilitate establishment. Average light levels were 500 μmol $m^{-2}s^{-1}$ with day/night temperature of 23/18°C. Once a sufficient number of plants was obtained, cultivars were arranged on benches in a randomized complete block design with four blocks and six plants in each experimental unit. Plants from the greenhouse were used to assess leaf pigments and certain photosynthetic variables.

Field trial. Plants from the greenhouse trial were cold acclimated at the end of 2005 and placed in a cooler for 6 weeks to meet chilling requirements. In May 2006 these potted plants were transplanted into the field in Ithaca, NY (Lat. 42.4N, Long. 76.5W). The trial was set up in a randomized com-

Table 1. Cultivar name, date of release, parentage and breeding program of 20 strawberry cultivars. Cultivar names followed by * indicate selections used in carotenoid analysis.

Cultivar	Release date	Parentage	Origin
Royal Sovereign*	1891	Noble x King of the Earliest	United Kingdom
Marshall	1893	American selection of unknown pedigree	Massachusetts
Dunlap*	1900	Likely Cresent x Cumberland	Illinois
Klondike*	1901	Pickerproof x Hoffman	Louisiana
Aberdeen*	1924	Likely Late Stevens x Chesapeake	NewJersey
Blakemore	1929	Missionary x Howard 17	Maryland
Fairfax*	1933	Royal Sovereign x Howard 17	Maryland
Sparkle*	1942	Fairfax x Aberdeen	New Jersey
Jerseybelle*	1955	NJ953 x NJ925	New Jersey
Surecrop	1956	Fairland x USMD1972	Maryland
Raritan*	1968	Redglow x Jerseybelle	New Jersey
Guardian*	1969	NC1768 x Surecrop	Maryland
Earliglow	1975	MDUS2359 (Fairland x Midland)	Maryland
Honeoye*	1979	Vibrant x Holiday	New York
Allstar	1981	US4419 x (NCUS1768 x Surecrop)	Maryland
Jewel*	1985	NY1221 x Holiday	New York
Northeaster*	1993	MDUS4380 x Holiday	Maryland
Cabot	1999	K87-5 x K86-19	Nova Scotia
L'Amour*	2003	(MDUS5252 x Etna) x Cavendish	New York
Ovation	2003	Lateglow x Etna	Maryland

plete block design with four replications and six plants in each experimental unit.

Photosynthesis. Simultaneous gas exchange and light adapted chlorophyll fluorescence data were collected using the LICOR 6400 gas analyzer with a fluorescence head attachment (LICOR, Lincoln, NE). Data were collected on a recently expanded leaf between the hours of 10:00 and 14:00. Light levels in the chamber were set to match ambient conditions for each trial; for the greenhouse trial light was set at 500 mmol PAR m^{-2} s^{-1} and the field trial at 1500 mmol PAR $m^{-2} \cdot s^{-1}$.

Chlorophyll Fluorescence. Chlorophyll fluorescence was measured using a LI-COR 6400 infrared gas analyzer with a chlorophyll fluorescence head attached to allow simultaneous gas exchange and fluorescence measurements. Measurements were made under full sunlight at noon to attain the steady state fluorescence of leaves adapted to ambient light conditions and again when exposed to a supersaturating pulse of light. The proportion of light absorbed by photosystem II (PSII) that is used in photochemistry is expressed as Φ_{PSII}, a measure of the rate of linear electron transport that reflects overall photosynthesis. Ambient fluorescence minus dark-adapted fluorescence divided by saturated fluorescence (Fv'/Fm') is a measure of the maximum quantum yield of PSII when all reaction sites are open.

Carotenoid/chlorophyll content. A subset from the greenhouse trial of 12 cultivars and one genotype of *F. chiloensis* and *F. virginiana* were used for pigment analysis. Leaf discs were collected between 11:00-13:00 from the center leaflet of the last fully expanded leaf, weighed and placed in liquid N_2. Pigments were extracted under low light from 50-100 mg of leaf tissue. Tetrahydrofuran and methanol (MeOH) were used as solvents with butylated hydroxytoluene as an antioxidant. Pigments were then transferred to ether via phase partitioning, the ether removed *in vacuo*, and the extract resuspended in MeOH/methyl t-butyl ether (1:1). HPLC was performed with an ASI-100 automated sample injector linked to a P680 HPLC pump and a PDA-100 photodiode array detector (Dionex Corp., Sunnyvale, CA). Pigments were eluted from a C30 reverse-phase column (Waters Inc., Milford, MA) via a linear solvent gradient (20 min) consisting of ammonium-acetate in MeOH (1 $g \cdot L^{-1}$) and methyl t-butyl ether.

Growth analysis. Flower counts were recorded on three plants in every field plot during the spring of 2007. After harvest, three plants per plot were excavated from the field and used for destructive growth analysis. Plants were washed and separated into individual growth components (leaves, crown, roots) then dried at 75°C for 3 days to a constant weight before recording. Leaf area was measured on all plants with a leaf area meter (Model 3000, LICOR Inc., Lincoln, NE) before drying leaves. Leaf area ratio (LAR) was calculated by dividing leaf area by the dry weight of the plant at harvest.

Fruit quality. Subsamples of 10 primary fruits from each plot were collected during the fruiting period and used to measure berry firmness using a Wagner Force Rive FDV-30 force gauge (Wagner Instruments, Greenwich, CT) with a 15 mm tip. Twenty g subsamples of fruit were collected during the fruiting period to measure soluble solids with a digital refractometer (ATAGO USA Inc., Bellevue, WA).

Anthocyanins and phenolics. Whole fruits were extracted in 80% methanol and 0.2% folic acid buffer solution. Total anthocyanins were measured using the pH differential method and total phenolics were measured using the Folin-Ciocalteu procedure (Singleton et al., 1999). All samples were analyzed in triplicate.

Fruit yield. All fruit was harvested from each plot twice per week during the harvest season and weighed. Marketable and unmarketable fruit were weighed separately. A random subsample of 1-L per plot was counted and this number was

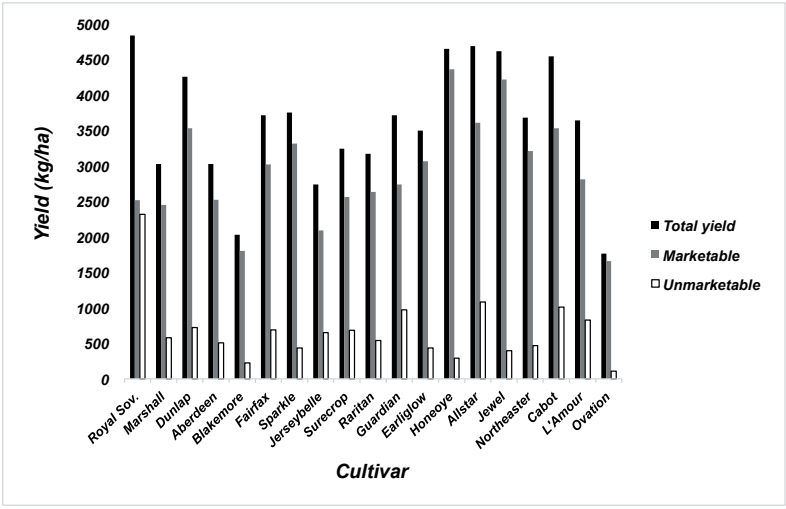

Fig. 1. Total, marketable and unmarketable (5 g or less or misshapen fruit) of 20 cultivars released over the last century. Cultivars listed in order of release date. Standard errors: total = 390; marketable = 380; unmarketable = 129.

divided into total fresh weight to determine average individual fresh fruit weight.

Results

Total, marketable and unmarketable yield were highly variable across cultivars; however, there was no significant correlation between yield and decade of release. Cultivars released in the 1970s and 1980s had the highest marketable yields (Fig. 1). Although there were no significant trends in yield, average weight of both primary fruit and lower order fruit (2º, 3º and 4º) increased over time (Fig. 2) whereas average fruit number per plant decreased (Fig. 3).

Fruit firmness increased significantly with year of release with current cultivars being almost 50% firmer than those released in the

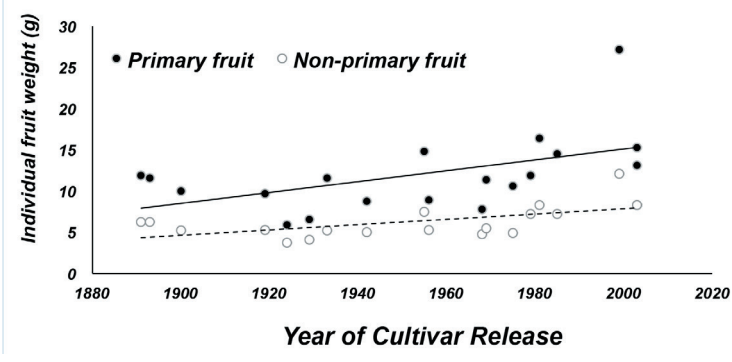

Fig. 2. Average individual fruit weight of primary (king, 1º) vs. 2º, 3º, and 4º berries.
Regression equations: Primary berry, y=106.3+0.0606*Year (r=0.41, p<0.0001); 2º, 3º, 4º y=-57.3 + 0.033*Year (r=0.54, p<0.0001)

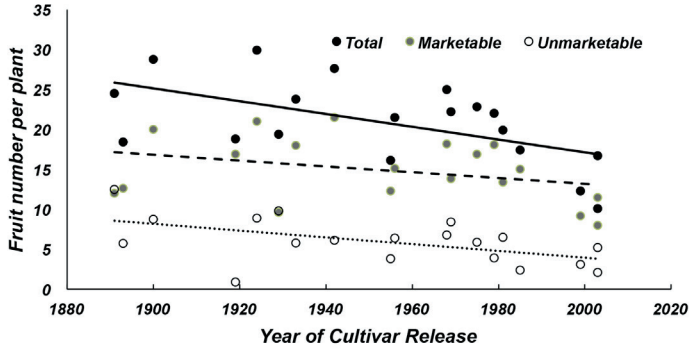

Fig. 3. Average total, marketable and unmarketable (<5g or misshapen) fruit number per plant for 20 cultivars grown in a matted row field trial in Ithaca, NY regressed against year of cultivar release. Regression equations: Total, y= 150 - 0.067*Year (r=-0.37, p=0.004), solid line; Marketable = 82.2 - 0.035*Year (r=-0.22, p=0.04), dashed line; Unmarketable, y= 64.2 - 0.03*Year (r=-0.37, p=0.004), dotted line.

early 1900s (Fig. 4A). Average percent soluble solids decreased slightly over time from approximately 8.7% to 7.6% (Fig. 4B).

Anthocyanin content ($mg \cdot g^{-1}$ fruit fresh weight) varied almost three fold from the lowest in 'Klondike' (25 mg/100 g) to the highest in 'Northeaster' (72 mg/100 g). Phenolic content also varied between cultivars with the lowest value of 224 mg/100 g observed in 'Cabot' to a high of 409 mg/100 g in 'Aberdeen' (Table 2). There were no significant trends over release year in either anthocyanin content or phenolic content, except for leutin.

Cultivars increased in total dry matter accumulation over time, primarily due to increased crown dry weight and a slight increase in root dry weight (Fig. 5). There was no significant change in leaf dry weight or leaf area observed in our study, no significant correlations between growth characteristics and yield, but Leaf Area Ratio (LAR) significantly decreased over time (Fig. 6).

