JOURNAL OF THE AMERICAN POMOLOGICAL SOCIETY

April 2016 **Volume 70** **Number 2**

AMERICAN POMOLOGICAL SOCIETY

FOUNDED IN 1848

INCORPORATED IN 1887 IN MASSACHUSETTS

2015-2016

PRESIDENT
P. HIRST

FIRST VICE PRESIDENT
M. WARMUND

SECOND VICE PRESIDENT
M. PRITTS

SECRETARY
T. EINHORN

RESIDENT AGENT MASSACHUSETTS
W. R. AUTIO

BUSINESS MANAGER/TREASURER
P. HIRST

EDITOR
R. P. MARINI

EXECUTIVE BOARD

K. POMPER
Past President

P. HIRST
President

M. WARMUND
1st Vice President

M. PRITTS
2nd Vice President

T. EINHORN
Secretary

E. FALLAHI
('13 - '15)

M. OLMSTEAD
('14 - '16)

N. BASSIL
('15 - '17)

ADVISORY COMMITTEE

2014-2016

D. KARP C. KAISER G. PECK J. OLMSTEAD D. LAYNE

2015-2017

E. CONEVA M. EHLENFELDT S. MUSACCHI G. REIGHARD F. TAKEDA

2016-2018

P. CONNER E. HELLMAN R. HERMA D. CHAVEZ L. WASKO DEVETTER

CHAIRS OF STANDING COMMITTEES

Editorial
M. WARMUND

Shepard Award
F. TAKEDA

Membership
T. ROBINSON

Wilder Medal Awards
J. CLARK

Nominations
K. POMPER

U. P. Hedrick Award
E. FALLAHI

Registration of New Fruit and Nut Cultivars
K. GASIC & J. PREECE

Website
M. OLMSTEAD

JOURNAL OF THE AMERICAN POMOLOGICAL SOCIETY

A Publication of the American Pomological Society

July 2016 **Volume 70** **Number 3**

CONTENTS

Published by

THE AMERICAN POMOLOGICAL SOCIETY

Journal of the American Pomological Society (ISSN 1527-3741) is published by the American Pomological Society as an annual volume of 4 issues, in January, April, July and October. Membership in the Society includes a volume of the Journal. Most back issues are available at various rates. Paid renewals not received in the office of the Business Manager by January 1 will be temporarily suspended until payment is received. For current membership rates, please consult the Business Manager.

Editorial Office: Manuscripts and correspondence concerning editorial matters should be addressed to the Editor: Richard Marini, 203 Tyson Building, Department of Plant Science, University Park, PA 16802-4200 USA; Email: richmarini1@gmail.com. Manuscripts submitted for publication in *Journal of the American Pomological Society* are accepted after recommendation of at least two editorial reviewers.

Postmaster: Send accepted changes to the Business office.

Business Office: Correspondence regarding subscriptions, advertising, back issues, and Society membership should be addressed to the Business Office, ASHS, 1018 Duke St., Alexandria, VA 22314; Tel 703-836-4606; Email: ashs@ashs.org

Page Charges: A charge of $65.00 per page ($32.50 per half page) will be made to authors for those articles constituting publication of research.

Society Affairs: Matters relating to the general operation of the society, awards, committee activities, and meetings should be addressed to the Secretary, Dr. Todd Einhorn, Mid-Columbia Agricultural Research and Extension Center, 3005 Experiment Station Drive, Hood River, OR 97031 USA; Email: todd.einhorn@oregonstate.edu

Society Web Site: http://americanpomological.org

Journal of the American Pomological Society 70(3): 114-123- 2016

Cutting Type and Time-of-Year Affect Rooting Ability of Hardy Minnesota *Prunus* Species

Neil O. Anderson, Emily Hoover, Sarah A. Kostick, Emily Tepe, and John Tillman

Additional index words: *Prunus cerasus, Prunus armeniaca, Prunus ×cistena*
Chemical abbreviations: EtOH, ethanol; IBA, indole-3-butyric acid; K-IBA, indole-3-butyric acid potassium salt

Abstract

Many species within the genus *Prunus* are difficult to root, and most cultivated accessions are grafted for propagule production. The University of Minnesota *Prunus* germplasm and cultivar releases include a variety of ornamental and edible fruit types that have received little research focus. Many accessions have never been evaluated for the ability to root, even though at least one sour cherry, *P. cerasus* 'Northstar', is sold on its own root system. Two experiments were conducted to evaluate if cutting position, time of year, or auxin treatment are important for terminal and basal softwood-semi-hardwood rooting success of: I) *P. ×cistena* (control); *P. armeniaca* 'Westcot', and 'Hardygold'; *P. cerasus* 'Northstar', and 'Meteor'; *P. domestica* 'Superior', and *P. spp*. 'Alderman' treated with 0.0041 M (1000 ppm) or 0.017 M (4000 ppm) indole-3-butryric acid-potassium salt (K-IBA) and II) *P. ×cistena* (control) and *P. spp*. 'Alderman' treated with 0.00033 M (80 ppm) of K-IBA and 80 ppm Indole-3-caprioc acid (ICapA). Cuttings were taken biweekly (5 June-11 Sept.. 2012) and then monthly until 4 Dec. 2012. After six weeks in the mist, cuttings were scored for callus formation, root development, and bud break (leaves, flowering). The highest frequency of rooting occurred in June and again in Oct.-Dec. for *P. ×cistena* and July, Sept.-Oct. for *P. armeniaca* 'Westcot'. All other cultivars had very low or no rooting. On average, regardless of genotypic variability, all *Prunus* analyzed had ≤60% rooting, which is less than commercially acceptable. The highest mean percent rooting ranged from 1.1% (*P. spp*. 'Alderman') to 24.1% (*P. armeniaca* 'Westcot') and 40.2% (*P. ×cistena*), although many had 100% rooting in specific cutting harvest weeks. Unexpected leaf and/or flowering of cuttings occurred as early as early June for 'Westcot', 'Superior', 'Alderman', and *P. ×cistena* (Growing Degree Days (GDD) = 837) or late June for 'Hardygold', 'Meteor', and 'North Star' (GDD =1070) was unprecedented. The reasons for such a quick release from dormancy, often without the accumulation of chilling units, are unknown.

The large and economically important genus *Prunus* includes many species that serve a variety of commercial functions throughout the world including fruit crops (apricots, peaches, plums, and sweet and tart cherries, ornamental and landscape uses as well as medicinal plants (*P. africana* Kalkman*)* (Potter, 2012*)*. However, in USDA Zones 3 and 4, low temperatures, lack of winter hardiness, and spring frosts limit the *Prunus* species that can be successfully cultivated (Andersen and Weir, 1967). Even if winter hardy, the majority of cultivars in this genus were often reported to be short lived (20-25 years; Taylor, 1965).

Since many *Prunus* cuttings do not reliably root, the majority of genotypes used for fruit production are propagated via grafting onto a rootstock (Hartmann et al. 1997), but some may benefit from planting on their own root system (Tworkoski and Takeda, 2007). Propagation via rooted cuttings is inexpensive in comparison to other vegetative means, and many rootstocks and ornamental plants are propagated using this method (Hartmann et al. 1997; Tworkoski and Takeda, 2007). However, little is known about the capability of *Prunus* cultivars hardy to northern latitudes to form adventitious roots and produce rooted cuttings reliably (Reigard et al., 1990).

There are many factors that impact the rooting of cuttings including genotype, auxin

Department of Horticultural Science, University of Minnesota, St. Paul, MN 55108

type and concentration, juvenility, time of cutting collection, level of hardening of cutting, as well as physiological and environmental conditions (Hartmann et al., 1997; Pijut and Espinosa, 2004; Strauch et al., 1985; Tworkoski and Takeda, 2007). Previous studies have demonstrated that the time of year in the growing season impacts rooting success in some *Prunus* species. For example, *P. serotina* Ehrhart (black cherry) achieved the highest rooting success in March, prior to flowering (Dehgan and Sheehan, 1990). In contrast, when peach (*P. persica* Batsch.) cuttings were collected in both Aug. and Oct., only the Aug. cuttings rooted (Tworkoski and Takeda, 2007), indicating that different physiological stages can be important for rooting success of different species or genotypes.

For many species, softwood cuttings have greater rooting success than hardwood cuttings (Couvillon, 1988; Hartmann et al., 1997). Easy and fast rooting make softwood cuttings an attractive method of propagation in species that do not generally root easily, although hardwood cuttings do not desiccate as easily, are easier to transplant, and less expensive than softwood cuttings (Hartmann et al., 1997). The rooting potential of interspecific cherry hybrids was often determined with the use of softwood cuttings (Wagner et al., 1985; Strauch et al., 1985).

The addition of auxin often aids in increasing the percentage of rooting in species that are difficult to root (Hartmann et al., 1997). The effect of IBA concentration is species- and genotype-specific. Indole-3-butyric acid (IBA) has been shown to be effective in peach and sweat cherry cultivars (Coulvillon, 1985; Strauch et al., 1985). However, the concentration of auxin and type of cutting affects rooting success (Tworkoski and Takeda, 2007). Reighard et al. (1990) utilized 0.0099 M (2000 ppm) IBA when testing the rooting of 400 genotypes of different *Prunus* species, whereas de Oliveira et al. (2003) showed that for some peach cultivars 0.0062 M (1500 ppm) IBA was effective. Strauch et al. (1985) concluded 0.0041 M IBA was effective for the *Prunus* species and cultivars tested. Adventitious rooting with IBA application on cuttings placed in heated soil under intermittent misting has occurred in cultivars of sweet and sour cherry (Larsen, 1982; Schimmelpfeng, 1965; Schönberg, 1963) and ornamental cherry species (Lamb and Nutty, 1971; Monin and Trefois, 1967; Rowe-Dutton, 1959). Rooting of some rootstocks via softwood cuttings was productive for "vigorously growing species", e.g. *P. cerasifera* Ehrh. 'Myrobalan', a common plum rootstock (Jeremin et al., 2002). In other cases, hardwood cuttings rooted better for *Prunus* rootstocks 'Ishtara', 'St. Julien', and 'Mahaleb' (Christov and Koleva, 1995; Szecsko et al., 2002).

Although, 'North Star' and some of the ornamental cultivars are not grafted, very little is known about the rooting of cuttings from winter-hardy genotypes. The objectives of this study were to determine whether cutting (node) position, time of year during growing season (softwood vs. hardwood cuttings), and concentration or type of auxin alters rooting success of seven winter-hardy *Prunus* genotypes.

Materials and Methods

Experiment 1: Genotypes. Cuttings of seven *Prunus* cultivars were taken every two weeks (n=10 cuttings/genotype/week) beginning 5 June 2012 (week 23) at the University of Minnesota's research center in Excelsior, MN (44°52'06.5"N lat., 93°38'03.9"W long.; Table 1). The frequency was reduced to once per mo. beginning with the collection in week 37, with the final collection in week 49. Week number is defined as the number of weeks from 1 Jan. (week 1). All cuttings from a genotype were collected from one or two trees at the research center. Trees from which the cuttings were harvested were managed for fruit not cutting production.

Rooting Treatments. The cuttings were wrapped in damp towels, placed in plastic bags, and stored at 4°C for 2 to 4 h before processing. Approximately 0.5 cm of both

Table 1: Species, fruit type (apricot, tart cherry, plum, sand cherry), and genotypes of *Prunus* germplasm used in Experiments I and II. Collection location of trees used for cuttings in these experiments was the Horticultural Research Center, University of Minnesota, Excelsior, MN.

Species	Fruit type	Genotypes	Experiment
P. armeniaca	Apricot	'Hardygold'	I
		'Westcot'	I
P. cerasus	Tart Cherry	'Meteor'	I
		'Northstar'	I
Prunus spp.	Plum	'Alderman'	I & II
		'Superior'	I
P. ×cistena	Purpleleaf Sand Cherry	*P. ×cistena*	I & II

the distal and proximal ends of each cutting was removed. Each cutting was then cut in half (length-wise), yielding distal and proximal sections from which the leaves were removed from each proximal end (to 5-6 cm from the base of each cutting). This created n=10 distal and n=10 proximal cuttings per genotype per week. Each of these was then scored twice vertically with a blade for 2.5 cm on the proximal end of each cutting for increased surface area for auxin uptake. Two auxin concentrations of Indole-3-butyric acid-potassium salt (K-IBA) were used in this experiment: 0.0041 M and 0.017 M (1000 and 4000 ppm, respectively). The control treatment was 0.0041 M (1000 ppm) K-IBA instead of 0 M K-IBA, since previous studies showed rooting of all cuttings at 0.0041 M K-IBA to the same as 0.0 M K-IBA (Nečas and Krška, 2013). The scored ends were then submerged in 3 cm K-IBA solution for 10 s, yielding four treatment groups: basal and distal cuttings with 0.0041 M and 0.017 M K-IBA. Following treatment, the proximal end of each cutting was inserted to a depth of approximately 3 cm into washed, pasteurized river sand in 10 cm deep web flats (20 cm x 20 cm). The flats were then placed under an intermittent mist system in a greenhouse (Saint Paul, MN; 44°59'17.8" N lat., -93°10'51.6"W long.) for six weeks of rooting (21$\pm$0.8 / 21$\pm$0.7°C, day / night, 16 h, 0600–2200 HR) high intensity discharge lighting at a minimum set point of 150 µmol m^{-2} s^{-1}, a mist frequency of 10 minute intervals (mist nozzles, reverse osmosis water) during 0600-2200 HR with a 7 s duration. Cuttings were randomized, blocking by week number they were taken and auxin treatment (RCBD design). As a result, only cuttings treated with one of the auxin treatments were in a given flat. Rating of cuttings occurred immediately after the end of each 6-week rooting period.

Experiment 2: Genotypes. Cuttings of *P. ×cistena* and *P. spp*. 'Alderman' were taken every 2 weeks beginning in week 23 (2012; Table 1). Similar to Experiment I, the frequency was reduced to once per mo beginning in week 37 with the final collection occurring in week 49.

Rooting Treatments. Cuttings were physically prepared following the protocol outlined in Experiment I. The scored, proximal ends of the distal and proximal cuttings were then submerged in 3 cm of either 0.00033 M (80 ppm) K-IBA or 0.000236 M (80 ppm) Indole-3-caprioc acid (ICapA) solutions. Cuttings soaking in the auxin solution treatments were then covered with plastic bags and kept at 4° C for 22 h. The same greenhouse and mist system conditions were used as outlined in Experiment I for a 6-week duration, after which rating of cuttings occurred immediately.

Data Collection. Prior to the rooting experiments, the number of nodes and total

cutting lengths were recorded. Following the six-week rooting period, cuttings from Experiments I and II were evaluated for viability, callus formation, root development, and flower or leaf bud break. Root ratings were assessed on a six point scale (0-5): 0 = dead, no callus; 1 = alive, no callus; 2 = callus, no roots; 3 = root initials; 4 = roots; 5 = well-developed, branching root system (Fig. 1). Percent rooting within genotypes for each week was equal to ([the number of cuttings / week that ranked in the 3-5 point scale]/20 replications)*100. Overall cutting health was also noted for the condition of leaves (turgid, limp), leaf abscission, bud break (vegetative or flowering) and extension growth of any shoots into leaves and/or flowers. In the case of leaf and/or flower bud break, we calculated the growing degree day (GDD) accumulations for each rooting week with the Katz et al. (1982) formula using the North Carolina model and the *P. armeniaca* base temperature of 4.4°C (Valentini et al., 2004). Mar. 15 was the date of bud break in 2012 for GDD calculations to begin. For chilling units (CU) accumulation, the Richardson et al. (1974) Utah temperature model was used with the beginning CU start date of 15 Sept. 2012, the date of first frost (Valentini, et al., 2004). No CUs accumulated prior to the start date.

Statistical Analyses. Root rating data from each experiment (I, II) were analyzed with univariate, repeated measures general linear model Analysis of Variance (ANOVA) along with mean separations using Tukey's Honestly Significance Difference (HSD) tests at α=0.05 (Statistical Package for the Social Sciences, SPSS, version 22, University of Chicago, Chicago, IL). Repeated measures was required since root ratings occurred on the same clones and/or ramats repeatedly over time (weeks); the same shoots could not be assessed since none were long enough (i.e. lacking sufficient node numbers) to harvest for the entire duration of either experiment. Rooting data within genotypes were pooled to calculate % rooting but were not statistically analyzed due to the lack of reps.

Results

Experiment I. In the repeated measures ANOVA for root ratings, the main effects

Fig. 1: Cutting rating scale (1-5) for rooting of *Prunus cerasus* 'Meteor'. Numerical scores are 1, 2, 3, 4 or 5, from left to right, representing poor (1) no callus or roots), callus only (2), and callus with increasing quantity of roots (3-5).

genotype ($P \leq 0.001$) and treatment ($P=0.004$) were highly significant whereas cutting position ($P=0.266$) was not. All interactions that included position were not significant ($P>0.074$ Genotype x Position, Treatment x Position, Genotype x Treatment x Position.) The only significant interaction was Genotype x treatment ($P=0.003$). Since cutting position was not significant, the distal and proximal positions were pooled.

Average root ratings for the 0.0041 M K-IBA treatment (control) ranged from 0.0 to 3.6 with the majority of genotypes having ≤ 2.0 (no roots with or without callus) at any given point in the experiment (Table 2). *Prunus* ×*cistena* had significantly higher mean root ratings in weeks 25, 45 and 49 than all other tested genotypes (Table 2). While this still held true in weeks 23 and 41, *P.* ×*cistena* overlapped with 'Westcot' (week 41) or 'Hardygold', 'Meteor', and 'Northstar' in week 23 (Table 2). In weeks 27, 29, 35 and 37, *P.* ×*cistena* overlapped with the majority of tested genotypes. The most divergent root rating responses were found in weeks 31 and 33 where *P.* ×*cistena* did not have the highest rating (Table 2).

Percent rooting for the 0.0041 M K-IBA treatment ranged from 0% to 100% (Table 3). Overall, *P.* ×*cistena* had the highest % rooting in weeks 23, 25, 27, 35, 37, 41, 45, and 49 with average root ratings of 0.6 to 3.6 during these weeks (Tables 2 and 3). However, *P.* ×*cistena* did not root in weeks 31 or 33 when 'Meteor' and 'Alderman' did (week 31 [20%] and week 33 [10%], respectively; Tables 2 and 3). No genotype had any rooting in week 29 with the 0.0041 M K-IBA (Table 3). In any given week, only three (weeks 23, 25, 37, 41, and 49) or four (weeks 45-49) genotypes rooted (Tables 2 and 3).

For the 0.017 M K-IBA, average root ratings ranged from 0.0 to 3.5 (Table 2). *Prunus* ×*cistena* followed a similar response to the

Table 2: Average root ratings (0 to 5 scale) of cuttings from seven *Prunus* genotypes treated with 0.0041 M and 0.017 M K-IBA for each week of collection in Experiment I[z]. Mean separations within treatments and week number, based on Tukey's 5% HSD.

	Mean Root Rating by Week Number										
Genotype	23	25	27	29	31	33	35	37	41	45	49
					0.0041 M KIBA Treatment						
P. ×*cistena*	2.7 a	3.6 a	0.6 ab	1.6 ab	0.6 bc	0.0 c	2.2 a	2.8 a	3.1 a	3.5 a	3.5 a
'Alderman'	1.8 bc	1.9 b	1.0 ab	1.5 ab	1.0 b	1.4 ab	1.6 ab	2.0 ab	1.9 bc	1.0 b	1.0 c
'Superior'	0.7 d	0.4 c	1.0 ab	0.8 b	0.2 c	0.6 bc	0.7 b	1.7 b	2.2 abc	1.2 b	1.7 bc
'Westcot'	1.3 cd	2.0 b	0.0 b	1.3 ab	1.1 b	1.1 ab	1.6 ab	2.8 a	2.4 ab	2.1 b	1.6 bc
'Hardygold'	2.0 abc	1.8 b	1.4 a	1.2 ab	0.0 c	1.3 ab	1.5 ab	2.1 ab	1.5 bc	1.7 b	1.9 b
'Meteor'	2.0 abc	1.6 bc	0.6 ab	1.4 ab	2.2 a	1.7 a	2.0 a	2.0 ab	2.0 bc	2.1 b	2.0 b
'Northstar'	2.1 ab	2.0 b	0.8 ab	2.0 a	2.0 a	1.4 ab	1.2 ab	1.6 b	1.2 c	0.8 b	2.0 b
					0.017 M KIBA Treatment						
P. ×*cistena*	2.9 a	3.5 a	0.2 b	0.2 c	1.4 abc	0.8 abc	0.4 b	2.7 a	3.2 a	2.6 ab	2.9 a
'Alderman'	1.5 bc	1.4 bc	0.0 b	0.3 bc	1.0 abc	0.6 bc	1.5 a	1.8 bc	1.9 bc	1.6 bc	0.9 bc
'Superior'	0.6 c	1.2 bc	1.1 ab	0.9 abc	0.6 bc	0.1 c	0.4 b	1.2 c	1.6 cd	1.1 cd	0.7 c
'Westcot'	1.2 bc	2.6 ab	0.6 ab	2.0 a	0.3 c	1.8 a	2.0 a	2.4 ab	2.8 ab	3.0 a	1.4 bc
'Hardygold'	2.1 ab	1.1 bc	1.5 a	1.5 ab	1.6 abc	0.4 bc	1.6 a	1.9 abc	1.2 cd	1.7 bc	1.4 bc
'Meteor'	3.0 a	0.7 c	0.2 b	2.0 a	2.0 a	1.3 ab	2.0 a	1.9 abc	2.0 bc	2.0 abc	2.0 ab
'Northstar'	2.0 ab	2.0 abc	0.5 ab	1.8 a	1.8 ab	1.3 ab	1.5 a	1.4 c	0.6 d	0.3 d	1.9 abc

[z] Cuttings collected every two weeks beginning 5 June 2012 (week 23) and once a mo. beginning week 11 September 2012 (Week 37) through week 49.

Table 3: Percent rooting for seven *Prunus* genotypes, rooting week[z] and (A) 0.0041 M and (B) 0.017 M K-IBA concentrations in Experiment I.

	% Rooting of *Prunus* Genotypes													
	P. ×*cistena*		'Alderman'		'Superior'		'Westcot'		'Hardygold'		'Meteor'		'Northstar	
Week	A	B	A	B	A	B	A	B	A	B	A	B	A	B
23	80	80	20	10	0	0	0	0	0	10	0	70	10	0
25	80	70	0	0	0	0	40	40	0	0	20	0	0	20
27	10	0	0	0	0	10	0	20	0	0	0	0	0	0
29	0	0	0	0	0	0	0	40	0	10	0	0	0	0
31	0	30	0	0	0	0	0	0	0	0	20	20	0	0
33	0	0	10	0	0	0	0	30	0	0	0	0	0	0
35	0	40	0	0	0	0	10	30	0	0	0	0	0	10
37	70	50	0	0	0	0	50	40	10	10	0	0	0	0
41	90	80	0	0	10	0	30	70	0	0	0	0	0	0
45	90	60	0	0	0	0	40	70	10	0	10	0	0	0
49	100	50	0	0	10	0	10	10	0	0	0	0	0	0

[z] Cuttings collected every two weeks beginning 5 June 2012 (week 23) and once a mo. beginning week 11 September 2012 (Week 37) through week 49.

0.0041 M K-IBA treatment, having a significantly higher root rating than some of the other tested genotypes. However, in weeks 27 and 29, *P.* ×*cistena* had significantly lower root ratings than other genotypes, unlike that found with 0.0041 M K-IBA treatment where this occurred in weeks 31 and 33 (Table 2).

Percent rooting ranged from 0% to 80% for the 0.017 M K-IBA treatment (Table 3). *Prunus* ×*cistena* cuttings treated with 0.017 M K-IBA had a maximum of 80% rooting in weeks 23 and 41 (Table 3) with root ratings of 2.9 and 3.2, respectively (Table 2). In contrast, for the 0.0041 M K-IBA treatment, *P.* ×*cistena* had 80% rooting in weeks 23 and 25 with average root ratings of 2.7 and 3.6, followed by 90% rooting in weeks 41 and 45 with average root ratings of 3.1 and 3.5, and 100% rooting in week 49 with an average root rating of 3.5 (Tables 2 and 3). Even across all genotypes the range of rooting success for 0.017 M K-IBA treatment was smaller (0-80%) than for 0.0041 M K-IBA (0-100%; Table 3). This did not mean that all genotypes had lower percent rooting with 0.017 M K-IBA, such as *P.* ×*cistena* (Tables 3 and 4). For example, 'Westcot' had 0% rooting in weeks 23 and 31, 10% in week 49, and 40% in week 25 for both K-IBA treatments (Table 3). However, 'Westcot' had a significant increase in rooting with 0.017 M K-IBA treatment in weeks 27, 29, 33, 35, 41, and 45 with average root ratings of ≥0.6 (Tables 2 and 3).

Leaf and flower break. Leaf break occurred at the very beginning of Experiment I, as early as weeks 23 with GDD = 837 ('Alderman', *P.* ×*cistena,* 'Superior', and 'Westcot') or week 25 at GDD = 1070 ('Hardygold', 'Meteor', and 'Northstar'; Table 4). This continued sporadically throughout the duration of this experiment with most genotypes; the notable exceptions were 'Alderman', *P.* ×*cistena* and 'Westcot' with one or more cuttings with leaf break occurring continuously in week 33 (GDD = 2208) through 49 (GDD = 2990; Table 4). Flowering occurred as early as week 37, before any chilling units accumulated in *P.* ×*cistena* and 'Westcot', and continued weekly thereafter through the experiment. *Prunus* ×*cistena* and 'Westcot' were the two genotypes with the highest rooting. The other genotypes flowered later in the experiment. 'Alderman began flowering during week 41 at GDD=2944 and CU=69 (Table 4). During week 41, in which

Table 4. Number of *Prunus* genotype cuttings with leaf[z] or flower[z] bud break during or following rooting, based on rooting week[y] number, growing degree day and chilling unit accumulations[x].

	Week number																					
	23		25		27		29		31		33		35		37		41		45		49	
Genotype	L	F	L	F	L	F	L	F	L	F	L	F	L	F	L	F	L	F	L	F	L	F
'Alderman'	3	0	1	0	7	0	0	0	0	0	2	0	11	0	2	0	11	11	13	20	8	29
'Hardygold'	0	0	1	0	0	0	1	0	0	0	0	0	1	0	0	0	6	0	8	4	8	5
'Meteor'	0	0	1	0	0	0	0	0	0	0	2	0	4	0	5	1	0	0	19	0	12	4
'Northstar'	0	0	1	0	0	0	0	0	0	0	0	0	3	0	1	0	0	0	2	0	8	6
P. ×*cistena*	1	0	2	0	2	0	2	0	0	0	3	0	4	0	2	1	2	1	25	18	31	25
'Superior'	2	0	0	0	1	0	0	0	0	0	1	0	0	0	1	0	2	0	10	6	7	8
'Westcot'	1	0	8	0	3	0	4	0	0	0	9	0	7	0	7	8	6	1	7	7	10	4
Total	7	0	14	0	13	0	7	0	0	0	17	0	30	0	18	10	27	3	84	55	84	81
GDD	837		1070		1256		1669		1965		2208		2441		2695		2944		3028		2990	
CU	0		0		0		0		0		0		0		0		69		449		755	

[z] L refers to leaf (vegetative) bud break and F refers to flower bud break.

[y] Cuttings collected every two weeks beginning 5 June 2012 (week 23) and once a mo. beginning week 11 September 2012 (Week 37) through week 49.

[x] The start date for GDD and CU calculations were 15 March and 15 September, 2012.

GDD=3028 and CU=449 had accumulated, both 'Hardygold' and 'Superior' began flowering (Table 4). Week 49 and onwards, 'Meteor' and 'Northstar' flowered (GDD=2990 and CU=755; Table 4). Leaf break occurred during week 22-25 and required GDD from 837-1070 (Table 4). In contrast, flower break did not occur until week 37 or later. Clearly, GDD for leaf break was much lower and occurred early than flower break (Table 4).

