JOURNAL OF THE AMERICAN POMOLOGICAL SOCIETY

APRIL 2018 Volume 72 Number 2

JOURNAL OF THE AMERICAN POMOLOGICAL SOCIETY

A Publication of the American Pomological Society

April 2018 Volume 72 Number 2

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. Guidelines for manuscript preparation are the same as those outlined in the style manual published by the American Society for Horticultural Science for HortScience, found at http://c.ymcdn.com/sites/www.ashs.org/resource/resmgr/files/style_manual.pdf.

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, C/O Heather Hilko, ASHS, 1018 Duke St., Alexandria, VA 22314; Tel 703-836-4606; Email: ashs@ashs.org

Page Charges: A charge of $50.00 per page for members and $65.00 per page ($32.00 per half page) will be made to authors. In addition to the page charge, there will be a charge of $40.00 per page for tables, figures and photographs.

Society Affairs: Matters relating to the general operation of the society, awards, committee activities, and meetings should be addressed to Michele Warmund, 1-31 Agriculture Building, Division of Plant Sciences, University of Missouri, Columbia MO 65211; Email:warmundm@missouri.edu.

Society Web Site: http://americanpomological.org

Journal of the American Pomological Society 72(2): 74-79 2018

A New Sweet Persimmon: 'Romang'

K.B. Ma, K.S. Cho, S.S. Kang, J.H. Han, Y.K. Kim, H.S. Hwana, and I.S. Shin

Additional index words: *Diospyros kaki,* breeding, fruit characteristics, physiological disorder

Abstract

'Fuyu' and 'Jiro' sweet persimmon were selected and introduced from Japan and account for over 90% of the persimmon production in Korea. The goal of our breeding program is to select domestic sweet persimmon to replace the cultivars from Japan and balance the unequal distribution of late maturing cultivars. 'Romang' was a cross of 'Ro-19' and 'Okugosho' in 1997 and it was selected as '97-23-29' in 2004 for its attractive eating quality and finally selected in 2008 since it had good characteristics with little yearly variation. 'Romang' is the first pollination-constant non-astringent (PCNA) persimmon in Korea. The intermediate growth habit is semi-spreading. Its maturation period as a mid-season cultivar was estimated at about 110 days after full bloom. Average fruit weight was 185 g and the fruit has oblate shape, red-orange skin color, and few physiological disorders such as fruit cracking at the apex.

Depending on characteristics of astringency, presence of seeds, and flesh color (Kajiura, 1946; Kikuchi, 1948), persimmon (*Diospyros kaki*) cultivars are classified into four types as follows: 1) pollination-constant and non-astringent (PCNA), 2) pollination-variant and non-astringent (PVNA), 3) pollination-variant and astringent (PVA), and 4) pollination-constant and astringent (PCA). The PCNA type is qualitatively different from the other three types in the level of tannin accumulation in the fruit because the PCNA-type fruit accumulates less tannin and the tannin cells are much smaller than in the other three types (Yonemori and Matsushima, 1985; Kanzaki et al., 2001). Persimmon is one of the major fruit crops and among the oldest cultivated fruits in Korea. Most cultivars are astringent, and the PVNA cultivar 'Johongsi' is generally known and appeared in Daeduck area of Damyang county, Jeon-nam province. In Korea, all of the sweet persimmons cultivated commercially were introduced from Japan in the 1900s. All nonastringent cultivars are of Japanese origin, except for 'Luo Tian Tian Shi', which is of Chinese origin (Yamada et al., 1993; Yamada et al., 1994; Wang, 1982). In 2014, persimmon production was 385,000 T and ranked second behind China, and is cultivated on 27,000 ha in Korea (Korea Statistical Information Service, 2016). Sweet persimmon's area has dramatically decreased from 20,000 ha in 2000 to 11,800 ha in 2015 (Korea Statistical Information Service, 2016). The main reason for declining production likely is the unequal distribution of cultivars 'Fuyu' and 'Jiro' which have poor fruit quality and ripen late, and are susceptible too cold injury in the fall. Therefore, we need to develop new cultivars that have good fruit quality and ripen earlier than 'Fuyu' and 'Jiro'. The PCNA genotype appears to be homozygous recessive for the natural loss of astringency, since the trait of natural astringency-loss in PCNA-type fruit is qualitatively inherited in the progenies and the PCNA genotype is recessive to the other three types (Ikeda et al., 1985; Kanzaki et al., 2001). Therefore PCNA-type cultivars are usually used to obtain PCNA-type seedings. We aim to develop persimmon cultivars of PCNA-type that are earlier maturing and have less physiological disorders than those introduced from Japan. In this study, we selected a new sweet persimmon cultivar that matures 15 days earlier and has less physiological disorders than 'Fuyu', and fruits had high sugar concentrations and soft juicy flesh.

Materials and Methods

In 1997 'Ro-19' was crossed with 'Okugosho' growing in the persimmon genetic resources orchard at the Pear Research Institute, National Institute of Horticultural and Herbal Science, Rural Development Administration in Korea. The seeds of F_1 progeny were collected as fully ripen fruits. The seeds were washed with water, dried for one day at room temperature, put in a polyethylene bag after treating with Benomyl wettable powder, and stored at 5℃ until used. Sprouted seeds were sowed in 10cm-diameter jiffy pot in May in 1998. Of the 260 seeding, 241 were planted in the breeding field located in Yeongam

National Institute of Horticultural & Herbal Science, RDA, Wanju, 55365, Korea
Corresponding author: Kyeong-Bok Ma, gbma@korea.kr

(34.51N, 126.36E) in April of the following year. A seedling labelled as '97-23-29' was initially selected in 2004 for its good eating quality. After initial selection, seven trees each of '97-23-29', 'R-19', 'Okugosho' and 'Shinsyuu' were propagated on the *D. kaki* seedling rootstock, and planted at a spacing of 5 m between rows and 4 m between trees. 'Shinsyuu' was considered the control for comparison because it ripens at a similar time in Korea and the fruit quality is best of cultivars of similar ripening time. All trees were trained to a central leader growth habit. Fruit traits were examined according to the Manual for Agricultural Investigation (RDA, 2003). We also investigated fruit cracking and fruit apex cracking with the naked eye, and divided cracking-severity into three levels of weak, medium and strong.

To prevent undesirable fruit setting, excessive flower buds were thinned to one bud per spur at 10-15 days before bloom to ensure a leaf-to-fruit ratio of > 20. For stable fruiting, the orchard consisted of about 5% pollinizers and bee hives were placed in the orchard just before bloom.

As trees came into bearing, five fruits were selected to investigate the fruit characteristics from each of seven trees per cultivar for 3 years from 2006 to 2008, and two or three times per season to ascertain the time of optimum maturity. The tree size was about 3.5 m height and 3 m width, and the yield per tree was about 40 kg. After the fruits were weighed, flesh firmness was measured on each side of the fruit with penetrometer (Zwick, DE/ZO 5T3 Kor.) equipped with an 8 mm diameter plunger. Thereafter, total soluble solids concentration was measured on each fruit by expressing juice from each side of the fruit onto a digital refractometer (Atago PR-101, Japan).

Data were analyzed as a completely randomized design with SAS's Proc GLM. When Analysis of Variance indicated that cultivars differed significantly ($P<0.05$) means were compared with Duncan's Multiple Range Test (DMRT) at 5% level using SAS statistical software (V 9.1, SAS Institute Inc., North Carolina, USA).

Description

'Ro-19' was released from a cross between 'Daigosho' and 'Hanagosho', which ripens in mid-Oct. (Naju region, Korea) (Fig.1). It has dense fresh texture, high sugar concentration, and fruit weight averaged 230g, but there is a little fruit cracking and fresh softening at the fruit apex. The other parent of 'Romang' is 'Okugosho', a medium size fruit, with rough flesh texture and a little cracking at the fruit apex. Although the parents have faults, we

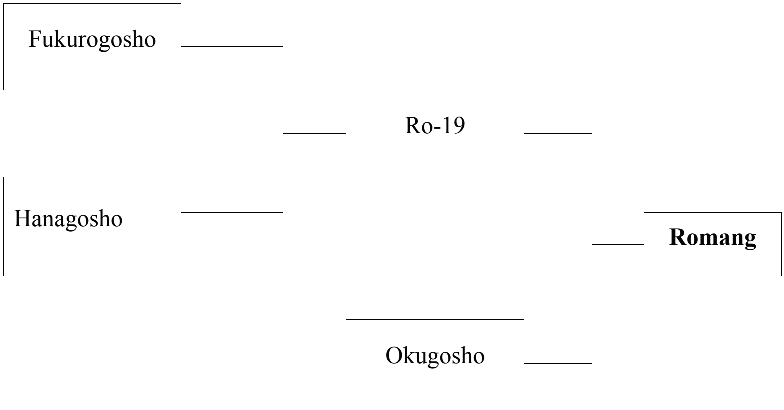


Fig. 1. Pedigree of 'Romang' persimmon

Table 1. Tree characteristics of 'Romang' persimmon compared to its parents, 'Ro-19' and 'Okugosho' and 'Shinsyuu' served as a control at Naju, Korea from 2006 to 2008.

Cultivar	Tree vigor	Tree form	One-Year Shoot					
			Length	Thickness	Color sunny side	Number of lenticels	Size of lenticels	Shape of lenticels
Romang	Medium	Semi-spreading	Medium	Medium	Brown	Many	Medium	Elliptic
Ro-19	Medium	Semi-spreading	Medium	Medium	Grey brown	Medium	Small	Circular
Okugosho	Medium	Semi-spreading	Medium	Medium	Brown	Few	Large	Elliptic
Shinsyuu	Medium	Semi-spreading	Medium	Thick	Brown	Medium	Medium	Elliptic

expected the cross to produce high quality F_1 progeny. The cross produced 241 progenies. We first selected PCNA-type, '97-23-29' strain and named it 'Wongyoba-01' at nine years after crossing in 2006 and in 2008 it was named 'Romang' because it showed little yearly variation and good fruit characteristics (see cover photograph).

'Romang' has moderate tree vigor and semi-spreading tree shape similar to 'Ro-19' and 'Okugosho'. The length of one-year-old shoots is medium, and bark on the sunny side is brown, similar to 'Okugosho'. The number of lenticels tends to be more than its parent and 'Shinsyuu', the size is medium, and the shape is elliptic (Table 1, Fig. 2). The characteristics of mid-shoot leaves were evaluated after shoot extension ceased. Following terminal bud development, mid-shoot leaves ware elliptic, which tended to differ from its parents, ovate for 'Okugosho', and obovate for 'Shinsyuu'. The leaf apex was obtuse, similar to 'Shinsyuu', but different from its parent, that were acute and the leaf base was round (Table 2, Fig. 3). Female flowers typically bloomed on 31 May, which is about three days later than 'Ro-19', and one day later than 'Shinsyuu' (Table 2).

Maturation of 'Romang' fruit was about

Fig. 2. One-year-old shoots of 'Romang' persimmon compared with 'Shinsyuu'.

Table 2. Leaf and flower characteristics of 'Romang', 'Ro-19', 'Okugosho' and 'Shinsyuu' persimmon at Naju, Korea from 2006 to 2008.

Cultivar	Full bloom date	Leaf blade			Flower type
		Shape	Shape of apex	Shape of base	
Romang	31 May.	Elliptic	Obtuse	Rounded	F[z]
Ro-19	28 May.	Ovate	Acute	Rounded	F
Okugosho	29 May.	Ovate	Acute	Obtuse	FM
Shinsyuu	30 May.	Obovate	Obtuse	Obtuse	F

[z] F indicate only female flowers; FM indicates female and male flowers.