In both the greenhouse and field trial, there was no difference in the rate of photosynthesis on a leaf area basis among the cultivars

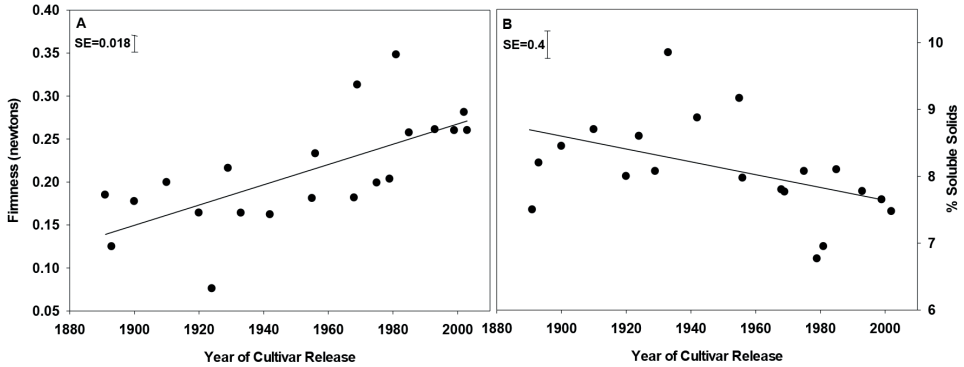

Fig. 4. Fruit firmness (newtons) and °Brix value of strawberry cultivars released over the last century. Points indicate mean of 20 berries. Regression equations: Firmness=-2.09 + 0.0012*Year (r=0.69; p=0.0006); Brix =26.84 - 0.0096*Year (r=-0.33, p=0.003).

Table 2. Anthocyanin and phenolic content (expressed in mg 3-phenyl-glucoside equivalents) of field-grown fruit from 20 cultivars and two progenitor species representing a range of release dates (FW=fresh weight).

Cultivar	Release Date	Anthocyanins (mg/100g FW)	Phenolics (mg 3-P-glu/100g FW)
Royal Sovereign	1891	27	225
Marshall	1893	60	278
Dunlap	1900	53	318
Klondike	1901	25	343
Aberedeen	1924	39	409
Blakemore	1929	29	265
Fairfax	1933	45	253
Sparkle	1942	69	275
Jerseybelle	1955	66	271
Surecrop	1956	57	345
Raritan	1968	46	308
Guardian	1969	42	350
Earliglow	1975	59	380
Honeoye	1979	59	326
Allstar	1981	32	305
Jewel	1985	62	301
Northeaster	1993	72	317
Cabot	1999	52	224
L'Amour	2003	36	266
Ovation	2003	37	341
SE		3	24
p-value		<0.0001	<0.0001
r-value		-0.32 (NS)	0.25 (NS)

(Fig. 7). Due to the higher light levels in the field during peak fruiting, A_{CO2} and g_s were higher compared to the greenhouse (Fig. 8). The mean of the cultivars in the greenhouse (14.1 µmol CO_2 $m^{-2} \cdot s^{-1}$) was intermediate to the photosynthetic rates of the progenitor species; *F. virginiana* (11.6 µmol CO_2 $m^{-2} \cdot s^{-1}$) and *F. chiloensis* (17.8 µmol CO_2 $m^{-2} \cdot s^{-1}$). In the field trial, photosynthetic data were collected at three phenological stages; flowering (11 May), peak fruiting (15 June) and late fruiting (31 June). The highest rates of A_{CO2}, g_s and ΦPSII (effective quantum yield of PSII) occurred during the peak fruiting stage. There was a trend of decreasing A_{CO2}, g_s, and ΦPSII with cultivars released over time (Fig. 7), but trends were not significant.

Regression analysis of the light-adapted fluorescence data indicates that there has been a decrease of 5% in the Fv'/Fm' over the last century of breeding as measured in the greenhouse (Fig. 8C). Initial mean Fv'/Fm' for the early cultivars (0.68) was intermediate to the two progenitor species; *F. chiloensis* (0.72) and *F. virginiana* (0.62) (Fig. 9C). Φ_{PSII} also decreased by 10% during the last century (Fig. 8D). Again, initial rates were intermediate to the progenitor species; *F. chiloensis* (0.61) and *F. virginiana* (0.50) and have become more similar to *F. virginiana*. α-carotene, β-carotene, zeaxanthin and violaxanthin levels of the cultivars have

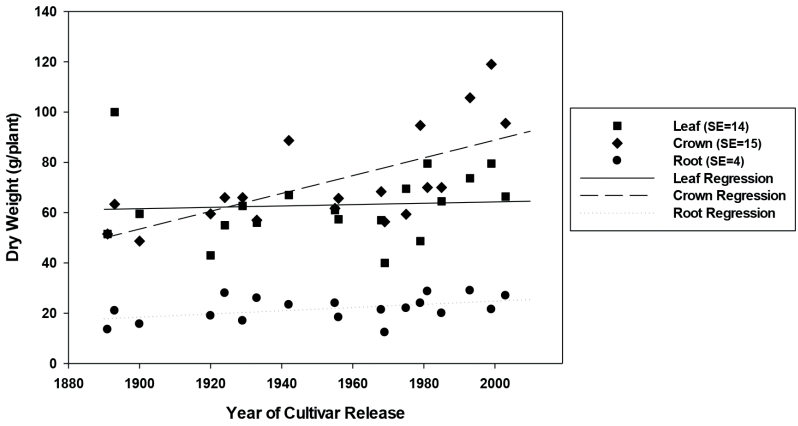

Fig. 5. Leaf, crown and root dry weights per plant of 20 strawberry cultivars grown in matted row field trial and regressed against year of cultivar release. Regression equations: Leaf (NS); Crown = -619 + 0.35*Year (r=0.45, p=0.0005); Root= -103 + 0.064*Year (r=0.28, p=0.03).

not changed directionally over time and are intermediate to the progenitor species that were evaluated (Table 3). The total amount of chlorophyll (a+b) on a leaf area basis is also intermediate to the progenitor species.

Discussion

Many cultivars have been released over the years in the northeastern United States with the goal of producing a higher yielding cultivar with superior fruit quality. However, data from this study suggest that there has been limited progress for several traits, particularly over the last three decades.

Fruit traits. Total, marketable and unmarketable yield were highly variable among cultivars grown under identical conditions and there were no significant trends over the last century (Fig. 1). This is consistent with New York census data (USDA, National Ag.

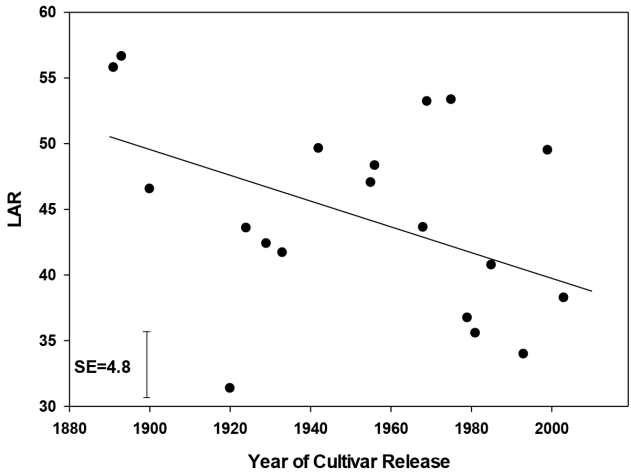

Fig. 6. Leaf area ratio (LAR= leaf area/plant dry weight) of 20 cultivars regressed against year of cultivar release. Regression equations: LAR= 236 - 0.098*Year (r=-0.36, p<0.01)

Table 3. Leaf chlorophyll and carotenoid content of greenhouse-grown fruit from 12 cultivars and two progenitor species representing a range of release dates.

Cultivar	Release Date	Chl A (g/cm^2)	Chl B (g/cm^2)	Chl A:B	α-carotene (mg/m^2)	β-carotene (mg/m^2)	Zeaxanthin (mg/m^2)	Violaxanthin (mg/m^2)	Leutin (mg/m^2)
Royal Sovereign	1890	44.8	12.4	3.6	87.8	1250	88.8	56.3	1136
Marshall	1893	39.9	10.7	3.6	79.5	1082	65.1	33.3	1090
Dunlap	1900	42.9	12.1	3.6	75.1	1154	63.7	45.8	1101
Aberdeen	1924	35.1	9.4	3.4	79.0	1059	81.8	44.0	1090
Fairfax	1933	48.6	14.1	3.5	91.5	1287	40.2	26.5	1366
Sparkle	1942	42.8	12.2	3.6	87.3	1215	52.9	36.4	1224
Jerseybelle	1955	46.3	13.6	3.6	102.2	1297	52.9	37.5	1400
Raritan	1968	44.9	12.2	3.6	81.7	1224	79.1	47.3	1252
Honeoye	1979	49.5	13.6	3.7	98.5	1356	64.7	54.0	1467
Jewel	1985	39.7	11.6	3.3	77.4	1126	78.7	47.0	1267
Northeaster	1993	50.3	15.1	3.0	96.2	1373	54.4	35.2	1548
L'Amour	2003	45.3	13.0	3.5	87.9	1226	72.8	42.5	1336
F. chiloensis		72.7	19.9	3.7	146.3	2113	68.6	44.0	1750
F. virginiana		31.3	9.3	2.8	57.3	853	111.8	66.7	884
SE		2.8	1.1	0.25	6.7	79.2	11.6	8	78.8
p-value		0.0083*	0.0189*	NS	<0.0001	<0.0001	0.0092	NS	<0.0001
r-value		0.41	0.48	-0.43	0.40	0.47	-0.10	0.07	0.77

[1] Significant linear regression over time ($p<0.001$).

Stat. Svc., New York) showing relatively stable yield per unit area over the past three decades. In fact, yield per ha in 2015 was almost identical to yield per ha in 1998. The highest marketable yields in our study were for 'Honeoye' (released in 1979), 'Allstar' (released in 1981) and 'Jewel' (released in 1985), three cultivars which continue to be widely planted in the northeastern U.S. This lack of yield improvement is in contrast to national strawberry yield averages which have increased steadily over the same period of time. The national average is heavily influenced by California and Florida which have benefited from the conversion to an annual production system and the introduction of day neutral cultivars in the mid-1980s (Pollack and Perez, 2005).

Although the overall yield of northeastern cultivars appears to have reached a threshold for short-day plants, significant changes in fruit size occurred during the last century of breeding. The average fruit size of both the primary and lower order berries steadily increased (Fig. 2); however, this has been accompanied by a reduction in fruit number per plant (Fig. 3). Fruit set also significantly increased over time as flower number decreased more (-50%) than the reduction in fruit number (-18%) per plant. Increases in fruit size can contribute to increased yield but will require a stable or increase in fruit number as well (Lacey, 1973). Yield increases based on fruit size have occurred in tomato (Grandillo et al., 1999) and several grain crops (Feil, 1992). Western strawberry cultivars are a potential source of greater individual fruit weight (Hancock et al., 1992).

The most pronounced change over time was fruit firmness. Increasing firmness was driven by market demand and does not seem to be related to any other physiological variable. Percent soluble solids decreased slightly over time (Fig. 4), but this variable is highly influenced by the environment so trends may not represent genetic changes. A study of Italian cultivars showed a negative relationship between soluble solids and productivity (Faedi et al., 2002). Heritability studies on California strawberry cultivars showed that the selection response for soluble solids is highly affected by the environment the selection occurs in, but it is possible to select for higher content (Shaw, 1990). The reduction in soluble solids observed over time may be due to a dilution effect of an increase in fruit size with the newer cultivars, although it is clearly possible to simultaneously achieve large fruit size and high soluble solids (e.g. 'Albion', 'Chandler'). Weather plays a role in soluble solids content, so this modest trend may not reflect a true genetic change in strawberry soluble solids content.