Experiment II. No treatments, genotypes, and cutting position were significant ($P>0.05$) in this experiment. Except for week 41, where one cutting rooted, 'Alderman' had 0% rooting, regardless of cutting position or treatment (Fig. 2). The rooting rates for 'Alderman' were lower than in Experiment I where three cuttings rooted in week 23 (Table 3). In contrast, *P.* ×*cistena*, had ~35% rooting for both cutting positions. Similar to 'Alderman', *P.* ×*cistena* had differing percent rooting from Experiment I. For example, the highest rooting in Experiment II was 85% and occurred in both weeks 23 and 41, rather than just week 41 in Experiment I. In other cases, rooting in week 37 was 60% in Experiment I and 0% in Experiment II. For both genotypes, the majority of cuttings had a rating between 0 and 2 in Experiment II. The proportion of *P.* ×*cistena* cuttings that scored 3 to 5 decreased (Fig. 2).

Discussion

Our study confirmed that previously documented factors that impact rooting of *Prunus* cuttings, e.g. species, genotype, auxin type and concentration, juvenility, time of cutting collection, as well as physiological and environmental conditions (Hartmann et al., 1997; Pijut and Espinosa, 2004; Strauch et al., 1985; Tworkoski and Takeda, 2007) were also true for winter-hardy types. Future studies might find differences over years, but these seven *Prunus* genotypes most likely would remain difficult-to-root. Even though some *Prunus* have escaped cultivation and become invasive in the wild, the likelihood that any of our tested genotypes would regenerate in the wild with adventitious root formation is unlikely (Deckers et al., 2008; Reichard and White, 2001; Vanhellemont et al., 2010). Based on these results, any new *Prunus* cultivar should be tested for all of these identified factors to determine the best

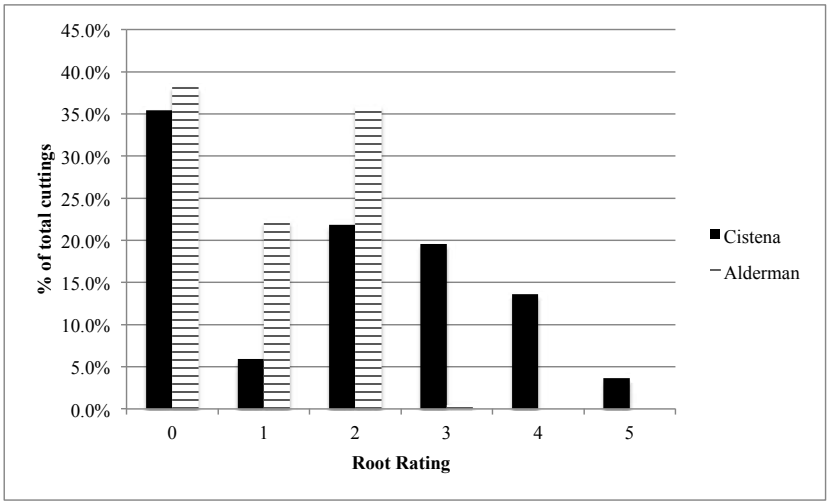

Fig. 2: Percent of cuttings of *Prunus spp.* 'Alderman' and *P.* ×*cistena* using 0.00033 M K-IBA or 0.00033 M ICapA (pooled by treatmeants and dates cuttings were taken in Experiment II); data are separated by root ratings and genotypes.

possible rooting method to mitigate the need for grafting.

Not all of the *Prunus* species or clonal cultivars tested formed adventitious roots when treated with K-IBA. *Prunus* ×*cistena* had the highest % rooting overall, but even this genotype did not root in weeks 29 or 33, and barely rooted at all during certain months of the year. Other types of sand cherries, *P. besseyi* (L.H. Bailey) Gleason and *P. pumila* L., had only an average of 31% rooting (Reighard et al., 1990). The commercially acceptable levels of asexual propagation of *Prunus* via cuttings is ≥60% rooting (Nečas and Krška, 2013). In addition to *P.* ×*cistena*, the only other genotypes with ≥60% rooting were 'Meteor' sour cherry during week 23 and 'Westcot' apricot in week 41 and 45. All other genotypes were consistently below commercially acceptable rooting levels of 60%. This was not unusual for *Prunus* genotypes where scions or rootstocks were difficult to root. Howard (1973) reported 0% rooting for *P. cerasifera* 'Myrobalan B' rootstock with 0 ppm IBA and only 35% 0.024 M (5000 ppm) IBA treatment. Reighard et al. (1990) found that European and Japanese plums (*P. cerasifera, P. domestica, P. institutoa, P. salicina,* and *P. munsoniana*) averaged only 42.5% rooting, whereas American plums (*P. americana, P. angustifolia* Marsh., and *P. hortulana*) were as low as 8.5%. Quantitative and qualitative differences in percent rooting could be the result of a variety of factors including genotypic differences, seasonality, degree of dormancy and maturity through the seasons.

In similar reports (Nečas and Krška, 2013), genotypes had a significant effect on root ratings in all possible respects, such that cultivars within and among species rooted significantly different. In the current study, genotypic effects within species were apparent with *P. armeniaca* 'Hardygold', where rooting was significantly lower than *P. armeniaca* 'Westcot'. Both apricot genotypes had higher percent rooting at 0.017 M K-IBA. Lower % rooting has been reported for *P. armeniaca* rootstocks (Reighard et al., 1990). Staniča et al. (2010) reported significant differences in rooting of *P. armeniaca* rootstocks using 0.0041 M K-IBA. In contrast to *P. armeniaca, P. cerasus* 'Meteor' and 'Northstar' had higher rooting with 0.0041

M K-IBA. The low levels of rooting for sour cherries in our study contrasts with Strauch et al. (1985) who reported that they rooted easily. Both plums (*P. spp.* 'Superior' and 'Alderman') followed the same trends as *P. cerasus*. Similar findings of genotypic differences both within and among species have been reported across the *Prunus* genus, although our findings for some species differ from that of Strauch et al. (1985).

The times of year cuttings were taken affected rooting. However, across the year (weeks), the concentration of K-IBA did not impact root ratings. Seasonality, reflective of the level of maturity (softwood vs. semi hardwood to hardwood cuttings) and degree of growth vs. dormancy has also been significant factors in past research. Strauch et al. (1985) found that cuttings with vigorously growing shoots rooted the worst, while the best were semi-hardwood cuttings. Nečas and Krška (2013) found that the critical factor for rooting of *Prunus* rootstocks was the date of harvest and growth status of cutting material. Reighard et al. (1990) also reported that percent rooting and cutting survival was highly influenced by time of year.

The structural difference between K-IBA and ICpA compounds is the number of carbon side chains; the former has four while the latter has six (Fawcett et al., 1960; Martinez, 2010). While this is the first report of using ICapA to root *Prunus*, it did not significantly affect rooting in either genotype. This was similar to other findings where the type of rooting compound tested had no significant effect (Nečas and Krška, 2013).

Some buds grew between week 23 and 25. No previous reports involving rooting of *Prunus* cuttings harvested during week 23-49 have ever reported the occurrence of such a quick release from dormancy. That this unexpected phenomenon continued sporadically in all genotypes with the exception of 'Westcot', 'Alderman', and *P. ×cistena* indicates this may be uniquely connected with these winter-hardy *Prunus.* Likewise, before the accumulation of any chilling units at week 41, flowering occurred as early as week 37. While GDD for leaf break was much lower and occurred earlier (GDD=837-1070, week 23-25) than for flower break (week 37 onwards; Table 4), neither were expected to occur in this experiment. Since an accumulation of CUs is required for deciduous *Prunus* to overcome endodormancy or rest period (Weinberger, 1967) the occurrence of leaf and flower bud break without any accumulated CUs was novel. For instance, *P. cerasus* 'Montmorency' grown in Michigan (USDA Zone 5-6) requires 954 chilling h to overcome endodormancy and begin growing (bud break) (Anderson et al., 1986; Richardson et al., 1974; Zavalloni et al., 2006). It would be reasonable to expect that winter-hardy *Prunus*, including *P. cerasus* tested herein, would require >954 chilling h since they are USDA Zones 3-4 winter hardy. This was clearly not the case with the seven genotypes tested. Further analyses are warranted to determine GDD and CUs for all *Prunus* hardy in Minnesota and their impact on early bud break in spring and risk of injury by spring frosts.

Acknowledgements

Funding in support of this publication was from the Minnesota Landscape Arboretum Land Grant Chair and the Minnesota Agricultural Experiment Station. We thank Dr. Jerry Cohen, University of Minnesota, for the generous donation of ICapA.

Literature Cited

Andersen, E.T., and T.S. Weir. 1967. *Prunus* hybrids, selections and cultivars at the University of Minnesota fruit breeding farm. Tech. Bull., Minn. Agric. Expt. Sta.

Anderson, J.L., E.A. Richardson, and C.D. Kesner. 1986. Validation of chill unit and flower bud phenology models for 'Montmorency' sour cherry. Acta Hort. 185:71-78.

Cristov, C. and A. Koleva. 1995. Stimulation of root initiation in hardwood sweet and sour cherry rootstocks (*Prunus mahaleb* L.). Bulgarian J. Plant Physiol. 21:68-72.

Couvillon, G.A. 1984. Propagation and performance of inexpensive peach trees from cuttings for high

density peach plantings. 1956. Propagating fruit trees. Int. Conf. Peach Growing 173:271-282.

Deckers, B., K. Verheyen, M. Vanhellemont, E. Maddens, B. Muys, and M. Hermy. 2008. Impact of avian frugiors on dispersal and recruitment of invasive *Prunus serotina* in an agricultural landscape. Biol. Inv. 10:717-727.

Dehgan, B., T.J. Sheehan, M.E. Kane, and F.C. Almira. 1990. Vegetative propagation of Florida native plants: V. *Prunus* spp. Proc. Florida State Hort. Soc. 103: 172-174.

Fawcett, C.H., R.L. Wain and F. Wightman. 1960. The metabolism of 3-indolylalkanecarboxylic acids and their amides, nitrites and methyl esters in plant tissues. Proc. Royal Soc. Lond. B Biol. Sci. 152:231-254.

Hartmann, H.T., D.E. Kester, F.T. Davies, and R.L. Geneve.1997. Plant Propagation: Principles and Practices. Sixth ed. Prentice Hall, New Jersey.

Howard, B.H. 1973. Factors affecting the rooting response of plants to growth regulator application. Acta Hort. 34:93-106.

Jeremin, G.V. 2002. Kostočkovyje kultury. Rostov na Donu. Feniks, p. 256.

Katz, R.W., A.H. Murphy, and R.L. Winkler. 1982. Assessing the value of frost forecasting to orchardists: a dynamic decision-making approach. J. Appl. Meteorology 21:518-531.

Lamb, J.G.D. and F. Nutty. 1971. Flowering cherries from cuttings. Garden Chronicle 169: 44-45.

Larsen, O.N. 1982. Propagation in Denmark of sour cherries by cuttings. Int. Plant Prop. Soc. Proc. 32:182-186.

Martinez, N. 2010. Insights into peroxisome matrix protein import and IBA metabolism through analysis of IBA-response mutations. Ph.D. Dissertation, Rice University, Houston, TX.

Nečas, T., I. Ondrasek, B. Krska, and T. Meisl. 2006. Simultaneously grafted softwood cuttings of rootstocks for apricots. Acta Hort. 985: 127-137

de Oliveira, A.P., A.A. Nienow, and E.O. de Calvete. 2003. Rooting potential capacity of peach tree cultivars of semi-hardwood and hardwood cuttings treated with IBA. Revista Brasileira de Fruticultura. 25:282-285.

Pijut, P.M. and C. Espinosa. 2004. Development of a rooted cutting propagation method for *Prunus serotina*. Combined Proc., Int. Plant Prop. Soc. 54: 513-515.

Potter, D. 2012. Basic information on the stone fruit crops, p. 1-21. In: Kole, C., and A. G. Abbot (eds.). Genetics, genomics, and breeding of stone fruits. CRC Press, New York.

Reichard, S.H. and P. White. 2001. Horticulture as a pathway of invasive plant introductions in the United States. BioSci. 51:103-113.

Reighard, G.L., D.W., Cain, and W.C Jr. Newall.1990. Rooting and survival potential of hardwood cuttings of 406 species, cultivars and hybrids of *Prunus*. HortScience 25:517-518.

Richardson, E.A., S.D. Seeley, and D.R. Walker. 1974. A model for estimating the completion of rest for 'Redhaven' and 'Elberta' peach trees. HortScience 9:331-332.

Rowe-Dutton, P. 1959. Mist propagation of cuttings. Digest No. 2, Commonwealth Bur. Hort. Plant Crops. East Malling, Maidstone, Kent.

Schimmelpfeng, H. 1965. Vermehrung von Sauerkirschen durch Grünstecklinge. Erwerbsobstbau 7:48-50.

Schönberg, G. 1963. The production of vegetatively-propagated rootstocks of *Prunus mahaleb* L. The rooting of cuttings in mist. Obstbau 3:90-92.

Stanică, F., M., Drumitascu, S.I.. Ancu, and I.. Dutu. 2010. Propagation by green cuttings of some new promising Romanian *Prunus* rootstocks. Acta Hort. 862:413-418.

Strauch, H., M. Roth, and W. Gruppe. 1985. Rooting softwood cuttings of interspecific cherry hybrids and *Prunus* species by mist propagation. Int. Workshop on Improvement of Sweet and Sour Cherry Varieties and Rootstocks. Acta Hort. 169:371-379.

Szecsko, V., A. Csikos, and K. Hrotko. 2002. Timing of hardwood cuttings in the propagation of plum rootstocks. Acta Hort. 577:115-119.

Taylor, N. 1965. The guide to garden shrubs and trees, including woody vines; their identity and culture. p. 202-208. Houghton Mifflin Company, Boston.

Tworkoski, T. and F. Takeda. 2007. Rooting response of shoot cuttings from three peach growth habits. Sci. Hort. 115.1:98-100.

Valentini N., G. Me, F. Spanna, and M. Lovisetto. 2004. Chilling and heat requirement of apricot and peach varieties. Acta Hort. 663:199-203.

Vanhellemont, M., K. Verheyen, J. Staelens, and M. Hermy. 2010. Factors affecting radial growth of the invasive *Prunus serotina* in pine plantations in Flanders. Eur. J. For. Res. 129:367-375.

Wagner, S.T., V. Oprita, and T. Panea. 1985. Sweet cherry interspecific hybrids propagation by soft cuttings using rooting stimulators. Acta Hort. 169:363-370.

Weinberger, J.H. 1967. Some temperature relations in the natural breaking of the rest of peach flower buds in the San Joaquin Valley, California. Proc. Amer. Soc. Hort. Sci. 91: 84-89.

Zavalloni, C., J.A. Andersen, and J.A. Flore. 2006. Phenological models for flower bud stages and fruit growth of 'Montmorency' sour cherry based on growing degree-day accumulation. J. Amer. Soc. Hort. Sci. 131: 601-607.

Journal of the American Pomological Society 70(3): 124-137 2016

Fruit Set Improvement of Highbush Blueberry *(Vaccinium corymbosum)* West of the Cascade Range in the Pacific Northwest: A Review

MATTHEW ARRINGTON[1] AND WASKO DEVETTER[2]

Additional index words: Flower morphology, pollination, calcium, boron, nutrient supplementation

Abstract

Highbush blueberry is an economically important crop across North America and has become important worldwide. Under certain climatic conditions experienced in various geographical regions, fruit set in blueberry remains relatively poor and contributes to reduced yields. The mechanisms contributing to fruit set are complex. Poor fruit set may be associated with improper or incomplete pollination, inadequate nutrient availability, disease pressure, or poor climatic conditions experienced during critical stages of flowering and fruit set. Improving fruit set in blueberry necessitates the evaluation of floral morphology and the impact of morphology on pollinator efficiency. Pollination requirements as they relate to floral morphology among specific cultivars of blueberry and pollen saturation, as well as the assessment of nutrient applications and their impact on reproductive processes pivotal for fruit set and development, are also in need of further investigation. The objective of this report is to provide a review of the literature addressing pollination and fruit set of highbush blueberry. The development of reproductive buds and flowers are impacted by nutrient availability and associated metabolic compounds and processes. Calcium and boron may be of specific importance for reproductive developmental processes and the requirements, roles, and potential application of these nutrients will be discussed. Variation exists in floral morphology across blueberry cultivars. Additional consideration is given to this variation and how it may impact the efficiency of pollination by primary commercial pollinators. Additionally, the efficacy of pollination may be influenced by pollinator species, the interaction between species of pollinator and cultivar of blueberry, and the environmental conditions during bloom time. These issues are important to understand, especially when considering the development of solutions for the improvement of fruit set for this economically important crop.

The production of highbush blueberries (*Vaccinium corymbosum*) has increased over the past ten years. Approximately 680 thousand metric tons (MT) of blueberries were produced worldwide in 2014 from 100,303 hectares of land (Brazelton, 2015). Cultivated production areas in the United States nearly doubled between 2004 (17,980 hectares) and 2014 (34,054 hectares) [United States Department of Agriculture (USDA), 2005 and 2015]. As a high-value and economically important crop, production efficiency is paramount. Regional climatic conditions impact the management of highbush blueberry and present a number of unique production issues. Pollination and subsequent fruit set remain among the most important challenges for ensuring sustained yields in various regions, including the Pacific Northwest (PNW), a major production region. Pollination of highbush blueberry is complicated by several factors, including: 1) a relatively short duration of pollen viability (5-12 days); 2) the limited effectiveness of traditional commercial pollinators; and 3) environmental conditions at bloom that are not conducive to honey bee (*Apis mellifera*) foraging (Kevan, 1990; Torchio, 1990b). Here, the contributions of flower development, pollination, and fruit development towards highbush blueberry productivity will be reviewed and suggestions will be made regarding future re-

[1] Graduate Research Assistant, E-mail: matthew.arrington@wsu.edu
[2] Assistant Professor and corresponding author, E-mail: lisa.devetter@wsu.edu
Department of Horticulture, Washington State University Northwestern Research and Extension Center, Mount Vernon, WA 98273

search directions to improve highbush blueberry yields and cropping efficiency.

Bud and flower development. Floral buds of highbush blueberry contain 5 to 12 flowers, with a wide variation in number and morphology between cultivars and little within (Pritts et al., 1992). Blueberries have both reproductive (flower) and vegetative (leaf) buds, which develop on one-year-old or older wood. Vegetative buds, which typically break from January to April, depending on accumulated growing degree days, region, and germplasm (e.g., northern versus southern highbush), only result in shoot and leaf growth. Flower buds, which are larger and rounder than vegetative buds, begin their formation in late-summer to early fall, and continue to develop until temperatures are excessively cold (approximately 7°C) (Kovaleski et al., 2015; Strik, 2015). Floral bud counts have been used to estimate crop yields (Salvo et al., 2012). Within flower buds are flower primordia, which can result in flower clusters that have the potential to become fruit. The development and occurrence of floral and vegetative buds is controlled in part by auxin and plant carbohydrate status (Darnell, 1991; Maust et al., 2000). Soluble carbohydrates regulate auxin synthesis; therefore, insufficient carbohydrate levels in some cultivars of blueberry may lead to decreased reproductive development, including floral bud development (Maust et al., 2000; Sairanen et al., 2012).

Additionally, photoperiod (day length) is an integral part of bud break. Plants exposed to chilling treatments without also experiencing short days were shown to not initiate bud break following chilling (Bañados and Strik, 2006).Prior to bud break, dormant buds are tightly closed and protected by bud scales. Typically a range from 200 to 300 chilling hours for southern highbush cultivars and over 800 chilling hours for northern highbush cultivars are needed to break endodormancy and approximately 4-8 weeks of short days are required to satisfy the photoperiod requirements (Bañados and Strik, 2006; Strik et al., 2014). Once requirements for flowering have been met, buds swell and break. These phenological stages are referred to as "bud swell" and "bud break", respectively. Bud swell can be discerned when the outer bud scales begin to separate as the flower buds enlarge and swell. Early green tip (referencing leaf buds) comes next and tends to develop in late winter or early spring. This stage is characterized by emerging vegetative tissues consisting of tightly rolled leaves. Bud break (referencing floral buds) often coincides with green tip and can be described as the opening of flower buds and increased visibility of individual flowers. Following bud break, plants progress into the late green tip and tight cluster stages, wherein leaves begin to unfold, but flowers remain closed and tightly packed together, respectively. Next, blueberry plants enter into a phase of shoot expansion in which multiple leaves emerge and begin to enlarge. The early and late pink bud stages represent a period of flower expansion and separation within the inflorescence, as well as the continued development of the corolla tubes. The corollas also transition in coloration from pink to white during this stage. The early bloom stage occurs when some corolla tubes are expanded and fully opened, but the majority of flowers remain closed. Early bloom eventually gives way to full bloom. During full bloom, a majority of the flowers are fully opened. This stage can last for 5-12 days depending on cultivar and climatic conditions (Pritts et al., 1992).

Flower morphology. Flowers in blueberry are characterized by their bell-shaped corollas, with stamens and anthers inside and a stigma sometimes protruding past the flower aperture (Eck and Mainland, 1971) (Figure 1). Blueberry flowers have elongated corolla tubes and poricidal anthers, which tends to make pollination by honey bees difficult (Ritzinger and Lyrene, 1999). Variation in flower size and morphology between cultivars has been shown to impact insect foraging activity and, as a result, fruit set (Courcelles et al., 2013). Corolla length and aperture diameter appear to be some of the most

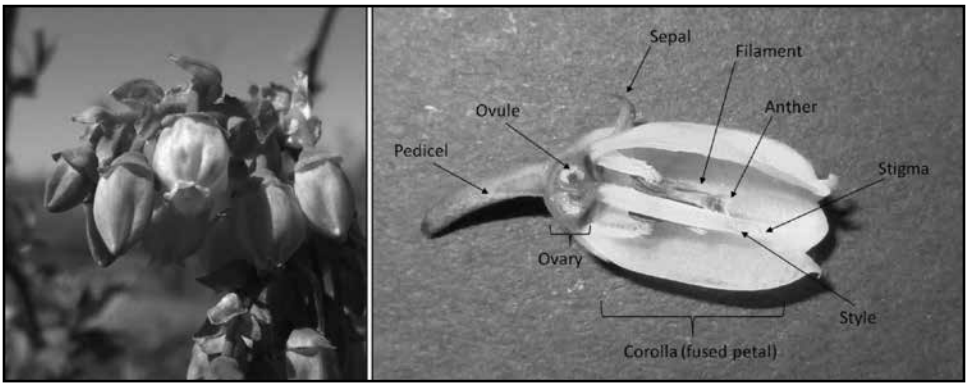

Fig. 1: (a) Flower of *Vaccinium corymbosum* 'Draper' (b) *V. corymbosum* 'Bluecrop' longitudinal cross-section with morphological features labeled.

important floral traits for pollen and nectar accessibility to honey bees, which impacts pollination and fertilization. As shown by Courcelles et al. (2013), there is a clear relationship between floral morphology and honey bee visitation rates in 'Duke', 'Bluecrop', 'Liberty', and 'Draper' blueberry. Both "legitimate visits" (through the flower entrance and thus, the pollen-bearing anthers) and "nectar robbing" (bypassing the entrance of the flower to harvest nectar at the base of the corolla) by honey bees visiting 'Bluecrop', 'Liberty', and to some extent, 'Draper' flowers, are common. 'Duke' flowers have a large aperture and flexible corolla, which allows honey bees to force their heads into flowers and access pollen. Flowers from 'Duke' are visited more often by honey bees relative to 'Draper' and 'Bluecrop'. Relative to 'Duke', shorter corolla lengths with smaller throat diameter does not facilitate efficient penetration of the flower by the head of honey bees to reach nectar and pollen rewards.

Outcrossing. The introduction of unrelated genetic material to the breeding cycle of a species or cultivar is known as outcrossing. Self-incompatible plants ensure genetic diversity by rejecting their own pollen and accepting unrelated pollen within the same species. In blueberry, variability exists between the effectiveness of fertilization with self- and outcrossed- pollen between cultivars. Cultivars of cultivated highbush blueberry are to a large extent self-fertile. However, it is well established that fertilization and yields are significantly increased when flowers are cross pollinated (Ehlenfeldt, 2001; Krebs and Hancock, 1990; Vander Kloet, 1984; Vander Kloet and Lyrene, 1987; Wood, 1968). Yields are also generally increased through cross pollination with the aid of insect pollinators (Ehlenfeldt, 2001; Krebs and Hancock, 1990). Generally, fewer tetrads of outcrossed pollen are required to reach pollen-load saturation than with self-pollination (Parrie and Lang, 1992). However, pollen tubes from self-pollinated plants grow at the same rate as outcrossed pollen once germination occurs (Krebs and Hancock, 1988; Vander Kloet and Lyrene, 1987). Only a fraction of ovules develop into seeds, with over 100 ovules being common to highbush blueberry fruit and fewer than 50 seeds forming (Pritts et al., 1992). Seed number is positively correlated to fruit size (until saturation occurs) and seed abortion is dependent on the level of self-fertility (Pritts et al., 1992; Vestrheim et al., 1996).

Pollination in highbush blueberry. In blueberry production, commercially managed honey bees, specifically the Italian honey bee (*Apis mellifera* 'ligustica'; commonly referred to as "honey bee") are primarily responsible for pollination. Wet and cool

spring weather limits honey bee activity and subsequently can negatively affect pollination and fruit set. The Northeastern region of the United States, including Michigan, is climatically similar to the PNW in average spring low and high temperatures [Baker et al., 1994; National Oceanic and Atmospheric Administration (NOAA), 2015]. However, highbush blueberry is native to the Northeastern United States and a variety of alternative insect pollinators have strong populations in the region (Garibaldi et al., 2013; Winfree et al., 2008). The co-evolution of some bee species with *Vaccinium* spp. may make them ideal blueberry pollinators relative to commercial honey bees (Tuell et al., 2009). The primary pollinators of wild highbush blueberry are bumblebees (*Bombus* spp.), mining bees (*Andrena* spp.), sweat bees (*Halictus* spp.), orchard/mason bees (*Osmia* spp.), leafcutter bees (*Megachile* spp.), and European sweat bees (*Lasioglossum* spp.), many of which exhibit sonication (Buchmann, 1985; Torchio, 1990b).

Honey bees are indigenous to Europe, with a number of subspecies inhabiting all parts of Europe and Africa (Jensen et al., 2005; Whitfield et al., 2006). Honey bees were introduced to North America from Europe in the early 1600's and have become vital to crop production. Pollination in many crops, like blueberry, is achieved through obtaining saturating populations of honey bees through the provision of commercially rented hives (Paini, 2004). In contrast to honey bees, many of the above species (including bumblebees) remain active and forage at lower temperatures (below 13°C), which can be advantageous in areas that are climacterically characterized as having cool and/or wet springs where honey bees are less active (Delaplane et al., 2000).