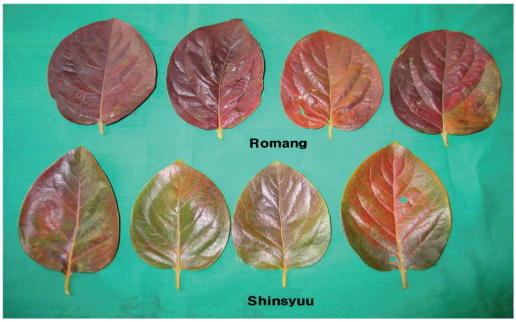


Fig 3. Leaf blade of 'Romang', 'Ro-19', compared with 'Shinsyuu' persimmon.

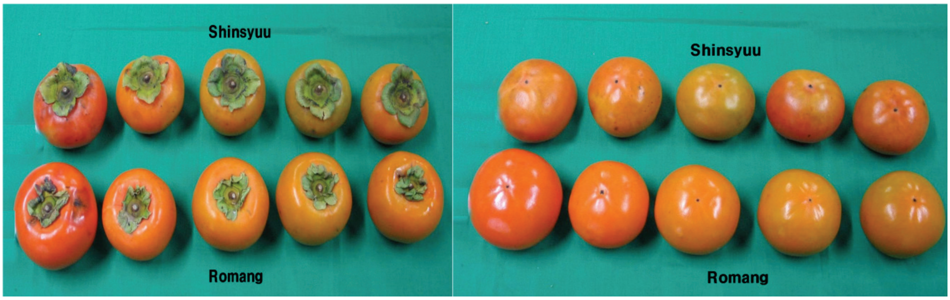


Fig. 4. Fruits of 'Romang' compared with 'Shinsyuu' persimmon.

110 days after full bloom, which was three days and seven days earlier than 'Ro-19' and 'Okugosho', respectively (Table 1, Table 3). The fruits were medium size (185 g), similar to 'Okugosho' (186 g), and smaller than 'Ro-19' (220 g), and 'Shinsyuu' (215 g). 'Romang' fruit shape was oblate, truncate in the apex of longitudinal section, the cross-section was

circular and the red-orange skin color darker than ‘Ro-19’ and ‘Okugosho’, and similar to ‘Shinsyuu’ (Table 2, Table 3). The soluble solids concentration was significantly higher (18.6 %) than ‘Ro-19’ (16.3 %), ‘Okugosho’ (16.2 %) and ‘Shinsyuu’ (16.4 %). ‘Romang’ had significantly softer fresh firmness (64 N) than ‘Ro-19’ (76 N), and ‘Okugosho’ (82 N), but similar to ‘Shinsyuu’ (59 N) (Table 2). Fruit cracking and fruit apex cracking were regarded as serious physiological disorders threatening stable production in Korea, but ‘Romang’ had less fruit cracking and fruit apex cracking than its parents and ‘Shinsyuu’ (Table 3).

Table 3. Ripening time and fruit characteristics of ‘Romang’ persimmon compared to its parents, ‘Ro-19’ and ‘Okugosho’ and ‘Shinsyuu’ served as a control at Naju, Korea from 2006 to 2008.

Cultivar	Maturity[z]	Fruit wt. (g)	Fruit shape	Skin color	Shape of apex in longitudinal section	General shape in cross section
Romang	110	185 b[y]	Oblate	Orange Red	Truncate	Circular
Ro-19	113	229 a	Oblate	Orange	Rounded	Circular
Okugosho	117	186 b	Oblate	Orange	Truncate	Circular
Shinsyuu	106	215 a	Oblate	Orange Red	Truncate	Circular

[z] Days after full bloom.
[y] Means within columns followed by common letters do not differ at the 5% level of significance, by Duncans multiple range test.

Table 4. Fruit characteristics and physiological disorder of ‘Romang’ persimmon compared to its parents, ‘Ro-19’ and ‘Okugosho’ and ‘Shinsyuu’ served as a control at Naju, Korea from 2006 to 2008.

Cultivars	SSC[z] (%)	Flesh firmness (N)	Juiciness	Cracking of fruit apex	Cracking of fruit	Persimmon type[y]
Romang	18.6 a[z]	64 c	High	Little	Little	PCNA
Ro-19	16.3 b	76 b	Moderate	Moderate	Little	PCNA
Okugosho	16.2 b	82 a	Low	Moderate	Severe	PCNA
Shinsyuu	16.4 b	59 c	High	Moderate	Moderate	PCNA

[z] Means within columns followed by common letters do not differ at the 5% level of significance, by Duncans multiple range test.
[y] *type = pollination constant non-astringent.

Availability

Protection for ‘Romang’ was applied for in Sept. 2009 and registered in 2015 (The No. 5520) after DUS (distinctness, uniformity and stability) test for two years by Korea Seed & Variety Service.

Acknowledgements

This work was carried out with the support of “Cooperative Research Program for Agriculture Science & Technology Development (Project No. PJ902919200801)” Rural Development Administration, Republic of Korea.

Literature Cited

Ikeda I., M.Yamada, and A. Kurihara. 1985. Inheritance of astringency in Japanese persimmon. J. Japan. Soc. Hort. Sci. 54:39-45.

Kajiura, M. 1946. Persimmon cultivars and their improvement (2) (in Japanese). Breeding Hort. 1: 175-182.

Kanzaki, S., Yonemori, K., Sugiura, A., Sato, A., Yamada, M. 2001. Identification of molecular markers linked to the trait of natural astringency loss of Japanese persimmon (Diospyros kaki) fruit. J. Amer. Soc. Hort. Sci. 126(1):51-55.

Kikuchi, A. 1948. Pomology-Part Ⅰ (in Japanese). Yokendo, Tokyo, Japan.

KREI. 2016. Agriculture in Korea. Korea Rural Economic Institute

Korea Statistical Information Service. 2016. Statistical Database. Agriculture, Forestry and Fishery.

RDA. 2003. Manual for agricultural investigation. Rural Development Administration, Suwon, South Korea.

Wang, R. 1982. The origin of ‘Luo Tian Tian Shi’. Chinese Fruit Tree 2:16–19.

Yamada, M., A. Sato, H. Yakushiji, K. Yoshinaga, H. Yamane, and M. Endo. 1993. Characteristics of ‘Luo Tian Tian Shi’, non-astringent cultivar of oriental persimmon (*Diospyros kaki* Thunb.) of Chinese origin, and its relativeness to non-astringent cultivars of Japanese origin. Bul. Fruit Tree Res. Sta. 25:19–32.

Yamada, M., H. Yamane, and Y. Ukai. 1994. Genetic analysis of Japanese persimmon fruit weight. J. Amer. Soc. Hort. Sci. 119(6):1298–1302.

Yonemori, K., Matsushima, J. 1985. Property of development of the tannin cells in non-astringent type fruits of Japanese persimmon (Diospyros kaki) and its relationship to natural deastringency. J. Japan. Soc. Hort. Sci. 54(2):201-208.

Journal of the American Pomological Society 72(2): 80-93 2018

Evaluation of Cold-climate interspecific Hybrid Wine Grape Cultivars for the Upper Midwest

Amaya Atucha[1*], Janet Hedtcke[2], and Beth Ann Workmaster[1]

Additional index words: cold-hardy, Wisconsin, Marquette, Frontenac, Brianna, La Crescent, La Crosse

Abstract

Cold-climate interspecific hybrid wine grape cultivars with largely *Vitis riparia* Michx. parentage, including several released since the early-1980s, have created opportunities for new and rapidly expanding grape and wine industries in the Northeast and upper Midwest of the United States. The objective of this study was to evaluate the viticulture performance of a selection of cold-climate wine grape cultivars grown in the upper Midwest, and to provide information on growth, yield, and fruit composition traits. Fruit yield (kg m^{-1} cordon) variation among years was not significant for red cultivars; however, white cultivars had significant differences in fruit yield produced during the extent of the study. 'Marquette', 'Maréchal Foch', and 'La Crescent' produced the most consistent yields among years, while 'Frontenac', 'Brianna', and 'La Crosse' were the top yielding cultivars. Fruit composition traits (soluble solids concentration (SSC) and titratable acidity (TA)) measured at harvest, varied among cultivars and years. 'Marquette' had the highest average SSC, while 'Léon Millot' had the lowest average levels of TA for all years of the red cultivars. Among white cultivars, 'Brianna' had the lowest average levels of TA in all years, and 'La Crosse' had the lowest average SSC. Differences in seasonal weather patterns among years influenced yield, vine vigor, and fruit composition data. 'Aromella' and 'Vignoles' were removed from the study due to poor winter survival, and these cultivars are not recommended for commercial production in growing regions with climate conditions similar to Wisconsin.

Introductions over the twentieth century, as well as into the early 2000s, of several interspecific hybrid wine grape (*Vitis* spp.) cultivars adapted to cooler climates has helped propel the expansion of the wine industry to upper Midwestern states, such as Michigan, Minnesota, Illinois, Indiana, Iowa, Ohio, and Wisconsin (Dami et al., 2005; Luby et al., 2006). The ideal cultivar for this northern temperate climate must be able to withstand low winter temperature extremes, in addition to grow with moderate vigor, produce substantial yields, and possess good fruit quality. Cultivars must also produce consistent and reliable crops. Consistency is key to the success of the wine grape industry in northern temperate zones by securing supplies of local and regional fruit. However, limiting factors, such as severe winter freezing temperatures, late spring frosts, adequate number of frost-free days, and high inter-seasonal variation in precipitation and temperature patterns, significantly challenge the goal of producing consistent high yields of quality fruit. Stress factors such as over- and under-cropping, excessive vegetative growth, disease infections, and drought can impact cold hardiness, overwintering capacities, and general growth of vines (Fennell, 2004; Howell, 2001), and pose an economic threat to growers and winemakers in cold-climate regions (Zabadal et al., 2007).

Cold-climate cultivars have been developed by breeders in France and the United States (Wisconsin, New York, and Minnesota) (Table 1) using primarily riverbank grape (*Vitis riparia* Michx.), as well as sand grape (*V. rupestris* Scheele), fox grape (*V. labrusca*

[1] Department of Horticulture, University of Wisconsin-Madison, 1575 Linden Drive, Madison, WI 53706
[2] West Madison Agricultural Research Station, University of Wisconsin-Madison, 8502 Mineral Point Road, Verona, WI 53593
[*] To whom reprint request should be addressed: Email address: atucha@wisc.edu

Table 1. Description of the nine cold-climate wine grape cultivars included in this study, grown at the West Madison Agricultural Research Station (WMARS) in Verona, Wisconsin (compiled from Smiley et al., 2016 and the National Grape Registry (http://ngr.ucdavis.edu/index.cfm).