The health components of fruit are becoming increasingly important to consumers, but most of these components have not been intentionally selected. A large variation in biochemical constituents was observed between cultivars, with an almost three-fold difference in anthocyanin content and almost two-fold difference in the phenolic content of the fruit. Previous studies indicate that cultivar can have a large influence on the content of phenolics and anthocyanins (Heinonen et al., 1998; Maas et al., 1991). However, these differences were not related to year of cultivar release. Previous work suggests that the composition of the specific compounds in cultivars is significantly different from the progenitor species (Aharoni et al., 2004) suggesting that some of this difference could be genetic. Additional analysis of metabolite profiles in the cultivars may elucidate changes in composition that have occurred over time.

Physiological Traits. Several crops which have undergone selective breeding have shown significant increases in yield; soybean (*Glycine max)* with 0.5-0.9% per year (Luedders, 1977), sorghum (*Sorghum bicolor* L.) with 1-2% per year (1950-1980) (Miller and Kebede, 1984), maize 1.4% per year (1930-1980) (Duvick, 1984), white clover (*Trifolium repens*) 0.6% per year (1930-1990) (Woodfield and Carandus, 1994) and tomatoes with 1.5% per year (Grandillo et al.,

1999). Similar trends have not occurred for northeastern strawberries despite many new cultivars being released. An expectation that direct-marketed northeastern cultivars will have high flavor may constrain significant yield improvement.

One possible avenue to increase yield is to improve canopy architecture and carbon partitioning. Optimal canopy architecture for light interception and carbon partitioning has been correlated with significant yield improvements in several other crops (Duncan et al., 1978; Duvick and Cassman, 1999; Feil, 1992; Irvine, 1975) although a dense plant canopy can result in greater disease pressure as well as greater interleaf shading within the canopy. Research on strawberry canopy characteristics showed large variability in dry matter partitioning between cultivars grown in a matted row (Strik and Proctor, 1988b). Studies investigating correlations between carbon allocation and yield also showed variable results. For example, a negative correlation was observed between yield and leaf number (Lacey, 1973), a positive correlation between leaf dry weight and leaf area in the fall with yield (Strik and Proctor, 1988c) and a positive correlation between crown dry weight and yield (Strik and Proctor, 1988a; Strik and Proctor, 1988b). Based on previous studies and the results of the current study, there has been no consistent change in canopy architecture or carbon partitioning suggesting that there has not been a focused effort to breed strawberry plants for a particular canopy architecture. This is in contrast to many crops that have shown significant increases in yield through changing plant architecture and carbon allocation patterns (Feil, 1992).

Yield and plant size also might be influenced by more efficient photosynthetic processes. The efficiency of PSII decreased over time, but only the results in the greenhouse were significant. The cultivated strawberry has photosynthetic rates that are intermediate to the progenitor species, *F. chiloensis* and *F. virginiana*, suggesting that these rates are heritable. Breeding studies also showed that the high photosynthetic characteristics of *F. chiloensis* may be quantitatively inherited (Hancock et al., 1989). Intentionally breeding for enhanced photosynthetic capacity or efficiency may be another route to improve yield of the cultivated strawberry, perhaps by incorporating more *F. chiloensis* genes into progeny.

Results from the greenhouse study showed that cultivars have maintained A_{CO2} rates that are intermediate to the two progenitor species (*F. chiloensis* and *F. virginiana*), similar to results of previous studies (Hancock, 1999; Hancock et al., 1989; Hancock et al,. 2002; Sedat et al., 1989). However, Hancock et al. (1992) found no relationship between CO_2 assimilation and yield in 34 strawberry cultivars. Other studies showed no correlation between yield and A_{CO2} (Evans, 1993). Similar observations in other crop species have led to the hypothesis that most crops are sink limited and that increasing A_{CO2} will not lead to increased yield. However, the relationship between carbon assimilation and dry matter production cannot be ignored. Dry weight accumulation of crops is related to the absolute amount of light intercepted by green foliage (Monteith and Moss, 1977); however, the effect that this has on yield is complicated by factors such as partitioning, interleaf shading, pest and disease pressure and respiration. Long et al. (2006) suggests that as photosynthesis is influenced by morphological characteristics, the potential influence that A_{CO2} has on yield may be masked by differing plant characteristics, particularly leaf area and canopy density.

Evidence that the strawberry plant also may be sink-limited is the observation that photosynthetic rates did not change significantly or slightly decreased over a century of breeding (Fig. 8 and 9) despite an increase in crown and root dry weight and a decrease in LAR (Fig. 7). If strawberry plants are source-limited, then photosynthetic rates per unit of leaf area would be expected to increase with a relative reduction in leaf area (LAR).

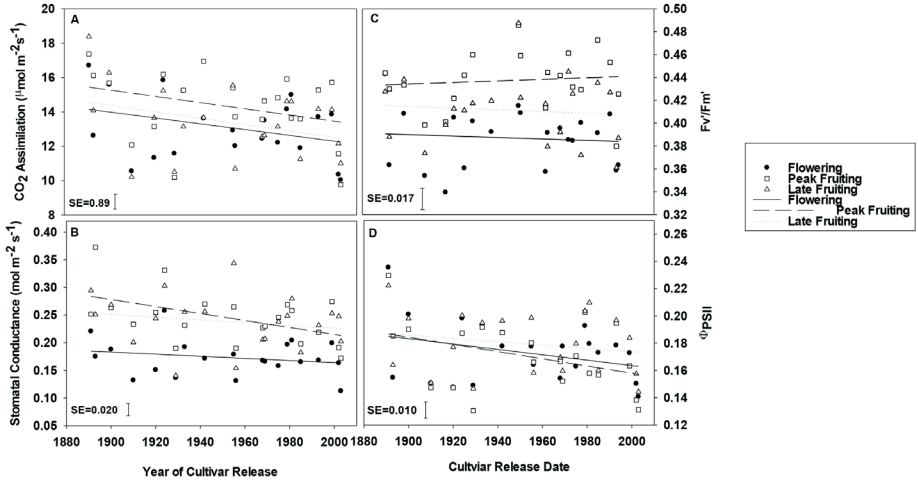

Fig. 7. Maximum CO_2 assimilation rates (A), stomatal conductance (B), Fv'/Fm' (C) and PSII efficiency (D) measured in the field on three dates for 20 cultivars and regressed against year of release. 1) Flowering (15 May 2008), 2) Peak Fruiting (11 June 2008) and 3) Late Fruiting (30 June 2008). Light level in chamber was 1500 µmol PFD $m^{-2}s^{-1}$. Regressions were not significant.

In the greenhouse trial, the progenitor species *F. chiloensis* had significantly higher rates of photosynthesis, higher photosynthetic efficiency Fv'/Fm' (Fig.), higher amounts of chlorophyll and lower xanthophyll content. *F. virginiana* had rates that were lower than cultivars with decreasing Fv'/Fm' and ΦPSII in cultivars over time. The results of

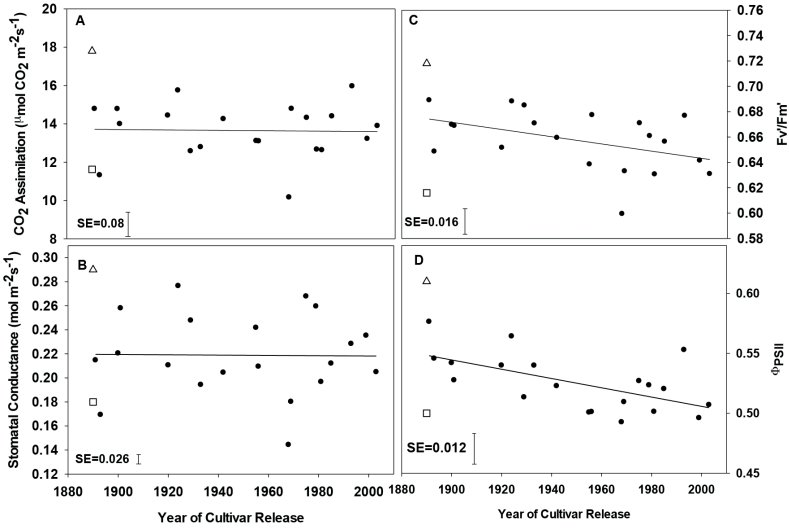

Fig. 8. Photosynthetic characteristics of greenhouse grown cultivars (mean of 6 points for each cultivar) and the progenitor species; *F. chiloensis* (open triangle) and *F. virginana* (open square) regressed against release date. CO_2 assimilation (A); stomatal conductance (B); Fv'/Fm' (C); PSII efficiency (D). Regression equations: Fv'/Fm' = 1.21 - 0.00028*Year (r=-0.39, P<0.002); **Φ**PSII = 0.23 - 0.00036*Year (r=-0.39, p<0.003).

this study suggest that *F. chiloensis* appears to have higher photosynthetic capacity and may provide a source for increasing carbon accumulation and yield of the cultivated strawberry. Serce et al (2002) also found that *F. chiloensis* genotypes have a higher assimilation rate than those of *F. virginiana*, but the former were affected more negatively when temperature increased. Such a response will make breeding for increased season long photosynthetic capacity difficult in a changing environment with warmer temperatures.

One of the major differences between strawberry production in California and Florida compared to the Northeast is the widespread use of day neutral cultivars and annual production systems. The development of improved day neutral cultivars that are well suited to the Northeast will contribute to increasing yield potential there.

Incorporation of new germplasm into the breeding stock will be an important component to improve both yield potential and fruit quality for northeastern strawberry cultivars by introducing traits such as high carbon assimilation and optimized partitioning, day neutrality, fruit number and improved fruit quality. Several studies (Dale and Sjulin, 1990; Hancock et al., 2002; Sjulin and Dale, 1987) have demonstrated that the genetic variability in the cultivated strawberry is narrow. Although there are a few breeding programs that have used wild species, the majority of programs rely on germplasm from *F.* x *ananassa* (Faedi and Coman, 2002). Introduction of new germplasm also will be important as growers are facing increasing challenges in dealing with increasingly variable environmental conditions.

Our study suggests several avenues might be available for productivity improvement including increasing fruit number per plant while maintaining fruit size, increasing fruit size while maintaining fruit numbers, modifying plant architecture and carbon allocation, improving carbon assimilation, and increasing photosynthetic efficiency. Incorporating day neutrality into adapted cultivars also could have a significant impact on yield. Increasing yield while maintaining the high flavor expectations for northeastern cultivars will be a significant challenge for breeders, particularly as temperatures warm.