It is common for commercial plantings of blueberry to be stocked with 2 to 6 honey bee hives per hectare in the PNW. Honey bee stocking rates are influenced by the strength of the colony at bloom and the level of attractiveness of a specific cultivar's flowers to honey bees (Marucci and Moulter, 1976; Sagili and Burgett, 2011; Wood, 1961). Honey bees introduced to plantings during early and late pink phenological stages commonly forage elsewhere and are slow to return to blueberries (Jay, 1986; Kevan et al., 1990). Consequently it is recommended to place honey bee hives between 5% to 25% bloom in blueberry and cranberry (*Vaccinium macrocarpon*), another related species (Delaplane et al., 2000; Moeller, 1973). Pollination by honey bees is carried out by nectar and pollen foragers. Foraging honey bees do not sonicate (otherwise known as buzz pollinate, which is the vibration of flight muscles causing dehiscence of poricidal anthers) and must consequently rub against anthers to obtain pollen. Several native and alternative pollinating species, including bumble bees, are able to sonicate and consequently can collect more pollen per flower visit than honey bees (Garibaldi et al., 2013). Honey bees also have an average tongue length of approximately 6.5 mm. This is short compared to some non-*Apis* bee species, such as bumble bees, that have tongues that are approximately 8.5 mm long (Balfour et al., 2013). The short tongue of honey bees can make it difficult to reach nectar sources in flowers with long corolla tubes that are present in some blueberry cultivars. Bumblebees enter through the opening of the flower and brush against stigmas and anthers, occasionally robbing nectar, but with less frequency than honey bees (Courcelles et al., 2013; Stubbs and Drummond, 1996). Significantly higher crop yields have been realized in plots where honey bees and bumblebees have been used together for pollination, making bumblebees an important resource for pollination and fruit set enhancement (Garibaldi et al., 2013).

Apiculture is the practice of maintaining honey bee colonies for their products and encompasses hive maintenance and promoting bee health. Nutrition can be promoted by increasing the diversity and availability of surrounding forage material (Toth and Robinson, 2005). Often hives are under significant

stress during pollination, having been stored and moved by semi-trucks across hundreds of kilometers. In transit, honey bees must rely on feeding trays with solutions of sucrose and nutrients for nourishment, which may be nutritionally lacking (Crailsheim, 1990; Standifer, 2003). Additionally, it has been reported that as high as 50% of queen honey bees are replaced within 6 months, with temperature fluctuations in shipment being linked to low sperm viability in queens and, as a result, the decreased ability of queens to lay fertilized eggs and maintain colony strength, leading to colony decline (Pettis et al., 2016). In evaluating hive health, the percent of filled or capped brood cells per frame (or uniform capping pattern) is indicative of overall hive health, and honey bees generally forage more with large brood populations to support (Eckert et al., 1994; Fewell and Winston, 1992; Free, 1967).

Foraging activity of honey bees can be increased when environmental conditions are ideal and by using brood pheromones to influence the hive activity (Pankiw et al., 1998). Brood pheromones are naturally produced by uncapped brood and increases honey bee foraging. There are two main types of brood pheromones: brood ester pheromone (BEP) and E-β-ocimene. Brood ester pheromones primarily regulate which bees can lay eggs through suppression of worker bee ovary development and egg laying, while E-β -ocimene influences the nurse-forage ratio, as well as the type and number of foragers. Another bee pheromone, queen mandibular pheromone (QMP), is produced by the queen and fed to attendants. Queen mandibular pheromone can be used to slow hive growth and deter reproductive swarming in order to increase hive strength and foraging activity (Pankiw et al., 1994; Winston et al., 1991). Both brood and QM pheromones are periodically used in commercial production of horticultural crops in order to increase honey bee visitations and resultant crop yields, but research on their potential benefits is lacking in blueberry.

While *Apis mellifera* 'ligustica' may be the most common commercial honey bee subspecies, others are also used for pollination in specific regions (Bailey and Ball, 2013). The Carniolan honey bee (*A. mellifera* 'carnica') is a subspecies of *A. mellifera* and maintains a higher level of activity early and late in the day (before 8am and after 4pm) and in cool and wet conditions as compared to 'ligustica' (Biasiolo and Comparini, 1990; Moritz and Hillesheim, 1985). Carniolan honey bees are native to Austria and Croatia, and are presently not widely utilized in North America. Carniolan bees have more foragers entering and leaving the hive in conditions of precipitation and cool temperatures than 'ligustica' [>18°C] (Alqarni, 1995). High levels of foragers entering and leaving hives is indicative of increased activity. The European black bee (*A. mellifera* 'mellifera') is also less sensitive to cool, wet conditions and has been known to overwinter well in small populations in northern United Kingdom (Corbet et al., 1993). The European black bee has a larger body than either the Italian or Carniolan honey bees, which may present problems with small, tubular blueberry flowers (Daly et al., 1995). European black bees may also be more susceptible to tracheal mites, which nearly destroyed 'mellifera' populations in the early 1900's (Baily and Ball, 2013). Regardless of general pollination efficiency, poor weather including cold temperatures, high winds, and steady rain can deter even the most effective pollinator species. The short bloom window of blueberry imposes an additional challenge, regardless of the pollinator chosen. Under these circumstances and to maximize pollination, there is an industry-wide preference for honey bee availability and field placement of hives in the early bloom stage (5% to 25% bloom), when only some flowers are fully open (Delaplane et al., 2000).

Adverse weather not only limits honey bee activity, but can also limit the rate of plant reproductive processes, ultimately reducing fertilization and subsequent yields (Hepler

and Yarborough, 1991; MacKenzie, 1997; Seely, 1994). Alternative pollinator species better adapted to foraging during the environmental conditions encountered during blueberry bloom time, as well as the morphology of blueberry flowers, may improve pollination in highbush blueberry (Bosch and Kemp, 2003; Courcelles et al., 2013; Heinrich, 1975; Seeley, 1994). Understanding the contributions of honey bees in blueberry, irrespective of the potential limitations, would benefit blueberry growers experiencing chronic pollination deficits and subsequent reduced yields. Mason and bumblebee species may be especially adapted to blueberry pollination, with higher levels of activity in cool and wet conditions and specialized physical traits (i.e. longer tongue, smaller body size, sonicating flight muscles) for pollen collection and transfer among the small, tubular flowers of blueberry (Balfour et al., 2013; Buchman, 1985; Desjardins and Oliveira, 2006; Sampson and Cane, 2000; Stubbs et al., 1994; Torchio, 1990a; Whidden, 1996).

Although native species of insect pollinators may be especially adapted for blueberry pollination, population dynamics and species habitat are also important considerations from a management perspective. Monoculture field situations that are intensively managed with frequent field maintenance can deter populations of native pollinators (MacKenzie and Winston, 1984). However, planting strips of wildflowers or greenbelts between fields can increase the attractiveness of field sites to pollinator habitation, as well as increase the diversity and abundance of pollinators (Blaauw and Isaacs, 2014; Kremen et al., 2002). Establishment of nesting boxes or hive systems for attracting and maintaining mason and bumblebee species has increased pollination and, as a result, fruit set in orchard crops (Bosch et al., 2000; Knight et al., 2005). In addition to habitat availability, the frequency of field cultivation practices decreases the permanent population of native bees in the area (Carré et al., 2009; Carvell et al., 2011). While investigations of native pollinator population decline are ongoing, causal factors continue to be confounding. Widespread use of broad spectrum insecticides is a danger to bees and other beneficial insects; however, the resilience of some species suggests we need continued research of additional factors beyond pesticides for causes of population decline (Gels et al., 2002; Goulson et al., 2008). Findings to date suggest that the successful utilization of native species as pollinators in blueberry production will likely have to be combined with habitat management practices that promote abundance and activity, such as reduced pesticide applications during critical periods of pollinator activity and reduced tillage. These management practices are complicated by the threat of spotted wing drosophila (SWD; *Drosophila suzukii*). In an effort to minimize the habitat for this insect pest, wooded areas are frequently reduced and pesticide applications utilized (Lee et al., 2011).

Fertilization and berry development. From early bloom through full bloom, pollination and fertilization in highbush blueberry can occur in as few as 5 to12 days (Dogterom et al., 2000). Fertilization of ovules and fruit development depends on successful pollination and fertilization. Reproductive tissues (i.e., pollen, ovules, and developing embryos) are sensitive to nutrient levels in the flower and stem tissue (May and Killingbeck, 1992). Pollen and ovary tissues are also sensitive to temperature and humidity. Warm and dry conditions can increase the speed of metabolic systems in plants (~20°C), thereby shortening the effective pollination period (De Vries and Dubois, 1987; Hedhly et al., 2004). In contrast, cool and wet conditions speed the decay of flower tissues and increase the potential for fungal and bacterial infections (Daykin and Milholland, 1990; Ngugi et al., 2002; Ngugi and Scherm, 2004; Ngugi and Scherm, 2006; Verma et al., 2006). Nutrients involved in pollen tube elongation, membrane integrity, and other reproductive processes critical for berry development, such as boron or calcium, could potentially

increase fruit set and decrease the number of berries lost to abscission under conditions that are otherwise limiting. Pollen viability is positively correlated in blueberry with seed counts and fruit size until a saturation threshold is reached (Vander Kloet, 1984).

Fertilized seeds contribute to fruit development and ultimate berry size in blueberry, although this is somewhat influenced by cultivar (Dogterom et al., 2000; Lang and Danka, 1991; Payne et al., 1989). Approximately 30 days following bloom, new fruit go through a period of rapid cell division (Cano-Medrano and Darnell, 1997). After initial growth by cell division, fruit enlargement occurs almost exclusively as the result of cell enlargement. In highbush blueberry, fruit size is positively related to seed number. Larger fruit generally have a greater number of cells, however, marketable size has been achieved with as few as 10-20 seeds per fruit (Coombe, 1976; Vorsa et al., 1991). The primary growth phase, which dictates ultimate fruit size, occurs during the initial 30 days immediately following bloom (Strik et al., 1993). Increased fruit size and seed number is reported to be due to the release of hormones from fertilized seeds, which stimulate fruit enlargement (i.e., auxins) (Pritts et al., 1992). Improvement of fruit size should target the periods of early cell division, as this is a critical stage that determines berry size. Provision of adequate moisture and mineral nutrients during early cell division are two strategies to ensure that this critical period goes unrestricted.

Following fertilization, fruits begin to enlarge, and competition for carbohydrates can manifest into fruit drop if carbohydrate resources are limiting (Racskó et al., 2007). The abscission of plant parts, including leaves and immature or ripe fruit, can occur naturally at multiple points throughout the season. However, under certain conditions, abscission of leaves and developing fruit can occur prematurely. Premature abscission of leaves and fruit often results from pest or pathogen pressures, nutrient deficiencies, and/or water stress (Arteca, 1996; Darnell and Birkhold, 1996; Hildebrand et al., 2001; McNicol and Williamson, 1989). In 'Draper' blueberry, plants can experience pre-mature fruit drop in western Washington and British Columbia. This phenomenon is commonly referred to as "Draper drop" and can result in severe crop losses. Similar fruit drop symptoms preceded by transient red coloration in young leaves and flowers is attributed to Fruit Drop Virus in blueberry (Martin et al., 2006). The cause of this premature fruit drop in 'Draper' is not known. Further discussion of plant nutrition in relation to fruit development is addressed in the section, "Nutrient Application".

Abscission may also result as a consequence of limited plant carbohydrates, with more sink material than source material, as occurs and described in pear (*Pyrus communis*) and apple (*Malus domestica*) (Byers et al., 1990; Dennis, 2000; McArtney et al., 2004; Wertheim, 2000). Developing fruit are strong carbohydrate sinks, leading to the diversion of soluble sugars from carbohydrate producing and storage organs to fruit. In apple and pear, fruit are thinned to increase the size of remaining fruit and to manage the overall carbohydrate source:sink ratio (Wertheim, 2000). Solar radiation and temperature can also influence photosynthesis and the production of carbohydrates. Consistent high light, above the light compensation point, drives carbohydrate production. Conversely, consistently low light conditions or prolonged high temperatures (>35°C) can reduce net photosynthesis and drive a carbohydrate deficit (Hancock et al., 1992). Adequate plant spacing between rows may also increase light interception, increasing overall photosynthates (Bryla and Strik, 2004).

Nutrient application. Nutrient management in blueberry production is crucial for successful and productive plantings. When various chemical elements are inadequate, the general health, yield, and quality of the crop may be reduced so long as other factors are not limiting. Severe nutrient deficiency

can lead to discolored leaves, wilted flowers, reduced yields, decreased cane initiation and growth, and plant death. Calcium and boron have been used as supplemental fertilizers in blueberry production and have the potential to positively influence fruit set and yield.

Calcium. Calcium is an essential macronutrient in blueberry production (Chen et al., 1998). The roles that calcium plays in plants are many and include structural (calcium bound to pectin cell walls), defense, and communication (between cells and tissues) (Dixon et al., 1994; Evans et al., 2001; Jarvis, 1984; Sanders et al., 1999). Calcium is particularly important for the function of calcium ion transporters and their subsequent role in environmental and biotic responses (Hepler and Wayne, 1985). Calcium is frequently applied as a soil amendment in the form of agricultural and/or dolomitic limestone or, when pH must remain stable, as calcium sulfate (gypsum) (Hart et al., 2006). Hanson and Berkheimer (2004) have shown that integration of soil-applied calcium into leaves and fruit is inconsistent and ineffective in blueberry. This response is similar to other fleshy fruits, including apple (*Malus domestica*) and tomato (*Solanum lycopersicum*) (Dong et al., 2005; Shear and Faust, 1970). Foliar applications of calcium chloride have been used to a smaller degree in blueberry. However, due to salt sensitivity, chlorine-containing nutrients are generally avoided in fertilizer programs (Korcak, 1988).

Nutrient levels vary across blueberry cultivars and the time of year, but levels remain most stable in late July or early August (Strik and Vance, 2015). Despite stability of macronutrients, high variability can occur in micronutrients. There is often a disparity between calcium content in leaves and in fruit, with higher levels of calcium being found in transpiring tissues, such as leaves (Strik and Vance, 2015; Wiersum, 1966). In addition, calcium movement and distribution in blueberry appears to vary greatly by cultivar (Strik and Vance, 2015). Plants which are not self-fertile and require cross-pollination with compatible genotypes have been found to produce pollen with lower levels of calcium relative to plants that do not require cross-pollination (Brewbaker and Kwack, 1963). Low calcium concentrations in pollen has been correlated with a reduced rate of pollen germination, with some dependence of germination on calcium levels found within the stigma (Bednarska, 1991; Brewbaker and Kwack, 1963; Ge et al., 2009; Taylor and Hepler, 1997). Post-harvest calcium chloride dips have also increased firmness in blueberries, a valuable trait in fresh market production; however, fruit was reported to taste salty and, as a result, fresh market blueberries are generally not dipped (Hanson et al., 1993). However, no significant increase in berry firmness has been realized when berries are treated with foliar applications of calcium chloride during the production season (Hanson, 1995). Mobility of calcium to fruit in blueberry is limited because fruit have low transpiration rates relative to the leaves, thereby limiting the movement of dissolved calcium in the xylem to fruit (Angeletti et al., 2010).

Boron. Boron is a micronutrient that is often deficient in blueberry production, particularly in western Washington and Oregon. Boron is important for pollen formation and health, with pollen germination, tube length, and speed of pollen tube growth all influenced by boron availability in reproductive tissues (Thompson and Batjer, 1950; Visser, 1955; Wang et al., 2003). Because of this, many commercial growers in western Washington and Oregon provide boron through foliar nutrient applications in order to improve pollen health and subsequent fruit set; however, research validating their use is lacking. Boron deficiency is common among soil types with coarse texture (Goldberg, 1997). Absorption and uptake of boron as undissociated boric acid (tetrahydroxyborate) occurs at the roots. Boron deficiency can result in reduced pollen viability, tube elongation, and lower germination rates of pollen in almond (*Prunus dulcis*) (Nyomora et al., 1997). Pollen tube growth rate and overall length are indicative

of viability and general health (Huang and Johnson, 1996; Knox and Friederich, 1974; Sahar and Spiegel-Roy, 1984). Evaluation of pollen germination and tube formation in the presence of given nutrients would allow for a more direct evaluation of reproductive responses to nutrients like boron in the environment and provide additional support for their commercial use. Even when leaf tissue samples indicate sufficient concentrations of boron, deficiencies can persist in meristematic and floral tissues (Shorrocks, 1997). However, there is a fine line between sufficiency and toxicity of boron in plants. Typically, boron is not applied in excess of 500 ppm in blueberry due to increased occurrences of phytotoxiciy. Culture media prepared with boron increases pollen germination rates *in vitro,* as well (Sotomayor et al., 2001). Significant differences between *in vitro* pollen germination of 'Rubel' and 'Jersey' blueberries were strongly correlated to fruit set, with higher germination positively correlated with increased fruit set (Brewer and Dobson, 1969). Boron deficiency can retard seed formation and reduce viability (Marschner, 1995; Marschner et al., 1996). While boron is readily absorbed and mobile within the xylem of plants, concentrating applications to the dynamic growth phase occurring during floral development, pollen germination, fertilization, and early berry development may increase absorption to target tissues (Angeletti et al., 2010; Brown and Hu, 1996; Lord and Russell, 2002). This intense period of development is metabolically demanding and plant nutrient levels at this stage may have far-reaching consequences on future fruit set, berry size, and fruit composition at harvest across different cultivars.

Conclusion

Pollination and fruit set are complex processes in blueberry production, being influenced by bud and flower development, pollinator activity, pollen compatibility, floral morphology, fertilization, and flower and fruit nutrient status. A systems-based approach is required to increase pollination and fruit set in blueberry, particularly in primary production regions west of the Cascades that experience chronically low fruit set. There is a positive relationship between productive cultivars with high fruit set and large, open flowers. Therefore, the link between floral morphology and cultivar attractiveness to pollinators should be investigated further, which may influence regional cultivar recommendations and pollination practices. Currently, the stocking rates for honey bee hives to achieve saturating conditions for pollination is derived from largely non-region-specific stocking density recommendations and may be unrealistic considering continually increased demand for honey bees. Optimization of honey bee stocking recommendations and understanding the role that alternative pollinator species can contribute to improving the overall success of pollination is important for difficult-to-pollinate crops, like blueberry. The potential benefits of supplementing additional boron and calcium are promising and are under investigation. Overall, further research and development of pollination and fruit set enhancement tools in highbush blueberry have great potential to close the regional production gaps in the PNW, which presently exist for this economically important crop.

Literature cited

Alqarni, A. S. 1995. Morphometrical and biological studies of the native honeybee race *Apis mellifera* L.; the carniolan *A. m. carnica* 'Pollmann' and their F1 hybrid. King Saud University, Riyadh, Saudi Arabia. Doctoral dissertation, MSc Thesis.

Angeletti, P., H. Castagnasso, E. Miceli, L. Terminiello, A. Concellón, A. Chaves, and A. Vicente. 2010. Effect of preharvest calcium applications on postharvest quality, softening and cell wall degradation of two blueberry (*Vaccinium corymbosum*) varieties. Postharvest Biol. Tech. 58(2): 98-103.

Arteca, R. N. 1996. Plant growth substances: principles and applications. Chapman & Hall, London, UK. 165-172.

Bailey, L. and B. V. Ball. 2013. Honey bee pathology (second edition). Academic Press/Elsevier, San Diego, CA. 1-5.

Baker, C.B., J.K. Eischeid, T.R. Karl, and H.F. Diaz. 1994. The quality control of long-term climatological data using objective data analysis. Preprints of AMS Ninth Conference on Applied Climatology, Dallas, TX.

Balfour, N. J., M. Garbuzov, and F. L. Ratnieks. 2013. Longer tongues and swifter handling: why do more bumble bees (*Bombus* spp.) than honey bees (*Apis mellifera*) forage on lavender (*Lavandula* spp.)? Ecol. Entomol. 38(4): 323-329.

Bañados, M. P. and B. Strik. 2006. Manipulation of the annual growth cycle of blueberry using photoperiod. In VIII International Symposium on Vaccinium Culture, Acta Hort. 715: 65-72.

Bednarska, E. 1991. Calcium uptake from the stigma by germinating pollen in *Primula officinalis* L. and *Ruscus aculeatus* L. Sex. Plant Reprod. 4(1): 36-38.

Biasiolo, A. and A. Comparini. 1990. Esterase-6 locus, a new enzyme polymorphism in *Apis mellifera*. Apidologie 21(2): 123-126.

Blaauw, B. R. and R. Isaacs. 2014. Flower plantings increase wild bee abundance and the pollination services provided to a pollination-dependent crop. J. Appl. Ecol. 51(4): 890-898.

Bosch, J., W.P. Kemp, and S. S. Peterson. 2000. Management of *Osmia lignaria* (Hymenoptera: Megachilidae) populations for almond pollination: methods to advance bee emergence. Environ. Entomol. 29(5): 874-883.

Bosch, J. and W. P. Kemp. 2003. Effect of wintering duration and temperature on survival and emergence time in males of the orchard pollinator *Osmia lignaria* (Hymenoptera: Megachilidae). Environ. Entomol. 32(4): 711-716.

Brazelton, C. 2015. World blueberry statistics and global market analysis. U.S. Highbush Blueberry Council, Folsom, CA.

Brewbaker, J. L. and Kwack, B. H. 1963. The essential role of calcium ion in pollen germination and pollen tube growth. Am. J. Bot. 50(9): 859-865.

Brewer, J. W. and R. C. Dobson. 1969. Seed Count and Berry Size in Relation to Pollinator Level and Harvest Date for the Highbush Blueberry, *Vaccinium corymbosum* 1. *J.* Econ. Entomol. 62(6): 1353-1356.

Brown, P. H. and H. Hu. 1996. Phloem mobility of boron is species dependent: evidence for phloem mobility in sorbitol-rich species. Ann. Bot. 77(5): 497-506.

Bryla, D. and B. C. Strik. 2004. Variation in plant and soil water relations among irrigated blueberry cultivars planted at two distinct in-row spacings. In VIII International Symposium on Vaccinium Culture. 715: 295-300.

Buchmann, S. L. 1985. Bees use vibration to aid pollen collection from non-poricidal flowers. J. Kans. Entomol. Soc. 58(3): 517-525.

Byers, R. E., J.A. Barden, R. F. Polomski, R. W. Young, and D. H. Carbaugh. 1990. Apple thinning by photosynthetic inhibition. J. Am. Soc. Hortic. Sci. 115(1): 14-19.

Cano-Medrano, R. and L. Darnell. 1997. Cell number and cell size in parthenocarpic vs. pollinated blueberry (*Vaccinium ashei*) fruits. Ann. Bot. 80(4): 419-425.

Carré, G., P. Roche, R. Chifflet, N. Morison, R. Bommarco, J. Harrison-Cripps, and J. Settele. 2009. Landscape context and habitat type as drivers of bee diversity in European annual crops. Agric. Ecosyst. Environ. 133(1): 40-47.

Carvell, C., J. L. Osborne, A. F. G. Bourke, S. N. Freeman, R. F. Pywell, and M. S. Heard. 2011. Bumble bee species' responses to a targeted conservation measure depend on landscape context and habitat quality. Ecol. Appl. 21(5): 1760-1771.

Chen, Y., J. M. Smagula, W. Litten, and S. Dunham. 1998. Effect of boron and calcium foliar sprays on pollen germination and development, fruit set, seed development, and berry yield and quality in lowbush blueberry (*Vaccinium angustifolium* Ait.). J. Am. Soc. Hortic. Sci. 123(4): 524-531.

Coombe, B. G. 1976. The development of fleshy fruits. Annu. Rev. Plant Physiol. 27(1): 207-228.

Corbet, S. A., M. Fussell, R. Ake, A. Fraser, C. Gunson, A. Savage, and K. Smith. 1993. Temperature and the pollinating activity of social bees. Ecol. Entomol. 18(1): 17-30.

Courcelles, D. M. M., L. Button, and E. Elle. 2013. Bee visit rates vary with floral morphology among highbush blueberry cultivars (*Vaccinium corymbosum* L.). J. Appl. Entomol. 137(9): 693-701.

Crailsheim, K. 1990. The protein balance of the honey bee worker. Apidologie 21(5): 417-429.

Daly, H. V., R. G. Danka, K. Hoelmer, T. E. Rinderer, and S. M. Buco. 1995. Honey bee morphometrics: linearity of variables with respect to body size and classification tested with European worker bees reared by varying ratios of nurse bees. J. Apic. Res. 34(3): 129-145.

Darnell, R. L. 1991. Photoperiod, carbon partitioning, and reproductive development in rabbiteye blueberry. J. Am. Soc. Hortic. Sci. 116(5): 856-860.

Darnell, R. L. and K. B. Birkhold. 1996. Carbohydrate contribution to fruit development in two phenologically distinct rabbiteye blueberry cultivars. J. Am. Soc. Hortic. Sci. 121(6): 1132-1136.

Daykin, M. E. and R. D. Milholland. 1990. Histopathology of Blueberry Twig Blight Caused by *Phomopsis vaccinii*. Phytopathology 80: 736-740.

De Vries, D. P. and L. A. Dubois. 1987. The effect of

temperature on fruit set, seed set and seed germination in 'Sonia'×'Hadley' hybrid Tea-rose crosses. Euphytica 36(1): 117-120.

Delaplane, K. S., D. R. Mayer, and D. F. Mayer. 2000. Crop pollination by bees. Cabi, Boston, MA.

Dennis, F. J. 2000. The history of fruit thinning. Plant Growth Regul. 31(1-2): 1-16.

Desjardins, È. C. and D. De Oliveira. 2006. Commercial bumble bee *Bombus impatiens* (Hymenoptera: Apidae) as a pollinator in lowbush blueberry (Ericale: Ericaceae) fields. J. Econ. Entomol. 99(2): 443-449.

Dixon, R. A., M. J. Harrison, and C. J. Lamb. 1994. Early events in the activation of plant defense responses. Annu. Rev. Phytopathol. 32(1): 479-501.

Dogterom, M. H., M. L. Winston, and A. Mukai. 2000. Effect of pollen load size and source (self, outcross) on seed and fruit production in highbush blueberry cv. 'Bluecrop' (*Vaccinium corymbosum*; Ericaceae). Am. J. Bot. 87(11): 1584-1591.

Dong, C. X., J. M. Zhou, X. H. Fan, H. Y. Wang, Z. Q. Duan, and C. Tang. 2005. Application methods of calcium supplements affect nutrient levels and calcium forms in mature tomato fruits. J. Plant Nutr. 27(8): 1443-1455.

Eck, P. and C. M. Mainland. 1971. Highbush blueberry fruit set in relation to flower morphology. HortScience 8(2): 494-495.

Eckert, C. D., M. L. Winston, and R. C. Ydenberg. 1994. The relationship between population size, amount of brood, and individual foraging behavior in the honey bee, *Apis mellifera* L. Oecologia 97(2): 248-255.

Ehlenfeldt, M. K. 2001. Self-and cross-fertility in recently released highbush blueberry cultivars. HortScience 36(1): 133-135.

Evans, N. H., M. R. McAinsh, and A. M. Hetherington. 2001. Calcium oscillations in higher plants. Curr. Opin. Plant Biol. 4(5): 415-420.

Fewell, J. H. and M. L. Winston. 1992. Colony state and regulation of pollen foraging in the honey bee, *Apis mellifera* L. Behav. Ecol. Sociobiol. 30(6): 387-393.

Free, J. B. 1967. Factors determining the collection of pollen by honeybee foragers. Anim. Behav. 15(1): 134-144.

Garibaldi, L. A., I. Steffan-Dewenter, R. Winfree, M. A. Aizen, R. Bommarco, S. A. Cunningham, and A. M. Klein. 2013. Wild pollinators enhance fruit set of crops regardless of honey bee abundance. Science 339(6127): 1608-1611.

Ge, L. L., C. T. Xie, H. Q. Tian, and S. D. Russell. 2009. Distribution of calcium in the stigma and style of tobacco during pollen germination and tube elongation. Sex. Plant Reprod. 22(2): 87-96.

Gels, J. A., D. W. Held, and D. A. Potter. 2002. Hazards of insecticides to the bumble bees *Bombus impatiens* (Hymenoptera: Apidae) foraging on flowering white clover in turf. J. Econ. Entomol. 95(4): 722-728.

Goldberg, S. 1997. Reactions of boron with soils. Plant and Soil 193:35-48.

Goulson, D., G. C. Lye, and B. Darvill. 2008. Decline and conservation of bumble bees. Annu. Rev. Entomol. 53: 191-208.