Cultivar	**Wine Color**	**Pedigree**	**Institution/Breeder**	**Release/ introduction**
Aromella	White	'Traminette' and Ravat 34	Cornell University	2013
Brianna	White	'Kay Gray' x E.S. 2-12-13 (includes *V. labrusca* and *V. riparia*)	Elmer Swenson of Osceola, Wisconsin	2001
Frontenac	Red	Landot 4511 x MN 89 (includes *V. riparia*, *labrusca*, *vinifera*, *aestivalis*, *lincecumii*, *rupestris*, *cinerea,* and *berlandieri*)	University of Minnesota	1996
La Crescent	White	'St. Pepin' x E.S. 6-8-25 (*V. riparia* x 'Muscat Hamburg') (includes *V. vinifera*, *riparia*, *labrusca*, *aestivalis*, and *rupestris*)	University of Minnesota	2002
La Crosse (ES 294)	White	(MN 78 x Seibel 1000) x 'Seyval' (includes *V. labrusca*, *lincecumii*, *riparia*, *rupestris*, *vinifera*)	Elmer Swenson of Osceola, Wisconsin	1983
Léon Millot	Red	Millardet et Grasset 101-14 O.P. x 'Goldriesling' [sibling of 'Maréchal Foch']	Eugene Kuhlmann of Alsace, France	1920
Maréchal Foch	Red	Millardet et Grasset 101-14 O.P. x 'Goldriesling' (*V. riparia* x *V. rupestris*) x *V. vinifera*) [sibling of 'Léon Millot']	Eugene Kuhlmann of Alsace, France	1920 (France); 1951 (USA)
Marquette	Red	MN 1094 (a complex hybrid of *V. riparia*, *V. vinifera*, and other *Vitis* species) x Ravat 262 (offspring of 'Pinot noir')	University of Minnesota	2006
Vignoles	White	Unclear, either 8-Seibel 6905 x 'Pinot de Corton' or Seibel 5455 ('Plantet') x Seibel 880 (includes *V. vinifera*, *lincecumii,* and *rupestris*)	J.F. Ravat, Montpellier, France	1949

L.), and other *Vitis* species. Many of the European wine grape (*V. vinifera* L.) -based cultivars, which comprise much of wine grape production worldwide, require more than 180 frost-free days to fully ripe fruit and cannot reliably survive the harsh winter climates of places like the upper Midwest. While the hybrids possess improved cold-temperature adaptation, as well as higher degrees of resistance to the pest phylloxera (*Daktulosphaira vitifoliae* Fitch), vineyard practices for optimal fruit production are still being determined. Appropriate cultivar selection and suitable management practices for economically sustainable production of these hybrids are proving to be different from those for *V. vinifera* cultivars and often require modification according to climate patterns, geography, and local site and soil conditions. These issues are being addressed in multi-institutional collaborative research efforts like the Northern Grapes Project (Particka and Martinson, 2016). In 2007, a survey from USDA-Risk Management Agency reported that 87% of Minnesota and 85% of Wisconsin growers had only been growing grapes for less than 10 years and more than 50% of current vineyard operators are looking for information on cultivar selection (Anonymous, 2007). Pest control, canopy management and pruning were among the top five issues cited by Wisconsin growers at commercial vineyards (USDA, 2013b). In Wisconsin, the most popular cultivars growers indicated they would like to plant in the future are 'Marquette' (17.5%), 'La Crescent' (8.5%), and 'Frontenac' (8.1%) (USDA, 2013c). Across the Midwest region, 'Marquette' (39%), 'Frontenac' (26%), and 'Maréchal Foch' (11%) are the most popular choices (Tuck and Gartner, 2013). The objective of this study was to evaluate the viticulture performance of a range of cold-climate wine grape cultivars under the growing conditions of southern Wisconsin, and to provide information on growth, yield, and vine winter survival.

Materials and Methods

Site description. The trial was established in 2008 at the West Madison Agricultural Research Station (WMARS) in Verona, Wisconsin (lat. 43°03'37"N, long. 89°31'54"W) in USDA Plant Hardiness Zone 5 (USDA, 2012). The soil is a deep, well-drained Griswold loam (fine loamy, mixed mesic, Typic Argiudoll) (USDA, 2013a), with 2 to 6% slope with moderate fertility. At the onset of the study, soil pH was 7.2 and organic matter level was 31 g kg^{-1}. Soils had high phosphorus (143 mg kg^{-1} Bray I), and high potassium (225 mg kg^{-1} exchangeable K). The mean annual number of frost-free days, and precipitation at WMARS are 157 days and 903 mm, respectively (1981–2010, NOAA National Center for Environmental Information). The average first and last frost dates (2000-2017) are 18 Oct. and 23 April , respectively (National Oceanic and Atmospheric Administration weather station at Charmany Farm, about 5 km east of the station).

Plant material and vineyard establishment. Vines of nine cold-climate interspecific hybrid cultivars were obtained from commercial nurseries and the University of Minnesota and Cornell University grape breeding programs as research material (details in Table 1): 'Aromella', 'Brianna', 'Frontenac', 'La Crescent', 'La Crosse', 'Léon Millot', 'Maréchal Foch', 'Marquette', and 'Vignoles'. Self-rooted vines were planted in 2008. Vines were cordon-trained with double trunks in year two and spur-pruned in year three. Each trunk was trained into a unilateral cordon (one meter/three feet high) utilizing the vertical shoot positioning (VSP) system on a three-wire trellis. The vines were planted in a randomized complete block design with four blocks with each cultivar replicate planted in 8.5 m (28 ft) long four-vine panels. Rows were oriented north-to-south with 3.4 m (11 ft) between rows and 2.1 m (7 ft) between vines and a total density of 1398 vines ha^{-1} (566 vines $acre^{-1}$).

The vineyard was subject to standard cultural practices for commercial vineyards (Dami et al., 2005; Wolf, 2008) with permanent sod alleyways and intra-row strips were maintained with post-emergence herbicide. Wood chip mulch was placed beneath the vines in order to minimize herbicide usage. The vineyard was not fertilized from the point of establishment through the timeframe presented here. Drip irrigation was installed at the time of planting and irrigation frequency was determined by tensiometer measurements. All vines were spur-pruned to two to three nodes. The double pruning method was utilized to minimize the effects of any spring frost injury (Dami et al., 2005). Winter survival of vines was noted, along with general assessments of bud cold damage, but specific bud damage ratings or quantification was not performed.

During 2010 and 2011 while vines were

establishing, crop level was reduced by thinning to one cluster per shoot. From 2012 onward, vines were thinned to two clusters per shoot. Bird netting was installed at veraison. Data sample collection times ranged from Aug. 2010 (yield) to March 2015 (pruning weights).

Pruning weights and crop load. Fresh dormant pruning biomass was measured by weighing one-year-old wood trimmings for individual vines from the initial pruning the winters following the 2011, 2013, and 2014 seasons, and expressed as kg of biomass per meter of cordon (kg m^{-1}). Individual vine pruning weights were not collected in 2012. The ratio of the weight of a season's yield to the cane biomass produced that season (taken as the pruning weights the following dormant season) is expressed as the Ravaz index (Ravaz, 1911). The Ravaz index was used as an expression of crop load and was calculated as the ratio of fruit-to-cane production of a given year, for the years 2011, 2013, and 2014. Balanced pruning for maintaining or adjusting vine balance was used according to the general growth-yield relationship formula '30+X': 30 buds kept for the first 0.45 kg (1.0 pound) of pruning biomass with an additional 5, 10, or 15 buds kept for every additional 0.45 kg (up to 1.8 kg) (based on general description by Dami et al., 2005) as follows: +0 buds for 'Maréchal Foch'; +5 buds for 'Aromella'; +10 buds for 'Frontenac', 'La Crescent', and 'La Crosse'; +15 buds for 'Brianna', 'Léon Millot', 'Marquette', and 'Vignoles'.

Fruit Yield. Data were collected from 2010 to 2014, as total kg of fruit per meter of cordon. Fruit from each cultivar was harvested as a single harvest event. Harvest occurred each year over a two to three-week period by criteria described below.

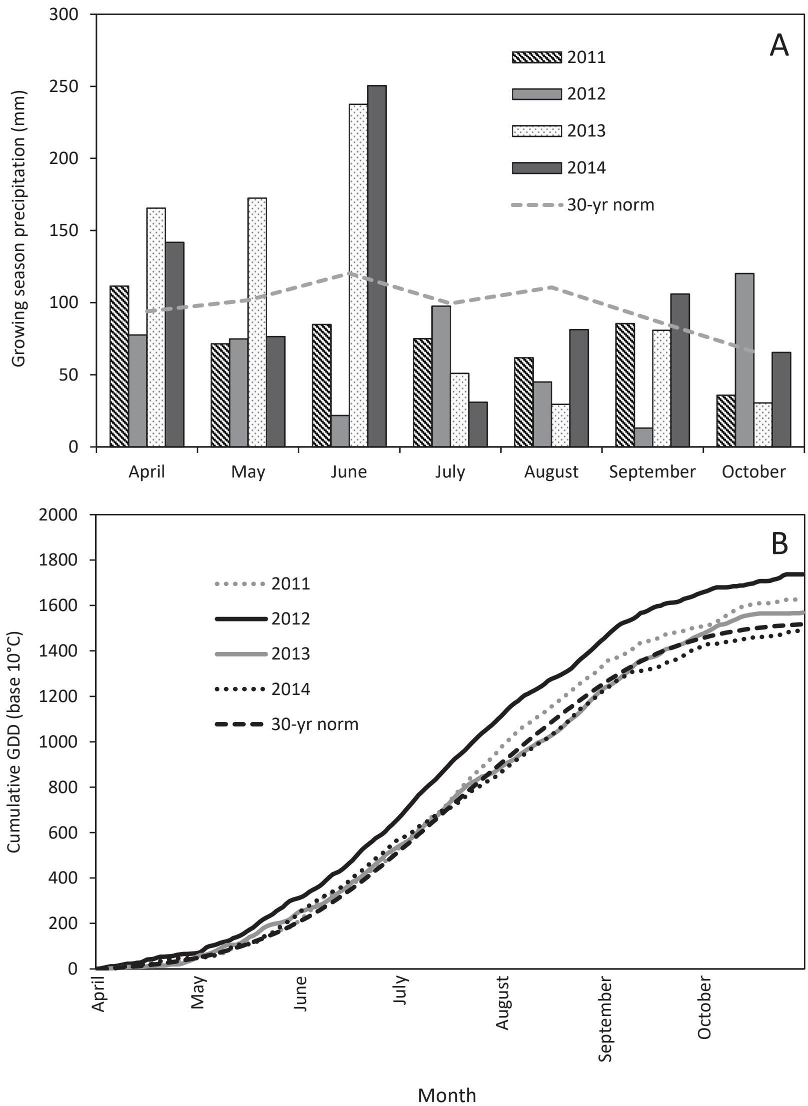
Fruit composition. Fruit composition data were collected in 2011, 2012, and 2014 as parameters of fruit maturity. Fruit harvest was determined by monitoring weekly soluble solids concentration (SSC), titratable acids (TA), and pH, as well as fruit condition, such as berry drop or degradation. SSC was the primary harvest indicator with a target of 22.0 %SSC. Additional targets of pH of 3.5 and TA of 6.0 g L^{-1} were considered, as well, based on local grower and winemaker advice. Berries were randomly selected from the four-vine panel and pooled to collect approximately 100 ml of juice weekly from veraison to harvest. Berries were transported to the laboratory on ice and subsequently kept in the refrigerator until analysis. Berries were crushed and juiced manually in plastic bags, and juice decanted. SSC was quantified with a HI96801 digital refractometer (Hanna Instruments, Woonsocket, Rhode Island) within 24 hours of sampling. Titratable acidity and pH values were determined with a HI902c titration system (Hanna Instruments, Woonsocket, Rhode Island) using a fixed end-point method of pH 8.2, 5 or 10 ml of sample, and 0.1 N sodium hydroxide titrant.

Weather conditions. Weather data were collected with an on-site weather station (WatchDog Micro Station, Spectrum Technologies, Inc., Aurora, Illinois) that recorded rainfall and hourly ambient temperature at cordon height (1 m). Additional temperature data (30-year norm (1981-2010) and daily minimum) were collected from the National Oceanic and Atmospheric Administration weather center at Charmany Farms (NOAA, 2014). Precipitation was summarized as monthly totals. Daily average temperatures were used to calculate growing degree-days (GDD) (base temperature 10 °C) for the growing season 1 April to 31 Oct. Dormant season daily minimum temperatures were summarized from 1 Nov. through 1 April.