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Correction

In volume 70(2), in the article by Hyun-Kil Jo[1], Jin-Young Kim[2*] and Hye-Mi Park[2] "Effects of pear orchards on carbon reduction", the following two additional footnotes were mistakenly omitted at the bottom of page 63:

[1] Department of Ecological Landscape Architecture Design, Kangwon National University, Chuncheon 200-701, Republic of Korea

[2] Department of Ecological Landscape Architecture Design, Graduate School, Kangwon National University, Chuncheon 200-701, Republic of Korea

Also, the footnote for Table 2 on page 67 should have been (the same with Table 3 and Fig. 3) (rather than Table 6 and Fig. 2 as shown)

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Bagging Technology Reduces Pesticide Residues in Table Grapes

Yizhen Wan[1,2,3], Qi-Rui Hou[1,2], Yan Wen[1,2], Lei Wang[1,2], and Quanyou Lu[1,2]

Additional index words: grape bagging, pesticide residue, table grape, berry appearance

Abstract

Most table grape growing regions in China have a continental climate. This climate is conducive for grape diseases. For this reason, our research group developed a bagging technology and a design product twenty years ago to protect the berries from damages by pests. This innovation was granted a patent in 2002 in China. Since then, this patent product has been dramatically extended to grape growers in China. Grape bagging is currently one of the most widely used viticultural practices in the table grape industry in China. However, effects of grape bagging on reduction of pesticide residues were not comprehensively evaluated. In this study, residues of seven pesticides, omethoate, cyhalothrin, mancozeb, methylthiophanate, chlorothalonil, metalaxyl mancozeb and triadimefon, were compared between the bagged and nonbagged berries of the two popular grape cultivars, 'Red Globe' and 'Kyoho', planted in China. The results showed that an annual program of 25 - 30 and 17-20 of pesticide applications were needed in Yangling, a typical region for growing table grapes in China to control diseases in 'Red Globe' and 'Kyoho', respectively. The recovery of the seven pesticides was over 96% and the precision of the determining method (RSD) was around 7% in most cases, indicating that the analytical methods were adequate. Pesticide residues in both of the nonbagged and bagged berries were lower than the grape maximum pesticide residue limits required by China government. The pesticide residues in the bagged berries were reduced to ~10 % of the residues in the nonbagged berries. This indicates that the bagging technology extensively reduced the pesticide residues in the berries.

Both production and planted area of table grapes in China are the largest among the countries in the world (He, 1999; Wan et al., 2008). The planted area and production were 715 kilo hectors and 11.6 million tons in 2013, respectively (FAO, data released in 2015). The table grape is one of the most favorite fruits appealing to both growers and consumers in China because of its short juvenility, relatively low cost and high return, high quality and rich nutrition in berries (He, 1999; Wan et al., 2008). However, most regions suitable for growing table grapes in China have a continental climate with the major rainy season from April to Oct., which is concurrent with the growing season (Wan et al., 2007; 2008). This climatic feature usually results in a high risk of disease epidemics in most grape production regions in China (Wan et al., 2007). As a result, a fungicide spray per week is suggested to the growers to control diseases in these regions and an annual estimation of 10 kilo tons of fungicides is needed to control the diseases for table grapes in China (Xiong et al., 2002a and 2002b; Li et al., 2012). Heavy reliance of pesticides for table grape production resulting in high pesticide residue in both of the vineyard and the grapes is a severe global environmental problem (Soles and Goldberg, 2000; De Melo Abreu et al. 2006; Dehouck et al., 2015). The pesticide residue in table grapes may be an even worse risk to consumers because some consumers eat the grapes directly without any processing, e.g. washing and scalding, to minimize the

[1] School of Biology and Technology, Jiangsu University of Science and Technology, Zhenjian, Jiangsu, P. R. China;
[2] Sericultural Research Institute, Chinese Academy of Agricultural Sciences, Zhenjiang, 212018, P R China,
[3] To whom reprint requests should be addressed; email:wyz689@hotmail.com; or: 1036520399@qq.com

pesticide residues before eating (Turgut et al., 2011; Grimalt and Dehouck, 2016).

Bagging viticulture has been successfully extended to the growers in China in the past decade (Wan, 2002; Zhou and Guo, 2005; Zheng et al. 2014). It is estimated that this technology is applied to 80% of the table grapes (~ 230,000 hectors in 2013) in China (Zheng et al. 2014). This is the first report to compare pesticide residues in bagged and nonbagged berries of the two most popular grape cultivars in China. This report will be very informative to the grape growers in other countries when they plan to apply this technology in their vineyards in the future.

Materials and Methods

Plant materials. Both 'Red Globe' and 'Kyoho' plants were obtained from the Experiment Station of Northwest A&F University, Yangling, Shaanxi, China. The grapevines were 10-12 years old, self-rooted and planted in the vineyard at a spacing of 1.2 × 2.5 m.

Bagging treatment and harvest. High quality paper bags (made by Tianjing Agriculture Preservation Technology Research Institute, Tianjing, China) were used for bagging. The twist wire was embedded near the mouth of the bags for efficient bagging (Wan 2002). The bags were made of the kraft white and soft paper without any coating on the paper. Holes were made in the bottoms of the bags for aeration and drainage. Prior to bagging, the grapevines were completely sprayed with chlorothalonil on a sunny day four weeks post full bloom using the normal pesticide spray method. After the spray, the berry bunches were promptly bagged after the clusters were dried. The nonbagged berry bunches used as the control were also sprayed with chlorothalonil at the same time. Bagging started on 20 June (at stage 31, with berries pea size), and ended 10 days before the harvest facilitating berry coloration (Wan 2002; Fig 1). After bag removal and before harvest, vines were not sprayed with pesticides. 'Kyoho' was harvested in the end of Aug., and 'Red Globe' in the end of Sept. from 2013 through 2015.

Pesticide sprays. Yangling has a typical continental climate. Vines were sprayed

Fig. 1. Bagged clusters with the bags pulled up 10 days before harvest for berry coloration.

every seven and 10 days to control pests in 'Red Globe', and 'Kyoho', respectively

Assessment of pesticide residues. Berries were harvested when fully ripened by berry color and taste. About 100 g of berries were randomly chosen from each of 10 bunches (one bunch from each of 10 vines) for one experiment. There were three biological replicates, where the experimental unit was a 10-vine section of row. The standard chemicals and other chemicals were bought from Sigma-Aldrich Co. (St. Louis, Missouri, USA).

Determination of mancozeb was based on the protocol by head space gas chromatography (Lin et al., 2013), in which mancozeb was transferred into carbon disulfide. The chemical transformation of mancozeb into carbon disulfide was carried out in the Head Space Injector (Chengdu Colintech Analysis Co., Chengdu, China) (Lin et al., 2013).

Determination of omethoate and metalaxyl mancozeb was carried out using Agilent 7890 Gas Chromatography plus a flame photometric detector with a column of DB-B 30 m × 0.22 mm × 0.35 μm (GC-FED, Santa Clara, CA, USA) (Li et al., 2012). Determination of cyhalothrin, chlorothalonil and triadimefon was using Agilent 7890 Gas chromatography plus an electron capture detector with a column of DB-B 30 m×0.22 mm×0.35 μm (GC-ECD, Santa Clara, CA, USA). Determination of methylthiophanate was done using Agilent 1100 High Performance Liquid Chromatography (HPLC, Santa Clara, CA, USA) (Li et al., 2012).

The recovery tests were carried out on six replicates (Pizzuttia et al., 2009).

Randomized block experiment design was used for this study. Three 10- vine plots from each of three blocks within the vineyard were used for each treatment, repeated for three years, were used for determination of a pesticide residue. Data were averaged from three blocks of a year and one-way ANOVAs were performed with a year as 'a replicate', using SPSS v13.0, for each combination of cultivar and pesticide to compare bagging treatments

Results

Frequency of the pesticides used in Yangling. The annual precipitation is 700-900 mm in Yangling, and about 65% of the yearly

Table 1. The pesticide frequency used in the growing season for control of pests in the two grape cultivars of 'Red Globe' and 'Kyoho' in this study.

Pesticides	Control of the major pests	Frequency of pesticides (g/hectare) Red Globe	Kyoho
Omethoate	Mites, aphids, planthoppers, leafhoppers	Twice or three times, 1200-1800g	Once or twice 600-1200g
Cyhalothrin	Thrips, aphids,	Once, 600g	Once, 600g
Mancozeb	Downy mildew, elsinoe anthracnose, white rot, black rot. brown rot	Five or six times, 3000-3600g	Three times, 1800g
Methylthiophanate	Anthracnose rot, white rot, grey mildew, brown rot	Five to seven times, 3000-4200g	Four or five times 2400-3000g
Chlorothalonil	Powdery mildew, elsinoe anthracnose, anthracnose rot, berry rot	Five to seven times, 3000-4200g	Four or five times, 2400-3000g
Metalaxyl mancozeb	Downy mildew, white rot, anthracnose rot	Four times, 2400g	Three times, 1800g
Triadimefon	Powdery mildew	Three times, 1800g	Once, 600g

rainfall concentrates from 20 May to the end of Sept. (Wan et al., 2008). 'Red Globe' is a cultivar derived from *Vitis vinifera*; and 'Kyoho' from a hybrid of *Vitis labrusca* ×*Vitis vinifera*. 'Red Globe' usually ripens in late Sept. or early Oct., and 'Kyoho' in late Aug. in Yangling. The species of *Vitis vinifera* is more susceptible to fungal pests than *Vitis labrusca* (He, 1999; Wan et al., 2008). Thus, 'Red Globe' is much more susceptible to fungi than 'Kyoho'. In addition, the berry growing period of 'Red Globe' is much longer than that of 'Kyoho'. For these two reasons, pesticides are applied more frequently to 'Red Globe' than 'Kyoho' (Table 1). Usually, 17 to 20 sprays are needed for disease control in 'Kyoho', and 25 to 30 sprays are needed for 'Red Globe' per season in Yangling.

For most of the fungicides, over 65% of the chemical functions were degraded within two days after spray (Li et al., 2012; Dehouck et al., 2015). The high moisture following a rain event potentially increases disease infection in the berries and vines. Thus, a prompt fungicide application following rain is suggested in this growing region and more pesticides may be used than in Table 1 for Yangling in some years with high rainfall.

Mean recovery and relative standard deviation (RSD.) To evaluate the recovery of the method, analyses were carried out in six replicates of "blank" grape samples at three different levels (20, 50 and 500μg/kg) and its RSD was calculated.

As shown in Table 2, the recovery was over 96% and RSD was around 7% in most cases, suggesting the analytical method in this study was reliable and its precision was

Table 2. Mean recovery and relative standard deviation (RSD) for determination of the seven pesticides

Pesticides	Standard concentrations	Recovery (%)	RSD (%)
Omethoate	20	101.8	6.6
	50	97.5	11.3
	500	96.3	5.7
Cyhalothrin	20	112.6	7.2
	50	96.6	12.6
	500	98.2	6.2
Mancozeb	20	103.2	7.3
	50	98.7	12.8
	500	99.4	6.9
Methylthiophanate	20	102.6	7.1
	50	97.3	11.6
	500	98.6	6.5
Chlorothalonil	20	105.6	7.3
	50	99.2	12.6
	500	98.4	6.5
Metalaxyl mancozeb	20	101.7	7.0
	50	97.6	10.6
	500	98.4	6.6
Triadimefon	20	106.2	6.5
	50	99.3	12.6
	500	98.7	7.2

Table 3. Pesticide residues (μg/kg) in the bagged and nonbagged berries of two grape cultivars[z].