Hancock, J. F., K. Haghighi, S. L. Krebs, J. A. Flore, and A. D. Draper. 1992. Photosynthetic heat stability in highbush blueberries and the possibility of genetic improvement. HortScience 27(10): 1111-1112.

Hanson, E. J., J. L. Beggs, and R. M. Beaudry. 1993. Applying calcium chloride postharvest to improve highbush blueberry firmness. HortScience 28(10): 1033-1034.

Hanson, E. J. 1995. Preharvest calcium sprays do not improve highbush blueberry (Vaccinium corymbosum L.) quality. HortScience 30(5): 977-978.

Hanson, E. J. and S. F. Berkheimer. 2004. Effect of soil calcium applications on blueberry yield and quality. Small Fruits Rev. 3(1-2): 133-139.

Hart, J. M., B. Strik, L. White, and W. Yang. 2006. Nutrient management for blueberries in Oregon. Oregon State University Extension Service. EM 8918.

Hedhly, A., J. I. Hormaza, and M. Herrero. 2004. Effect of temperature on pollen tube kinetics and dynamics in sweet cherry, *Prunus avium* (Rosaceae). Am. J. Bot. 91(4): 558-564.

Heinrich, B. 1975. Bee flowers: a hypothesis on flower variety and blooming times. Evolution 29(2): 325-334.

Hepler, P. K. and R. O. Wayne. 1985. Calcium and plant development. Annu. Rev. Plant Physiol. 36(1): 397-439.

Hepler, P. R. and D. E. Yarborough. 1991. Natural variability in yield of lowbush blueberries. HortScience 26(3): 245-246.

Hildebrand, P. D., K. B. McRae, and X. Lu. 2001. Factors affecting flower infection and disease severity of lowbush blueberry by *Botrytis cinerea*. Can. J. Plant Pathol. 23(4): 364-370.

Huang, Y. and C.E. Johnson. 1996. A convenient and reliable method to evaluate blueberry pollen viability. HortScience 31(7): 1235-1235.

Jarvis, M. C. 1984. Structure and properties of pectin gels in plant cell walls. Plant Cell Environ. 7(3): 153-164.

Jay, S. C. 1986. Spatial management of honeybees on crops. Bee World 67(3), 98-113.

Jensen, A. B., K. A. Palmer, J. J. Boomsma, and B. V. Pedersen. 2005. Varying degrees of *Apis mellifera*

ligustica introgression in protected populations of the black honeybee, *Apis mellifera* 'mellifera', in northwest Europe. Mol. Ecol. 14(1): 93-106.

Kevan, P. G. 1990. Pollination: keystone process in sustainable global productivity. In VI International Symposium on Pollination 288: 103-110.

Kevan, P. G., E. Clark, and V. G. Thomas. 1990. Insect pollinators and sustainable agriculture. Am. J. Alternative Agr. 5(01): 13-22.

Knight, M. E., A. P. Martin, S. Bishop, J. L. Osborne, R. J. Hale, R. A. Sanderson, and D. Goulson. 2005. An interspecific comparison of foraging range and nest density of four bumblebee (*Bombus*) species. Mol. Ecol. 14(6): 1811-1820.

Knox, R. B. and E. Friederich. 1974. Tetrad pollen grain development and sterility in Leschenaultia formosa (Goodeniaceae). New Phytol. 73(1): 251-258.

Korcak, R. F. 1988. Nutrition of blueberry and other calcifuges. Hortic. Rev. 10: 183-227.

Kovaleski, A. P., J. G. Williamson, J. W. Olmstead, and R. L. Darnell. 2015. Inflorescence bud initiation, development, and bloom in two southern highbush blueberry cultivars. J. Am. Soc. Hortic. Sci. 140(1): 38-44.

Krebs, S. L. and J. F. Hancock. 1988. The consequences of inbreeding on fertility in *Vaccinium corymbosum* L. J. Amer. Soc. for Hort. Sci. 113(6): 914-918.

Krebs, S. L. and J. H. Hancock. 1990. Early-acting inbreeding depression and reproductive success in the highbush blueberry, *Vaccinium corymbosum* L. *Theor.* Appl. Genet. 79(6): 825-832.

Kremen, C., N. M. Williams, and R. W. Thorp. 2002. Crop pollination from native bees at risk from agricultural intensification. Proc. Natl. Acad. Sci. U.S.A. 99(26): 16812-16816.

Lang, G. A. and R. G. Danka. 1991. Honey-bee-mediated cross-versus self-pollination of 'Sharpblue' blueberry increases fruit size and hastens ripening. J. Am. Soc. Hortic. Sci. 116(5): 770-773.

Lee, J. C., D. J. Bruck, H. Curry, D. Edwards, D. R. Haviland, R. A. Van Steenwyk, and B. M. Yorgey. 2011. The susceptibility of small fruits and cherries to the spotted-wing drosophila, Drosophila suzukii. Pest Manag. Sci. 67(11): 1358-1367.

Lord, E. M. and S. D. Russell. 2002. The mechanisms of pollination and fertilization in plants. Annu. Rev. Cell Dev. Biol. 18(1): 81-105.

MacKenzie, K. E. and M. L. Winston. 1984. Diversity and abundance of native bee pollinators on berry crops and natural vegetation in the lower Fraser Valley, British Columbia. Can. Entomol. 116(07): 965-974.

MacKenzie, K. E. 1997. Pollination requirements of three highbush blueberry (*Vaccinium corymbosum* L.) cultivars. J. Am. Soc. Hortic. Sci. 122(6): 891-896.

Marschner, H. 1995. Functions of mineral nutrients: macronutrients. Mineral nutrition of higher plants. 2: 379-396.

Marschner, H., E. A. Kirkby, and I. Cakmak. 1996. Effect of mineral nutritional status on shoot-root partitioning of photoassimilates and cycling of mineral nutrients. J. Exp. Bot. 47(Special Issue): 1255-1263.

Martin, R. R., I. E. Tzanetakis, M. Sweeney, and L. Wegener. 2006. A virus associated with blueberry fruit drop disease. Acta Hortic. 715: 497.

Marucci, P. E. and H. J. Moulter. 1976. Blueberry pollination in New Jersey. In Symposium on Vaccinium Culture in Europe. 61: 175-186.

Maust, B. E., J. G. Williamson, and R. L. Darnell. 2000. Carbohydrate reserve concentrations and flower bud density effects on vegetative and reproductive development in southern highbush blueberry. J. Am. Soc. Hortic. Sci. 125(4): 413-419.

May, J. D. and K. T. Killingbeck. 1992. Effects of preventing nutrient resorption on plant fitness and foliar nutrient dynamics. Ecology 73(5): 1868-1878.

McArtney, S., M. White, I. Latter, and J. Campbell. 2004. Individual and combined effects of shading and thinning chemicals on abscission and dry-matter accumulation of 'Royal Gala' apple fruit. J. Hortic. Sci. Biotech 79(3): 441-448.

McNicol, R. J. and B. Williamson. 1989. Systemic infection of black currant flowers by *Botrytis cinerea* and its possible involvement in premature abscission of fruits. Ann. Appl. Biol. 114(2): 243-254.

Moeller, F. E. 1973. Timing of placement of colonies of honey bees for pollination of cranberries. J. Econ. Entomol. 66(2): 370-372.

Moritz, R. F. and E. Hillesheim. 1985. Inheritance of dominance in honeybees (*Apis mellifera* capensis Esch.). Behav. Ecol. Sociobiol. 17(1): 87-89.

Ngugi, H. K. and H. Scherm. 2004. Pollen mimicry during infection of blueberry flowers by conidia of *Monilinia vaccinii-corymbosi*. Physiol. Mol. Plant Pathol. 64(3): 113-123.

Ngugi, H. K. and H. Scherm. 2006. Biology of flower-infecting fungi. Annu. Rev. Phytopathol. 44: 261-282.

NOAA's National Weather Service. (n.d.). Retrieved June 9, 2015, from http://ptwc.weather.gov/?region=1andid=pacific.TSUPAC.2014.11.15.0304

Nyomora, A. M., P. H. Brown, and M. Freeman. 1997. Fall foliar-applied boron increases tissue boron concentration and nut set of almond. J. Am. Soc. Hortic. Sci. 122(3): 405-410.

Paini, D. R. 2004. Impact of the introduced honey bee (*Apis mellifera*) (Hymenoptera: Apidae) on native

bees: a review. Austral Ecol. 29(4): 399-407.

Pankiw, T., M. L. Winston, and K. N. Slessor. 1994. Variation in worker response to honey bee (*Apis mellifera* L.) queen mandibular pheromone (Hymenoptera: Apidae). J. Insect Behav. 7(1): 1-15.

Pankiw, T., R. E. Page Jr, and M. K. Fondrk. 1998. Brood pheromone stimulates pollen foraging in honey bees (*Apis mellifera*). Behav. Ecol. Sociobiol. 44(3): 193-198.

Parrie, E. J. and G. A. Lang. 1992. Self-and cross-pollination affect stigmatic pollen saturation in blueberry. HortScience 27(10): 1105-1107.

Payne, J. A., J. H. Cane, A. A. Amis, and P. M. Lyrene. 1989. Fruit size, seed size, seed viability and pollination of rabbiteye blueberries (Vaccinium ashei Reade). Acta Hortic. 241(3): 38-43.

Pettis, J. S., N. Rice, K. Joselow, and V. Chaimanee. 2016. Colony failure linked to low sperm viability in honey bee (Apis mellifera) queens and an exploration of potential causative factors. PloS one 11(2): 1-10.

Pritts, M. P., J. F. Hancock, B. C. Strik, M. Eames-Sheavly, and D. Celentano. 1992. Highbush blueberry production guide. Northeast Regional Agricultural Engineering Service, Ithaca, NY.

Racskó, J., G. B. Leite, J. L. Petri, S. Zhongfu, Y. Wang, Z. Szabó, and J. Nyéki. 2007. Fruit drop: The role of inner agents and environmental factors in the drop of flowers and fruits. Int J Hortic Sci. 13(3): 13-23.

Ritzinger, R. and P. M. Lyrene. 1999. Flower morphology in blueberry species and hybrids. HortScience 34(1): 130-131.

Sagili, R.R. and D.M. Burgett. 2011. Evaluating honey bee colonies for pollination: A guide for commercial growers and beekeepers. Pacific Northwest Extension publication. PNW 623.

Sahar, N. and P. Spiegel-Roy. 1984. In vitro germination of avocado pollen. HortScience 19(6): 886-888.

Sairanen, I., O. Novák, A. Pěnčík, Y. Ikeda, B. Jones, G. Sandberg, and K. Ljung. 2012. Soluble carbohydrates regulate auxin biosynthesis via PIF proteins in Arabidopsis. Plant Cell 24(12): 4907-4916.

Salvo, S., C. Muñoz, J. Ávila, J. Bustos, M. Ramirez-Valdivia, C. Silva, and G. Vivallo. 2012. An estimate of potential blueberry yield using regression models that relate the number of fruits to the number of flower buds and to climatic variables. Sci. Hortic. 133: 56-63.

Sampson, B. J. and J. H. Cane. 2000. Pollination efficiencies of three bee (Hymenoptera: Apoidea) species visiting rabbiteye blueberry. J. Econ. Entomol. 93(6): 1726-1731.

Sanders, D., C. Brownlee, and J. F. Harper. 1999. Communicating with calcium. Plant Cell 11(4): 691-706.

Seeley, T. D. 1994. Honey bee foragers as sensory units of their colonies. Behav. Ecol. Sociobiol. 34(1): 51-62.

Shear, C. B. and M. Faust. 1970. Calcium transport in apple trees. Plant Physiol. 45(6): 670-674.

Shorrocks, V. M. 1997. The occurrence and correction of boron deficiency. Plant Soil 193(1-2): 121-148.

Sotomayor, C., H. Silva, and J. Castro. 2001. Effectiveness of boron and zinc foliar sprays on fruit setting of two almond cultivars. In III International Symposium on Pistachios and Almonds, 591: 437-440.

Standifer, L. N. 1980. Honey bee nutrition and supplemental feeding. Beekeeping in the United States Agricultural Handbook 335: 39-45.

Strik, B., C. Brun, M. Ahmedullah, A. Antonelli, L. Askham, D. Barney, and D. Havens. 1993. Highbush blueberry production. Pacific Northwest Extension Publication. PNW 215.

Strik, B.C., C.E. Finn, and P.P. Moore. 2014. Blueberry cultivars for the Pacific Northwest. Pacific Northwest Extension Publication. PNW 656.

Strik, B. 2015. Blueberry plant physiology – Why it's important to understand the plant to manage it well. Proc. Oregon State Univ. Blueberry School: 57-61.

Strik, B. C. and A. J. Vance. 2015. Seasonal variation in leaf nutrient concentration of northern highbush blueberry cultivars grown in conventional and organic production systems. HortScience 50(10): 1453-1466.

Stubbs, C. S., F. A. Drummond, and E. A. Osgood. 1994. *Osmia ribifloris* biedermannii and *Megachile rotundata* (Hymenoptera: Megachilidae) introduced into the lowbush blueberry agroecosystem in Maine. J. Kans. Entomol. Soc. 67(2): 173-185.

Stubbs, C. S. and F. A. Drummond. 1996. Blueberry and cranberry (*Vaccinium* spp.) pollination: a comparison of managed and native bee foraging behavior. In VII International Symposium on Pollination. 437: 341-344.

Taylor, L. P. and P. K. Hepler. 1997. Pollen germination and tube growth. Annu. Rev. Plant. *Biol.* 48(1): 461-491.

Thompson, A. H. and L. P. Batjer. 1950. The effect of boron in the germinating medium on pollen germination and pollen tube growth for several deciduous tree fruits. P. Am. Soc. Hortic. Sci. 56: 227-30.

Toth, A. L. and G. E. Robinson. 2005. Worker nutrition and division of labor in honeybees. Anim. Behav. 69(2): 427-435.

Torchio, P. F. 1990a. Bees as crop pollinators and the role of solitary species in changing environments. In VI International Symposium on Pollination 288: 49-61.

Torchio, P. F. 1990b. Diversification of pollination

strategies for US crops. Environ. Entomol. 19(6): 1649-1656.

Tuell, J. K., J. S. Ascher, and R. Isaacs. 2009. Wild bees (Hymenoptera: Apoidea: Anthophila) of the Michigan highbush blueberry agroecosystem. Ann. Entomol. Soc. Am. 102(2): 275-287.

United States Department of Agriculture, 2005. Noncitrus fruits and nuts 2005 summary. 14 Jan. 2016. http://usda.mannlib.cornell.edu/usda/nass/NoncFruiNu//2000s/2005/NoncFruiNu-07-06-2005.pdf>.

United States Department of Agriculture, 2014. Noncitrus fruits and nuts 2014 summary. 14 Jan. 2016. <http://usda.mannlib.cornell.edu/usda/nass/NoncFruiNu//2010s/2015/ NoncFruiNu-07-17-2015.pdf>.

Vander Kloet, S. P. 1984. Effects of Pollen Donors on Seed Production, Seed Weight, Germination and Seedling Vigor in *Vaccinium corymbosum* L. Am. Midl. Nat. 112(2): 392-396.

Vander Kloet, S. V. and P. M. Lyrene. 1987. Self-incompatibility in diploid, tetraploid, and hexaploid *Vaccinium corymbosum*. Can. J. Bot. 65(4): 660-665.

Verma, N., L. MacDonald, and Z. K. Punja. 2006. Inoculum prevalence, host infection and biological control of *Colletotrichum acutatum*: causal agent of blueberry anthracnose in British Columbia. Plant Pathol. 55(3): 442-450.

Vestrheim, S., K. Haffner, and K. Grønnerød. 1996. Highbush blueberry production and research in Norway. In VI International Symposium on Vaccinium Culture 446: 177-180.

Visser, T. 1955. Germination and storage of pollen. Landbouwhogeschool, Wageningen. PhD dissertation.

Vorsa, N. and J. R. Ballington. 1991. Fertility of triploid highbush blueberry. J. Am. Soc. Hortic. Sci. 116(2): 336-341.

Wang, Q., L. Lu, X. Wu, Y. Li, and J. Lin. 2003. Boron influences pollen germination and pollen tube growth in *Picea meyeri*. Tree Physiol. 23(5): 345-351.

Wertheim, S. J. 2000. Developments in the chemical thinning of apple and pear. Plant Growth Regul. 31(1-2): 85-100.

Whidden, T. L. 1996. The fidelity of commercially reared colonies of *Bombus impatiens* Cresson (Hymenoptera: Apidae) to lowbush blueberry in southern New Brunswick. Can. Entomol. 128(05): 957-958.

Whitfield, C. W., S. K. Behura, S. H. Berlocher, A. G. Clark, J. S. Johnston, W. S. Sheppard, and N. D. Tsutsui. 2006. Thrice out of Africa: ancient and recent expansions of the honey bee, *Apis mellifera*. Science 314(5799): 642-645.

Wiersum, L. K. 1966. Calcium content of fruits and storage tissues in relation to the mode of water supply. Acta. Bot. Neerl. 15(2): 406-418.

Winfree, R., N. M. Williams, H. Gaines, J. S. Ascher, and C. Kremen. 2008. Wild bee pollinators provide the majority of crop visitation across land-use gradients in New Jersey and Pennsylvania, USA. J. Appl. Ecol. 45(3): 793-802.

Winston, M. L., H.A. Higo, S. J. Colley, T. Pankiw, and K. N. Slessor. 1991. The role of queen mandibular pheromone and colony congestion in honey bee (*Apis mellifera* L.) reproductive swarming (Hymenoptera: Apidae). J. Insect Behav. 4(5): 649-660.

Wood, G. W. 1961. The influence of honeybee pollination on fruit set of the lowbush blueberry. Can. J. Plant Sci. 41(2): 332-335.

Wood, G. W. 1968. Self-fertility in the lowbush blueberry. Can. J. Plant Sci. 48(4): 431-433.

Journal of the American Pomological Society 70(3): 138-148 2016

Variation in Fruit Constituent Sugar Concentrations and their Stability during Processing of Selected Genotypes in *Prunus domestica*

S.B. Castro[1*], C.H. Crisosto[1] and T.M. DeJong[1]

Additional index words: *Prunus domestica,* soluble solids, sorbitol

Abstract

The *Prunus domestica* breeding program at the University of California, Davis has several selections which produce fruit that many processors find difficult to dry, sort, rehydrate, and pit using current processing machinery. Unlike the commercial 'Improved French' cultivar, UC Davis breeding program germplasm selections have highly variable ratios of various sugar constituents. The goal of this project was to determine if the different ratios of soluble sugars and sorbitol correlate with difficulties in processing. Thus, the objectives of this research were to select cultivars with distinctly different sugar profiles and then determine what changes in sugar and sorbitol concentrations occur as the fresh fruit is dried and subsequently processed. This research could help determine what sugar ratios in plums are preferential for industrial processing. Based on previously published information, it was anticipated that sucrose would more readily hydrolyze during drying and processing than sorbitol and that higher concentrations of sorbitol would be more stable and potentially act as a preservative, inhibiting degradation of sucrose and reducing sugars. In 2010 and 2011, fruit of 17 and 10 prune genotypes, respectively, were analyzed for glucose, fructose, sucrose, and sorbitol concentrations before and after drying. In 2011, the dried fruit were also rehydrated and pitted using commercial machinery. As anticipated, sucrose concentrations generally decreased during drying and processing while glucose, fructose, and sorbitol concentrations remained relatively stable. There was no apparent concentration-related preservative effect of sorbitol on the other sugars, however the rate of change in fructose and glucose differed among cultivars. The change in sugar profiles from fresh to dried was much greater than the change from dried to processed. Additionally, sugar profiles of the ten genotypes dried in both years were consistent between years.

The *Prunus domestica* cultivar development program at the University of California, Davis, was established in 1985 with the support of the California Dried Plum Board. The program was initiated to develop new European plum cultivars which produce fruit with characteristics similar to the industry standard, 'Improved French'. Currently, California produces about 160,000 metric tons, 60% of the world market and 99% of the US market of dried plums, often called prunes (California Dried Plum Board, 2013). The California industry has over 24,000 hectares and is essentially composed of one cultivar, 'Improved French'. The industry's monocultural structure is a potential problem if catastrophes related to weather and/or disease were to occur.

Dried plum processing in California involves harvesting with mechanical trunk shakers and partially drying the fruit for 20 to 25 hours at 74 °C to change the moisture content from about 80% to approximately 20%. Processing includes rehydration of the dried fruit in steam for 12-20 minutes until it reaches ~32% moisture. After rehydrating, the fruit is pitted with a machine that uses a metal probe to punch out the pit and potassium sorbate is applied as a preservative. In some large processing facilities, the prunes are put through multiple machines for sizing, pitting, steaming and bagging.

In the past three decades, the UCD breeding program has produced several high qual-

[1] Present address: University California Davis, 1 Shields Ave., 1035 Wickson Hall, Davis, CA 95616
[*] Author for correspondence

ity candidate prune selections for production in California, but most have had problems enduring commercial handling procedures. A current obstacle for the program is predicting which selections will be able to withstand commercial processing.

The four major soluble carbohydrates found in prune fruit and examined in this research are glucose, sucrose, fructose, and sorbitol. Different ratios of these three sugars and one sugar alcohol can greatly influence fruit taste. Fructose is three times sweeter than sorbitol, 2.3 times sweeter than glucose, and 1.7 times sweeter than sucrose (Genard et al., 2003). Additionally, these soluble carbohydrates vary in their susceptibility to degradation and caramelization upon heating. Sucrose can hydrolyze upon heating, producing glucose and fructose (Wilford et al., 1997). Thus, since drying involves heating, sucrose in fresh fruit can hydrolyze during dehydration and increase the concentrations of fructose and glucose which are the reducing sugars (Stacewicz-Sapuntzakis et al., 2001). These reducing sugars are the main ingredients in non-enzymatic browning, also called the Maillard Reaction or MR (Fayle and Gerrard, 2002). Reducing sugars in French 'd'Agen' prunes decrease less when the fruit is dried at 70 °C rather than 80 °C, suggesting that using lower temperatures during drying helps reduce sugar degradation and caramelization (Wilford et al., 1997). Sorbitol sugar alcohol is not a reactant molecule in the MR because it does not have the necessary carbonyl group. Thus, fruit with high sorbitol concentrations exhibit less browning during dehydration. However, upon drying, when water loss is ~ 60% and the temperature is high, sorbitol reportedly also undergoes some caramelization (Wilford et al., 1997).

Sorbitol is a sugar alcohol unique to the Rosaceae family, and sorbitol and sucrose are the major components involved in translocation of carbohydrates in the phloem as it moves from leaves to fruit (Seymour et al., 1993; Layne and Bassi, 2008). In *Prunus* sp. fruits, sorbitol moves into the fruit and is either stored or is metabolized to glucose and fructose by sorbitol oxidase and sorbitol dehydrogenase, respectively (Layne and Bassi, 2008; Kim et al. 2015). A review of sugar compositions of Rosaceae fruits from eight sources, indicates that *P. domestica* fruit contain much higher sorbitol concentrations than most other Rosaceae genera and fruits of other *Prunus* species (Richmond et al., 1981; Van Gorsel et al., 1992).

Sorbitol is a desirable consumer product because of its digestive benefits and low glycemic index (Stacewicz-Sapuntzakis et al., 2001). It is also non-cariogenic, meaning that it does not promote tooth decay (Dried Plum Board, 2013). High-sorbitol fruit provides consumers with a natural laxative and sweet flavor (Stacewicz-Sapuntzakis et al., 2001). Fruit with a low glycemic index are particularly attractive in diabetic-friendly diets. It is ironic that while plums have a uniquely beneficial sugar profile, "in general, plums have attracted the curiosity of biochemists to a remarkably small degree" (Seymour et al., 1993).

We suspect that sorbitol is an important factor in determining fruit process-ability and how the sugars react to heat. It is thought to act as a preservative, a humectant, and a preventative against excessive fruit browning (Cinquanta et al., 2002; Forni et al., 1992). Adding sorbitol before dehydration preserves functional protein properties in egg (Yoo and Lee, 1993). Forni et al. (1992) stressed that the sorbitol concentration in a plum should be a factor in determining suitability for drying and processing, because sorbitol does not degrade in the MR like glucose and fructose.

During this research, other questions arose concerning yearly variation in soluble solids content. Information from private prune processors suggested that differences in sugar concentrations of 'Improved French' occur from year to year (Steve Rasmussen, personal communication). Similarly, Brooks et al. (1993) reported a significant difference in total soluble solids between two different

years in *P. persica* (L.) Batch germplasm. However a different, three-year study found no significant differences in fructose and sorbitol concentrations in *P. persica* among years (Cantin et al., 2009). The same study noted that while environmental conditions often influence sugar concentrations, the sugar profile of sucrose, glucose, fructose and sorbitol are relatively constant across environments. One aspect of this study was to test if sugar profiles remained relatively constant when sugar concentrations changed by year.

The UCD plum breeding program relies on a very diverse germplasm to produce a wide array of fruit characteristics. Its selections produce fruit that are superior to 'Improved French' in traits such as flavor, fruit size, small pit size, strong pit, field heat tolerance, and fresh to dry fruit ratios (DeJong et al., 2011 and DeJong et al., 2012). However, many of the new selections produce fruit that does not remain intact during processing. Prune cultivars are known to vary in total sugar content and the relative proportions of the four major sugars (Wrolstad and Shallenberger, 1981; Forni et al., 1992; Seymour et al., 1993). Therefore, we suspected that there are substantial variations in constituent sugars among selections in the UCD breeding program, although the sugar ratios of fruit from the germplasm and active selections had not previously been tested. Preliminary sugar analyses showed that the superior-flavored genotypes had different sugar and sorbitol ratios than 'Improved French', and often also did not withstand processing well.

The overall goal of this project was to determine whether differences in sucrose, glucose, fructose, and sorbitol ratios were correlated with a fruit's ability to withstand processing. We first characterized the constituent sugars in fruit from multiple selections. Next, we determined how the different sugars reacted to heat during drying and processing. Finally, we determined if initial sugar concentration ratios were correlated with the amount of change that occurred in the sugar profile during drying, and the processing durability of the fruit. This project also determined what sugars degrade upon drying to 20% moisture and during subsequent partial rehydration to 30% moisture and processing, and whether high sorbitol concentrations influence the degradation of reducing sugars. We expected sucrose concentrations to decrease substantially during heating due to hydrolysis and that fructose and glucose concentrations would remain relatively stable or increase, since sucrose hydrolysis produces fructose and glucose. The loss of fructose or glucose would depend on the extent of their involvement in the MR or browning. Just as sorbitol can retain properties of proteins (Yoo and Lee, 1993), we anticipated that sorbitol might inhibit breakdown of reducing sugars. Thus, we hypothesized that plums with higher fresh sorbitol concentrations would lose less glucose, fructose, and sucrose than plums with lower sorbitol concentrations.

Materials and Methods

The experiments were conducted over two years (2010 and 2011). In 2010, we determined sugar concentrations in fresh and dried fruit from 17 plum genotypes. In 2011, we examined fresh, dried and processed fruit of ten genotypes: nine selected from among the original 17 and one additional genotype that was suspected of having an unusually high sorbitol concentration.

Fruit harvest and sampling. The trees used in this experiment were grown in one of two experimental plum blocks, one in Fresno County, CA, and the other in Solano County, CA. Both orchards were managed with semi-conventional farming methods, with foliar fertilization, winter pruning, irrigation, and pest control methods such as herbicide and dormant oil sprays. Fruit were harvested from trees that were five to eight years old. Trees were propagated by planting rootstocks, then top-grafting scions onto the base of each scaffold in the second or third year after planting the rootstocks. In both years, fruit was harvested when the average fruit

pressure was 3 to 4 PSI. In 2010, two boxes of fruit from one tree of each genotype were harvested: ~ 20 to 27 kg of fresh fruit. Ten fruit were randomly selected from the bulk sample for fresh sugar analysis. The remainder of the fruit was dried.