Statistical analysis. Statistical analysis was conducted using the mixed model analysis of variance with covariance structures (SAS, Version 9.3. SAS Institute, Cary, NC). Model assumptions were confirmed with the UNIVARIATE procedure. Year was considered random for fruit yield analysis. For all other analyses, year and cultivar were fixed and block was random. If there was a significant year x cultivar interaction, the analysis was performed by year. The repeated measures statement was used to account for correlation of plots being repeatedly sampled every year and degrees of freedom were adjusted using Kenward-Roger (Gbur et al., 2012; Littell et al., 2006; Loughin, 2006; Schabenberger and Pierce, 2001). Significance was determined using α = 0.05 and Tukey's HSD test was used for mean separation. For statistical analysis and discussion purposes, cultivars were separated into red or white categories, the color referring to the product (i.e., wine), not the berry.

Results

Weather conditions. Since the establishment of the vineyard in 2008, there was a wide range in weather conditions across seasons (Fig. 1). The numbers of frost-free days were 186, 178, 167, and 171 for



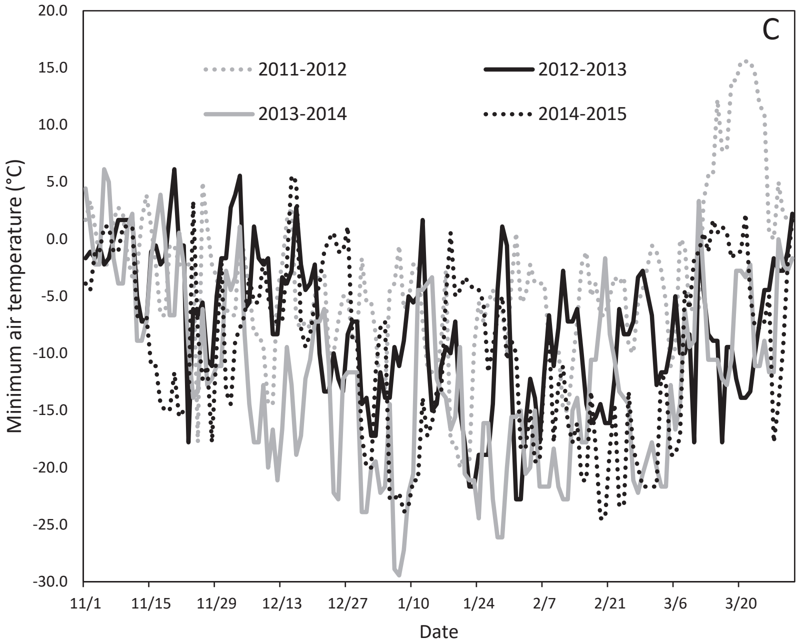


Figure 1. Summary of weather conditions at or near the West Madison Agricultural Research Station (WMARS), Verona, Wisconsin from 2011-2014 and the corresponding 30-year norm data (1981-2010, from the National Weather Service): A) Monthly precipitation totals (mm) from April through Oct.; B) Cumulative growing degree-days (GDD, base 10°C), 1 April through 31 Oct.; C) Daily minimum temperatures recorded at Charmany Farm (about 5 km east of WMARS) for NOAA Online Weather Data.

2011, 2012, 2013, and 2014, respectively (WMARS weather station). A chart of growing degree-days (Fig. 1B) shows that the entire 2012 season was the warmest, while Aug. through Oct. 2011 experienced warmer temperatures than in 2013 or 2014. In 2012, there was lack of rainfall, particularly in June and Sept. (Fig. 1A), as well as record high temperatures. The springs of 2013 and 2014 had higher precipitation than the 30-year normal, with June receiving more than twice the 30-year normal in both years (Fig. 1A). Of the four years considered here, the highest late summer (Aug. + Sept.) rainfall occurred in 2014 with a total of 187 mm. The winter of 2013-2014 was the most severe since the vines were planted (Fig. 1C). There were eight days with minimum temperature below -24 °C, with the lowest temperature experienced at -29 °C. Although there were periods of severe cold in each winter, the season of 2013-2014 had three-fold more days with temperatures below freezing than any other winter during this study.

Vine establishment. By 2010, cordons filled the trellis at the 2.1 m (7 ft.) vine spacing. Differences in vine vigor were noted across years and cultivar (Fig. 2). For all cultivars, pruning biomass was equal or higher in 2013 and 2014 vs. 2011 ($p < 0.0001$ for reds and for whites), which reflects that the vines continued to mature and gain vigor. Among the red cultivars, 'Maréchal Foch' had the lowest pruning weight each year and maintained a low level of vigor with age (Fig. 2A). There were no pruning biomass differences among red cultivars in 2011, while in both 2013 and 2014 'Maréchal Foch' was significantly lower than 'Léon Millot' and 'Marquette' ($p <$ 0.0136 and 0.0156, respectively). Among the

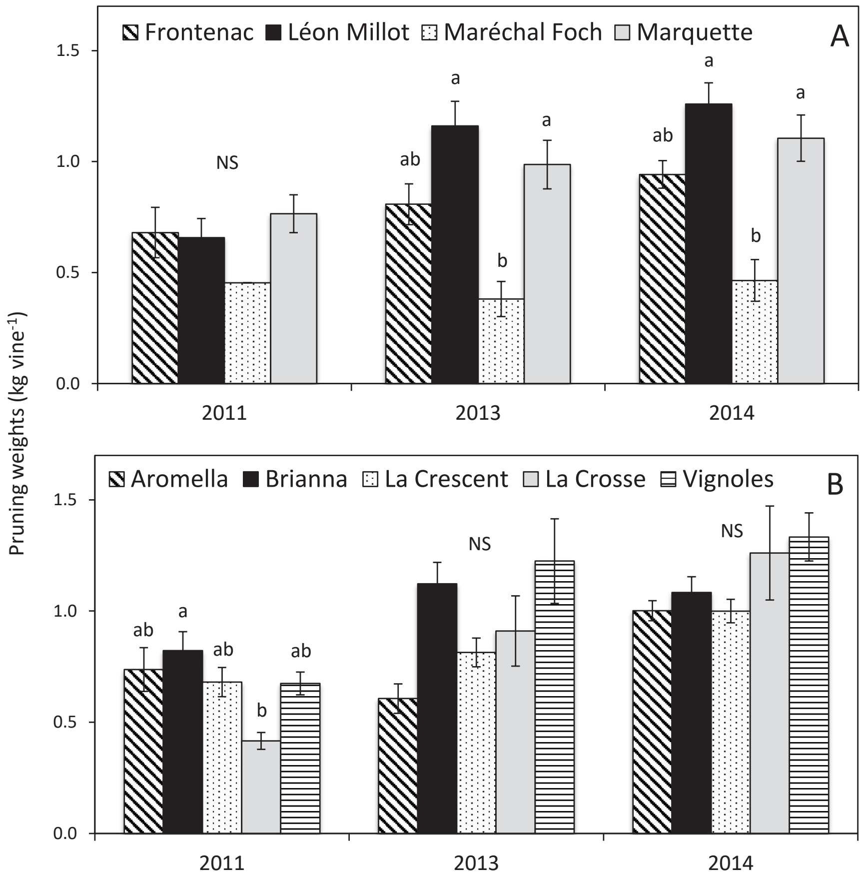


Figure 2. Mean pruning weights (kg m^{-1} of cordon) of four red (A) and five white (B) wine grape cultivars grown at the WMARS in Verona, Wisconsin from 2011, 2013, and 2014. Pruning weights were not collected in 2012. Means ± SEM (n=4), averaged over four blocks. Lower case letters indicate statistically significant differences among cultivars within years, according to Tukey's HSD test (α=0.05). For red cultivars (A), significant differences occurred among cultivars during 2013 (p=0.0136) and 2014 (p=0.0156), while no differences were observed in 2011 ($p \geq 0.05$). For white cultivars (B), significant differences occurred among cultivars during 2011 (p=0.0361), while no differences were observed in 2013 and 2014 (p>0.05).

white cultivars, 'La Crosse' showed slower establishment than the others in 2011 ($p < 0.0361$), especially compared to 'Brianna' (Fig. 2B). By 2013 and 2014 there were no pruning biomass differences among the white cultivars. 'Marquette' grew vigorously at our site, particularly as lateral shoots.

Fruit yields. Across all years among the red cultivars, 'Frontenac' was significantly higher yielding (average of 3.9 kg m^{-1}) than 'Maréchal Foch', 'Léon Millot', and 'Marquette' ($p<0.0001$) by nearly two-fold but there was no difference among those latter cultivars (Fig. 3A). 'Marquette' was the most consistent, yielding an average 2.1 kg m^{-1} yr^{-1} (range 1.6 to 2.7 kg m^{-1}; Fig. 3A). Across all years among the white cultivars, 'Brianna' and 'La Crosse' were the most productive, each yielding an average of 3.3 kg m^{-1} (Fig. 3B). 'Aromella' and 'Vignoles' produced

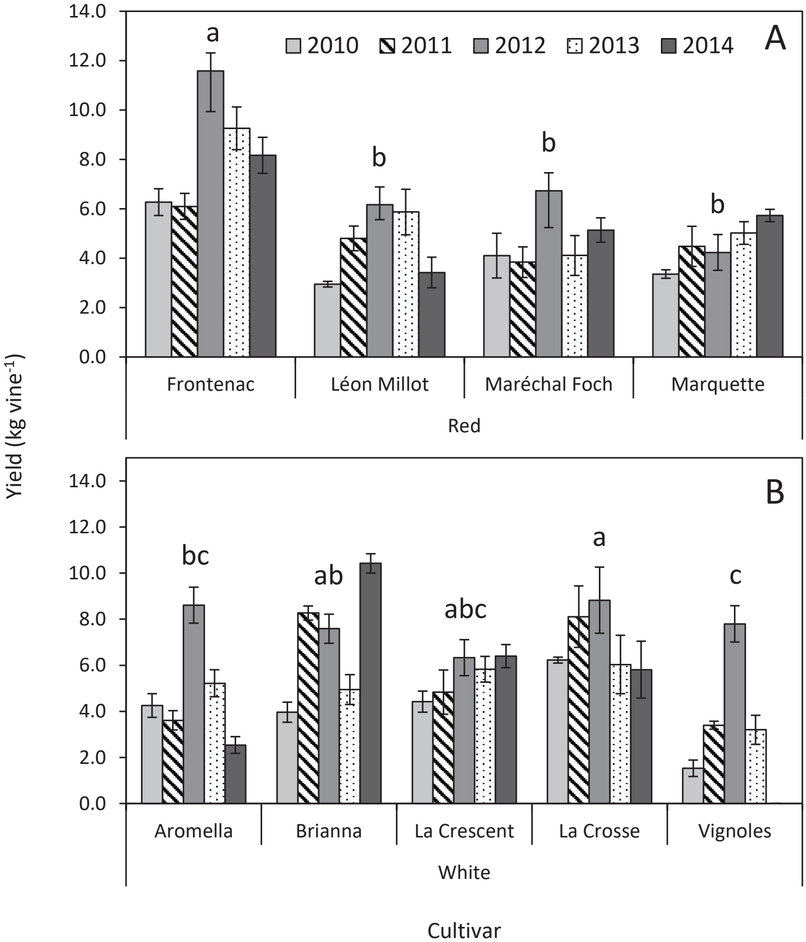


Figure 3. Mean fruit yield (kg m^{-1} of cordon) of four red (A) and five white (B) wine grape cultivars grown at the WMARS in Verona, Wisconsin over five years (2010-2014). Means ± SEM (n=4), averaged over four blocks. Lower case letters indicate statistically significant differences among cultivars for a repeated measures model that includes the five years of observation, according to Tukey's HSD means separation test (α=0.05).

lower yields with averages of 2.3 and 1.5 kg m^{-1}, respectively (Fig. 3B). These were also the most inconsistent yielding cultivars; both only had moderate yields in 2012 and otherwise were the lowest yielding. 'Vignoles' was not cropped in 2014, as a management decision due to severe winter injury. 'La Crescent' ranked midway (average 2.6 kg m^{-1}) among the whites with very consistent yields from year to year (2.1 to 3.0 kg vine^{-1}; Fig. 3B).