Cultivars	Pesticides	Pesticide residues										
		2013	2014	2015	Avg.	S.D.[y]	2013	2014	2015	Avg.	S.D.	p values [w]
Red Globe [x]	Omethoate	ND	ND	ND	-	-	ND	ND	ND	-	-	-
	Cyhalothrin	ND	ND	ND	-	-	16.4	17.7	18.3	17.5	1.0	- or very low
	Mancozeb	12.7	16.2	13.8	14.2	1.8	154.3	163.4	172.3	163.3	9.0	9.49×10^{-6}
	Methylthiophanate	12.8	16.8	13.1	14.2	2.2	134.3	148.6	126.7	136.5	11.1	4.84×10^{-5}
	Chlorothalonil	17.4	14.3	18.4	16.7	2.1	190.6	187.3	178.3	185.4	6.4	1.67×10^{-6}
	Metalaxyl mancozeb	47.6	64.5	50.8	54.3	9.0	187.6	166.8	174.5	176.3	10.5	1.07×10^{-4}
	Triadimefon	10.5	12.8	13.7	12.3	1.7	144.4	156.3	147.6	149.4	6.2	3.10×10^{-6}
Kyoho [x]	Omethoate	ND	ND	ND	-	-	ND	ND	ND	-	-	-
	Cyhalothrin	ND	ND	ND	-	-	10.2	11.6	12.7	11.5	1.3	- or very low
	Mancozeb	8.6	6.3	9.5	8.1	1.7	90.7	78.6	80.3	83.2	6.6	4.29×10^{-5}
	Methylthiophanate	12.6	9.7	11.2	11.2	1.5	106.7	120.5	97.6	108.3	11.5	1.33×10^{-4}
	Chlorothalonil	12.5	10.3	14.6	12.5	2.2	130.5	112.8	117.9	120.4	9.1	3.71×10^{-5}
	Metalaxyl mancozeb	8.6	6.2	7.8	7.5	1.2	50.3	55.7	60.2	55.4	5.0	8.41×10^{-5}
	Triadimefon	10.2	11.8	13.6	11.9	1.7	132.5	126.3	147.2	135.3	10.7	3.93×10^{-5}

Note: [z] One-way ANOVA was performed for each combination of pesticide and cultivar
[y] S.D. was the standard deviation from the average values of three years' data.
[x] P-value for difference of pesticide residue in the bagged berries between two cultivars was 0.01075, and P-value was 0.004444 for the nonbagged berries. For this analysis, two cultivars were used as 'factors'.
[w] P- values for residue differences between the bagged and nonbagged berries.

relatively high (Dehouck et al., 2015; Grimalt et al., 2016).

Pesticide residues in the real berry samples. For the bagged or nonbagged berries, the pesticide residues in 'Red Globe' were significantly higher than those in 'Kyoho' ($p < 0.02$) (Table 3). However, the pesticide residues in both treatments and cultivars in this study (Table 3) were lower than the grape maximum pesticide residue limits made by the China government and the Codex Alimentarius Commission (Shu et al., 2005; Yang et al., 2007). The pesticide residues in the nonbagged berries were ten fold higher than for bagged berries in most cases (Table 3), indicating the bagging technology was able to extensively reduce the pesticide residues in the berries ($p < 0.001$). However, the reduction differed among the pesticides. The reduction of metalaxyl mancozeb in 'Red Galobe' was relatively small by bagging compared to other pesticides. But the reason for this phenomenon is unclear.

Discussion and Conclusion

Establishment of reliable analytical methods is prerequisite to precisely determine pesticide residue in grapes. In the past two decades, Chinese viticulturists surveyed reliable methods to determine pesticide residue in grapes (Li et al., 2012). The standard methods for analysis of pesticide residues in agriculture have been established and published in government documents in China. This study was performed according to these standard methods. Our results were compatible with previous reports (Pizzuttia et al., 2009; Li et al., 2012).

Fifteen years ago, the bagging technology started to extend to Chinese grape growers (Wan, 2002; Zhou and Guo, 2005; Zheng et al. 2014). Currently, over 80% of the table grapes are bagged and the technology is important to improve berry quality (Zheng et al. 2014) and berry appearance (Wan, 2002; Zheng et al., 2014). Little dust is deposited in the bags (Fig 1), thus the appearance of the bagged berries was brighter than nonbagged berries and the brighter appearance appeals to consumers (Zheng et al., 2014).

Most of the pesticide residues in 'Red Galobe' were much higher than those in 'Kyoho', particularly in nonbagged berries (Table 3). As aforementioned, 'Red Globe' is more susceptible to disease than 'Kyoho' (Wan et al., 2008). The berry growth period of 'Red Globe' was longer than for 'Kyoho'and at harvest 'Red Globe' usually is severely infected with downy mildew in Yangling (Wan et al., 2008). For these reasons, pesticides were more frequently used in 'Red Globe' than 'Kyoho', possibly resulting in a high pesticide residue in 'Red Globe' (Table 3). However, other factors, such as berry size or cluster compactness, may also cause differences between cultivars.

The results of this study reflect the pesticide residues in the whole berries. However, the pesticide residues in the berry skins should be higher than those of the entire fruits. Washing can remove most of the pesticide residues on the berry skins (Zheng et al., 2014). Thus, consumers are still advised to wash grapes before eating, though trace of pesticide residues was deposited on the bagged berries (Wan, 2002; Zheng et al., 2014).

In conclusion, the berry bagging technology is currently a popular viticulture practice used in the Chinese table grape industry and greatly decreased the pesticide residues in the grape berries.

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Book Review: Pomegranate Production and Marketing

Ibrahim Kahramanoglu and Serhat Usanmaz. CRC Press, Taylor and Francis Group, 6000 Broken Sound Parkway NW, Suite 300, Boca Raton, FL33487-2742. Hardcover. ISBN 13:978-1-4987-6850-4. $149.95.

This book is relatively short, with 134 pages, 15 chapters, 52 figures, and 20 tables. It ranges from cultivar descriptions, production, biotic and abiotic challenges to production, to postharvest, aril and juice production, health benefits, and international trade. It contains great information and can be a rich reference for information on pomegranates. However, the book appears not to have been carefully edited for scientific content and English, making parts confusing and somewhat difficult to read. Therefore, in several places, nouns and verbs do not agree, there are misspellings, such as: "seeds are oil reach" rather than the correct "seeds are oil rich." The pointed branches on pomegranates are modified stems, and therefore are thorns, whereas in the book, they are called "spines," which are modified leaves, such as seen on cacti. Some parts of the book are uneven, such as taste only being mentioned for some of the cultivar descriptions. The authors are from Cyprus, and cultivars, pests, and many descriptions are focused on this general area of Europe. For example, the leaf-footed bug is an important pomegranate pest in California, but is not mentioned in the book.

Even with these shortcomings, this is a valuable book on pomegranates. The authors have much practical experience with growing, harvesting, postharvest, and marketing this crop. Chapters in which the authors drew on their own practical experiences have few references; these include Important Cultivars, Ecological Needs (a two-paragraph chapter), Production, Pomegranate Pests, Pomegranate Diseases, Weed Management, Physiological Disorders, Fruit Thinning, and Harvest and Fresh Fruit Processing. The other six chapters are written more as literature reviews, and include the Introduction, Postharvest Biology and Storage, Aril Production, Juice Production, Pomegranate and Health (Review), and Pomegranate Trade.

Both the chapters written based on the authors' experiences and the literature review chapters are valuable as reference materials. There are many useful tables in this book that are original and have practical importance. For example, there is a table on the daily irrigation water requirements of pomegranates for the eight-month growing season for plants of various age categories. There is a table on the nitrogen-phosphorus-potassium requirements of pomegranates of various ages. The authors even provide the optimum leaf mineral concentrations for pomegranate. For chemical control of pests, diseases, and weeds on pomegranates, tables are provided with recommended concentration, harvest interval, and the EU maximum residue level (MRL) of each chemical.

The Introduction does a good job setting up the book and explaining that the history of pomegranate cultivation dates back to 3000 BCE, thus documenting 5000 years of production. Facts – such as pomegranates being berries; explaining that the three types of flowers, hermaphrodite, male, and intermediate forms, mainly occur on spurs; normal fruit size range (200-1000 g) and extremes, up to 1800 g/fruit; and the story about there being 613 pomegranate seeds in one pomegranate fruit (the range is 200-800), making one for every commandment in the Jewish Bible – are all very interesting and enrich the book.

Especially in chapters drawn from the authors' experiences, the information present-

ed is very practical and should be valuable to both new and experienced pomegranate growers. For example, good detail is presented on when and how to thin pomegranate fruit, including a before-and-after thinning photograph. The explanation of how and when to harvest pomegranate fruit is equally practical and useful. For example, the authors recommend that any dew be completely dry before harvest to avoid blemishes, that shears be used for harvest and they show a photograph of the stem end of properly harvested fruit. They correctly emphasize that as soon as the pomegranate fruit is harvested, weight reduction begins as moisture is lost, and why coolness is important to begin the postharvest life of pomegranates.

Because the USDA does not have any quality standards for pomegranates, the authors explain the EU standards based on the Codex standards for classification of pomegranates. They explain the differences between "Extra," "Class I," and "Class II and also present a table on packing sizes for pomegranate fruit.

The authors do a good job explaining quality attributes for pomegranates, externally and internally. Color, size and shape are components of external quality, whereas, texture, soluble solids, titratable acidity, anthocyanins, phenolics, ascorbic acid, volatiles, and nutritional quality are components of internal fruit quality. These are each explained and a table is presented on the nutrient components of 'Wonderful' arils and juice.

Postharvest deterioration of pomegranates is explained and storage recommendations are given to reduce this deterioration. One example is modified atmosphere storage, which is explained along with modified atmosphere packaging, which increases aril shelf life to about 14 days.

Challenges with efficient aril removal are explained. Tools and a machine for removing arils are described; however, hand extraction is the traditional method. The challenges and inefficiencies of removing arils have contributed to the popularity of juice, which is much easier to extract from the fruit. Juice production and handling are described in some detail.

An interesting review chapter is on pomegranates and health. The review is well done and literature from quality scientific journals is cited. From reading this chapter, it appears that pomegranates are a fountain of youth and are a major health benefit. Consumption of pomegranates and their products helps with cardiovascular health, hyperlipidemia, and hypertension. Pomegranates have antioxidant properties, anti-carcinogenic benefits, anti-microbial properties, anti-inflammatory activity, anti-diabetic properties, and antiviral properties. Pomegranates also help with oral and skin health, obesity, erectile dysfunction, sperm quality, and Alzheimer's disease.

I recommend this book as a reference for pomegranate growers and researchers. Although there are some confusing sentences because of the apparent lack of editing, there is much practical information packed into this small book. It is a service to have this information together in one reference.

John E. Preece
Supervisory Research Leader/Horticulturist
National Clonal Germplasm Repository
USDA-ARS
One Shields Avenue
University of California
Davis, CA 95616-8607

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Drought and Biostimulant Impacts on important Attributes of Perennial Ryegrass for Orchard and Vineyard Floor in the Intermountain West Region of the United States

Shahla Mahdavi[1] and Esmaeil Fallahi[2]

Additional index words: Abscisic acid, glycine betaine, digoxin, *Lolium perenne*

Abstract

Water shortage is a critical issue worldwide, mandating the use of efficient irrigation systems (i.e. drip and micro-jet) in modern orchards and vineyards to irrigate only the target trees and vines. Therefore, this issue may adversely impact orchard floor vegetation, which is needed to prevent tractor traffic compaction. Thus, the impact of two levels of evapotranspiration-based (ETc) water stresses and biostimulants consisting of abscisic acid (s-ABA), Glycine betaine (GB), digoxin, nano silica and some of their combined applications on perennial ryegrass (*Lolium perenne,* L.*)* under climatic and soil conditions of the Intermountain West, USA were studied. Comparing two levels of irrigation, grass clippings with 50% ETc had higher electrolyte leakage and proline but lower visual performance, chlorophyll index (CI) and chlorophyll b than those with 75% ETc. With the exception to the digoxin at 0.5 $mg \cdot l^{-1}$, clippings from all biostimulant-treated plots had significantly better visual performance and higher CI, proline, chlorophyll a and b and potassium (K) concentration than those from the un-treated control. These results underscore the value of these biostimulants for improving the orchard and vineyard floor grass covers under drought stress conditions that prevail in the western United States.