In 2011, fruit from nine genotypes were harvested from the same trees as in 2010, along with several other trees of the same genotypes located in the experimental blocks. Fruit from one additional genotype were also harvested. The genotypes harvested were chosen on the basis of the sugar profiles determined in 2010 and whether there would be enough fruit to send through commercial prune processing equipment. Twenty-seven to 46 kg fruit were harvested from each genotype. Fresh fruit sugar analysis was conducted on three randomly selected subsamples of ten fruit each from each of the ten genotypes. After the fruit were dried, three 10-fruit subsamples of each genotype were again randomly selected for dried sugar analysis and the rest were processed using commercial machinery. Three more 10-fruit subsamples of processed fruit from each variety were also analyzed for sugar contents.

Fresh Juice Preparation. The fresh fruit juice preparation in 2010 and 2011 was the same except for the numbers of subsamples and genotypes tested. From the 10-fruit sample or subsample, 10 fruit slices were blended in a standard food blender. The resultant juice was strained from the flesh using cheesecloth mesh and centrifuged at 4°C at 17,000 × g for 10 min. The clarified supernatant was removed and submitted to the UCD Analytical Lab for high-performance liquid chromatography (HPLC) sugar analysis as described by Richmond et al., (1981).

Dried Fruit Preparation. In 2010 and 2011, the remainder of the fresh fruit that was not used for fresh sugar analysis was dried in a Harvest Saver R-4 dehydrator (Commercial Dehydrator Systems, Inc., Eugene, OR). Fruit from each genotype was dried for 20 to 25 hr at 73.8 °C to ~ 20% moisture. The dried fruit was separated according to screen size according to industry practice. Screen sizing used screens with specific sized holes to sort dried fruit by size. The largest A screen designation separated fruit with a dried mass of 9.07 grams or larger.

Three 10-fruit subsamples from each genotype were selected from the A screen or larger for sugar analysis. In both years, fruit flesh and skin were removed from the pit and ground in liquid nitrogen to obtain a homogenous sample. The ground dried flesh was then submitted to the UCD Analytical Lab for sugar and sorbitol profiling. In 2011, when more than one tree was harvested per genotype, each tree was tested separately. Thus, if two trees were harvested for a specific genotype, six subsamples were taken for that genotype, three from each tree.

Analysis of Processed Fruit. In 2011, the dried fruit remaining after sampling for sugar analysis were combined to create one large dried fruit sample for each genotype. The bulk samples were then sent through conventional processing machinery at Taylor Brothers Farms (Yuba City, CA). Processing consisted of a high-pressure steam bath for 12 minutes followed by pitting in a PP prune pitter (Ashlock Company, San Leandro, CA) and a spray application of 50% potassium sorbate sufficient to coat each fruit. Potassium sorbate is used by the prune industry to prevent growth of mold and yeast. The fruit was then stored in sealed plastic bags for several weeks to allow the moisture to equilibrate. Subsequent to storage three 10-fruit subsamples per genotype were tested for sugars as explained above.

Data Analysis. The sugar concentrations provided by the UCD Analytical Lab were on a "percent of a given sample" basis. Values were normalized according to their respective sample sizes to provide fresh weight (FW) concentrations of g/100g. The term FW is used to describe the fruit at a non-100% dry weight basis, for example: 80% moisture juice; 20% moisture dried fruit; or 30% moisture processed fruit.

Results and Discussion

2010 Fresh Sugar Analysis. Both the total sugars and the ratios between glucose, fructose, sucrose, and sorbitol varied substantially among fruit from different genotypes (Table I). 'Improved French', the primary prune cultivar grown in California, had the most sorbitol. Interestingly, 'Sutter' had relatively high sorbitol concentrations but even higher sucrose concentrations; this cultivar

Table 1. Total sugar and proportions of glucose, fructose, sucrose, and sorbitol in 17 genotypes of fresh (FW) and dried plum (FW) in 2010.

Variety	Fruit State	Fresh Glucose (g/100g)	Fresh Fructose (g/100g)	Fresh Sucrose (g/100g)	Fresh Sorbitol (g/100g)	Total Sugars[y] (g/100g)
Imp. French	Fresh	7.40	2.30	5.10	7.30	22.10
	Dried[z]	23.10	10.30	3.28	23.98	60.70
Sutter	Fresh	5.50	1.90	9.80	6.40	23.60
	Dried[z]	17.77	10.83	12.40	18.80	59.80
Muir Beauty	Fresh	4.30	1.60	9.60	4.80	20.30
	Dried[z]	17.10	10.37	14.10	17.77	59.30
D6N-103	Fresh	6.90	3.10	4.00	4.60	18.60
	Dried[z]	23.93	12.57	4.33	17.73	58.60
D2N-76	Fresh	6.30	2.10	4.70	5.70	18.80
	Dried[z]	20.93	10.17	3.63	20.90	55.60
D18S-12	Fresh	3.40	1.20	5.10	4.30	14.00
	Dried[z]	14.27	7.27	14.47	20.70	56.70
F9N-21	Fresh	6.00	2.60	3.30	4.90	16.80
	Dried[z]	24.27	12.63	2.00	22.47	61.40
F13S-46	Fresh	4.00	1.20	8.30	4.70	18.20
	Dried[z]	19.83	10.40	12.37	17.20	59.80
F13N-24	Fresh	3.30	1.60	6.80	4.90	16.60
	Dried[z]	18.07	11.17	6.20	18.67	54.10
G3S-2	Fresh	5.30	2.60	6.50	3.60	18.00
	Dried[z]	19.63	12.50	11.70	14.37	58.20
D10S-8	Fresh	5.00	1.90	6.20	5.90	19.00
	Dried[z]	20.23	11.23	9.57	18.63	59.70
F2N-32	Fresh	9.60	4.70	3.80	4.50	22.60
	Dried[z]	22.33	11.43	8.10	14.90	56.80
Sugar	Fresh	6.00	2.20	4.70	4.70	17.60
	Dried[z]	24.90	13.13	0.40	18.57	57.00
Burton	Fresh	4.00	1.30	5.70	3.50	14.50
	Dried[z]	18.07	8.50	14.67	14.37	55.60
3-8E-46RR	Fresh	4.40	2.60	4.00	5.00	16.00
	Dried[z]	17.67	13.10	0.27	17.67	48.70
F11N- 27	Fresh	3.10	1.60	13.40	6.40	24.50
	Dried[z]	11.87	7.80	23.17	16.73	59.60
E6S-12	Fresh	4.60	1.70	13.00	5.00	24.30
	Dried[z]	16.83	10.27	27.17	10.17	64.40

[z] n=3

[y] Sum of glucose, fructose, sucrose and sorbitol

was discovered to have problems withstanding rough handling during drying and processing a few years after its release in 2000. The Glu/Fru ratio has been widely used as a taxonomic trait and the ratios among the genotypes in this study were similar to other reports (vanGorsel et al., 1992; Wrolstad and Shallenberger, 1981). The Glu/Fru ratios ranged from 1.69 ('3-8E-46RR') to 3.33 ('F13S-46'), within the range of 0.9 to 4.1 reported for Rosaceous species in general.

2010 Dried Fruit Sugar Analysis. As expected, the concentrations of sugars in the dried fruit samples were approximately three times greater than the concentrations in the fresh fruit, reflecting water loss during drying (Table I). However, fresh and dried fruit sugar ratios changed upon dehydration. For example, sucrose in 2-8E-46RR went from 4.0 g/100g in the fresh fruit to 0.27 g/100g in the dried fruit, despite the water content in the fruit decreasing by 60%. This suggests that sucrose was indeed hydrolyzed into glucose and fructose. There was a consistent slight decrease in the Glu/Fru ratio from fresh to dried across all genotypes, thus suggesting that fructose degraded faster than glucose.

2011 Fresh, Dried, and Processed Fruit Sugar Analysis. The fresh weight (FW) sugar concentrations normalized to a fresh weight basis for fresh, dried, and processed fruits from ten plum genotypes in 2011 are shown in Table 2. FW Sugar concentrations increased from fresh to dried and decreased slightly from dried to processed due to the decrease in water content upon drying and increase upon processing rehydration. In 2011, the total sugars (the sum of glucose, fructose, sucrose, and sorbitol) were generally lower than in 2010. An extreme example was FW fresh juice of 'Muir Beauty' which had 20.3 g total sugars/100 g in 2010, but only 14.5 in 2011. The difference between years varied with the variety; e.g. fresh juice of 'F13N-24' had a less extreme difference of 16.6 g total sugars/100 g in 2010 and 15.8 in 2011. Except for 'F9N-21', total sugars were lower in 2011 than in 2010. The genotypes with the most sorbitol, 'F9N-21', 'D2N- 76', 'F13N-24', 'D13N-53'and 'Improved French', were highest in both years. Similarly, the genotypes with low sorbitol were consistently low in both years. For example, 'E6S- 12' had the least sorbitol and most sucrose. As with sorbitol, the genotypes with the most sucrose were consistent in both years ('E6S-12', 'Muir Beauty', 'D10S- 8', and 'F13N-24'). Due to the loss of water, a majority of the high fresh juice sucrose genotypes gained in g/100g of sucrose upon dehydration. Despite the drastic decrease in water, low sucrose items had a decrease in sucrose when going from fresh to dried to processed. More specifically, the 4 genotypes with some of the lowest sucrose, 'D6N-103', 'Improved French', 'F9N-21' and 'D2N-76' had some of the largest changes in the ratio between sorbitol and sucrose between fresh and processed fruit. The sum of reducing sugars changed very little in relationship to the sorbitol from fresh to dried to processed. Apparently the sucrose lost in hydrolysis was compensated for by the increase in glucose and fructose. Despite the change in water status, the various genotypes had consistent sugar proportions as fruit were dried and processed. The sugar with the most variation between fresh and processed fruit was sucrose while fructose exhibited the least variation.

For a more uniform sugar comparison, the moisture in each fruit type was subtracted from the sugar concentrations to determine an estimated molar sugar concentration in 100% Dry Weight (DW) fruit. There were substantial variations in soluble solids among genotypes. The fresh juice of 'E6S-12' had the most sucrose and least sorbitol, glucose, and fructose while F9N- 21 had the highest amount of sorbitol. Even on a DW basis, the concentrations of sugars changed from fresh to dried: glucose and fructose increased, sucrose decreased, while sorbitol remained relatively stable. The sugars changed independent of each other and there was little correlation between changes in sugars and initial sorbitol concentrations (Fig. 1). The

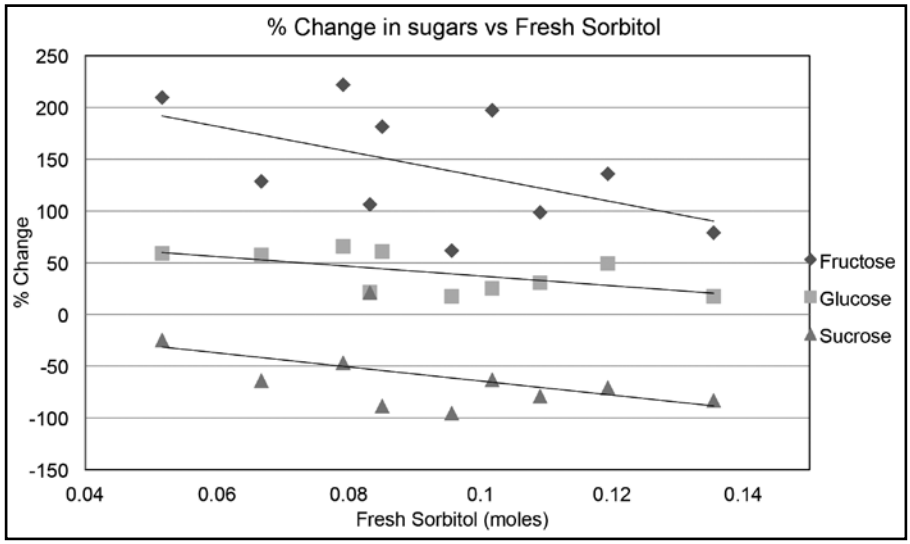

Fig. 1: Correlation between percent change in reducing sugars from fresh to processed fruit and fresh fruit sorbitol concentration in 2011. R squared values were R^2=0.2804, R^2=0.3516 and R^2=0.233 for fructose, glucose and sucrose respectively.

change in DW molar sugar concentrations from dried to processed fruit in 2011 was much smaller than the change observed from fresh to dried fruit in both years. This was not surprising, since fruit experienced just 15 minutes of heat during processing compared to 20 hours during drying. In general, processing increased fructose concentrations and decreased sucrose concentrations. The sorbitol and glucose concentrations changed least and no pattern was detected.

The total molar DW of sugars of most genotypes increased as the fruit went from fresh to dried to processed, independent of the initial ratio. 'E6S-12' and 'Improved French', with the highest and lowest sucrose to sorbitol ratios, respectively both had increased total sugars after drying and processing. Since no additional sugar was added at any time during this experiment, it is possible that upon dehydration and rehydration some other non-sugar, non-water part of the fruit also degraded. If so, calculating sugar concentrations from percentages could give the illusion that the sugar is increasing when instead other parts of the fruit are being lost. Plum fruit does release volatile compounds through enzymatic reactions when heated (Stacewicz-Sapuntzakis et al., 2001). The disappearance of certain phenolic compounds from *P. domestica* upon drying has been reported (Raynal et al., 1989). Sabarez et al. (2000) identified changes in volatiles that occur in prunes during dehydration due to MR and/or caramelization.

The fruit quality of the processed fruit was different according to the genotype. Despite this, there was no direct correlation between processing quality and sugar profiles. Fruit size, flesh texture and skin thickness are all factors that might influence processing as much as high sorbitol. The fresh flesh texture and skin thickness are two influential factors in processing tolerance that need to be addressed in future research.

Sorbitol and sucrose effects on sugar changes. The soluble solid ratios changed during dehydration and processing as sucrose likely hydrolyzed and created more glucose and fructose. Glucose and fructose were also likely degraded in the MR, but the actual rate of degradation was impossible to

quantify in this study since the breakdown of sucrose likely added more reducing sugars at the same time. Contrary to expectations, high sorbitol fruit concentrations did not seem to inhibit sugar degradation. Under the conditions of this experiment, high fresh fruit sorbitol concentrations had little to no influence on sucrose hydrolysis during drying and processing and fruit with low sorbitol concentrations exhibited less change in sucrose concentrations between fresh and processed fruit (Fig. 1). Another surprise was the greater variability in the percent change in fructose than in glucose between fresh and processed fruit. There was an apparent positive correlation between fresh fruit sucrose concentration and the change in fructose during processing (Fig.2). This is especially interesting because a similar relationship was not observed with the other reducing sugar, glucose. This suggests that glucose may be more susceptible to the Maillard reaction than fructose or that more fructose than glucose is produced when sucrose is hydrolyzed. The greater changes in fructose may correspond with the differences in observed changes from dried to processed fruit. Fructose consistently increased during rehydration while glucose did not.

The 17 California selections that were tested in 2010 had slightly different sugar concentrations than 15 previously tested European cultivars (Forni et al., 1992). The European cultivars had total sugars ranging from 9.3% to 26.6%. The total sugars reported here for California cultivars in 2010 ranged from 19.9% to 33.0% (Table 2). The same cultivars often produce fruit with higher sugar in California's climate than when grown elsewhere in the U.S. or in Europe. The cultivar 'Sugar', common to both studies, produced 11.28 g total sugars /100 g in Europe but 17.6 g total sugars/100 g in California (Table I). In both studies, glucose was 34% of the total sugars in this cultivar. Fructose, sucrose, and sorbitol were 8.3%, 41.5%, and 16.0% of total sugars in Europe, respectively, but 12.0%, 26.7% and 26.7%, respectively, in California. These data, along with the 2010 and 2011 comparison of genotypes, suggest the relative sugar makeup of a plum fruit genotype is consistent among years and environments. There was greater variability in the specific fruit sugar ratio between European and California cultivars. The European cultivars had higher sucrose and glucose concentrations than fructose and sorbitol. In

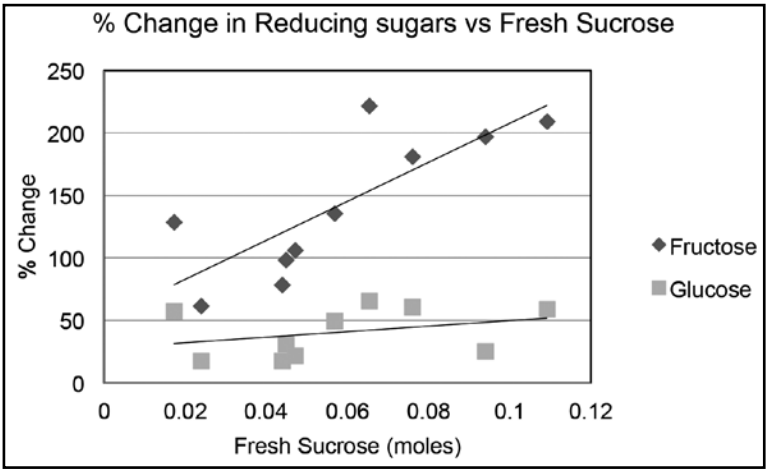

Fig. 2: Correlation between percent change in reducing sugars from fresh to processed fruit and fresh fruit sucrose concentration in 2011. R squared values were R^2=0.6321, R^2=0.1063 for fructose, and glucose respectively.

Table 2. Fruit sugar composition dry weight (DW) concentrations in fresh, dried and processed plums from 10 genotypes in 2011. Concentrations were calculated from the percentage of estimated DW.

Genotype	Fruit type	Glucose		Fructose		Sucrose		Sorbitol	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE
Muir Beauty	Fresh Juice	20.17	0.46	5.49	0.15	32.16	0.54	18.53	0.64
	Dried	23.01	0.31	12.47	0.19	14.86	0.56	18.35	0.75
	Processed	25.89	0.48	16.7	0.13	12.21	1.33	18.54	0.76
F9N-21	Fresh Juice	26.17	0.67	8.80	0.23	15.02	0.65	24.66	0.72
	Dried	29.81	0.78	13.70	0.23	3.23	0.49	24.20	0.59
	Processed	31.24	1.28	15.95	0.42	2.57	0.72	25.86	0.30
D2N-76	Fresh Juice	22.06	0.62	7.26	0.12	15.33	1.00	19.86	1.20
	Dried	27.83	0.37	12.55	0.40	4.47	0.44	23.11	0.75
	Processed	29.26	0.73	14.63	0.17	3.25	0.48	23.40	0.15
D10S-8	Fresh Juice	17.93	0.67	6.54	0.43	16.11	1.76	15.15	0.78
	Dried	22.73	0.29	14.13	0.12	19.79	1.35	16.18	0.29
	Processed	22.11	1.83	13.70	0.96	19.77	4.62	16.69	1.73
F13N-24	Fresh Juice	15.46	0.72	6.66	0.32	19.46	0.59	21.73	1.18
	Dried	21.31	0.40	13.15	0.31	9.13	0.80	25.40	1.63
	Processed	23.47	0.52	15.95	0.47	5.79	0.40	23.66	1.06
Imp. French	Fresh Juice	19.83	0.71	7.47	0.28	5.91	0.86	12.15	1.38
	Dried	31.88	0.30	13.70	0.16	2.06	0.37	18.18	0.82
	Processed	31.70	0.35	17.34	0.87	2.17	0.08	23.94	1.19
D6N-103	Fresh Juice	29.69	0.90	11.59	0.73	8.18	1.13	17.42	0.95
	Dried	34.26	0.86	16.13	0.62	1.26	0.27	17.62	0.27
	Processed	35.44	0.47	19.02	0.44	0.39	0.10	17.30	0.85
Burton	Fresh Juice	15.36	0.84	4.56	0.45	22.39	1.021	14.42	1.21
	Dried	23.83	0.81	12.51	0.59	12.44	1.41	17.76	1.00
	Processed	25.87	0.74	14.93	0.63	12.16	1.36	18.72	0.96
E6S-12	Fresh Juice	12.84	0.62	4.35	0.21	37.36	2.318	9.40	0.64
	Dried	18.70	0.65	10.65	0.36	28.98	1.03	11.26	0.68
	Processed	20.72	1.06	13.67	1.31	28.54	1.29	14.58	1.95
D13N- 53	Fresh Juice	19.64	6.07	7.50	2.14	16.1	4.032	21.19	7.09
	Dried	24.60	0.50	14.20	0.80	3.81	0.93	24.47	0.37
	Processed	25.84	0.05	16.60	0.25	3.03	0.13	25.32	0.62

contrast, the California genotypes were more variable: some selections had high glucose and sucrose, others had high sucrose and sorbitol, and yet others had high glucose and sorbitol (Table 1). Only one California genotype, 'F2N-32', had a greater percent fructose than sucrose.

Sugar concentrations among genotypes changed from 2010 to 2011, confirming that sugar concentrations were influenced by environment, as reported for peaches (Brooks et al., 1993; Cantin et al., 2009). Spring and summer temperatures differed substantially between the two years of the study. 2010 had a cool spring and summer, while 2011 had a very wet spring and hot summer. These factors and others like crop load could have influenced the differences in sugar concentrations in 2010 and 2011. Despite these differences, there was a positive correlation

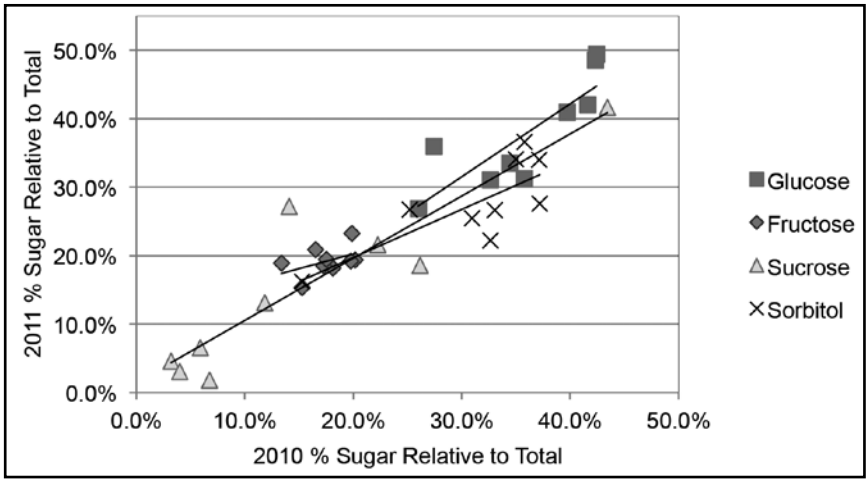

Fig. 3: Correlation between the percentages of four sugars in dried plums from nine genotypes in 2010 and 2011. Each data point is a mean of at least three subsamples.

between the data from 2010 and 2011, suggesting that the relative ratios of fruit sugars remained relatively stable from year to year (Fig. 3). This consistency can help the breeding program predict the ratio of sugars a genotype will produce under commercial cultivation. The length of the trend lines in Figure 3 also indicates the variability of each sugar type between years. For example, fructose only varied from ~ 14% to 23%, producing a very short trendline, while sucrose had a much wider variation from ~ 4% to 45%. These data on germplasm variability in specific sugars may indicate the breeding potential for increasing or decreasing different fruit sugars. For example, breeding a high-fructose fruit would be more challenging than a high-sucrose fruit because of the lower variation in fruit fructose content the germplasm.

Differences in the acidity of the plums might explain why fructose and glucose did not degrade at the same rate during processing. When heated, fructose and glucose stability is dependent on pH (Shallenberger and Mattick, 1983). Therefore, changes in sugar ratios may be influenced by fruit acidity. Fructose is most stable between pH 4 and 6, while glucose is more stable between pH 2 and 6 (Shallenberger and Mattick, 1983). Malic acid is a primary acid in fresh plums. Malic acid combined with fructose increases the tendency for fruit browning (Livingston, 1953). Fructose and glucose did not change at the same rate during processing in this experiment. It is possible that fruit pH and titratable acidity could have influenced the rates of sugar catabolism in the diverse California germplasm used in this project however details on titratable acidity at each step in the processing were not determined.

This experiment was conducted to determine what effect different sorbitol concentrations had on the other sugars within the fruit. The expectation was that genotypes with higher sorbitol concentrations would have less degradation of sucrose, fructose, and glucose when heated during drying and processing. The various concentrations of sorbitol in the tested genotypes did not affect the rates of change in sucrose, fructose, and glucose upon dehydration. Since fruit of genotypes with high sorbitol concentrations reacted during processing the same as low-sorbitol genotypes, it is not likely that sorbitol affects the rate of hydrolysis or caramelization of other fruit sugars. Sucrose hydrolyzed upon heating regardless of sor-

bitol concentration, which did influence the percent change in fructose concentration. While sorbitol did not stabilize other fruit sugars as expected, it was relatively resistant to breakdown during heating and thus was the most stable sugar compound in the fruit during processing. Thus, while high fruit sorbitol may not help stabilize other sugars during fruit processing, selecting for genotypes with high sorbitol may still increase a fruit's ability to withstand processing if the sorbitol concentration relative to that of other sugars is high. Sorbitol's known preservative qualities likely assist in other aspects of dried fruit quality.

Literature Cited

Brooks, S.J., J.N. Moore, and B.J. Murphy. 1993. Quantitative and qualitative changes in sugar content of peach genotypes [*Prunus persica* (L.) Batsch.] J. Amer. Soc. Hort. Sci. 118: 97-100

Cantin, C.M., Y. Gogorcena, and M.A. Moreno. 2009. Analysis of phenotypic variation of sugar profile in different peach and nectarine [Prunus persica (L.) Batsch] breeding progenies. J. Schi Food Agric. 89:1909-1917.

Cinquanta, L., M. De Matteo, and M. Esti. 2002. Physical pre-treatment of plums (*Prunus domestica*). Part 2. Effect on the quality characteristics of different prune cultivars. Food Chem. 79:233-238

California Dried Plum Board 2013. Nutrition 15 May 2013. Nutrition. <http://www.californiadried-plums.org/nutrition/nutrition-composition>

DeJong, T.M., S.J. Castro and C.J. DeBuse. 2012. Dried plum cultivar development evaluation 85 CPB 3. Research Report submitted to the California Prune/Dried Plum Board, Sacramento, CA.

DeJong, T.M., S.J. Castro and C.J. DeBuse. 2011 Dried plum cultivar development evaluation 85 CPB 3. Research Report submitted to the California Prune/Dried Plum Board, Sacramento, CA.

Fayle, S.E., and J. A. Gerrard. 2002. The maillard reaction.The Royal Society of Chemistry.

Forni, E., M.L. Erba, A. Maestrelli, and A. Polesello. 1992. Sorbitol and free sugar content in plums. Food Chem. 44:269-275.

Genard, M., F. Lescourret, L. Gomez, and R. Habib. 2003 Changes in fruit sugar concentration in response to assimilate supply, metabolism and dilution: a modeling approach applied to peach fruit (Prunus persica). Tree Physiology 23: 373-385

Kim, H.Y., M. Farcuh, Y. Cohen, C. Crisosto, A. Sadka, and E. Blumwald. 2015. Non-climacteric ripening and sorbitol homeostasis in plum fruits. Plant Science 231 (2015) 30-39.

Layne, R.D., and D. Bassi. 2008.The peach, botany production and uses. CAB International.

Livingston, G.E. 1953. Malic acid-fructose reaction. J. of the Am. Chem. Soc. 75:1342-1343

Raynal, J., M. Moutounet, and J. Souquet. 1989. Intervention of phenolic compounds in plum technology. 1.changes during drying. J. Agric. Food Chem. 37: 1046-1050

Richmond, M., S. Brandao, J. Gray, P. Markakis, and C. Stine. 1981. Analysis of simple sugars and sorbitol in fruit by high-performance liquid chromatography. J. Agric. Food Chem. 29:4-7

Sabarez, H.T., W.E. Price, and J. Korth. 2000. Volitile changes during dehydration of d'Agen prunes. J. Agric. Food Chem. 48:1838-1842.