Crop load. Ravaz index values are shown in Table 2 for 2011, 2013, and 2014. Among the red cultivars, there was a significant year x cultivar effect ($p < 0.0002$). 'Maréchal Foch' had a higher index (16.5) than 'Léon Millot' (3.0) and 'Marquette' (5.8) in 2014 ($p < 0.0022$), but no differences among red cultivars were found in 2011 or 2013. There was a significant year x cultivar effect for the

Table 2. Crop load ratios using the Ravaz index (ratio of fruit to cane production of a given year) for 2011, 2013 and 2014 (n=36).

	Ravaz Index			
Cultivar	2011	2013	2014	
Reds				
Frontenac	9.7	12.4	9.1	ab[z]
Léon Millot	7.4	6.2	3.0	b
Maréchal Foch	7.8	17.0	16.5	a
Marquette	5.8	6.3	5.8	b
p-value	0.2143	0.0601	0.0022	
Whites				
Aromella	5.2 b	9.5	2.6	b
Brianna	10.3 b	4.6	10.2	a
La Crescent	5.8 b	8.3	6.6	ab
La Crosse	23.2 a	8.5	7.8	a
Vignoles	5.1 b	7.9	- -	
p-value	0.0001	0.7802	0.0060	

[z] Values within columns and color followed by common letters do not differ, by Tukey's HSD test (α=0.05).

white cultivars ($p < 0.0001$). All the white cultivars had an index less than 10 except for 'La Crosse' in 2011 (23.2) and 'Brianna' in 2011 (10.3) and 2014 (10.2). 'La Crosse' had exceptionally low vine vigor (Fig. 2B) but high fruit yield (Fig. 3B) in 2011, while 'Brianna''s crop load was the highest of all in 2014 (Fig. 3B).

Fruit composition. Among the red cultivars and across all years, 'Marquette' had the highest SSC (p<0.0001, =0.0003, and =0.0054 for 2011, 2012, 2014, respectively), with no significant difference between the others. 'Léon Millot' had the lowest TA and highest pH values during 2011 (p<0.0001 and =0.0001) and 2014 (p<0.0001 and =0.0026) among the red cultivars (Fig. 4). 'Maréchal Foch' had the next lowest TA levels in 2011 and 2014, while TA levels in 'Frontenac' and 'Marquette' were among the highest. There were no significant differences among the red cultivars for TA or pH during 2012 (p=0.3634 and =0.0576). For the years in which 'Vignoles' was cropped (2011 and 2012), those fruit had the highest SSC levels (p=0.0059 and =0.0008). Each year 'La Crescent' had some of the highest SSC levels (2014 p=0.0253). 'La Crosse' had the lowest values of SSC of the white cultivars, however not significantly different from 'Aromella' or 'Brianna'. 'Brianna' had the lowest TA levels for 2011, 2012, and 2014 (p=0.0004, =0.0003, and <0.0001, respectively), followed by 'La Crosse'. In 2011 and 2014, 'Vignoles', 'Aromella', and 'La Crescent' had TA levels greater than 11.0 g L^{-1}, more than twice that of 'Brianna'. 'Brianna' reached the highest pH values each year, as well (p=0.0021, =0.0008, and <0.0001, 2011, 2012, and 2014, respectively).

Discussion

Successful performance of cold-climate interspecific hybrid wine grape cultivars in the U.S. upper Midwest includes, along with strong winter survival, a moderate vigor for ease of canopy management, high and consistent yields, and fruit quality traits of good sugar accumulation, yet low to moderate titratable acidity, as berries mature. The climate experience over the four years reported here, 2011-2014, varied notably in

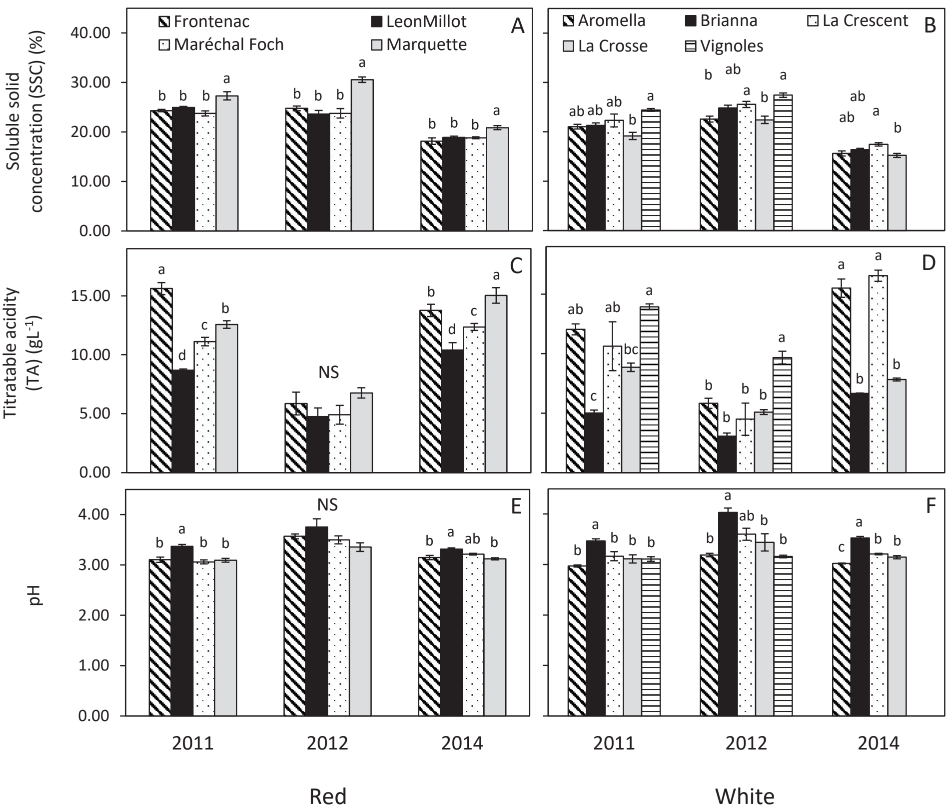


Figure 4. Fruit quality indexes at harvest (soluble solid concentration (SSC), titratable acidity (TA), and pH) of four red (A, C, and E) and five white (B, D, and F) wine grape cultivars grown at the WMARS in Verona, Wisconsin, for 2011, 2012, and 2014. Means ± SEM (n=48), averaged over four blocks. Lower case letters indicate statistically significant differences among cultivars, according to Tukey's HSD test (α=0.05).

both temperature and precipitation over the growing seasons, as well as in winter low temperature (Fig. 1), and as such, has proven to be a good test of cultivar performance.

Variability existed among the cultivars in their ability to thrive, despite their selection for being cold hardy. Although we did not fully evaluate cold damage of buds during the span of the study, 'Vignoles' and 'Aromella' exhibited cold damage most years, which contributed to the highly significant cultivar x year interaction for yield. Likewise, at a more northern sister UW Agricultural Research Station (Zone 3b), 'Vignoles' and 'Aromella' suffered >75% primary bud loss in 2014 (Volenberg, 2014), that was in addition to significant injury in previous winters (M. Stasiak, personal communication). 'Maréchal Foch' and 'Léon Millot' were also reported to have suffered notable primary bud injury in 2014, but secondary and tertiary buds were largely still viable (Volenberg, 2014). Zabadal et al. (2007) categorizes 'Vignoles' as moderately hardy (-23 to -26 °C) and 'Frontenac', 'Maréchal Foch', and 'La Crescent' at very hardy (-29 to -34 °C). Our observations confirm that the Wisconsin winter climate is too severe to reliably produce 'Vignoles' and 'Aromella', while 'Frontenac' and 'La Crescent' can be successfully grown in Southern Wisconsin. It is interesting to note that 'Frontenac and 'La Crescent' were also identified as being two of the most popular and sought after cultivars by Wisconsin grape growers (Rochester, 2011; Tuck and Gartner, 2013; USDA, 2013c).

Despite these overwintering challenges, most of the cultivars continued to gain vigor over time, except for 'Maréchal Foch', which had the lowest pruning weight each year while maintaining a consistent level of vigor with age (Fig. 2). Increased vigor that is the result of on-going establishment of the vine is qualitatively different from the vigor that is stimulated in response to non-lethal stress, such as severe midwinter temperatures. We hypothesize the high vigor of 'Léon Millot', 'Vignoles', and 'Aromella' in the years after establishment may be due to the stimulation of increased vegetative growth after notable winter injury. The low Ravaz indexes (3.0 or less; Table 2) for these cultivars in 2014 reflect the combination of low (or no, in case of 'Vignoles') yields and high vegetative biomass production. Moderate vigor, such as demonstrated by 'La Crescent' and 'Frontenac', is desirable to produce a balanced growth to support fruit production, but also to keep canopy management tasks (e.g., thinning, shoot positioning, and leaf pulling) reasonable. 'Marquette' demonstrated vigorous growth at our site, largely marked by abundant lateral growth.

Two important aspects of yield when considering successful cultivars for a growing region are: total yield and year-to-year consistency. In this trial, 'Frontenac', 'La Crosse', and 'Brianna' were the highest producing cultivars (Fig. 3). Among the white cultivars, 'La Crescent' had the least variation from year to year, but had overall yields that were not statistically different from those of 'Vignoles', and 'Aromella'. Yields of 'La Crescent' were reduced likely due to its nature of shelling (premature berry drop) (Thull and Luby, 2016). The steady and large yield decline of 'Aromella' and 'Vignoles' since 2012 is indicative of the poor winter survival of these cultivars in the severe winter of the U.S. upper Midwest. 'Marquette' was the most consistently producing red cultivar, while 'Frontenac' was the highest yielding, as well as most variable, over the years of this trial.

Recommended ranges of the fruit maturity parameters for wine production from *V. vinifera* grapes are widely used (Amerine et al., 1967; Winkler et al., 1974; Boulton et al., 1996; Dami et al., 2005). However, there is a critical need to develop similar criteria for cold-climate cultivars that will provide guidance to growers in establishing optimal harvest times. In both 2011 and 2012, all red cultivars produced higher SSC than the *V. vinifera* recommended range of 20.5-23.5 %SSC (as summarized in Rolfes et al., 2015), while in 2014 all but 'Marquette' were below this range (Fig. 4). The recommended TA concentration for juice of red wine cultivars (6.0-8.0 g L^{-1}) (Rolfes et al., 2015) was exceeded in both 2011 and 2014. Only in the warmer year of 2012 were these concentrations close to the recommended *V. vinifera* range, and those for 'Léon Millot' and 'Maréchal Foch' were both below this (4.57 and 5.42 g L^{-1}, respectively). The shorter ripening period of the cold-climate cultivars relative to *V. vinifera* and the often lack of abundant heat units in the upper Midwest climate contribute to fruit maturity profiles characterized by higher titratable acidity with often moderate sugar accumulation (Haggerty, 2013; Teh et al., 2014). Researchers in central Iowa found similar fruit ripening profiles for 'Marquette', 'Frontenac', and 'La Crescent' to those in our study (Rolfes et al., 2015). Bavougian et al. (2013) studying 'Frontenac' in Nebraska under various trellis training systems, including those utilizing VSP, reported lower sugars and higher TA levels than in this trial. The Northern Grapes Project reported higher sugars but also much higher TA in 2012 and 2013 in New York state than at WMARS for

'Marquette' and 'Frontenac' (Martinson and Particka, 2015).