The population increase, meshed with the worldwide water shortages is becoming an increasingly critical issue that mandates minimum use of irrigation water with maximum efficiency and productivity in all agricultural crops. Under such conditions, priority for water use will be given to food crops and as a result landscape plants like turf will be subjected to additional water shortage. At the same time, orchard and vineyard floor cover grasses are needed to reduce soil erosion and compaction from tractors, while maintaining herbicide strips (Rowley et al., 2012).

To conserve water, it is necessary to adopt efficient irrigation systems such as drip and micro-jet in modern orchards (Fallahi et al., 2011), and these irrigation methods may adversely impact vegetation in row middles. Among the possible solutions for maintaining a bio-mulch in these alleyways is the use of drought resistant grasses such as crested wheat grass (*Agropyron cristatum*) (Fallahi et al, 2015) and osmoprotectants or biostimulants (Farooq et al., 2009). Various supplemental chemicals, including plant biostimulants, enhanced survival and recovery of various plants after dehydration. Since the discovery of abscisic acid as a growth retardant by Addicott et al. (1964), a synthetic form was used to induce dormancy (Goggin et al., 2009), flower induction (Greene et al., 2011), fruit set in apples (Greene. 2012), and color enhancement in grapes (Peppi et al., 2006). Plant biostimulants such as abscisic acid play an important role in the regulation of plant tolerance to various environmental stresses, especially drought stress (Quarrie, 1989). Both natural (Lopez-Garbonell et al., 1994) and synthetic forms of plant biostimulants increase tolerance to abiotic stresses (Farooq

[1, 2] PhD graduate and Professor of Pomology, respectively, University of Idaho, Parma Research and Extension Center, 29603 U of I Lane, Parma, ID, USA

et al., 2009). Elevated ABA levels in xylem and root during dehydration can induce closure of stomata as a mechanism to conserve water (Wilkinson and Davis, 2002), inhibit leaf growth or transpiration (Alves and Setter, 2000) and adjust osmotic enhancement (Zhaolong, 2002). Tworkoski et al. (2011) reported that s-ABA was effective in delaying dehydration-induced wilt of shoot tips in apple trees. Recently the role of s-ABA for reducing stress in landscape plants has received particular attention.

Glycine betaine (GB) is an ammonium compound in plants and was discovered in the 19th century. This compound increases in response to dehydration stress (Ashraf and Foolad, 2007). Glycine betaine accumulates under abiotic stress conditions in many plants including barely *(Hurdeum vulgare L.*) (Nomura et al., 1995). In many plants endogenous GB is not sufficient to reduce abiotic stresses dehydration (Subbarao et al., 2000) and exogenous application of GB improves the growth and production of plants under stress (Hussain et al., 2008). Foliar application of GB enhanced the growth of plants under drought conditions by maintaining osmotic pressure and improving stomatal conductance, leading to an enhanced carboxylation efficiency of Rubisco and photosynthesis (Sakamoto and Murata, 2002).

Next to oxygen, silicon is the most abundant element on the surface of the earth. Although not considered as an essential element for higher plants, silicon has major roles in leaf turgidity of monocots and cell wall structure (Gong et al., 2003). Silicon can be effective against both biotic and abiotic stresses (Epstein, 1999). Gong et al. (2003) suggested that application of silicon could improve growth of wheat in arid or semiarid areas. Hattori et al. (2005) reported that nano silicon enhanced drought tolerance in two cultivars of sorgum (*sorgum bicolour*). Agarie et al. (1998) reported that silicon decreased electrolyte leakage and increased cell walls polysaccharides in rice, suggesting that silicon could be involved in thermal stability of lipids in cell membranes and prevent structural and functional deterioration of cell membranes when rice plants are exposed to abiotic stresses.

Digoxin (Dig) has been exclusively used in human tissues. According to Lelievre and Lechat (2007), digoxin is a cardiac glycoside that binds to and inhibits sarcolemma-bound (Na^+/K^+-) Mg^{2+}-ATPase in human. The inhibition induced by Dig leads to an efflux of K from the cell and, in proportion to the extent of inhibition of the ATPase, an increase in internal sodium ion concentration ($[Na^+]$) at the inner face of the cardiac membranes. This local accumulation of sodium causes an increase in free calcium concentrations via the Na^+–Ca^{2+} exchanger. This free cellular Ca is responsible for the inotropic action of Dig, secondary to the release of Ca^{2+} from the sarcoplasmic reticulum, including the clinical and molecular basis (Lelievre and Lechat, 2007). Edner et al. (1993) studied the influence of Dig on muscular and symphatoadrenergic activity and the serum potassium concentration. The role of this compound is not known in plant tissues.

Grasses are the most common cover crops in apple orchards. Newhouse and Dana (1989) used perennial ryegrass in strawberries. They reported that this living mulch significantly increased strawberry yield and quality and protected strawberry crowns from spring and winter winds and cold temperatures. Granatstein et al. (2010) reported that using living mulch with a mix of perennial ryegrass and tall fescue (*Schedonorus phoenix* (Scop) Holub) in a modern 'Gala' apple orchard system provided good weed control, soil quality benefits and meaningful N contribution. Stefanelli et al. (2009) used a mixture of mammoth red clover (*Trifolium pratense* Var. perenne, L.) and endophytic perennial ryegrass as living mulch for orchard floor management and reported this as a suitable combination in an organic apple production.

Perennial ryegrass is a popular cool season grass, which is commonly used alone and in mixture with other species in modern orchard

and vineyard floors. However, information on the use of various levels of water stress and biostimulants on this grass, particularly as living mulch for orchard floors is lacking. Thus, our objective in this experiment was to study the effect of two levels of ET-based drought in combination with exogenous s-ABA, glycine betaine (GB), s-ABA plus GB, two levels of digoxin, two levels of nano silica and digoxin plus nano silica on performance, and other attributes of perennial ryegrass climatic conditions of the southwest Idaho in the Intermountain West Region of United States.

Materials and Methods

Experimental Site and Turf Establishment. The experimental site was located at the Pomology and Viticulture Orchards and Vineyards of the University of Idaho, Parma Research and Extension Center, Parma, Idaho, USA. Perennial ryegrass (*Lolium perenne* L.*)* was planted in May of 2013. The history of cultural practices and maintenance of this lawn was well recorded and kept at the University of Idaho Research and Extension Center. This was considered a well-established and perfectly suitable lawn, resembling floor cover (alley way) in many commercial orchards and vineyards in the region. The experiment was performed during spring and summer of 2015.

Calculations for ETc, Water Applications. The University of Idaho Parma Research and Extension Centre Weather station (Agri-Met) was located at the experimental site and recorded ET data for all agricultural crops including lawns. There were two irrigation systems to create water deficiency at 50% ETc or 75% ETc of a full ETc. Water requirement was calculated based on ETc where ETc = ETr x Kc (Allen et al., 1998) and the details of irrigation calculations were similar to an earlier reports by Fallahi et al. (2011) and Mahdavi et al. (2016).

Biostimulant Treatments. Biostimulant treatments were prepared and applied twice. The first application was made between 8 a.m. and 1 p.m. on 28 July 2015. On this day, temperatures during application ranged from 21° C to 28° C with a clear sky and wind was calm (less than 2 $km \cdot hr^{-1}$). The second applications were made on 11 Aug. 2015. On this day, temperatures during application ranged from 29° C to 37° C with a clear sky, and the wind was calm (about 1 km/hr). Each solution was uniformly sprayed at the rate of 0.78 $l \cdot m^2$. The non-treated control plots were also sprayed with water at the 0.78 $l \cdot m^2$. The treatment at each application were as follows:

1. ProTone SG® (s-abscisic acid ; s-ABA; 20% soluble granule formulation; Valent BioScience Inc, Libertyville, Illinois, USA) at 100 µM.
2. Glycine betaine (GB; ≈ 99% purity, Sigma Life Science, Sigma-Aldrich Louis, MO, USA) at 100 mM.
3. S-ABA at 100 µM and GB solutions at 100 mM applied separately at each application time.
4. Digoxin (Dig; Powder; DSM Pharmaceuticals Inc. Greenville, NC, USA) at 0.25 $mg \cdot l^{-1}$ (Dig_1) or 0.5 $mg \cdot l^{-1}$ (Dig_2).
5. Nano silica (NanSi; ≈ 99% purity, 20-30 nm, amorphous, US Research Nanomaterials Inc., Houston, TX, USA) at 1 mM ($NanSi_1$) or at 2 mM ($NanSi_2$).
6. Dig at 0.25 $mg \cdot l^{-1}$ and NanSi solutions at 1 mM prepared and applied separately (not in the same solution) at each application time.
7. Un-treated control (water application only).

Measurements. Soil moisture (volumetric water content; VWC) was measured with a fully computerized soil moisture meter, equipped with two 7.5-cm rods, designed for lawn moister (FieldScout Digital Soil Moisture Meter, Model TDR 300, Spectrum Technologies, Aurora, IL, USA) before and after each irrigation and sometimes in between irrigations. At each time, VWC from three different locations within the same plot was measured and averaged. Although ETc was

the bases for irrigation scheduling, the soil VWC was measured to monitor and compare the ETc-based water application with that shown with a soil moisture meter. Soil moisture measurements were made between 27 July and 7 Sept.

Performance of the lawn was visually rated based on a combination of several factors, including greenness, growth, density and appearance on a scale of 1 to 9, according to the guideline recommended by Morris (2002); where 1 is poor performance, 7 is acceptable and 9 is outstanding. Visual performance ratings for 7 different dates were averaged and reported.

Chlorophyll indices between 10:00 a.m. to 2:00 p.m. were determined on 12 dates between 29 July and 3 Sept. 2015, using equipment based on a new technology (FieldScout Chlorophyll Meter, Model CM 1000, Spectrum Technologies, Aurora, IL, USA). With this instrument, the ambient and reflected 700 nm and 840 nm wavelengths are used to calculate the relative chlorophyll index. It measures conical viewing areas between, 30 and 180 cm from the lens. The instrument measures index of relative chlorophyll content ranges from 0 to 999. At each time, chlorophyll indices of three different locations per plot were measured and averaged. The average values of chlorophyll indices over these 12 dates are presented in this report.

A composite grass-clipping sample from three locations per plot was taken at a height of approximately 3 to 4 cm from the soil level on 24 Aug., 2015. Clippings were washed in a mild solution, containing 1% Liqui-Nox anionic detergent (AlcoNox Inc., White Plains, NY, USA), rinsed in three different 25-l containers of distilled water, and dried in a forced-air oven at 65°C. Clippings fresh weight (FW) and dry weight (DW) were used to calculate dry weight percentage (DW %). The dried leaves were ground to pass a 40-mesh screen using a Cyclotec Sample Mill (Model 1093; Tecator, Hoganas, Sweden). For mineral analysis, specific guidelines and methods described by Gavlak et al. (2005) were used. Leaf tissue was analysed for potassium (K) by dry ashing at 500°C, nitric/perchloric digestion and the use of Inductively Coupled Plasma Atomic Emission Spectrometer (ICP-AES) (Perkin-Elmer; Norwalk, CT, USA).