Seymour, G.B., J.E.Taylor, and G.A. Tucker. 1993. Biochemistry of fruit ripening. Chapman & Hall, London. p.390.

Shallenberger, R.S., and L.R. Mattick. 1983. Relative stability of glucose and fructose at different acid pH. Food Chemistry 12:159-165.

Stacewicz-Sapuntzakis, M., P.E. Bowen, E.A. Hussain, B.I. Damayanti-Wood, and N.R. Farnsworth. 2001. Chemical composition and potential health effects of prunes: a functional food?, Critical Reviews in Food Science and Nutrition, 41:4, 251-286

van Gorsel, H., C. Li, E.L. Kerbel, M. Smits, and A.A. Kader. 1992. Compositional characterization of prune juice. J. Agric. Food Chem. 40:784-789.

Wilford, L.G., H. Sabarez, and W.E. Price. 1997. Kinetics of carbohydrate change during dehydration of d'Agen prunes. Food Chem. 59: 149-155.

Wrolstad, R.E., and R.S. Shallenberger. 1981. Free sugars and sorbitol in fruits— a compilation from the literature. J. Assoc. of Official Analy. Chemists. 64:91-103

Yoo, B., and M. Lee. 1993. Thermoprotective Effect of sorbitol on proteins during dehydration. J. Agric. Food Chem. 71: 190-192.

Journal of the American Pomological Society 70(3): 149-157 2016

SEM Observations, Pollen Viability and Germination in Some Selected Plum Genotypes Cultivated in Romania

Ioana Mitre Jr.[1], Viorel Mitre[1], Erzsebet Buta[1], Rodica Pop[1], and Radu E. Sestraş[1]

Additional index words: *Prunus domestica,* pollen tube, pollen grain

Abstract

The pollen morphology, viability, germination and pollen tube growth of six wide-spread plum genotypes (*Prunus domestica*), were studied. 'Toptaste', 'Topfive' and 'Gras ameliorat' had the largest pollen grains (> 50 µm), whereas 'Jojo', 'Anna Späth' and 'Stanley' cultivars were represented by slightly smaller pollen size (45.28 - 48.41µm). Pollen shape was prolate-sheroidal (L/W ratio 1.18-1.02 µm) in the polar view and spherical-triangular in the equatorial view. Pollen viability varied with the genotype: the highest percentage was observed for 'Jojo' (40.96%) and 'Toptaste' (40.87%), the lowest was recorded for 'Gras ameliorat' (26.33%). The highest pollen germination (87.0%) at the end of 24 h incubation at 10°C was recorded for 'Stanley'. 'Anna Späth' developed the longest pollen tubes (1061.1 µm) when the pollen grains were incubated at 15°C for 24 h.

Plum is an important stone fruit species for industrial and domestic processing and for fresh consumption (Glišić, 2012; Nikolić, 2012). In Romania, plum is the major fruit species cultivated on 67,478 ha area with a yield of 424,468 tonnes (according to FAO, 2012). Romania holds the 4th place in the production of plums following China, Serbia and USA and the 2nd spot in Europe after Serbia (Coman, 2010; www.faostat.fao.org). Regarding this, many breeding initiatives are focused on developing new genotypes with favorable morphological traits, self-fertility, resistance to drought, frost and pest/diseases (Sharafi, 2013). To carry our breeding research, determination of the viability and germination potential of pollen is one of the most important factors. Conserving and cryopreserving pollen of the highest viability and germination potential from desirable genotypes/cultivars is important for the further improvement of valuable plums cultivars (Ćalić, 2013). Breeders, as well as growers, are often interested in the pollen viability status of their crops (Pline, 2002; Cosmulescu, 2015) to facilitate breeding programs.

Many studies evaluated pollen grain quality through morphological observations (mainly pollen size determination), staining for viability estimation and *in vitro* germination analyzes (Ćalić, 2013; Nikolić, 2012). Scanning electron microscopy (SEM) can provide information about the surface of pollen. This method is used to identify, discriminate and classify different genotypes (Komai, 2014). Pollen viability is crucial in breeding programs, in understanding the sterility issues, while providing important data for taxonomy, systematics and evolutionism (Ćalić, 2013; Nikolić, 2012; Sharafi, 2011b).

Knowledge of pollen germination potential is instrumental not only in setting the value of a cultivar as pollinator in infertile combinations, but also in establishing the biological condition of pollen as a result of the influence of some exogenous factors (preservation, pesticides, virus and fungal diseases). Generally, in order to obtain successful pol-

[1] University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Faculty of Horticulture, 3-5 Mănăştur 400372, Romania, Fax. +40-264-593.792, ioana_mitre@yahoo.com, mitreviorel@yahoo.com, ebuta2008@yahoo.com, prodica64@yahoo.com

[2] rsestras@usamvcluj.ro (corresponding author)

lination, the pollen must have a germinability of 30% (Cordea, 2014).

Viability and germination percentage depends on genotypes and are strongly influenced by temperature, humidity and physiological processes (Petrişor, 2012) with direct impact on yield (Glišić, 2012; Sorkheh, 2011b). Several staining method were used and tested to determine, pollen viability of plums and other species (Bolat, 1999; Ćalić, 2013; Tosun, 2007). However, there are few studies in the literature where pollen viability was evaluated using potassium iodide staining.

The aim of this communication is to describe pollen morphology and to determine the pollen viability and germination rate of six plum genotypes ('Jojo', 'Toptaste', 'Topfive', 'Anna Späth', 'Stanley' and 'Gras ameliorat'). In addition, the effect of temperature, incubation time upon pollen germination and pollen tube length was studied.

Materials and Methods

The experiment was conducted at the University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Romania. Pollen grains of six plum genotypes ('Jojo', 'Toptaste', 'Topfive', 'Anna Späth', 'Stanley' and 'Gras ameliorat') were studied. In early April 2015, 10 white flower buds (balloon stage) were harvested from 15 trees of each genotype, and brought to the laboratory where anthers (pre-anthesis stage) were extracted. Petals and sepals were separated and the anthers were wrapped in tracing paper and left to dry for 24 hrs at room temperature (20-24ºC), which allowed the anther to dehisce. Pollen grains thus collected were used immediately.

SEM observations. SEM studies were conducted to highlight any morphological differences in pollen of the six plum genotypes. For the pollen grain morphological characterization different aspects were studied: shape, length, width and number of colpi in the polar and equatorial view, as reported by Ćalić (2013). Scanning electron micrograph images were taken at 140 x and 900 x using VEGA SEM LSU microscope (TESCAN, Ltd.). Pollen grains were placed directly on the microscope stubs and were studied by 2D and 3D details. Ten pollen grains were observed for each genotype.

Confocal microscope (Zeiss CLSM 710 Confocal Laser Scanning Microscope, supported by Zeiss ZEN 2009 software) was used to find depth information of pollen ornamentation. Small quantities of pollen grains were placed by a brush on the microscope slide. Ten pollen grains per genotype were observed. Exine architecture of pollen grains were taken at 10,000 x.

Pollen viability. Pollen viability was estimated after the anthers were kept in a Carnoy's solution for 2 hrs, after which they were washed in 80% ethyl alcohol. Potassium iodide (25%) stain was used to detect pollen viability or sterility (Bolat, 1999; Cordea, 2014). Five Petri dishes were used per genotype. The pollen grains of each genotype were observed in 4 fields (microscopic area) per Petri dish (20 fields/genotype). In each field, 40-60 pollen grains were counted, therefore a total of 4800 to 7200 grains were observed. The brown pollen was considered viable, and the colorless unviable. The percentage of viable pollen was determined with a digital microscope (LCD Aigo Digital Microscope EV5610).

Pollen germination. To assess germination, pollen grains were cultured in a medium containing 1.5 g agar, 15 g sucrose and 100 ml distilled water heated until the mixture become transparent (but not boiled), according to the methodology describe by Cordea (2014) and used in the same studies by Radičević (2013) and Firmage (2001). Solidification of medium occurs in 10-15 min depending on the prepared layer thickness. When the medium became cold, the pollen grains were scattered uniformly with a brush on the solid medium. Five Petri dishes was used for each genotype. The pollen grains of each genotype were observed in 4 fields (microscopic area) per Petri dish (20 fields/genotype). In each field, 30-45 pollen grains

were counted and a total of 3600 to 5400 pollen grains were observed. Germination was evaluated one, three and 24 hrs after sowing, by recording the total number of germinated and non-germinated grains (Cordea, 2014; Sestraş, 2004), using a digital microscope (Aigo Digital Microscope EV5610 - Beijing Research Institute of Precision Instrument Aigo Co., Ltd). To determine the effect of temperature, pollen was tested at 10, 13, 15 and 20ºC. A pollen grain was considered germinated when the pollen tube was equal to or greater than the diameter of the pollen grain (Sorkheh, 2011a; Wang, 2005).

Pollen tube length. Pollen tube length (PTL) was observed as the germination capacity in the same medium at various temperatures (10, 13, 15 and 20ºC) and time exposures (1, 3 and 24 hrs). PTL was recorded directly with an ocular micrometer fitted to the eyepiece of the microscope based on the micrometer scale (µm). Mean pollen tube length was calculated as the average length of 15 pollen tubes measured randomly on four microscopic areas per Petri dish. The microscope area was 50.24 mm^2.

Statistical analysis. Experimental design was a completely randomized design and the treatment structure was a factorial with six temperature/time combinations and six genotypes (plum cultivars), and five replicates per each variant. A 2-way ANOVA, including the interaction term, had illustrated that the interactions were significant; an LSD was used to compare temperature/time combinations within each genotype. A one-way ANOVA for each genotype was performed in Excel, and Duncan's New Multiple Range Test (Sestras et al., 2012), was used to compare genotypes within each temperature/time combination.

Results and Discussion

SEM observations. Results described in this study regarding the pollen morphology and exine sculpturing are similar to the results reported by Arazani (2005), Ćalić (2013), Gilani (2010), Youshihiro (1989) for pollen of the genus *Prunus*. Morphological observations of the genotypes are presented in Figures 1 and

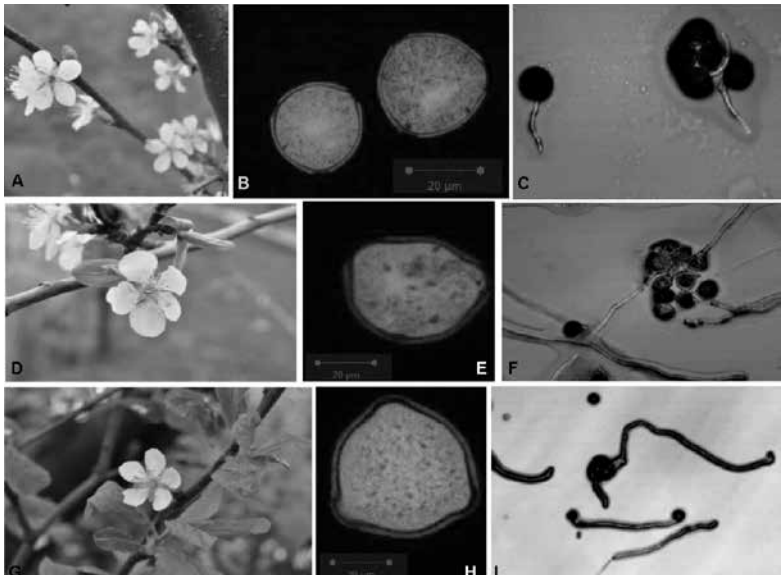

Fig. 1. (A, B, C). Mature flowers, pollen grain shape and germination in 'Jojo'. (D, E, F) Mature flowers, pollen grain shape and germination in 'Toptaste'. (G, H, I) Mature flowers, pollen grain shape and germination in 'Topfive' (Bars=20 µm)

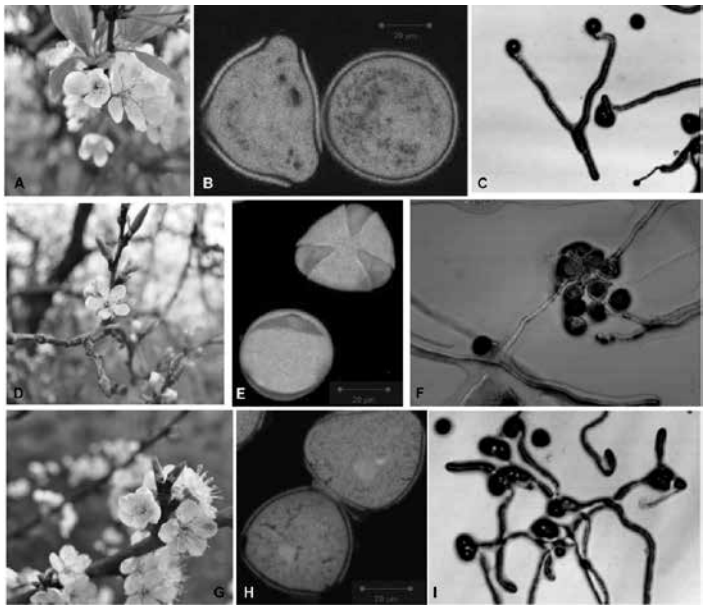

Fig. 2. (A, B, C). Mature flowers, pollen grain shape and germination in 'Stanley'. (D, E, F) Mature flowers, pollen grain shape and germination in 'Anna Späth'. (G, H, I) Mature flowers, pollen grain shape and germination in 'Gras ameliorat' (Bars=20 µm)

2. All had isopolar, symmetrical and tricolporate pollen grains. 'Toptaste', 'Topfive' and 'Gras ameliorat' had large pollen (> 50µm), whereas 'Jojo', 'Anna Späth' and 'Stanley' had medium pollen size (48.41-45.28 µm). Microscopic analysis reveals that the pollen shape was prolate-sheroidal in the polar view and spherical-triangular in the equatorial view. The length – width in the polar view was 1.18-1.02 µm. Polar diameter (length) varied from 45.26 µm ('Anna Späth') to 55.45 µm ('Topfive'). The horizontal distance (width) was between 38.18 µm ('Anna Späth') and 54.17 µm ('Topfive'). The length – width ratio in the equatorial view was 0.84 µm and 0.97 (Table1). The exine surface was striate confirmed by Ćalić (2013) with three colpi as reported by Gilani (2010) (Fig. 1 and 2).

Pollen viability. Pollen viability is important in the pollination process and seed production (Rodriguez – Riano, 2000). For most species, the viability deteriorated more rapidly if strict storage conditions were not maintained (Adhikari, 1998; Kearns 1993).

Previous results showed that pollen viability estimation depends on staining method, genotypes and environmental changes (Cavusoglu, 2013; Sorkheh, 2011a). Pollen viability differed for various genotypes (Bayazit, 2011; Sorkheh, 2011a) of plum and almond.

In the current study, pollen viability varied among the six genotypes. Pollen viability was significantly higher (<38%) for 'Jojo', 'Toptaste' and 'Anna Späth's than for the other genotypes (26.3 to 34.4%). 'Gras ameliorat' had the lowest viability (26.33%) and 'Stanley' had the lowest germination.

Pollen germination. Temperature is the important factor controlling plant growth and development and could affect pollen performance. Pollen germination temperature is a major factor in fertilization success and implicitly in crop production (Sorkheh, 2011b).

Temperature ranges and the influence of temperature on germination was studied for different plant species, including almond (Sorkheh, 2011b), sweet cherry (Radičević,

Table 1. Morphological characteristics (length, width and L/W ratio) of pollen grains for six plum genotypes (μm)

Morphological traits (μm) of pollen	Plum genotypes					
	Jojo	Toptaste	Topfive	Anna Späth	Stanley	Gras ameliorat
Length	48.41	54.25	55.45	45.26	45.28	52.13
Width	41.36	52.03	54.17	38.18	40.90	45.71
L/W ratio polar view	1.17	1.04	1.02	1.18	1.10	1.15
L/W ratio equatorial view	0.85	0.95	0.97	0.84	0.90	0.87

2013; Tosun, 2007), plum (Sharafi, 2013), medlar (Cavusoglu, 2013), pecan (Peng, 2015) and sour cherry (Blidariu, 2008). The optimum temperature required for pollen germination was about 15°C to 20°C for apricot, sour cherry and sweet cherry (Bolat, 1999).

The results of pollen germination (%) at various time exposures and temperatures are summarized in the Table 2. The current study demonstrates that the pollen's optimum germination temperature was 20°C for 24 h, for four of the six genotypes. Pollen germination at 20°C for 24 h ranged from 39.2% ('Topfive') to 85.6% ('Jojo'), whereas some authors (Botu, 2002; Glišić, 2012) reported lower values. Lower temperatures were more effective for 'Stanley' and shorter exposure time was effective for 'Gras ameliorat'.

In this study, the higher germination percent (87.0%) was recorded for 'Stanley' (10°C, 24 h) followed by 'Anna Späth' with 84.5% (20°C, 24 h). 'Anna Späth' is able to germinate rapidly only in one hr after sowing, at a temperature of 20°C (61.8%), but the means were not significantly different than other genotypes. 'Gras ameliorat' achieved a low germination percent (9.20%) at the same exposure time and temperature level. 'Gras ameliorat' had a high germination level (58.7%), if the exposure time was longer (3 h) at the same temperature (20°C).

'Anna Späth' had the higher germination rate (78.3%), followed by 'Jojo' (75.2%) and 'Stanley' (75.5%) and differences were significant. Surprisingly, at 10°C (24 h exposure) 'Stanley' achieved the highest germination percent (87.0%), germination for 'Jojo' was 67.7% (Table 2) and the remaining genotypes exceeded the minimum germination level (25-30%) according to Cordea (2014) and Nikolić (2012).

Increasing the germination temperature to 15°C had a negative influence on germination of 'Toptaste' (28.6%), 'Topfive' (39.2%), 'Gras ameliorat' (23.6%) and 'Stanley' (57.1%). 'Jojo' (75.2%) and 'Anna

Table 2. The influence of temperature for varying lengths of time on pollen germination (%) of six plum genotypes

Temp.		Pollen germination (%)[z]											
(°C)	Hrs.	Jojo		Toptaste		Topfive		Anna Späth		Stanley		Gras ameliorat	
20	1	43.8f	B	4.1e	D	7.0b	D	61.8cd	A	32.1c	C	9.2d	D
20	3	45.8e	CD	70.5a	AB	35.5a	D	80.3ab	A	68.5b	AB	58.7a	BC
13	3	4.1d	B	47.2bc	C	26.4ab	D	78.3abc	A	73.9ab	AB	24.2c	D
10	24	67.7c	B	33.8cd	C	36.1a	C	60.0d	B	87.0a	A	43.8b	C
15	24	75.2b	A	28.6d	C	39.2a	BC	65.9bcd	AB	57.1b	AB	23.6c	C
20	24	85.6a	A	59.7ab	BC	39.2a	D	84.5a	A	75.5ab	AB	56.2ab	CD
Average		**63.70**		**40.38**		**30.79**		**71.90**		**65.67**		**35.97**	

[z] Values are means of five replicates per each variant: genotype x treatment combination (temperature and time). Mean separated by different letters denote significant differences (Duncan test, $P < 0.05$) for treatment combination across rows (upper case letters) and for genotypes down columns (lower case letters).

Table 3. The influence of temperature for varying lengths of time on pollen tube length (PTL) of six plum genotypes

Temp.		Pollen tube length (μm)[z]											
(°C)	Hrs.	Jojo		Toptaste		Topfive		Anna Späth		Stanley		Gras ameliorat	
20	1	514.7b	A	447.5b	BC	428.1b	C	488.2c	AB	492.2c	AB	62.1d	D
20	3	847.6a	A	824.5a	B	620.7a	D	535.0b	E	806.8a	C	458.6b	F
13	3	169.7e	D	104.1e	E	450.8b	C	453.4c	C	578.0b	A	523.0a	B
10	24	331.6c	A	141.1d	B	79.1d	D	71.8d	E	109.4e	C	63.2d	F
15	24	246.0d	C	218.0c	CD	289.3c	B	1061.1a	A	202.0d	D	130.5c	E
Average		**421.96**		**347.05**		**373.85**		**521.95**		**437.74**		**247.52**	

[z] Values are means of five replicates per each variant: genotype x treatment combination (temperature and time). Mean separated by different letters denote significant differences (Duncan test, $P < 0.05$) for treatment combination across rows (upper case letters) and for genotypes down columns (lower case letters).

Späth' (65.9%) had an increased germination rate comparatively with 67.7%, respectively 60.0% at 10°C (Table 2). Germination was improved when pollen was exposed to 20°C for 24 hrs.

Pollen tube length (PTL). Germination and pollen tube length are the most important properties in fruit trees related to pollen quality and reproduction capacity (Sharafi 2011b; Moshtagh, 2015). According to Sharafi (2011b) for some *Prunus* genotypes high pollen germination was not associated with high pollen tube length (PTL), however for some genotypes high germination percentage was accompanied by high PTL.

Pollen tube length, in the current study, was measured at several temperature levels and time exposures (Table 3). Due to rapid development, pollen tube length at 20°C for 24 h exposure couldn't be measured. PTL varied from 62.1μm ('Gras ameliorat' 20°C, 1h) to 1061.1 μm ('Anna Späth' 15°C, 24 h). According to other studies (Sharafi, 2013) PTL varied from 353.6-642.4 μm (24 h, 24°C) in some plum genotypes. PTL measured by Sharafi (2011c) was between 70-1201 μm (24 h, 22°C) in 20 loquat cultivars. According to Moshtagh (2015) apple pollen tube length ranged between 99.65 to153.62 μm (36 h, 24°C).

In the current study, maximum pollen PTL (1061.1 μm) was observed for 'Anna Späth' at 15°C after 24 h. Ćalić (2013) recorded a maximum PTL of 822 μm (exposure 24 h) in *Prunus domestica* cv. Požegača.

'Jojo' had the significantly highest PTL (514.7 μm) at 20°C for 1 h whereas 'Gras ameliorat' had the shortest pollen tubes (62.1μm). 'Toptaste' had higher PTL (824.5 μm) after 3 h at a temperature of 20°C but exposure to 13°C resulted in the lowest PTL (104.1 μm). Similar results were reported in other studies for *Prunus persica* (Sharafi, 2011a) and *Prunus domestica* (Sharafi, 2013). 'Topfive' had the highest PTL (620.7 μm) when pollen grains are held at 20°C for 3 hrs and lower temperatures (10°C) caused shorter PTL (79.1 μm).

The highest PTL (1061.1 μm) in this study was for 'Anna Späth' when pollen were exposed to 15°C for 24 hrs. However, the most studied cultivars develop the highest PTL at 20°C, while Sorkheh (2011) found that PTL was greatest at 25°C.

If the pollen grains were kept a longer time (24 h), but at a lower temperature (10°C), the PTL of 'Anna Späth' and 'Gras ameliorat' was significantly shorter (71.8 and 63.2 μm, respectively). 'Stanley' can develop rapidly (after 3 h) the highest PTL (806.8 μm) when the pollen grains are kept on solid medium at 20°C, but in case of 'Gras ameliorat' the PTL can become higher (523.0 μm) after 3 h even if the temperature is lower (13°C). These results corresponds well with results in plum found by Ćalić (2013) and our results indi-

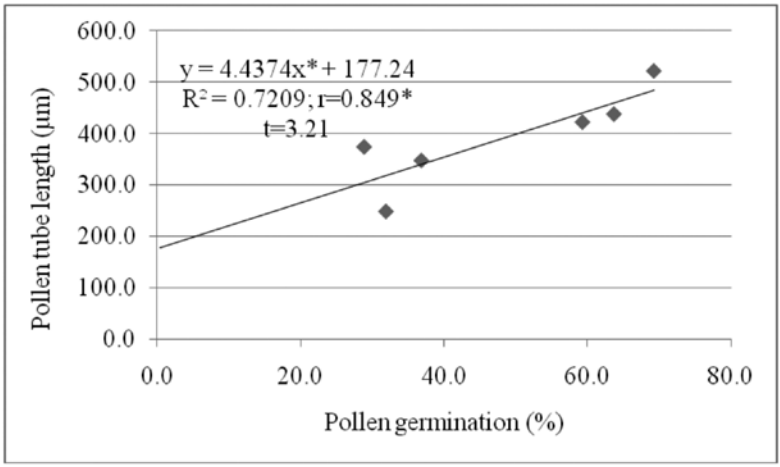

Fig. 3. Relationship between pollen tube length and pollen germination.

cate that pollen tube length can be genotype dependent.

When pooled over all six combinations of temperature and exposure time there was a significant and positive linear relationship between pollen germination and pollen tube length ($R^2 = 0.72$; Figure 3).

Conclusions

This study is important to understanding and improving plum reproduction biology, particularly pollen production, fruit set and fruit quality (Asma, 2008; Sutyemez 2011). Some size and index differences were measured among the genotypes; the pollen was generally similar in shape and exine structure. Pollen grain sizes varied depending on genotype (1.02 µm L/W for Topfive to 1.17 µm L/W for 'Jojo').

The results show that pollen germination can be influenced by temperature as found by Sorkheh (2011b). The germination rate was positively related to exposure time. Pollen germination responded to temperature differently for genotypes reported by Nikolić (2012).

Pollen of self-fertile genotypes, such as 'Jojo', 'Anna Späth', 'Stanley' can germinate rapidly (after 3 h at 20ºC), and pollen of these genotypes could germinate at 10ºC and 15ºC, respectively. For 'Stanley' the highest germination percentage (87.0%) occurred at 10ºC for 24 h.

Pollen tube length (PTL) varied significantly according to genotype and environmental conditions (exposure time and temperature). 'Anna Späth' had the highest PTL (1061.1 µm), after 24 h at 15°C. Similar PTL was recorded for 'Stanley' (578.0 µm) and 'Gras ameliorat' (523.0 µm) after 3 h at 13°C. These results can be helpful in future breeding programs.

Acknowledgements

This paper was published under the frame of European Social Fund, Human Resources Development Operational Programme 2007-2013, project no. POSDRU/159/1.5/S/132765.

Literature Cited

Adhikari, K.N., and C.G. Campbell. 1998. In vitro germination and viability of buckwheat (*Fagopyrum esculentum* Moench) pollen. Euphytica 102(1):87-92.

Arazani, K., M.A. Nejatian, and G. Karimzadeh. 2005. Apricot (*Prunus armeniaca*) pollen morphological characterization through SEM, using the multivariate analysis. New. Zeal. J. Crop. Hort. 33:381-388.

Asma, B.M. 2008. Determination of pollen viability,

germination ratios and morphology of eight apricot genotypes. Afr. J. Biotechnol. 7(23):4269-1273.

Bayazit, S., O. Çalişkan, and I. Burhanettin. 2011. Comparison of pollen production and quality characteristics of cultivated and wild almond species. Chil. J. Agr. Res. 71(4).

Blidariu, A., O. Iordanescu, R. Micu, and A. Dragunescu. 2008. Researches concerning pollen's germination and viability of some sour cherries varieties in the plain area of Banat. Bul. Hort. 65(1):505.

Bolat, I., and L. Pirlak. 1999. An investigation on pollen viability, germination and tube growth in some stone fruits. Turk. J. Agric. For. 23:383-388.

Botu, M., C. Sarpe, S. Cosmulescu, and I. Botu. 2002. The genetic control of pollen fertility, pollenizing and fruit set for the *Prunus domestica* L. plum cultivars. Acta Hort. 577:139-145.

Ćalić, D., N. Devrnja, I. Kostić, and M. Kostić. 2013. Pollen morphology, viability, and germination of *Prunus domestic cv*. Požegača. Sci. Hortic. 155:118–122.

Cavusoglu, A. and M. Sulusoglu. 2013. In vitro pollen viability and pollen germination in medlar (*Mespilus germanica* L.). Int. Res. J. Biological Sci. 2(5):49-53.