The harvest fruit maturity targets for this trial (SSC 22 %SSC, TA 6.0 g L^{-1}, and pH 3.5) were met inconsistently over the seasons reported. Variability in weather conditions experienced in southern Wisconsin during the study strongly affected fruit quality traits of these cold-climate cultivars. Higher accumulation of GDD during 2012 affected primarily the reduction of TA while only slightly affecting the accumulation of SSC. In comparison, the more moderate warm temperature experience in 2011 resulted in comparable sugar accumulation to those of 2012, but conditions were not sufficient to modulate high organic acid levels (Lakso and Kliewer, 1975). The coolest (and wettest) year, 2014, led to the lowest concentrations of SSC coupled with high TA values. In a study in Minnesota, Teh et al. (2014) reported that TA levels in cold-climate cultivars decline only gradually toward harvest time, and that during 2012, ratios of tartaric to malic acid were fairly constant in 'Frontenac' and 'La Crescent', while this ratio increased in 'Marquette' due to a significant decrease in malic acid concentration. Also in that study, the attainment of fruit maturity corresponded with the accumulation of 1400 to 1500 GDD (base 10°C). At our location, 1400 GDD accumulations were not reached until late Sept. in 2013 and 2014. In contrast, relatively rapid heat unit accumulation occurred in the latter part of the 2012 season, such that 1400 GDDs was reached by the beginning of Sept. In 2011, this GDD threshold was attained in early Sept., but did not reach 1500 GDD until the end of the month. Based on our observations, greater heat accumulation during the growing season has a significantly higher impact on acid degradation than on the production and accumulation of sugars.

Another aspect of vineyard management for the production of substantial and consistent yields of higher quality is decisions on the amount of fruit to carry per unit of canopy, or vine balance. Crop load (i.e., fruit:cane biomass, Ravaz index) ratios are often between 4 to 12 for most *V. vinifera* cultivars and above 12 for hybrids (Zabadal et al., 2007). Reynolds and Wolf (2008) state that for many cultivars crop load ratios between 10 to 12 are optimum, while in areas where heat accumulation is more of a challenge, crop load ratios as low as 5 may be reasonable. Most Ravaz indexes for the cultivars in this study were around 10 or below, with only a few below 5 (Table 2). Crop load ratio values for 'Maréchal Foch' and 'Frontenac' were 2-2.5 times higher than either 'Marquette' or 'Léon Millot' in 2013 and 2014, which is interesting given the fact that 'Léon Millot' is a sibling of 'Maréchal Foch'. Vos (2014) found that a range of crop load treatments from 2 to 14 did not have associated negative effects on future vine productivity in 'Frontenac' grown in Iowa. In our study 'La Crosse' had an exceptionally high crop load in 2011, but more moderate values in 2013 and 2014. In 2011 and 2014, 'Brianna' had relatively high crop load ratios among the white cultivars. The generally low (<10) Ravaz values may indicate that many of these cultivars can handle greater fruit load, especially 'Marquette' and 'La Crosse'. This would require a revision of the growth-yield relationship formula for calculating bud counts.

Conclusion

Our evaluation of cold-climate interspecific hybrid wine grape cultivars considered a balance of yield and fruit maturity parameters, along with a concern for vine balance. 'Aromella' and 'Vignoles' have been removed from the WMARS vineyard due to their poor winter survival and resulting lack of production. These cultivars are not recommended for commercial production in Wisconsin, unless local winter minimum temperatures warmer than at WMARS are feasible. Of the other cultivars being grown at WMARS, no one cultivar was both the highest and most consistent producer; however, several are promising. While not the highest yielding of the red cultivars, 'Marquette' had consistent yields, as well as the ability to reliably produce higher sugars than the other reds, although it is also typically high in TA. 'Marquette' may be able to carry heavier crop loads, which may help to control its shoot vigor. 'Frontenac' possessed moderate vigor and was the best yielding red cultivar. However, it promises to be consistently high in TA, save in exceptionally warm growing seasons. No one white cultivar stood out as exceptional in sugar production. 'Brianna' was a high yielding white cultivar whose fruit composition was comparable to the other whites in SSC production, while consistently low in TA, even in the cool growing season of 2014. 'La Crosse' was only moderate in SSC production, but had relatively lower levels of TA, high yields, and consistent vine balance. 'La Crescent' was consistent and moderate in yields, and its popularity among growers indicates possesses other desirable qualities than its fruit composition traits (as reported here) would indicate. Further evaluation is required, but the fruit quality and control of vegetative vigor of several of these cold-climate interspecific hybrids may be influenced by carrying larger crop loads. 'Frontenac', 'Marquette', and 'La Crescent' showed the most stable vine balance, based on the Ravaz index.

Acknowledgments

We thank Judith Reith, Brian Emerson, and Rebecca Harbut for their work in the design and establishment of the WMARS vineyards and oversight of data collection. Also, we thank all the UW-Madison undergraduate interns and graduate students who assisted in the daily and seasonal tasks of vineyard management and data collection. Funding for this project was provided by the UW-Madison College of Agriculture and Life Sciences, UW-Madison ARS, and USDA Specialty Crop Block Grant #10-012.

Literature Cited

Amerine, M.A., Berg, H.W., and Cruess, W.V. 1967. The Technology of Wine Making. Avi Publishing Company, Westport, Conn.

Anonymous. 2007. Risk management tools for the grape industry in Minnesota and Wisconsin. 24 June 2016. http://www.agmrc.org/media/cms/MNandWISurveyResults_894C57D3D946F.pdf

Bavougian, C.M., Read, P.E., Schlegel, V.L., and Hanford, K.J. 2013. Canopy light effects in multiple training systems on yield, soluble solids, acidity, phenol and flavonoid concentration of "Frontenac" grapes. HortTechol. 23:86-92.

Boulton, R.B., Singleton, V., Bission, L.F., and Kunkee, R.E. 1996. Principles and Practices of Winemaking. Springer Publishing Co., New York.

Dami, I., Bordelon, B., Ferree, D.C., Brown, M., Ellis, M.E., Williams, R.N., and Doohan, D. 2005. Midwest grape production guide, Bulletin 919. Ohio State University, Columbus, Ohio.

Fennell, A. 2004. Freezing tolerance and injury in grapevines. J Crop Improv. 10:201-235.

Gbur, E.E., Stroup, W.W., McCarter, K.S., Durham, S., Young, L.J., Christman, M., West, M., and Kramer, M. 2012. Analysis of Generalized Linear Mixed Models in the Agricultural and Natural Resources Sciences. ASA, CSSA, and SSSA, Madison, WI.

Haggerty, L. 2013. Ripening Profile of Grape Berry Acids and Sugars in University of Minnesota Wine Grape Cultivars, Select Vitis vinifera, and Other Hybrid Cultivars. University of Minnesota, St. Paul, MS thesis.

Howell, G.S. 2001. Sustainable grape productivity and the growth-yield relationship: A review. Am. J. Enol. Vitic. 52:165-174.

Lakso, A.N. and Kliewer, W.M. 1975. The influence of temperature on malic acid metabolism in grape berries I. Enzyme responses. Plant Physiol. 56:370-372.

Littell, R.C., Stroup, W.W., Milliken, G.A., Wolfinger, R.D., and Schabenberger, O. 2006. SAS for mixed models. SAS institute, Cary, NC.

Loughin, T.M. 2006. Improved experimental design and analysis for long-term experiments. Crop Sci. 46:2492-2502.

Luby, J., Mansfield, P., Hemstad, P., and Beam, B. 2006. Development and evaluation of cold-hardy wine grape breeding selections and cultivars in the upper Midwest, Progress Report for Viticulture Consortium-East. Cornell University, Geneva, NY.

Martinson, T. and Particka, C. 2015. A Tale of Two Crops: Impact of late-spring frost on Frontenac Fruit Composition. 29 June 2016. http://northerngrapesproject.org/wp-content/uploads/2015/12/Martinson-presentation-PDF.pdf

NOAA. 2014. National Climate Data Center. 16 March 2015. http://www.ncdc.noaa.gov/oa/ncdc.html

Particka, C. and Martinson, T. 2016. Year 4 Progress Report-Northern Grapes: Integrating Viticulture, Winemaking, and Marketing of New Cold-Hardy Cultivars Supporting New and Growing Rural Wineries. 20 January 2017. http://northerngrapesproject.org/wp-content/uploads/2016/02/Year-4-Northern-Grapes-Project-Progress-Report.pdf

Ravaz, M.L. 1911. L'effeuillage de la vigne. Annales de l'Ecole Nationale d'Agriculture de Montpellier 11:216-244.

Reynolds, A.G. and Wolf, T.K. 2008. Pruning and Training, p. 98-123. In: Wolf, T. K. (ed.), Wine Grape Production Guide for Eastern North America. Plant and Life Sciences Publishing, Ithaca, NY.

Rochester. 2011. Report of Wisconsin Grape Growers Survey. 30 June 2016. https://fruit.wisc.edu/wp-content/uploads/sites/36/2016/06/Report-of-the-Wisconsin-Grape-Growers-Survey.pdf

Rolfes, D., Nonnecke, G., and Domoto, P.A. 2015. Effects of canopy management practices on cold-hardy grape cultivars. 24 June 2016. http://northerngrapesproject.org/wp-content/uploads/2015/03/2015MarchNGPnewsletter.pdf

Schabenberger, O. and Pierce, F.J. 2001. Contemporary statistical models for the plant and soil sciences. CRC Press, Boca Raton, FL.

Smiley, L., Domoto, P.A., and Cochran, D. 2016. A Review of Cold Climate Grape Cultivars. 20 Jan 2015. http://www.extension.iastate.edu/viticulture/cold-climate-cultivars

Teh, S., Haggerty, L., Hegeman, A., and Luby, J. 2014. Fruit Ripening Profiles of Cold Climate Wine Grape Cultivars. 24 June 2016. http://northerngrapesproject.org/wp-content/uploads/2014/02/FruitRipeningProfiles.pdf

Thull, J. and Luby, J. 2016. Brianna and La Crescent: Ten Viticulture Tips. June 13. http://northerngrapesproject.org/wp-content/uploads/2016/03/Brianna-and-La-Crescent-Viticulture-enology-and-tasting.pdf

Tuck, B. and Gartner, W. 2013. Vineyards and Grapes of the North: A status report. 30 December 2014. http://www.extension.umn.edu/community/economic-impact-analysis/reports/docs/2013-Vineyards-Grapes-North.pdf

USDA. 2013a. Web Soil Survey. 15 January 2015. http://websoilsurvey.sc.egov.usda.gov/App/HomePage.htm

USDA, A. 2012. USDA Plant Hardiness Zone Map, 2012. May 15 2015. http://planthardiness.ars.usda.gov/.

USDA, N.A.S.S. 2013b. 2011 Grape and wine industry survey. 5 February 2015. http://www.nass.usda.gov/Statistics_by_State/Wisconsin/Publications/Crops/WIGrapeWine

USDA, N.A.S.S. 2013c. 2011 Grape and wine industry survey addendum. 2 January 2015. http://www.nass.usda.gov/Statistics_by_State/Wisconsin/Publications/Crops/WIGrapeWineAddendum.pdf

Volenberg, D. 2014. Vineyard IPM scouting report for week of 19 May 2014 UW-Extension Door County and Peninsular Agricultural Research Station. 24 June 2016. https://fruit.triforce.cals.wisc.edu/wp-content/uploads/sites/36/2014/06/IPM-Report-week-1-5.19.2014.pdf

Vos, R. 2014. Stage of maturation, crop load, and shoot density affect the fruit quality of cold-hardy grape cultivars. PhD Diss.Dept. of Horticulture, Iowa State Univ., Ames.