Free proline content was determined according to the method described by Bates et al. (1973). Fully expanded leaves (0.1g) were extracted with 10 mL of 3% (w/v) sulphosalicylic acid. The extract was centrifuged for 10 min. at 3000 RPM. Two mL of supernatant was transferred to fresh test tubes and 2 mL acid ninhydrin and 2 mL glacial acetic acid were added and incubated for 1 hour in a boiling water bath followed by an ice bath. Three mL toluene was added and mixed vigorously until the chromosphere phase separated from the aqueous phase. A calibration curve was prepared from a set of standards consisting of 0, 20, 40, 80 and 100 ppm proline (≈ 99.5% purity, Sigma Life Science, Sigma-Aldrich Louis, MO, USA), using the same procedures as described for samples. The absorbance was read at 520 nm using a spectrophotometer (Bio Mate UV Visible, ThermoScientific, Madison, Wisconsin). This instrument estimated concentrations of proline in unknown samples from a standard curve.

Electrolyte leakage (EL) of leaves was measured according to the method of Valencovic et al. (2006). With this method, 0.5 g of leaves were excised and cut into 2 cm segments after being rinsed three times with distilled deionized water. Leaf segments of each sample were placed in a sterile test tube containing 25 mL of distilled deionized water. Test tubes were shaken on a shaker for 24 hours and the initial conductivity (EC_1) was measured using EC meter (FieldScout Direct Soil EC Meter w/8in Probe, Spectrum Technologies, Aurora, IL, USA). Leaf samples were then autoclaved at 121°C for 1 hour in hot water bath followed by an ice bath and conductivity of killed tissues (EC_2) was measured after tubes cooled to room temperature. The percent electrolyte leakage

was calculated as $(EC_1/EC_2)_*100$.

Photosynthetic pigments from turfgrass leaves were extracted as described by Arnon (1949). For this purpose, 0.25 g fresh leaves were ground and completely homogenized in 2 ml of 80% (v/v) acetone using a mortar and pestle, and then centrifuged 10 minutes and 6000 RPM. Absorbance of the resulting extracts was measured at three wavelengths 663, 470 and 646 nm for chlorophyll a (Chl a) and chlorophyll b (Chl b), using Bio Mate Uv Visible, ThermoScientific (Madison, Wisconsin) and converted to mg/g leaf fresh weights.

Layout, Experimental Design, and Statistical Analyses. The experimental design was a randomized-complete-block split-plot design with two levels of irrigations as the main plot (main-effect) and stress-inducing biostimulants, either alone or in some combinations, and an un-treated control as sub-plots, each with four blocks. The size of each sub-plot was 2.0 m x 1.5 m Sufficient buffer zones were kept within each block and between different blocks to prevent cross contamination of irrigation regimes. Analyses of variance and all possible correlation coefficients among all attributes were conducted using SAS Version 9.4 Programme (SAS Institute, Cary, NC, USA), with PROC GLM. Means were compared by Duncan's multiple range test at $P \leq 0.05$.

Results and Discussion

Interaction. There was no significant interaction between water stress levels and biostimulant treatments for any of the parameters measured in this study.

Soil Volumetric Water Content (VWC). Irrigation was scheduled based on daily ETc data. However, an approximately 25% difference in VWC existed between the 50% ETc and 75% ETc treatments during the course of this study (Fig.1), which confirms the validity of the use of ETc for creation of these two levels of stress in this study.

Water Stress Effects. Grass clippings with 50% ETc had significantly higher proline and electrolyte leakage but lower visual performance, chlorophyll index (CI), and chlorophyll b than those with 75% (Table 1) and the positive correlation coefficient between chlorophyll index and visual rating was extremely strong (r = 0.97). Grass clippings with 75% ETc tended to have higher K concentration than those with 50% although differences were not significant (Table 1). Although we did not have a 100% ETc treatment in this study, high visual ratings (as high as a rating of 9 out of 9) in the 75% ETc indicated that

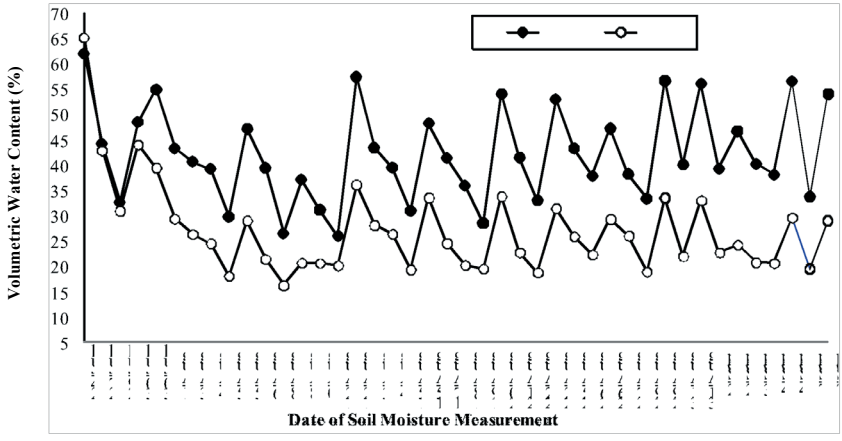

Fig. 1. Soil volumetric water content of 50% ETc and 75% ETc plots during the experiment

Table 1. Effect of water stress and biostimulants on some attributes in orchard and vineyard floor cover perennial rye grass.

Treatment	Visual performance (1-9)*	Chlorophyll Index (1-999)*	Proline (µM/gr FW)	EC (%)	Chlorophyll a (mg/gr FW)	Chlorophyll b (mr/gr FW)	K (% dwt)
Water stress level†							
50% ETc	5.97 b ‡	349 b	13.8 a	28.3 a	0.671 a	0.258 b	1.10 a
75% ETc	8.34 a	510 a	7.7 b	10.4 b	0.703 a	0.340 a	1.41 a
Biostimulant†							
s-ABA	7.33 a	445 a	11.9 ab	17.1 c	0.68 abc	0.273 bc	1.35 a
GB	7.21 a	428 ab	11.8 ab	16.4 c	0.700 ab	0.327 ab	1.24 ab
s-ABA& GB	7.38 a	448 a	13.2 a	17.1 c	0.805 a	0.385 a	1.36 a
Dig_1	7.34 a	440 a	10.1 bc	14.9 c	0.678 abc	0.312 ab	1.32 a
Dig_2	6.68 b	403 bc	8.4 cd	25.2 b	0.615 bc	0.269 bc	1.11 b
$NanSi_1$	7.30 a	436 a	11.7 ab	15.1 c	0.716 ab	0.327 ab	1.27 a
$NanSi_2$	7.15 a	433 ab	11.6 ab	20.2 bc	0.740 ab	0.258 bc	1.27 a
Dig_1NanSi_1	7.31 a	438 a	10.4 abc	16.3 c	0.697 abc	0.318 ab	1.33 a
Control	6.71 b	396 c	7.3 d	32.2 a	0.555 c	0.219 c	1.10 b

* Visual performance rating: 1= poor performance, progressively to 9 = highest (best) performance. Chlorophyll index= Index of relative chlorophyll content= 0 to 999.

† Abbreviations: ETc = Evapotranspiration of lawn; s-ABA= s-abscisic acid; GB= glycine betaine; Dig= Digoxin; NanSi= nano-silica; FW= fresh weight; dwt= dry weight; EC %= Percentage electrolyte leakage; K= Leaf potassium Content.

‡ Mean values within each column of irrigation or biostimulant treatments followed by different letter (s) were significantly different at $P \leq 0.05$ by Duncan's multiple ranges test.

this level of irrigation was sufficient. However, application of water at 50% ETc over the period of this study was unacceptably stressful.

Effects of Biostimulants. With the exception to the Dig_2 treatment, application of each biostimualnt alone or in combination, significantly increased visual performance, chlorophyll index, proline, chlorophyll a and b, and K concentration and decreased percentage electrolyte leakage as compared to control (Table1) and this observation underscores the value of these biostimulants in drought stress reduction. Our study was conducted on a large scale and under field conditions, similar to a realistic commercial orchard and vineyard floor conditions. However, our results with GB was consistent with Hu et al. (2012) who reported a higher chlorophyll content in perennial ryegrass under abiotic stress in greenhouse conditions. They did not include s-ABA in their study. It is essential to conduct an economical analysis to see if the use of these biostimulants, particularly digoxin, to reduce lawn stress is justifiable. Also, further study to understand the mode of action of digoxin spray in plants is necessary.

Higher K in the s-ABA-treated clippings is in agreement with a previous report in Cathamus plants (Gadallah, 1996). Concentrations of K in clippings were negatively correlated with lawn temperatures (r=-0.70) and with clipping percentage electrolytes leakage (r = -0.69), which confirms the positive relationship between K and drought resistance (data not shown). Since application of most biostimulants that resulted in a better orchard floor performance also increased leaf K concentration, it is important to study the physiological and biochemical relationship between K and drought tolerance in more detail. For example, it is essential to see if a simple potassium spray could induce and increase

drought tolerance ofperennial ryegrass. If this theory is proven to be correct, a simple K spray can replace application of rather expensive biostmulants, while saving water.

Conclusions

High visual ratings in the 75% ETc is a clear indication that this level of irrigation was sufficient to maintain a reasonable turf. However, 50% ETc treatment was too stressful and long-term application of this level of irrigation may lead to poor turf quality in alleyways of orchards and vineyards. Nevertheless, based on the results of this study, application of biostimulants can slow the process of grass quality decline under extremely severe drought conditions (i.e. 50% ETc). This area deserves further study to see if applications of other stress levels such as 65% ETc with these biostimulants can further reduce stress and maintain turf visual quality.

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Exploring the Growth and Cropping Potential of Pierce's Disease Resistant *Vitis vinifera* L. Selections for Enhanced Viticultural Sustainability in Alabama and the Southeast

Andrej W. Svyantek[1], Elina D. Coneva[1*], J. Raymond Kessler[1], James D. Spiers[1], Edgar L. Vinson III[1], and James A. Pitts[2]

Additional index words: viticulture, Pierce's disease, subtropical, vigor, yield

Abstract

Cultivation of *Vitis vinifera* L. grapevines has not been economically feasible in the southeastern U.S. due to the major limiting factor, Pierce's disease (PD), caused by the endemic, xylem-limited bacterium *Xylella fastidiosa.* Heretofore, hybrids of *V. vinifera* x *V. arizonica/candicans* have not been evaluated in the hot and humid subtropical climate of central Alabama. In 2010, an experimental vineyard consisting of three UC Davis developed PD resistant 87.5% *V. vinifera* selections ('U0501-12', 'U0502-10', and 'U0501-12') was planted at the Chilton Research and Extension Center, (CREC), Clanton, AL for the purpose of investigating the survival rate and overall performance of these selections in the southeastern U.S. Preliminary studies in our lab suggest the *V. vinifera* selections responded well to local conditions and were free of PD infection. This report focuses on recent two-year assessment of vegetative growth, cropping potential, and fruit quality of *V. vinifera* advanced selections during the period of vine establishment. Our results suggest 'U0501-12' had the smallest trunk cross-sectional-area in both years. Pruning weights for all selections ranged between 1.7 and 2.0 kg/vine in both study years. Total yield in 2015 was 8.7, 10.7 and 10.9 kg/vine for 'U0501-12', 'U0502-01', and 'U0502-10', respectively. Furthermore, 'U0502-10' consistently had the largest cluster size and lowest cluster number per vine. The PD resistant *V. vinifera* selections demonstrated high cropping potential and plant vigor in both study years, indicating they can sustain viticulture in the southeast while enhancing opportunities for the grape growing industry in the region. Further work to thoroughly characterize the viticultural performance of PD resistant *V. vinifera* selections in Alabama's environment is critical.