Coman, M., M. Butac, D. Sumedrea, I. Dutu, M. Iancu, C. Mazilu, and C. Plopa. 2010. Plum culture in Romania - current status and perspectives. Acta Hort. 968:25-32.

Cordea, M.I. 2014. Plant breeding - Practical works (in Romanian). ed. AcademicPres, Cluj-Napoca, Ro.

Cosmulescu, S., I. Trandafir, and V. Nour. 2015. Chemical Composition and Antioxidant Activity of Walnut Pollen Samples. Not. Bot. Horti. Agrobo 43(2):361-365.

Dalkiliç, Z., and H.O. Mestav. 2011. In vitro pollen quantity, viability and germination tests in quince. Afr. J. of Biotechnol. 10(73):16516-16520.

Firmage, H. D., and A. Dafni. 2001. Field Tests for Pollen Viability; a Comparative Approach. Acta Hort. 561.

Gasparino, E.C., M.A.V. Da Cruz-Barros, A. Chautems. 2013. Pollen morphology in Brazilian species of *Codonanthe* (Mart.) Hanst. and *Nematanthus* Schrader (Gesneriaceae). Grana. 52(4):258-274.

Gilani, S.A., R.A. Qureshi, A.M. Khan, and D. Potter. 2010. Morphological characterization of the pollens of the selected species of genus *Prunus* Linn. from Northern Pakistan. Afr. J. Biotechnol. 9(20):2872-2879.

Glišić, I., C. Radosav, N. Milošević, M. Đorđević, and S. Radičević. 2012. Initial and final fruit set in some plum (*Prunus domestica* L.) hybrids under different pollination types. Genetika 44(3):583-593.

Kearns, C. A., and D. W. Inoyue. 1993. Techniques for pollination biologists. Uni. Press, Colorado.

Komai, F., O. Kanako, I. Yuko, Y. Mitsunori, T. Osamu and K. Susumu. 2014. SEM observation of wet lily pollen grains pretreated with ionic liquid. J. Jpn. Soc. Hortic. Sci. 83(4): 317–321.

Moshtagh, F., A. Dadar, G. E. Moghadam and A. Ahmad. 2015. Investigation on pollen viability, germination, and tube growth in some apple cultivars in climate conditions of Shirvan. J. Appl. Environ. Biol. Sci. 4(12S):295-302.

Nikolić, D.T., V.S. Rakonjac, and M.M. Fotirić-Akšić. 2012. The effect of pollenizer on the fruit set of plum cultivar Čačanska Najbolja, J. Agri. Sci. 57(1):9-18.

Peng, H. Z., Q.Y. Jin, H.L. Ye, and T.J. Zhu. 2015. A novel in vitro germination method revealed the influence of environmental variance on the pecan pollen viability. Sci. Hortic. 181:43–51.

Petrişor, C., V. Mitre, I. Mitre, L. Jantschi, and C.M. Balan. 2012. The rate of pollen germination and the pollen viability at ten apple cultivars in the climatic conditions of Transylvania. Bulletin UASMV 69(1): 417-418.

Pline, W.A., K.L. Edmisten, T. Oliver, J.W. Wilcut, R. Wells, and N.S. Allen. 2002. Use of digital image analysis, viability stains, and germination assays to estimate conventional and glyphosate-resistant cotton pollen viability. Crop Sci. 42(6):2193-2200.

Radičević, S., D. Nikolić, R. Cerović, and M. Đorđević. 2013. *In vitro* pollen germination and pollen grain morphology in some sweet cherry (*Prunus avium* L.) cultivars. Rom. Biotech. Lett. 18(3):8341-8349.

Rodriguez-Riano, T., and A. Dafni. 2000. A new procedure to asses pollen viability. Sex. Plant Repro. 12:241–244.

Sestraş, R.E. 2004. Plant breeding (in Romanian). Ed. AcademicPres, Cluj-Napoca, Ro.

Sestraş, R. E., M. Ardelean, I.M. Cordea, and A.F. Sestraş. 2012. Biostatistics and Forestry Experimental Technique - Support for practical work (in Romanian). Ed. AcademicPres, Cluj-Napoca, Ro.

Sharafi, Y. 2011a. An investigation on the pollen germination and tube growth in some *Prunus persica* genotypes and cultivars. Afr. J. Microbiol. Res. 5:2003-2007.

Sharafi, Y. 2011b. Pollen viability and longevity in some selected genotypes of peach, plum, prune and sour cherry. J. Med. Plants. Res. 5(2):275-279.

Sharafi, Y., A.R. Motallebbi-Azar, and A. Bahmani. 2011c. In vitro pollen germination, pollen tube growth and longevity in some genotypes of loquat (*Eriobotria japonica* Lindl.) Afr. J. Biotechnol. 10(41):8064-8069.

Sharafi, Y., A. Ghanbari, A.M. Naji, A. Kordenaeej, and A. Rezaei. 2013. Comparison of pollen traits of

some plum cultivars of Iran. Intl. J. Agron. Plant. Prod. 4(2):314 -318.

Sorkheh, K., B. Shiran, V. Rouhi, M. Khodambashi, J.N. Wolukau, and S. Ercisli. 2011a. Response of in vitro pollen germination and pollen tube growth of almond (*Prunus dulcis* Mill.) to temperature, polyamines and polyamine synthesis inhibitor. Biochem. Syst. Ecol. 39:749–757.

Sorkheh, K., S.H. Behrouz, R. Vahid, and K. Mahmood. 2011b. Influence of temperature on the in vitro pollen germination and pollen tube growth of various native Iranian almonds (*Prunus l*. spp.) species. Trees 25:809–822.

Sutyemez, M. 2011. Pollen quality, quantity and fruit set of some selfcompatible and self-incompatible cherry cultivars with artificial pollination. Afr. J. Biotechnol. 10(17):3380-3386.

Tosun, F., and F. Koyuncu. 2007. Investigations of suitable pollinator for 0900 Ziraat sweet cherry cv.: pollen performance tests, germination tests, germination procedures, *in vitro* and *in vivo* pollinations. Hortic. Sci. 34(2): 47–53.

Wang, Q., L. Kong, H. Hao, X. Wang, J. Lin, J. Šamaj, and F. Baluška. 2005. Effects of brefeldin A on pollen germination and tube growth. Antagonistic effects on endocytosis and secretion. Plant. Physiol. 139(4):1692–1703.

Journal of the American Pomological Society 70(3): 158-164 2016

A History of Bunch Grapes Research in Mississippi

ERIC T. STAFNE

Abstract

Bunch grapes research in Mississippi has helped shape the current grape industry in the southeastern United States. Cultural management studies, cultivar and rootstock trials, processing (wine, juice, raisin), and Pierce's disease research all contributed to the base knowledge of the region. Research began at Mississippi A&M College as early as 1888 and presently continues at Mississippi State University. The most impactful research over the years was primarily in the areas of Pierce's disease identification and plant response and cultivar development. Norman Loomis, United States Department of Agriculture, pushed grape research forward in Mississippi for decades. His work was influential and eventually led to the release of three bunch grape cultivars, Miss Blanc, Miss Blue, and MidSouth. Today, the grape industry in Mississippi is very small, but with rapidly growing industries in surrounding states it seems only a matter of time before Mississippi follows and grape research once again becomes a substantial area of interest.

It is not often that the bunch grapes (*Vitis* spp. subgenus *Euvitis* Planch.) research done in Mississippi is cited or even recalled in current literature. Mississippi is better known for muscadines (*Vitis rotundifolia* Michx. syn. *Muscadinia rotundifolia* subgenus *Muscadinia* Planch.), but several native species of bunch grapes also exist throughout Mississippi. Bunch grapes have played a significant role in Mississippi horticultural research over the past 125 years, most notably the work of Norman Loomis who worked at the United States Department of Agriculture (USDA) in Meridian, Mississippi, as well as several researchers at Mississippi A&M College (later Mississippi State University). Loomis performed much of the formative research in the early to mid-20th century with contributions from other researchers before and after that time.

For many years bunch grapes research was emphasized by horticultural researchers, especially in the work of trialing new cultivars for the state. At one time or another several experiment stations around Mississippi had bunch grape trials, including those in Beaumont, Crystal Springs, McNeill, Meridian, Ocean Springs, Starkville, Stoneville, and Verona. The primary areas of research can be divided into five main areas: Cultural Studies, Pierce's Disease (PD) (*Xylella fastidiosa* Wells et al.), Cultivar and Rootstock Trials, Cultivar Development, and Processing.

Cultural Studies

Pruning, tipping, thinning, and fertilizer studies were performed to understand how those practices would affect different grape cultivars under Mississippi growing conditions (Overcash, 1950). As in many grape growing regions, one specific concern in Mississippi is frost. Even in south Mississippi frost is a concern, especially with early budbreak cultivars. In one study with 'Extra', Loomis (1939) found that late winter pruning (early to mid-March) retarded growth and may be of use in frost avoidance. Even though late pruning delayed foliation and flowering, it did not affect fruit ripening or subsequent vine growth.

A later study by Loomis (1942) with 'Champanel' examined cane pruning vs. spur pruning. 'Champanel' was known to yield poorly when cane pruned. The study also included a treatment where the tip of the shoot was pinched off after the point of the last in-

[1] Associate Extension and Research Professor, Mississippi State University

This publication is a contribution of the Mississippi Agricultural and Forestry Experiment Station.

florescence. Yields of 'Champanel' improved significantly when spur pruned. The pinching treatment improved cluster weights as well, but not yields. Thus, spur pruning was recommended for 'Champanel', but the value of the pinching treatment was questioned.

Loomis (1949) followed up the earlier 'Champanel' study by expanding on the pinching aspect of the first study. The earlier study showed no significant differences in yield and vine growth between pinched and nonpinched treatments, although the data showed an increase in yield of 3.08 kg with pinching. Although not statistically significant, further examination was warranted. In the later study, more vines were used and the study encompassed five seasons. This additional work showed that pinching 'Champanel' shoots at or just after flower increased fruit yields and decreased pruning weights. Loomis suggested that pinching be used for only very vigorous cultivars and when vines were unbalanced.

Uneven ripening is another problem for many red grape cultivars in the South, especially 'Concord'. Cluster thinning is one strategy to combat uneven ripening. Ragland (1939) cluster thinned 'Campbell's Early', 'Concord', and 'Delaware' in hopes of improving uniformity in fruit ripening. Leaving one cluster per shoot reduced yields by 25% and leaving two clusters per shoot reduced yields by 10%; however, cluster size was increased significantly as was ripening uniformity. For 'Concord', clusters had 20.3% and 9.8% fewer green berries when one and two clusters per shoot were retained, respectively. Thinning also hastened harvest by approximately one week. Although undiagnosed at the time, Ragland described symptoms of "leaf scorch" which was probably PD. Cluster thinning dramatically reduced these symptoms and improved the overall vine appearance, likely due to an overall reduction in vine stress. Overcash (1955a) performed similar studies on 'Concord' and 'Delaware' when grafted to 'Dog Ridge' rootstock.

Another method to alter ripening is through the use of plant growth regulators (PGRs). Overcash (1955b) applied several PGRs to 'Concord', but failed to enhance ripening uniformity. Four PGRs (sodium thiocyanate (NaCNS), 2,4,5-trichlorophenoxypropionic acid (2,4,5-TP), 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), and maleic hydrazide (MH)) were applied at several rates, but no treatment increased ripening uniformity. However, some were phytotoxic to the leaves and shoots, especially 2,4,5-T, which often led to berry shatter.

Much of this work done by Loomis, Overcash, and others, along with research in surrounding states, especially in Florida, helped shape current recommendations for Mississippi (Stafne, 2016). Past research also played a role in determining new research needs. Current vine cultural management research includes crop forcing, compensatory fruit yields, and timing of fruit removal as it affects vine establishment.

Pierce's Disease

Pierce's disease was largely unknown for the first half of the 20th century. It was seen as a mysterious "degeneration". In the 1950s and 1960s it was still considered a virus, not a bacterium (Hewitt et al., 1958). Even as late as the early 1980s the exact cause of PD was undetermined (Stojanovic et al., 1980). Loomis (1958, 1961) reported symptom expression on several cultivars and species of bunch grape. The disease was found in most cultivars, but some lived longer and displayed greater vigor. Lack of these traits was linked to PD infection. Very few cultivars showed no symptoms over a two year period, including 'Dog Ridge' and 'Herbemont'. *Vitis palmata* Vahl was the only species that did not exhibit symptoms or evidence of infection when indexed. 'Beta', 'Concord', and 'Niagara' all showed symptoms and were considered to be short-lived cultivars in areas where PD was prevalent. Loomis opined that it was resistance or klendusity. The study found that seasonal weather conditions played a substantial role in the symptom expression of

the disease. Pierce's disease continues to be a major limitation, because so few commercial quality cultivars are resistant to the disease.

Cultivar and Rootstock Trials

Trialing unproven cultivars, rootstocks, and their numerous combinations were a point of emphasis for Mississippi researchers from the late 19th to the middle of the 20th century. Bunch grapes were widely grown in the eastern U.S., especially 'Concord', but hybrid and *V. vinifera* L. grapes were also making in-roads. The climate of Mississippi is certainly warm enough and with a long ripening season such that grape cultivars can ripen adequately; however, high night temperatures and high humidity make management a challenge. Therefore, it was important to identify cultivars that could survive in these conditions.

An early report from the Mississippi Agricultural Experiment Station (MAES) (1890) stated that 86 cultivars of grapes had been planted for evaluation in 1888. The following year's report (MAES, 1891) described the first harvest of several cultivars. Interestingly, the report said that 10 lb (4.5 kg) of bones obtained from a nearby slaughterhouse were buried under the ground where each vine was planted. 'Brilliant' and 'Niagara' were considered the best after one harvest. Later reports continued to describe the progress of grapevine cultivar trials, with descriptions of fruit quality and disease incidence the primary descriptors (MAMCES, 1892, 1897, 1903). By 1894 the trial had grown to 122 cultivars and two locations, Starkville and Ocean Springs. Fungal diseases such as black rot (*Guignardia bidwellii* (Ellis) Viala and Ravaz) and ripe rot (*Glomerella cingulata* (Stoneman) Spauld. & H. Shrenk) were reported, but no problems with mildews were observed (MAMCES, 1894).

Mississippi A&M College released the first bulletin on growing grapes in Mississippi in 1892 (Tracy and Earle, 1892). The authors recommended grape production in Mississippi as the vines grew well and most diseases could be controlled, aside from bitter rot (*Greeneria uvicola* Berk. & M.A. Curtis). The best performing cultivars in south Mississippi were 'Ives', 'Champion', 'Delaware', 'Niagara', and 'Concord' and best in the north were 'Moore's Early', 'Delaware', 'Brilliant', 'Niagara', 'Eaton', 'Triumph', 'Rommel', and 'Herbemont'. Numerous European (*V. vinifera*) cultivars were tried but were susceptible to spring frost as well as mildew diseases. At this time PD was also unknown and may have contributed to the failure of these cultivars.

By 1895 the cultivar trial grew to 152 (MAMCES, 1895). Insect pests were not mentioned until Earle (1896) described sawfly larvae (*Erythraspides vitis* Harris) and grape leaffolder (*Desmia funeralis* Hübner) as minor problems near the Gulf Coast. The trial was terminated in 1898 and a final report was published by Moore (1899). A total of 124 grape cultivars were described with details of good and bad attributes, but only 11 were recommended as being suitable for shipping and or local market production. Tracy (1900) presented a slightly different list broken down by fruit color and maturity time.

Grapevines were planted again in 1902 at the McNeill Branch Station, with intentions to expand if they proved successful (Ferris, 1903). By 1905, up to 40 more cultivars were being trialed by Mississippi A&M College researchers (MAMCES, 1905). A later publication by Ferris (1922) stated that attempts to establish a rootstock trial with *V. vinifera* cultivars was a complete failure. The losses were attributed to poor growing conditions and later-than-desired planting. Cultivar testing continued after prohibition began on a modest scale, as reported by Anderson (1924) and later by Loomis (1948), but new trials shifted toward evaluating rootstocks.

Many popular grape cultivars, such as 'Concord' and some *V. vinifera*, perform poorly in Mississippi due to factors including high heat, humidity, and disease pressure. To combat these conditions, rootstocks were tri-

aled as an attempt to improve vine productivity, fruit quality, and overall vine health and survival. An early trial (Loomis and Lutz, 1937) focused on the poor ripening of own-rooted 'Concord'. The purpose of the study was to determine if grafting 'Concord' could improve the amount of leaf area produced on the vine, as an earlier study showed promise in yielding better quality fruit when leaf area was greater than 20,000 cm^2 per vine. After testing 10 different rootstocks, they concluded that the use of a rootstock could influence 'Concord' fruit quality via vine vigor and that grafted vines were superior to own-rooted vines. Therefore, they recommended using grafted 'Concord' to improve uneven ripening and lack of vigor.

Soil-borne problems, such as diseases, insects, and excess salinity, may also exist, necessitating the need for tolerant rootstocks (Loomis, 1943). A study in the Gulf Coast region of Mississippi (Poplarville) evaluated 42 rootstocks for their survival and commercial potential (Magoon et al., 1937). Results were not consistent. Some *V. riparia* Michx. x *V. rupestris* Scheele rootstocks performed well and others were failures. Those that contained *V. champini* Planch. (i.e. 'Barnes', 'Joly', 'De Grasset', and 'Dog Ridge') all performed well. In a concurrent study in Meridian, MS (Loomis et al., 1939), 'Dog Ridge' performed well and 'Aramon' x rupestris Ganzin 2 was also rated highly, whereas at Poplarville it was a complete failure. 'Solonis' x Othello No. 1613 also failed at Poplarville, but acceptable at Meridian. The studies differed in that the Poplarville study only evaluated rootstocks while the Meridian study had grafted vines. Several cultivars improved when grafted, depending on the rootstock; some cultivars performed better on certain rootstocks only to die on others. Only eight cultivars out of 58 proved successful when grown on their own roots over a six-year period: 'Champanel', 'Champion', 'Delaware', 'Extra', 'Herbemont', 'Lenoir', 'Marguerite', and 'R.W. Munson'. The study was continued another two years (Loomis et al., 1943) whereupon 'Dog Ridge' was declared the clear superior rootstock; however, they cautioned that scion-rootstock interactions occurred and continued evaluation was necessary to elicit the best combinations for each cultivar.

Two further rootstock trials (Loomis, 1952; Loomis, 1965) also found that the proper scion-rootstock combination was key to success. Of particular note in the 1952 study, a triploid muscadine hybrid proved very successful. It was described in the 1965 study as B-4 5, a 'Scuppernong' x 'Louisiana' hybrid. 'Dog Ridge' again performed well for many cultivars, especially 'Concord' where yields were twice that of other rootstocks in the 1952 trial. In the 1965 study, Loomis suggested that 'Dog Ridge' was the best overall option because it had resistance to PD and nematodes.

In 1972 grape and wine research became a major focus (Hegwood, 1987). By 1975 Mississippi State University researchers were testing 80 grape cultivars at four locations in the state: Crystal Springs, Richton (Beaumont), Stoneville, and Verona. Hegwood (1987) stated that many of the hybrid cultivars tested, including 'Seyval blanc', 'Vidal blanc', 'Chancellor', 'De Chaunac', 'Aurora', 'Baco Noir', 'Chelois', 'Rosette', 'Alwood', 'Moored', 'Carman', and all *V. vinifera* cultivars (Cabernet Sauvignon, Pinot Noir, Chardonnay, Chenin blanc, Sauvignon blanc, and Semillion) died in fewer than 10 years and some by the third growing season. Only 'Villard blanc' was still alive after 10 years. Trials continued on into the 1980s with inclusion of the Florida hybrid grapes, such as 'Lake Emerald' and 'Blue Lake' (Tyner et al., 1982, 1983). Many of the cultivars were removed in 1984 to facilitate more planting space for muscadine vines (Tyner et al., 1984).

Cultivar Development

Three bunch grape cultivars were released from Mississippi State University in the early 1980s: 'MidSouth', 'Miss Blue', and 'Miss

Blanc' (Overcash et al., 1981; 1982). These three cultivars are resistant to PD, making them among the few bunch grape cultivars that can be grown successfully in the Deep South. Presently, they are difficult to obtain and are not available through the nursery trade. However, enterprising hobbyists and some research stations have kept them in circulation. The grape breeding program was originally part of the USDA Horticultural Field Station near Meridian, Mississippi under the leadership of Norman Loomis. The USDA and Mississippi State University collaborated to test new grape selections from the 1940s to the 1970s. 'Miss Blue', resulting from a 'Dog Ridge' x 'Moore Early' cross, has a 'foxy' aroma and flavor from the *V. labrusca* L. parent. 'MidSouth' was also reported to have this characteristic, but it has no *V. labrusca* in its parentage, but rather *V. champini*, *V. vinifera*, *V. rupestris*, *V. berlanieri* Planch., and *V. lincecumii* syn. *V. aestivalis* var. *lincecumii* Buckley (Munson) (Stafne, 2015b). Neither of these grapes were recommended for wine production upon release although Overcash et al. (1981) indicated they might produce wines in the same class as 'Concord' and 'Cynthiana'. Recently, 'MidSouth' has found new life when used in blends with mead (honey wine) (Stafne, 2015a). 'Miss Blanc' was released as a juice grape with potential for use in wine blends (Overcash et al., 1982).

Processing

The A.B. McKay Food and Enology Laboratory was constructed on the campus of Mississippi State University in 1974 to support new agricultural enterprises. It began operation in 1975 which was followed by the Native Wine Act in Mississippi that allowed for commercial production and sale of wine made from grapes produced solely within the state (MSU, n.d.). Wine research by Boris Stojanovic was well under way by the late 1970s and many *V. labrusca*, *V. vinifera*, and French-American hybrid grapes were evaluated for their wine quality when grown in Mississippi environments (Vine et al., 1982). The lab still exists today, but has not made wine for some time.

There is no substantial raisin production outside of California in the United States. However back in the early 1940s, American grapes were dried in an attempt to make raisins. Loomis (1946) stated that this type of production was suggested (without saying by whom), but it could have been part of wartime agricultural efforts. In a study done during 1943 and 1944 on grapes grown in Meridian, MS and Beltsville, MD few of the grapes made high quality raisins. 'Seneca' was deemed the most palatable, but many of those with high amount of *V. labrusca* were described as having an objectionable taste (i.e. foxy). Sun drying was an acceptable method, especially when dipped in lye for a short period of time prior to drying. The conclusion was that American-type grapes could be useful for home use, but not likely for commercial purposes.

Future Directions

Bunch grapes are not big business in Mississippi. There are very few wineries and those in existence primarily use muscadine fruit to produce wine. One of the biggest needs is better cultivars. As Stafne et al. (2015) stated, the South may be the region in the most need of grapevine breeding. Other factors in need of improvement, outside of political challenges, include disease resistance (especially anthracnose (*Elsinoë ampelina* (de Bary) Shear), seedlessness, reduced fruit cracking, higher quality fruit for wine production (both white and red, but particularly red), and increased length of fruit storage.

Currently, several studies are being conducted with bunch grapes in south Mississippi. Cultivar trials are comparing standard PD-resistant and tolerant cultivars (e.g. 'Blanc Du Bois', 'MissBlanc', 'Villard blanc') with newer genotypes that have not been tested under Deep South conditions. From these cultivars, a small-scale breeding program has been initiated to develop new

selections with a focus on disease resistance (PD and anthracnose), loose cluster architecture, and high fruit yields. Other concurrent studies include vine spacing, crop forcing, and different pruning strategies to achieve the best quality fruit in the hot, humid Gulf South climate.

Nearby states like Tennessee, Alabama, and Georgia are enjoying strong, vibrant grape and wine industries that continue to grow year after year. Areas of Mississippi can produce good quality fruit for the processing or fresh markets. The current research projects are on a small-scale, but the pieces are in place to expand if stakeholders dictate the need for it.

Literature Cited

Anderson, W.S. 1924. Some results of grape variety tests at South Mississippi Experiment Station. Miss. Agric. Expt. Sta. Circ. 52.

Earle, F.S. 1896. Fruits and vegetables on the Gulf Coast. Miss. Ag. Mech. Coll. Exp. St. Bull. No. 37.

Ferris, E.B. 1903. Report of work at McNeill Branch Station for 1902. Miss. Agr. Expt. Sta. Bull. No. 79.

Ferris, E.B. 1922. Report from South Mississippi Branch Experiment Station for 1921. Miss. Agr. Expt. Sta. Bull. 201.

Hegwood, C.P. 1987. Viticulture research update, p. 7-9. In: K.H. Remy (ed.). Proc. Second Viniculture Short Course 1987. Miss. Agr. & For. Expt. Sta.

Hewitt, W.B., N.H. Loomis, J.P. Overcash, and G.K. Parris. 1958. Pierce's disease virus in Mississippi and other southern states. Plant Dis. Report. 42:207-210.

Loomis, N.H. 1939. Note on grape foliation as affected by time of pruning. Proc. Amer. Soc. Hort. Sci. 37:653-654.

Loomis, N.H. 1942. The performance of Champanel vines using different pruning treatments. Proc. Amer. Soc. Hort. Sci. 40:310-312.

Loomis, N.H. 1943. Rootstocks for grapes in the South. Proc. Amer. Soc. Hort. Sci. 42:380-382.

Loomis, N.H. 1946. Drying of American-type grapes. Proc. Amer. Soc. Hort. Sci. 47:195-198.

Loomis, N.H. 1948. Trials of grape varieties in Mississippi. Miss. Agri. Expt. Sta. Cir. 144.

Loomis, N.H. 1949. The effect of pinching off the terminals on yield and cane growth of Champanel grapes. Proc. Amer. Soc. Hort. Sci. 54:181-182.

Loomis, N. H. 1952. Effect of fourteen rootstocks on yield, vigor, and longevity of twelve varieties of grapes at Meridian, Mississippi. Proc. Amer. Soc. Hort. Sci. 59:125-132.

Loomis, N.H. 1958. Performance of *Vitis* species in the South as an indication of their relative resistance to Pierce's disease. Plant Dis. Rep. 42(7):833-834.

Loomis, N.H. 1961. Symptom expression and occurrence of Pierce's Disease virus at Meridian, Miss. Proc. Amer. Soc. Hort. Sci. 77:331-336.

Loomis, N. H. 1965. Further trials of grape rootstocks in Mississippi. Proc. Amer. Soc. Hort. Sci. 86:326-328.

Loomis, N.H. and J.M. Lutz. 1937. The relationship of leaf area and leaf area fruit ratios to composition and flavor of Concord grapes. Proc. Amer. Soc. Hort. Sci. 35:461-465.

Loomis, N.H., J.R. Magness, and C.A. Magoon. 1939. Studies on the adaptability of some American grape varieties to southern conditions when grown on their own roots and on certain stocks. Proc. Amer. Soc. Hort. Sci. 37:639-644.

Loomis, N.H., C.A. Magoon, and J.R. Magness. 1943. Further studies on the adaptability of some American grape varieties to southern conditions when grown on their own roots and on certain stocks. Proc. Amer. Soc. Hort. Sci. 42:391-394.

Magoon, C.A., J.R. Magness, W.S. Anderson, and S.R. Greer. 1937. Investigations on the adaptability of grape root stocks to Gulf Coast conditions. Proc. Amer. Soc. Hort. Sci. 35:466-470.

Mississippi Agricultural Experiment Station (MAES). 1890. Second annual report of the Mississippi Agricultural Experiment Station 1889. Agr. College, Miss.

MAES. 1891. Third annual report of the Mississippi Agricultural Experiment Station 1890. Agr. College, Miss.

Mississippi Agricultural and Mechanical Experiment Station (MAMCES). 1892. Fourth annual report of the Mississippi Agricultural and Mechanical College Experiment Station 1891. Agr. College, Miss.