Winkler, A.J., Cook, J.A., Kliewer, W.M., and Lider, L.A. 1974. General Viticulture. Univ. of California Press, LTD, London.

Wolf, T.K. 2008. Wine grape production guide for eastern North America. Plant and Life Sciences Publishing, Ithaca, NY.

Zabadal, T., Dami, I., Goffinet, M., Martison, T., and Chien, M. 2007. Winter injury to grapevines and methods of production, p. 16, Michigan State Extension E2930. Michigan State Univ., East Lansing, MI.

Journal of the American Pomological Society 72(2): 94-100 2018

Breeding for Brown Rot (*Monilinia* spp.) Tolerance in Clemson University Peach Breeding Program

Wanfang Fu[1], Ralph Burrell[1], Cassia Da Silva Linge[1], Guido Schnabel[1]
and Ksenija Gasic[1]

Additional index words: disease, fruit, RosBREED, QTL, haploblock

Abstract

Brown rot, caused by *Monilinia* spp., is one of the most economically important diseases of stone fruits. The fungus mainly affects the blossoms and fruit, and the resulting disease can lead to significant pre- and postharvest yield losses. Estimated yearly cost to the U.S. stakeholders for chemical protection against the disease can reach $170M. Although some degree of resistance in peach landraces ('Bolinha') and interspecific material (almond × peach) has been reported, genetic resistance to brown rot in peaches is still lacking. In commercial peach production, the disease is managed by practicing sanitation and the application of fungicides. The Clemson University peach breeding program within the RosBREED project aims to understand the genetics behind the peach fruit response to brown rot with the ultimate goal of combining disease resistance with high fruit quality via DNA informed breeding. To this end, 26 cultivars /advanced selections and 138 progeny, representing 9 breeding families, with 'Bolinha' source of resistance have been phenotyped for fruit response to brown rot using wounded and non-wounded disease assays in 2015 and 2016. Previously obtained genotypic data, and reported QTLs associated with brown rot response in peach fruit, were used to obtain preliminary information on variability in brown rot associated genomic regions. Phenotypic performance or trait values of these alleles/ haplotypes were discussed. The data presented here provide a foundation for developing predictive DNA information that has potential for immediate application in U.S. peach breeding.

Brown rot, caused by *Monilinia* spp. is one of the most important diseases concerning stone fruits. As a polycyclic disease, brown rot may cause severe yield losses by affecting peaches in two phases: blossom and twig blight caused by ascospore infection in spring and pre- and postharvest fruit decay caused by conidia infection in summer (Zehr et al., 1982; Tate et al., 2000). Although some degree of resistance has been identified in the Brazilian cultivar 'Bolinha' (Feliciano et al., 1987) and some interspecific hybrids (almond × peach) developed in the peach breeding program in California (Gradziel et al., 2003), most of the commercial peach cultivars are susceptible to brown rot (Martinez-Garcia et al., 2013). The disease is still mainly controlled by routine fungicide applications in conventional production systems, which can cause environmental and health issues (Sharma, 2005; Rungjindama et al., 2014). Consumer demand for fewer chemical treatments in fruit production has been increasing over the last years. In addition, new *Monilinia* strains with fungicide resistance have been reported, suggesting that the chemical approach may become less efficient (Luo et al., 2008; Chen et al., 2013, 2015). Thus, the main goal for current peach breeding programs is to develop new cultivars with enhanced disease resistance/tolerance and high-quality fruit by enhancing appearance, along with improved flavor and aroma.

Previous studies have shown that brown rot resistance in peach is inherited as a polygenic and quantitative trait (Gradziel et al., 1993, 2002; Martinez-Garcia et al., 2013; Pacheco et al., 2014). QTLs associated with brown rot have been reported in peach × almond (Martinez-Garcia et al.,

[1] Department of Plant & Environmental Sciences, Clemson University, Clemson, South Carolina, USA
[*] Corresponding author: Ksenija Gasic, kgasic@clemson.edu

2013) and in peach (Pacheco et al., 2014). Martinez-Garcia et al. (2013) detected 2 QTLs (*QTL1.1* and *QTL1.2*) associated with brown rot resistance/tolerance in peach fruit on linkage group (LG) 1 by assessing an F_1 progeny from controlled cross between the peach cultivar Dr. Davis (female) and the almond × peach F_2BC_2 introgression line 'F8, 1–42'. Analyzing an F_1 progeny from the cross between the two commercial cultivars 'Contender' (moderate resistance) x 'Elegant Lady' (susceptible), Pacheco et al. (2014) mapped two QTLs, one for skin resistance, *SK-if_2009*, on LG2, and another for flesh resistance, *FL-rd_2009*, on LG3. Once the QTLs were mapped, the associated genomic regions could be further analyzed to detect haplotypes associated with brown rot resistance. Thus, the objective of this study was to evaluate the brown rot infection responses in peach fruits from the Clemson University breeding program, identify haploblocks/haplotypes in previously reported QTLs associated with brown rot and determine the phenotypic performance/trait values of detected haplotypes/alleles.

Materials and Methods

Phenotypic evaluations for fruit responses to infection with *Monilinia fructicola* were performed in two years (2015 and 2016) using 8 cultivars/advanced selections and 131 progeny from 8 crosses with 'Bolinha' as source of resistance to brown rot. Additional 18 cultivars and 7 progeny from 1 cross were evaluated only in 2016. For each genotype, 40 fruits were randomly selected and bagged at 'pit hardening', to prevent pesticide contact. The fruits were harvested at commercial maturity, stored at 4°C for 2-4 days until the day of the assay, and were allowed to warm to room temperature for 24h before inoculation. Fruit surface was sterilized by 30sec immersion in 10% bleach (0.6% NaOCl), rinsed in deionized water, and air dried. Out of 40 bagged fruits, 20 unblemished fruits of similar maturity determined by I_{AD} (Ziossi et al., 2008) were used for inoculations. Parallel inoculations, 10 fruits each, for both wounded and non-wounded treatment were performed following the protocol of Martinez-Garcia et al. (2013). Non-wounded fruits were inoculated by adding a 10μL droplet of inoculum with the concentration of 2.5×10^4 conidia per ml of *M. fructicola* isolate KH-13. This highly virulent single-spore isolate was obtained from nectarine trees in Seneca, SC in 2013. Wounded treatments were accomplished by applying a 10μL droplet of inoculum to an intact fruit surface and then breaching the cuticule through the droplet using a 22 gauge needle and creating an injury about 2 mm deep. Inoculated fruits were incubated in dark under humid condition at room temperature (22±1°C). Lesion diameters (mm) were recorded after 72h incubation and disease severity index (DSI) for each individual was calculated as the product of average lesion diameter × disease incidence (proportion of lesions greater than 3mm). The phenotyped individuals were genotyped using the 9K peach SNP array (Verde et al., 2012). SNP-based haploblock/haplotypes were determined at previously reported brown rot associated QTL regions (Martinez-Garcia et al., 2013; Pacheco et al., 2014). Statistical differences among different genotypes and haplotypes/alleles were detected by performing ANOVA and Dunnett's T3 test in SPSS v. 23 (IBM®) at the significance of $p<0.05$.

Results and Discussion

In this study, 164 genotypes were evaluated for fruit responses to brown rot inoculation with wounding and non-wounding treatment. The results of the phenotypic evaluations were significantly different between the two treatments, suggesting that wounding increases DSI in the analyzed peach material (Figures 1 and 2). In addition, DSI was positive and significantly correlated between wounded and non-wounded treatments ($r = 0.369$, $p = 0.000$). Lowest DSI, <15, with wounding was observed in the advanced selection 'NC97-45', and 'Contender' and 'June Gold' (Fig. 1). 'NC97-45' has

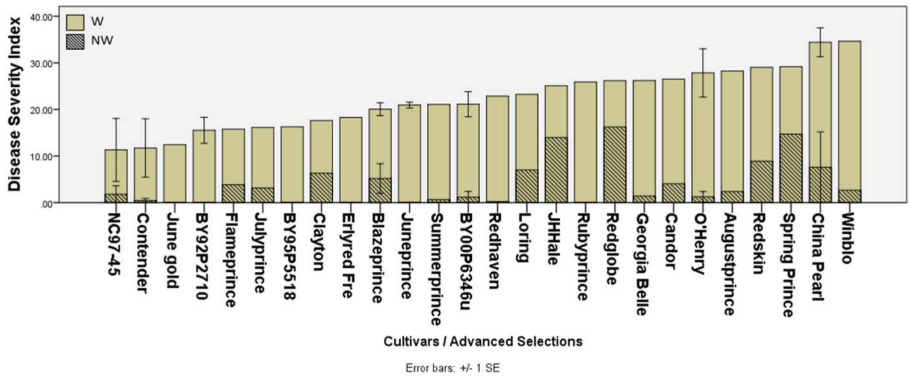


Figure 1. Brown rot disease severity index (DSI) observed in wounded (W) and non-wounded (NW) fruit of peach cultivars and advanced selections. DSI = average lesion diameter incidence (# of lesions greater than 3mm/total # lesions). Bars with standard error indicate average DSI of two years.

'Contender' as a parent which supports the findings of Pacheco et al. (2014) that 'Contender' is a source of resistance/tolerance to brown rot in peach. Advanced selection 'BY00P6346u' from the Byron peach breeding program, is a descendant of 'Bolinha' and is used for introgression of 'Bolinha' resistance/tolerance to brown rot in peach breeding programs (Gradziel et al., 1997). 'BY00P6346u' showed higher DSI with wounding than 'Contender', supporting previous observations that 'Bolinha' resistance/tolerance is mostly skin related, and once fruit ripens flesh becomes susceptible to brown rot (Gradziel and Wang, 1993). The pedigree analysis shows no connections between 'June Gold' ('Flamingo' × 'Springtime') and 'Contender' or 'Bolinha'. In addition, 'Juneprince' ('FV325-58' × 'June Gold'), descendant of 'June Gold', showed similar DSI as 'BY00P6346u' when wounded and better response when not wounded , suggesting that 'June Gold' could also be used as a source of brown rot tolerance in peach.

'Bolinha' was used as a direct (in C1, 6, 7, 8, 9) or indirect (via 'BY00P6346u' in C2, 3,

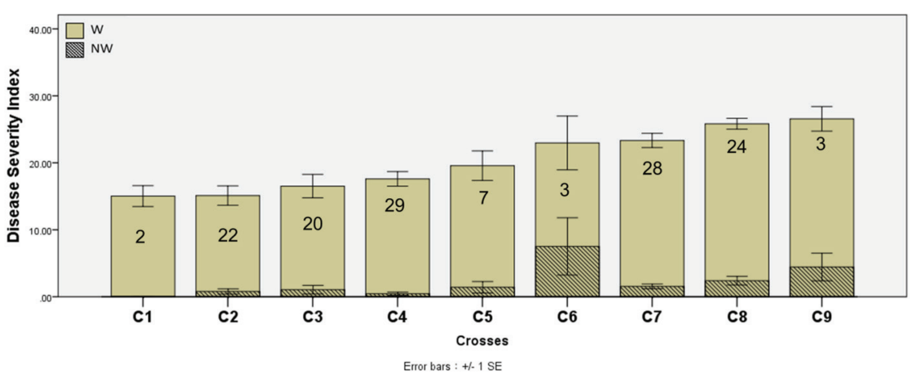


Figure 2. Average brown rot severity for fruits of crosses (C) with 'Bolinha' source of resistance over two seasons (2015-2016). Number of progeny in each cross were shown on the bars. W, wounded; NW, non-wounded.