Bunch grapes (*Vitis* sp.) represent an economically and culturally important fruit crop with global production increasing from 63 million metric tons in 2003 to over 77 million tons in 2013, representing a 22% increase (FAOSTAT, 2013). In the U.S., production exceeded 8 million metric tons in 2015 (USDA-NASS, 2016). In the southeastern U.S., bunch grape production has been limited by Pierce's disease (PD), caused by an endemic xylem-limited bacterium, *Xylella fastidioa* (Hopkins and Purcell, 2002; Wells et al., 1987). As a result, Alabama's viticulture industry has evolved around the cultivation of PD resistant hybrid bunch grapes (American and French-American) and muscadine grapes (*Muscadinia rotundifolia* Small) (Keller, 2010). Increasing local interest in grape production is evident in the number of bearing acres in Alabama which grew from 215 in 2002 to 426 in 2012, a 198 % increase (USDA-NASS, 2016).

Recent breeding work conducted at the UC Davis identified a single gene, PdR1, responsible for PD resistance in a *V. arizonica/candicans* hybrid selection (b43-17) (Krivanek et al., 2006). Using marker assisted selection, 87.5% *V. vinifera* selections were developed

[1] Department of Horiculture, 101 Funchess Hall, Auburn University, Auburn, AL 36849;
[2] Auburn University, Chilton Research and Extension Center, 120 Co Rd 756, Clanton, AL 35045
[*] Corresponding author to whom reprints requests should be addressed: edc0001@auburn.edu

after three *V. vinifera* crosses starting with F8909-08, an offspring of a *V. rupestris* (A. de Serres) x b43-17 cross bearing the PdR1b resistance locus (Riaz et al., 2009).

Until recently, *V. vinifera* vines have not been successfully grown in the high PD pressure of Central Alabama. Based on results from the UC Davis breeding program, and the encouraging preliminary results from our lab (Coneva, 2016), it is hypothesized that PD resistant selections should be capable of producing premium quality European wine grapes in the southeastern U.S., where sustainable production of *V. vinifera* grapes has previously been prevented. To accomplish the goal of evaluating the performance and feasibility of producing *V. vinifera* grapes in Alabama, an experimental vineyard consisting of advanced UC Davis developed PD resistant 87.5% *V. vinifera* selections was established. Data were collected to determine their productivity and vigor in a previously unexplored environment of Central Alabama, considered as a high-risk PD zone (Anas et al., 2008).

Materials and Methods

Three PD resistant *V. vinifera* selections were planted at the Chilton Research and Extension Center near Clanton, AL, USA (32°55'11.6" N, 86°40'25.4" W) on December 9, 2010. Experimental design was a randomized complete block with six blocks and the experimental unit was five plants per selection per block. Experimental vines were grafted on 'Dog Ridge' rootstock and planted in a Dothan fine-loam (kaolinitic, thermic Plinthic Kandiuudults) soil with pH adjusted to 6.2 prior to planting. Vines were spur-pruned and trained to a Vertical Shoot Positioning (VSP) system with three catch wires. Supplemental drip irrigation was provided. Rows were oriented North to South and the planting distance was 2.1 x 3.66 m.

Data were collected to determine the vine vigor and growth characteristics. The traits measured included vine pruning weight and trunk cross-sectional-area (TCSA). Vines were dormant pruned annually in early spring of 2014 and 2015 and prunings were weighed with an Adam CPWplus-35 scale (Adam Equipment Inc, Danbury, CT, USA). TCSA was measured for each vine at 25 cm above the graft union using a digital caliper (Mitutoyo Corporation, Kawasaki, Japan).

Total yield per vine was recorded at harvest for each experimental vine using an Adam CPWplus-35 scale. Vines were harvested by hand. In 2014, soluble solid concentration, expressed as Brix (°), was determined on a 10 berry subsample/vine using a RF-15 hand refractometer (Ade Advanced Optics). Following harvest in 2015, soluble solid content was determined using a digital refractometer (Pal-1 Atago, Co., Tokyo, Japan) at room temperature based on a homogenized 50-berry subsample per vine.

Analysis of variance was performed on all responses using PROC GLIMMIX in SAS version 9.4 (SAS Institute, Cary, NC). The experimental design was randomized complete block. Where residual plots and a significant COVTEST statement using the HOMOGENEITY option indicated heterogeneous variance among treatments, a RANDOM statement with the GROUP option was used to correct heterogeneity.

Results and Discussion

Dormant pruning conducted in early 2014 revealed 'U0502-01' had significantly greater vigor than 'U0501-12', but both selections had similar vigor compared to 'U0502-10' (Fig. 1). In 2015, vine vigor was lowest for 'U0502-10' based on pruning weights. For both years, all selections' pruning weights exceeded 1.7 kg/vine, indicating vigorous plant growth. Selection 'U0501-12' had the smallest trunks in both years (Fig. 2).

In 2014, the experimental location experienced abnormal wet and cold spring and summer conditions which may have contributed to the development of powdery mildew (*Uncinula necator* (Schwein.) Burrill) infection that potentially impacted grape yields. No powdery mildew infection occurred in

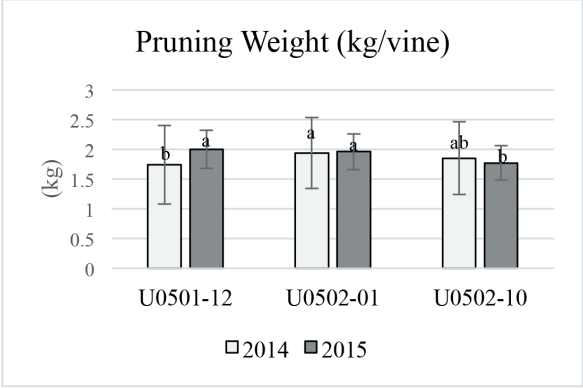

Figure 1. Pruning weight of three PD resistant 87.5% *V. vinifera* selections grown at CREC, Clanton, AL in 2014 and 2015. Error bars indicate ± SE of the mean. Least squares means within year with common letters do not differ at the 5% level of significance, by Shaffer-simulated method.

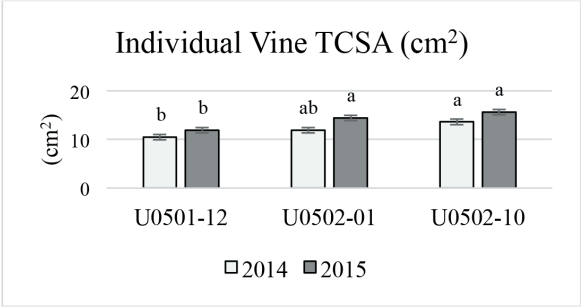

Figure 2. Trunk cross sectional area (cm^2) of PD resistant 87.5% *V. vinifera* selections grown at CREC, Clanton, AL in 2014 and 2015. Error bars indicate ± SE of the mean. Least squares means within year with common letters do not differ at the 5% level of significance, by the Shaffer-simulated method.

2015, and yields were higher. In both years, total yield was highest for selections 'U0502-10' and 'U0502-01' (Table 1). Although 'U0501-12' had the lowest yield per vine (8.7 kg/vine) in 2015, when the potential yield per acre was calculated based on planting density, 'U0501-12' crop was equivalent to 11.1 t/ha. The remaining two selections exceeded 13.7 t/ha in 2015.

'U0502-10' produced the largest and fewest clusters per vine in both study years. Selections 'U0501-12' and 'U0502-01' had a similar number of clusters per vine, whereas 'U0501-12' produced the smallest clusters in both years. Average cluster weight was highest for 'U0502-10' in 2015, with clusters weighing 406 g. Similar to their performance in California, Alabama-grown clusters ranked from smallest for 'U0501-12,' to largest for 'U0502-10,' with 'U0502-01' producing intermediate size clusters in both locations (Walker and Tenscher, 2008; Walker and Tenscher, 2009).

To date, PD infection has not been detected in any of the *V. vinifera* grapes tested in the current planting. Overall berry sugar content ranged from 17.0 to 21.9 ° Brix. 'U0502-01' and 'U0502-10' had the lowest sugar content

Table 1. Yield characteristics of PD resistant 87.5% *V. vinifera* selections grown at the CREC, Clanton, AL, 2014 and 2015.

Selection	Total yield (kg/vine)	Cluster number/vine (No.)	Cluster weight (g)	Soluble Solids (%)
		2014		
U0501-12	2.1 b[z]	41.5 a	49.9 c	21.9 a
U0502-01	3.4 a	42.4 a	80.9 b	18.9 b
U0502-10	3.4 a	25.8 b	132.1 a	19.5 b
		2015		
U0501-12	8.7 b	45.3 a	189.2 c	21.1 a
U0502-01	10.7 a	47.8 a	225.8 b	19.8 b
U0502-10	10.9 a	26.5 b	406.4 a	17.0 c

[z] Least squares means within column and year followed by common letters do not differ at the 5% level of significance by the Shaffer-simulated method.

in 2014 and 2015 respectively. In both years, 'U0501-12' grapes had the highest sugar content.

The successful growth and development of these three selections offered the unique opportunity to examine viticultural characteristics of *V. vinifera* in the high PD pressure environment of central Alabama. The promising preliminary results of our study indicate the potential opportunity for sustainable production of a new, high-value horticultural crop for the southeastern U.S. Increasing our understanding of crop load management, high fruit quality and balanced vine vigor will be of critical importance in future studies aiming to develop proper management techniques to maximize economic and environmental sustainability of *V. vinifera* production in the southeastern U.S.

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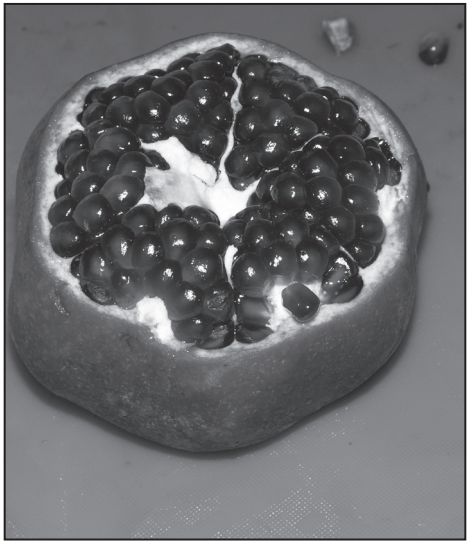
About The Cover:

'Parfianka' (Garnet Sash) has dark red, large to very large fruit and arils with soft seeds. The taste is sweet with balanced acidity offering interest to the flavor (Kennedy, 2010). It tested with 15.2 % SSC and a TA of 1.04 (Table 2), attesting for the sugar-acid balance. It was selected in Turkmenistan by O.F. Mizgiryova, N.I. Zaktrager, and A.D. Strebkova, VIR Kara-Kala Experiment Station, Turkmenistan. There, it is a traditional cultivar (Kennedy, 2010). It was received by the NCGR in 1995 as some of the original material from Turkmenistan. It is reported to be Dr. G.M. Levin's favorite from the Turkmenistan collection. Dr. Levin was a botanist who worked most of his career at the Kara-Kala Experiment Station where he managed the world's largest pomegranate collection. The fruit is easily harvested and ripen with or before Wonderful (Kennedy (2010). Hardwood cuttings root readily and it produces moderately dense trees. In informal taste tests at the NCGR fruit tastings, fresh 'Parfianka' consistently outscores 'Wonderful,' the primary cultivar grown in the USA.



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