MAMCES. 1894. Sixth annual report of the Mississippi Agricultural and Mechanical College Experiment Station 1893. Agr. College, Miss.

MAMCES. 1895. Eighth annual report of the Mississippi Agricultural and Mechanical College Experiment Station. Agr. College, Miss.

MAMCES. 1897. Tenth annual report of the Mississippi Agricultural and Mechanical College Experiment Station. Agr. College, Miss.

MAMCES. 1903. Sixteenth annual report of the experiment station of the Mississippi Agricultural and Mechanical College. Agr. College, Miss.

MAMCES. 1905. Eighteenth annual report of the experiment stations of the Mississippi Agricultural and Mechanical College. Agr. College, Miss.

Mississippi State University (MSU). n.d. A. B. McKay

Food and Enology Laboratory. Miss. Coop. Ext. Pub. M1492.

Moore, J.S. 1899. Grapes. Miss. Agr. Expt. Sta. Bull. No. 56.

Overcash, J.P. 1950. Pruning and thinning of grapes studied at Experiment Station. Miss. Farm Res. 13(6):7-8.

Overcash, J.P. 1955a. Pruning, cluster thinning, and potash fertilizer experiments with Concord and Delaware grapes growing on Dog Ridge rootstocks. No. 41. Mississippi State College, Miss. Agr. Expt. Sta.

Overcash, J.P. 1955b. Some effects of certain growth-regulating substances on the ripening of Concord grapes. Proc. Amer. Soc. Hort. Sci. 65:54-58.

Overcash, J.P., C.P. Hegwood, and B.J. Stojanovic. 1981. 'MidSouth' and 'Miss Blue' – two new bunch grape cultivars. Miss. Ag. & For. Expt. Sta. Res. Rep. 6(18).

Overcash, J.P., C.P. Hegwood, and B.J. Stojanovic. 1982. 'Miss Blanc' a new bunch grape cultivar. Miss. Ag. & For. Expt. Sta. Bull. No. 909.

Ragland, C.H. 1939. A preliminary report on the effect of cluster thinning on the maturity, yield, and cluster size of grapes. Proc. Amer. Soc. Hort. Sci. 37:661-662.

Stafne, E.T. 2015a. A southern revival? Bunch grapes in Mississippi. American Society for Horticultural Science (ASHS) Community Blogs. 30 March 2016. <http://www.ashs.org/blogpost/1288786/223074/A-Southern-Revival-Bunch-Grapes-in-Mississippi>.

Stafne, E.T. 2015b. MidSouth grape. In: E.T. Stafne (ed.). Mississippi Fruit and Nut Blog. 30 March 2016. <https://msfruitextension.wordpress.com/2015/05/13/midsouth-grape/>.

Stafne, E.T. 2016. Fruit and Nut Review: Bunch Grapes. Miss. St. Univ. Ext. Serv. Inf. Sheet IS-1608.

Stafne, E.T., S. Sleezer, and J.R. Clark. 2015. Grapevine breeding in the southern U.S, p. 379-408. In: A.G. Reynolds (ed.). Grapevine breeding programs for the wine industry. Woodhead Publishing Ltd., Cambridge, UK.

Stojanovic, B.J., J.P. Overcash, and C.P. Hegwood. 1980. Pierce's disease of grapevines in Mississippi. Is it a virus, bacterium, or mycoplasma? Vinifera Wine Growers J. 7(2):96-105.

Tracy, S.M. and F.S. Earle. 1892. Grapes. Miss. Ag. Mech. Coll. Exp. St. Bull. No. 22.

Tracy, S.M. 1900. Grape growing in the South. USDA Farm. Bull. No. 118.

Tyner, F.H., A.J. Laiche, Jr., B. Graves, C.H. Hovermale, and J.D. Davis. 1982. Report of research progress at the Mississippi Agricultural & Forestry Experiment Station South Mississippi Branch 1981. Miss. Agr. & For. Expt. Sta. Info. Bull. 29.

Tyner, F.H., A.J. Laiche, Jr., B. Graves, C.H. Hovermale, J.D. Davis, and I.W. Savelle. 1983. Report of research progress at the Mississippi Agricultural & Forestry Experiment Station South Mississippi Branch 1982. Miss. Agr. & For. Expt. Sta. Info. Bull. 39.

Tyner, F.H., A.J. Laiche, Jr., B. Graves, C.H. Hovermale, J.D. Davis, and I.W. Savelle. 1984. Report of research progress at the Mississippi Agricultural & Forestry Experiment Station South Mississippi Branch 1983. Miss. Agr. & For. Expt. Sta. Info. Bull. 55.

Vine, R.P., G.M. Brooks, and J. Burgin. 1982. Making wines at home. Miss. Agr. & For. Expt. Sta. Info. Bull. 26.

Journal of the American Pomological Society 70(3): 165-168 2016

DNA Markers Based on *PSY* Genes Can Differentiate Yellow- and White-Fleshed Loquats

Weilin Wei[1], Muhammad Qasim Shahid[2], Zhike Zhang[1], Hongyan Song[1], Xianghui Yang[1,3], and Lin Shunquan[1,3]

Additional index words: *Eriobotrya japonica,* fruit flesh color, marker-assisted selection

Abstract

White-flesh is one of the most appreciated traits in loquat. The development of new white-fleshed cultivars is costly and time consuming, involving the generation and selection of thousands of progeny each year. Marker-assisted selection (MAS) is an excellent technique to accelerate conventional breeding approaches. In this study, a specific DNA marker based on the sequence of phytoene synthase (*PSY*) gene was identified as an ideal DNA marker for the identification of fruit flesh color. We screened 31 cultivars derived from different countries and 24 F_1 hybrids developed from 4 cross combinations, and analyzed the relationships between flesh color and the DNA marker. The results showed that the DNA marker not only can distinguish yellow- and white-fleshed cultivars accurately, but can be used for accurate and early selection of yellow and white flesh progeny derived from controlled hybridizations.

Loquat (*Eriobotrya japonica* Lindl.), a member of the Rosaceae, accumulates carotenoids as the main pigments in mature fruit. The fruit can be divided into two groups, yellow- and white-fleshed, according to the color of the flesh (Lin, 2008; Deng et al., 2009). White-fleshed cultivars have become more popular because of their delicious flesh and higher retail selling price. However, breeding loquat is still limited at the seedling selection stage because of the long juvenile period (Wang et al., 2012). Marker-assisted selection (MAS) can shorten the breeding process and enhance breeding efficiency, and is widely utilized in many crops (Lahogue et al., 1998; Dalbo et al., 2001; Chu et al., 2011; Kong et al., 2013; Xu, 2013). With the development of loquat specific markers, DNA markers are widely used in loquat for various purposes, such as assessing genetic diversity(Vilanova et al., 2001; Chen et al., 2007; Wang et al., 2010; Wu et al., 2015), genetic linkage mapping (Gisbert et al., 2009) and germplasm identification (Watanabe et al., 2008; Fan et al., 2012). However, few DNA marker studies have focused on fruit color. Studies by Sheng et al. (2006) and Xie et al. (2012) only studied several white-fleshed cultivars as experimental materials. Phytoene synthase (*PSY*) gene is a very important structural gene of the carotenoid biosynthesis pathway, many studies showed that an increase in the expression of *PSY* leading to a massive carotenoids accumulation (Giuliano et al., 1993; Lois et al., 2000; Kato et al., 2004). Fu et al. (2014) revealed that the white-fleshed loquats contain only the mutant sequence *EjPSY2A*^{d} and the yellow-fleshed loquats may contain both normal sequence *EjPSY2A* and mutant sequence *EjPSY2A*^{d} or only normal sequence *EjPSY2A*. However, this hypothesis requires additional support because only 14 Chinese loquat cultivars (7 yellow-fleshed and 7 white-fleshed), were used as experimental materials. In this study, we hypothesized that sequence diversity of *PSY* gene may provide a means of developing a molecular marker for early selection of

[1] College of Horticulture, South China Agricultural University, 510642, Guangzhou, China
[2] College of Agriculture, South China Agricultural University, 510642, Guangzhou, China
[3] Corresponding author, Email: gzyxh@scau.edu.cn; loquat@scau.edu.cn

yellow- and white-fleshed loquat. A total of 31 cultivars and 24 F_1 hybrid progeny from 4 cross combinations were used as experimental materials to test our hypothesis.

Materials and Methods

Plant materials. Thirty-one cultivars, 21 yellow-fleshed and 10 white-fleshed, were collected from the Loquat Germplasm Resources Garden, College of Horticulture, South China Agricultural University (SCAU). The details of these cultivars are listed in Table 1.

In 2010, 4 cross combinations (Zaozhong No.6×Javierin, Zaozhong No. 6×Peluches, Zaozhong No.6×Ullera, and Zaozhong No. 6×Marc) were hybridized and 24 F_1 hybrid plants were selected and kept for desirable traits. Among them, there were 11 yellow-fleshed and 13 white-fleshed selections.

DNA extraction and PCR amplification. DNA was extracted from young leaves according to the manual of a DNA extraction kit (Beijing Kangwei Biotech Inc., Beijing, China). A biophotometer was used to assess DNA concentration and purity.

A pair of gene specific primers EjPSY-2AUP1 5′-TATGAACCATTGATTAGT-CTAGC-3′ and EjPSY2ADP1 5′-GTTATT-GTCACCGTAGTCGC-3′ were used for amplification (Fu et al., 2014). PCR amplification was performed in a total volume of 25µl, including 0.5µl of each primer (10µM), 1µl template DNA, 10.5µl sterilized ddH_2O, and 12.5µl of 2× Mix buffer (Shanghai Shenggong Inc., Shanghai, China). The PCR profile was initiated with a preliminary step of 5 min at 95°C, followed by35 cycles at 95°C for 30 s, 55°C for 30 s, and 72°C for 1 min, and was terminated with a final extension for 7 min at 72°C. All amplification products were separated on 1.2% (W/V) agarose gels, and stained with ethidium bromide. Photos of the gels were taken and saved with BioradGelDoc XR system (Bio-Rad, USA).

Results

Marker genotyping of loquat cultivars. The results clearly showed that PCR amplification of the *PSY* gene yielded two fragments: a long fragment, 1013bp and a short fragment, 319 bp (Fig.1). All white-fleshed cultivars investigated possessed only the short fragment, indicating these cultivars contained only the mutant sequence *EjPSY-2A*^{d}. However, all 21 yellow-fleshed cultivars showed the long (1013bp) fragment. Of these 21 yellow-flesh cultivars, 13 also showed the

Table 1. Name of loquat cultivars, flesh color and country of origin used in the study.

Code	Cultivar	Flesh color	Origin	Code	Cultivar	Flesh color	Origin
1	Dawuxing	yellow	China	16	Ullera	yellow	Spain
2	Guangrongben	yellow	China	17	Javierin	yellow	Spain
3	Huabao No.3	yellow	China	18	Peluches	yellow	Spain
4	Luoyangqing	yellow	China	19	Dazhong	yellow	China
5	Wanzhong	yellow	China	20	Baozhuqingzhong	yellow	China
6	Meihuaxia	yellow	China	21	Jiefangzhong	yellow	China
7	Zhongzhong No.6	yellow	China	22	Baiyu	white	China
8	Xiangzhong	yellow	China	23	Baili	white	China
9	Moeiowase	yellow	Japan	24	Wugongbai	white	China
10	Golden Nugget	yellow	USA	25	Ruantiaobaisha	white	China
11	Mogi	yellow	Japan	26	Shiromogi	white	Japan
12	Dahongpao	yellow	China	27	Ninghaibai	white	China
13	Chuannao	yellow	China	28	Biqizhong	white	China
14	Nagasakiwase	yellow	Japan	29	Bingtangzhong	white	China
15	Marc	yellow	Spain	30	Tianzhong	white	China
31	Guifei	white	China				

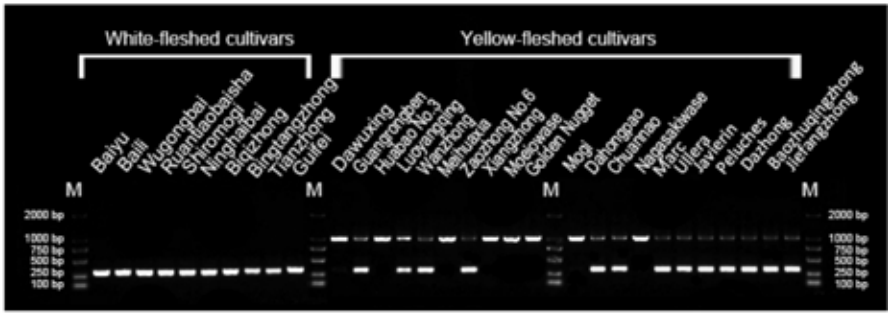

Fig. 1. PCR amplifications of 31 loquat cultivars using *PSY* gene specific marker. M represents DNA Ladder.

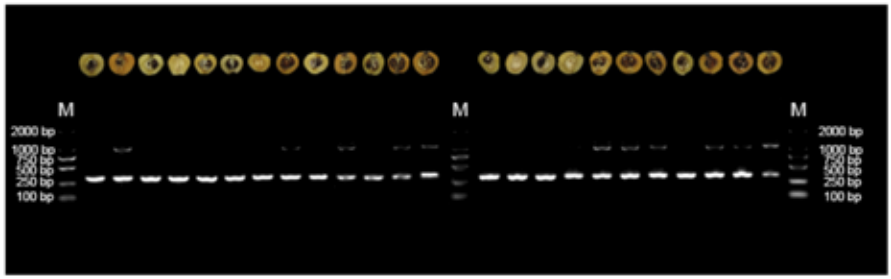

Fig. 2. PCR amplifications of 24 loquat plants derived from F_1 using *PSY* gene specific marker. M represents DNA Ladder.

319bp fragment as well, indicating these cultivars were heterozygous for this gene. The differences in amplification products clearly differentiated the 31 cultivars into yellow- and white-fleshed phenotypic groups.

Marker genotyping of F_1 hybrid plants. In this study, 24 F_1 hybrid plants derived from 4 different cross combinations, which had flowered and bore fruit, were used for marker genotyping. The results revealed that hybrid plants with white-fleshed fruit yielded only one short fragment, while hybrid plants with yellow-fleshed fruit produced two fragments (Fig.2). The PCR amplification results of F_1 hybrid selections were consistent with the known cultivars. This evidence supports our hypothesis that the polymorphisms revealed in the *PSY* gene amplification products using the specific primers described can be used as a molecular marker for early selection of yellow- and white-fleshed loquats.

Discussion

There are also yellow-fleshed and white-fleshed varieties in peach, the yellow color of leaf vein can serve as a user-friendly marker for selection of yellow-fleshed varieties (Ma et al., 2014). However, there is no agronomic/phenotypic character that can differentiate between yellow-and white-fleshed cultivars at the seedling stage in loquat, so the presence of molecular markers will be helpful for accelerating the breeding of loquat. MAS is a very effective technique for fruit trees because it assists in accurate and rapid selection at the seedling stage for various important agronomic traits. The availability of DNA markers is of great significance in a species like loquat with a long period of juvenility. At present, an increasing number of growers prefer to plant white-fleshed cultivars for its high market price, and it is of paramount interest to develop a suitable method to select white-fleshed progeny from controlled cross-

es at the seedling stage. Our study demonstrated that the molecular DNA marker based on the structure of *PSY* gene can successfully distinguish yellow- and white-fleshed cultivars, and F_1 hybrid progeny with 100 % accuracy.

Acknowledgments

This research was partially supported by Guangzhou science and technology project (201504010028). Professor Jules Janick helped with the language editing of this manuscript.

Literature cited

Chen, Y.Y., Z.X. Lai, J.Y. Chen, Y.Q. Cai, Y.Q. Guo, and J.S. Wu. 2007. RAPD analysis of 65 accessions of loquat germplasm (*Eriobotrya* spp.). Chinese J. Tropical Crops 28:65-71.

Chu, Y., C.L. Wu, C.C. Holbrook, B.L. Tillamn, G. Person, and P. Ozian-Akins. 2011. Marker-assisted selection to pyramid nematode resistance and the high oleic trait in peanut. Plant Genome 4:110-117.

Dalbo, M.A., G.N. Ye, and N.F. Weenden. 2001. Marker-assisted selection for powdery mildew resistance in grapes. J. Amer. Soc. Hort. Sci.126:83-89.

Deng, C.J., Z.F. Chen, X.Y. Zhang, L.J. Zhang, L.X. Xie, S. Zheng, X.J. Zhang, Q.H. Li, X.Q. Wei, and Q.Z. Xu. 2009. Diversity analysis of fruit description characteristics of loquat germplasm resources. Fujian Fruits 2:42-47.

Fan, F.H., Y.S. Yang, Q.H. Li, Y. Deng, and X.P. Wen. 2012. Identification of new germplasms of Guizhou loquat using ISSR markers. J. Beijing Forestry Univ. 34:52-57.

Fu, X.M., C. Feng, C.Y. Wang, X.R.Yin, P.J. Lu, D. Grierson, C.J. Xu, and K.S. Chen. 2014. Involvement of multiple phytoene synthase genes in tissue- and cultivar-specific accumulation of carotenoids in loquat. J. Exp. Bot. 65:4679-4689.

Giuliano, G., R.Tavazza, G. Diretto, P. Beyer, and M.A.Taylor. 1993. Metabolic engineering of carotenoid biosynthesis in plants. Trends Biotechnol. 26:139-145.

Gisbert, A.D., J. Martanez-Calvo, G. Llicer, M.L. Badenes, and C. Romero. 2009. Development of two loquat (*Eriobotrya japonica* (Thunb.) Lindl.) linkage maps based on AFLP and SSR markers from different Rosaceae species. Mol. Breed. 23:523-538.

Kong, F.M., J.J. Liu, Y.N. Chen, Z.B. Wan, and T.M.Yin. 2013. Marker-aided selection of polyploid poplars.Bioenergy Res.6:984-990.

Kato, M., Y. Ikoma, H. Matsumoto, M. Sugiura, H. Hyodo, and M. Yano. 2004. Accumulation of carotenoids and expression of carotenoid biosynthetic genes during maturation in citrus fruit. Plant Physiol. 134:824-837.

Lahogue, F., P. This, and A. Bouquet. 1998. Identification of a co-dominant SCAR marker linked to the seedlessness character in grapevine. Theor. Appl. Genet. 97:950-959.

Lin, S.Q. 2008. Loquat, In: Enclopedia of fruits and nuts. CABI, pp:643-651.

Lois, L.M., M. Rodríguez-Concepción, F. Gallego, N. Campos, and A. Boronat. 2000. Carotenoid biosynthesis during tomato fruit development: Regulatory-role of 1-deoxy-D-xylulose 5-phosphate synthase. Plant J. 22:503-513.

Ma, J.J., J. Li, J.B. Zhao, H. Zhao, F. Ren, L. Wang, C. Gu, L. Liao, and Y.P. Han. 2014. Inactivation of a gene encoding carotenoid cleavage dioxygenase (CCD4) leads to carotenoid-based yellow coloration of fruit flesh and leaf midvein in peach. Plant Mol. Biol. Rep. 32:246-257.

Sheng, L.M., H.K. Wang, C.M. Xu, and Z.Zhang. 2006. ISSR analysis of a new individual of white flesh loquat. Jiangsu Agri. Sci. 3:97-100.

Vilanova, S., M.L. Badenes, L. Marinez-calvo, and G. Llaver. 2001. Analysis of loquat (*Eriobotrya japonica* Lindl.) germplasm by RAPD molecular markers. Euphytica 121:25-29.

Wang, L.M., J.Z. Chen, L.X. Ou, and C.H. Cai. 2012. Research and progress on juvenile period in fruit trees. Guangdong Agri. Sci. 10:46-50.

Wang, Y.Q., Y. Fu, Q. Yang, N. Luo, Q.X. Deng, J. Yan, J.G. Zeng, and G.L. Ruan. 2010. Genetic diversity of *Eriobotrya* analyzed by ISSR markers. Sci. Silvae Sin. 46:49-60.

Watanabe, M., O.M. Yamamoto, and Y.S. Nishitanic. 2008. Cultivar differentiation identified by SSR markers and the application for polyploidy loquat plants. J. Jpn. Soc. Hort. Sci.77:388-394.

Wu, D., W.G. Fan, Q.G. Guo, J.S. Anthony, Y.Wang, P.T. Michael, and G.L. Liang. 2015. Genetic diversity of loquat [*Eriobotrya japonica* (thumb.)Lindl.] native to Guizhou province (China) and its potential in the genetic improvement of domesticated cultivars. Plant Mol. Biol. Rep. 33:952-961.

Xie, L.X., J.H. Xu, L.J. Zhang, X.Y. Zhang, S. Zheng, and T. Li. 2012. Genetic relationship analysis of 24 white-fleshed loquat (*Eriobotrya japonica*) germplasms. Fujian J. Agri. Sci. 27:261-266.

Xu, D.J. 2013. Pyramiding of two *BPH* resistance genes and *Stv-b*^{i}gene using marker-assisted selection in *japonica*rice.Crop Breed.Applied Biotech. 13: 99-106.

About The Cover:

Loquat

Loquat (*Eriobotrya japonica*) is an ancient crop that has been known for 2,000 years in China and has been distributed worldwide since the 18th century. Loquat, sometimes called Japanese Medlar or Japanese plum, is a member of the *Rosaceae* family and is probably native to the cooler hill regions of China to south-central China. Loquat was introduced to Japan about 1,000 years ago and quickly became naturalized. Japan is the leading producer of loquats followed by Israel and Brazil. In the U.S., loquat is produced commercially only in southern California, where the fruit is popular in ethnic markets when there are few other local fruits available. Loquat has become naturalized in regions with subtropical to mild temperate climates. In the U.S., Loquat is hardy in USDA hardiness zones 8 and above and will flower only where winter temperatures remain above -1°C. In cooler climates, the tree is grown as an ornamental but will not bear fruit. The best climate may be where trees cease vegetative growth in early fall, followed by cool, but non-freezing winters, and warm springs. It is a small evergreen tree or large shrub, about 5 to 10 m tall, and can be grown as an ornamental plant. Loquat is susceptible to the bacterial disease fire blight (*Erwinia amylovora*). The aromatic white flowers are 2 cm in diameter with five petals and 20 stamens arising from panicles of three to ten flowers. Loquats are considered self-fruitful, but cross pollination by various insects improves fruit set and size. The fruits, three to five cm long, grow in clusters, are oval, rounded or pear-shaped. As the fruit ripens, it changes from green to yellow or orange skin, sometimes with red blush. Each fruit contains one to ten ovules which mature into large brown seeds. Seeds comprise about one-third of the ripe fruit.

Loquat is an unusual crop. Unlike the more popular apple and pear, it is a subtropical species and blooms in autumn; the fruit develops in winter, and ripens in early spring. Loquat is considered a soft fruit and has a short storage life. However, it is versatile and can be consumed fresh or processed as fruit halves or pieces, juice, and wine. The fruit is typically harvested when fully ripe, about 90 days after full bloom. The fruit is sweetest when it is soft and orange and can be stored in refrigeration for one to two weeks. More than 800 cultivars exist in Asia, and cultivars are maintained by grafting onto seedling rootstocks. Based on flesh color, loquat can be divided into two groups: yellow-fleshed and white-fleshed. The fruit is high in sugar (7 to 17 brix), acid, pectin, vitamins (especially vitamin A) and antioxidants and is eaten fresh or is used to make jam, jelly, and chutney. Loquat syrup is used in Chinese medicine for soothing the throat and is an ingredient for cough drops and is thought to reduce vomiting. Leaves can be used to make a tea to relieve diarrhea and may reduce swelling.

Cover photograph: Loquat, courtesy of Xianghui Yang

Instructions to Authors

Journal of the American Pomological Society

The prime purpose of the Journal of the American Pomological Society is to provide a repository for information on all aspects of fruit and nut crops. The long-term emphasis of the journal on cultivars and rootstocks continues, but manuscripts reporting original research on a wide range of fruit and nut crops are welcomed. Acceptable areas of research including pruning, nutrition, growth regulators, cultural practices, economics, and pest control. Studies involving the interaction of one or more of these aspects with either cultivars and/or rootstocks are particularly appropriate. If in doubt about the suitability of a particular manuscript, please contact the Editor.

Reports on field studies are expected to contain data from multiple years. Reports are to be the result of adequately replicated trials and the data should be subjected to appropriate statistical analysis. Manuscripts submitted for publication in the Journal must not have been previously published, and submission implies no concurrent submission elsewhere.

Scientific names and authorities for plants, disease organisms, and insects should be include parenthetically when the organism is first mentioned. American spelling conventions and SI units should be used. The number system is used for literature citations. Manuscripts should be double spaced throughout. Typical organization is as follows: Title, Authors, Abstract, Introduction, Materials and Methods,Results, Discussion, Literature Cited,Tables, Figures. The Results and Discussion sections are often combined. Author addresses and acknowledgements are in footnotes on the first page. More detailed instructions for manuscript preparation can be found at: http://www.americanpomological.org/journal/journal.instructions.html

Prior to submission, manuscripts should be reviewed by at least two colleagues and revised accordingly. **At the time of submission, the corresponding author must attest in the covering letter to the Editor that all co-authors on the paper have had the opportunity to review it prior to submission, that it has not been published previously, and that it is not presently under consideration for publication elsewhere. In addition, the names and full contact information (mailing address, e-mail and telephone numbers) for three potential reviewers should be provided.** Submit three copies of the manuscript to the Editor: Dr. Richard Marini, 203 Tyson Building, Department of Plant Science, University Park, PA 16802-4200 USA; E-mail: richmarini1@gmail.com. Electronic submission is encouraged. Acceptable formats are MSWord or WordPerfect.

Manuscripts are sent to two reviewers competent to evaluate scientific content. Acceptance for publication depends upon the combined judgement of the two reviewers and the Editor. In unusual circumstances the Editor, without further review, may return a manuscript, which obviously does not meet Journal standards, to the author.

A charge of $65.00 per page ($32.50 per half page) will be made to authors for those articles constituting publication of research.

American Pomological Society
2016 Annual Meeting

to be held in conjunction with

The Annual Conference of the American Society for Horticultural Science

8-11 August • Atlanta, Georgia
Sheraton Atlanta Hotel

Call for Wilder Silver Medal Nominations

The Wilder Committee of the American Pomological Society (APS) invites nominations for the 2016 Wilder Silver Medal Award. All active members of APS are eligible to submit nominations. The award was established in 1873 in honor of Marshall P. Wilder, the founder and first president of APS. The award consists of a beautifully engraved medal which is presented to the recipient at the annual meeting of APS, held during the ASHS Annual Meeting.

The Wilder medal is presented to individuals or organizations that have rendered outstanding service to horticulture in the area of pomology. Special consideration is given to work relating to the origination and introduction of meritorious fruit cultivars. Individuals associated with either commercial concerns or professional organizations will be considered if their introductions are truly superior and have been widely planted. Significant contributions to the science and practice of pomology other than through fruit breeding will also be considered. Such contributions may relate to any important area of fruit production such as rootstock development and evaluation, anatomical and morphological studies, or noteworthy publications in any of the above subjects. Information about the award, past recipients, etc. can be found on the APS website at:
http://americanpomological.org/wilder1.html

To obtain nomination guidelines, please contact committee chairperson,

Dr. John R. Clark
Dept. of Horticulture, University of Arkansas
phone: 479-575-2810
fax 479-575-8619
e-mail: jrclark@uark.edu

Nominations must be submitted by 1 May 2016.

JOIN THE *American Pomological* SOCIETY!

Membership Benefits:

- Online and/or printed access to the Journal of APS
- Networking opportunities with world-renowned pomologists
- Award opportunities for superior pomology-related research and student papers
- Leadership opportunities

Individual, Student, and Library Rates Available

Join Online TODAY!

http://americanpomological.org