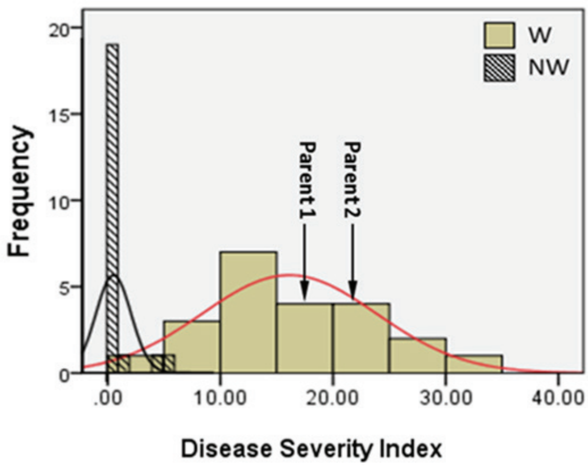


Figure 3. Brown rot disease severity index distribution in cross2 (C2). W, wounded; NW, non-wounded.

4, 5, 7) donor of brown rot resistance/tolerance in the evaluated crosses (C). The number of individuals in crosses varied from 2 to 29 due to already applied selection for fruit quality. Average fruit responses to brown rot infection in the crosses in the wounded treatment were similar to advanced selections and cultivars with narrower range (15 - 28 DSI), and non-wounded treatment elicited no to very low (<5) DSI in crosses (Fig. 2). C1 and C2 had the lowest DSI (>15) under wounded treatment and were among the lowest DSI for non-wounded treatment, while C8 and C9 had the highest DSI under wounded treatment (>25) and were among the highest DSI under non-wounded treatment. Under non-wounded treatment, most of the crosses showed similar DSI (<5), except for the C6 (Figure 2). Individual seedlings within crosses showed segregation for response to brown rot infections with individuals exhibiting lower and higher DSI than the average for the family (data not shown). Transgressive segregations were observed for brown rot DSI in the 'BY00P6346u' derived crosses. In the C2 most progenies showed no symptoms under the non-wounded treatment, while under the wounded treatment they exhibited lower DSIs than both parents (Figure 3). Phenotypic data analysis for C2 under both treatments, revealed advanced selections 5 and 7 with lower DSIs than most of the 'Bolinha' derived breeding material. Similar results were observed in other 'BY00P6346u'-derived crosses evaluated in this study, supporting 'BY00P6346u' as a good choice for incorporation of brown rot resistance/tolerance in peach breeding program (data not shown).

Analysis of the reported QTL regions revealed five and two haploblocks on LG1, *QTL1.1* and *QTL1.2*, respectively (Martinez-Garcia et al., 2013), and one haploblock for each of the QTLs reported on LG 2 and 3 (Pacheco et al., 2014) (Table 1). Number of haplotypes/alleles observed in haploblocks ranged from 3 in *QTL1.1* haploblock (H) 2 and 3 (*QTL1.1*_H2 and *QTL1.1*_H3) to 7 in *SK-if-2009* (Table 1). Analysis of phenotypic performance of each detected haplotype/allelic combination (diplotypes/genotypes) revealed significant differences in *QTL1.1, QTL1.2* and *SK-if_2009* under wounded and/or non-wounded treatment. Detailed analysis showed that the individual alleles provided different effects on brown rot resistance/tolerance in the four brown rot associated genomic regions (data not shown).

Seven different diplotypes/genotypes were identified in *QTL1.1*_H3 (Figure 4A). Significantly different (p<0.05) phenotypic performances were detected among different genotypes in this genomic region under the wounded treatment. The non-wounded treatment showed similar fruit responses for brown rot infection among the different genotypes. Trait value analysis of each individual haplotype/allele effect under wounded treatment suggested that the presence of allele 'b' significantly increases DSI (Figure 4B). Identified genotypes in *SK-if_2009* exhibited significantly different responses

Table 1. Haploblocks/haplotypes detected in QTLs associated with brown rot response in peach fruit.

Linkage group	QTL / Haploblock		Haploblock region (Mb)	Flanking SNPs	Number of SNPs	Number of alleles / Haplotypes
LG1	QTL1.1	H1	1.78-1.88	SNP_IGA_5258 SNP_IGA_5726	4	3
LG1	QTL1.1	H2	6.95-7.99	SNP_IGA_19818 SNP_IGA_22766	3	6
LG1	QTL1.1	H3	8.23-8.31	SNP_IGA_23251 snp_1_7856380	3	4
LG1	QTL1.1	H4	9.26-9.71	SNP_IGA_25403 SNP_IGA_26500	5	5
LG1	QTL1.1	H5	10.39-10.63	SNP_IGA_28112 SNP_IGA_28465	5	4
LG1	QTL1.2	H1	26.92-27.06	SNP_IGA_88104 SNP_IGA_88772	5	5
LG1	QTL1.2	H2	30.86-32.14	SNP_IGA_99110 SNP_IGA_101065	4	5
LG2	SK_if_2009		21.89-22.47	SNP_IGA_274142 SNP_IGA_276426	10	7
LG3	FL_rd_2009		9.28-9.8	SNP_IGA_320761 SNP_IGA_321596	5	5

[1] QTL1.1 and QTL1.2 were detected in peach × almond progeny (Martinez-Garcia et al., 2013).
[2] SK_if_2009 and FL_rd_2009, skin and flesh associated QTLs respectively, were detected in 'Contender' × 'Elegant Lady' progeny (Pacheco et al., 2014).
[3] H - haploblock.

to brown rot infection under both wounded and non-wounded treatments (Figure 4C). The absence of the allele 'c' suggested lower DSI under both treatments (Figure 4D). No significant differences of the phenotypic performance/trait values were observed among the different diplotypes/genotypes in the *FL-rd_2009*, regardless of the treatment. However, analysis of effect of presence or absence of individual haplotypes/alleles showed significant differences under wounded and/or non-wounded treatment and will be further investigated (data not shown). Individual haplotype/allele trait value analysis was hindered by the lack of genotypic data for all phenotyped material. We are currently acquiring additional genotypic and phenotypic ('Contender' derived crosses) data to strengthen our findings.

Even though the source of brown rot resistance in the Clemson peach breeding program is different than in those used to detected the QTLs reported by Martinez-Garcia et al. (2013) and Pacheco et al. (2014) this published information allowed dissection of these genomic regions in haplotypes/alleles relevant for peach. The *QTL1.1* and *QTL1.2* were detected in an interspecific cross using almond background (Martinez-Garcia et al., 2013) and the *SK-if_2009* and *FL-rd_2009* QTLs were detected in an intraspecific F_1 progeny using 'Contender' as source of resistance (Pacheco et al., 2014). Once haplotypes/alleles were determined phenotypic performance or trait values of each allele/genotype were elucidated and statistically significant differences among phenotypic performances of alleles/genotypes were found. In addition, phenotyping for disease by Pacheco et al. (2014) was different in that they analyzed the percentage of infected fruits in non-wounded assay and average rot diameter in wounded assay. The Clemson University peach breeding program used 'Bolinha' derived resistance/tolerance to brown rot as a main source and could offer new insights into genomic regions associated with this trait. Thus, to better understand the genetic mecha-

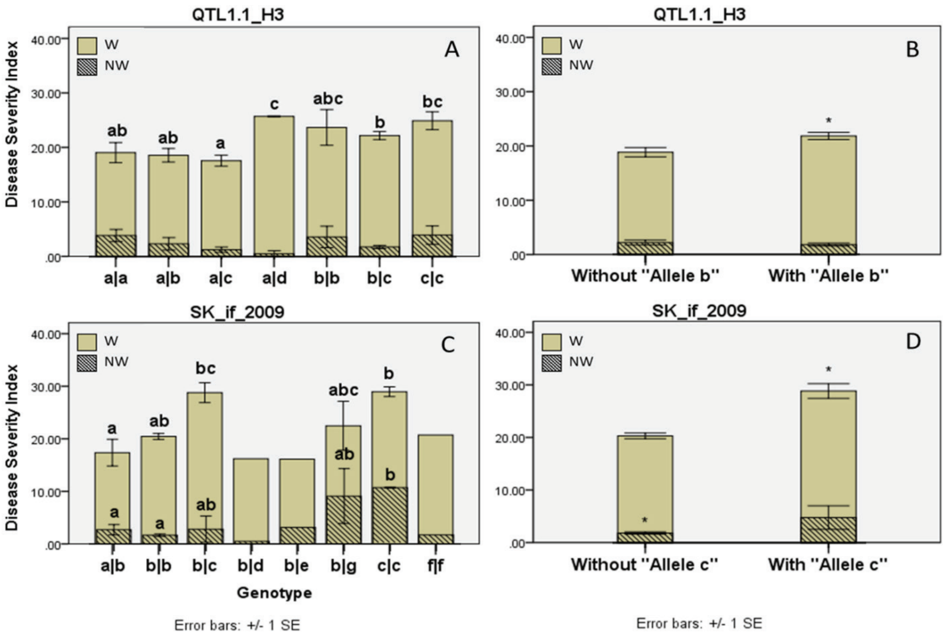


Figure 4. Trait values of brown rot associated genotypes (A) and haplotypes/alleles (B) detected in Clemson University peach breeding germplasm on *QTL1.1*_Haploblock3 (*QTL1.1*_H3) (Martinez-Garcia et al., 2013). Different letters/* indicate significant differences at P<0.05 according to Dunnett's T3 test. W, wounded; NW, non-wounded.

nisms that control brown rot fruit resistance/tolerance, further QTL mapping studies using pedigree based analysis (PBA) approach will be performed. This could uncover additional regions in peach genome associated with brown rot DSI and or provide additional resolution in elucidating trait values of brown rot associated haplotypes/alleles.

Conclusion

In this study, we presented the responses of 164 pedigreed germplasm from the Clemson University peach breeding program to inoculations with *Monilinia fructicola*. Significant differences in brown rot fruit infection responses were observed. Genotypes/diplotypes with different phenotypic performance/trait values were detected for three published brown rot associated QTLs, *QTL1.1*, *QTL1.2* and *SK-if_2009*, and haplotypes/alleles with trait values were detected in all reported brown rot associated genome regions (*QTL1.1*, *QTL1.2*, *Sk-if_2009* and *FL-rd_2009*). The analyzed peach germplasm exhibited sufficient brown rot tolerance/resistance variability for novel detection of genomic regions associated with brown rot tolerance/resistance in peach applying PBA approach. This work represents an important basis for developing predictive DNA information tools for brown rot resistance / tolerance.

Acknowledgments

This work was supported by USDA's National Institute of Food and Agriculture for the Specialty Crop Research Initiative through the competitive project "RosBREED: Combining disease resistance with horticultural quality in new rosaceous cultivars" (2014-51181-22378). The authors thank Musser Farm staff for their technical assistance.

Literature cited

Chen, F., S.E. Everhart, P.K. Bryson, C. Luo, X. Song, X. Liu, and G. Schnabel. 2015. Fungicide-induced transposon movement in *Monilinia fructicola*. Fungal Genet. and Biol. 85: 38-44.

Chen, F., X. Liu, and G. Schnabel. 2013. Field strains of *Monilinia fructicola* resistant to both MBC and DMI fungicides isolated from stone fruit orchards in the eastern United States. Plant Dis. 97: 1063-1068.

Feliciano, A., A.J. Feliciano, and J. Ogawa. 1987. *Monilinia fructicola* resistance in the peach cultivar Bolinha. Phytopathol. 77: 776-780.

Gradziel, T., R. Bostock, and J.P. Adaskaveg. 2002. Resistance to brown rot disease in peach is determined by multiple structural and biochemical components. Acta Hort. 622: 347-35.

Gradziel, T. and D. Wang. 1993. Evaluation of brown rot resistance and its relation to enzymatic browning in clingstone peach germplasm. J. Amer. Soc. Hort. Sci. 118: 675-679.

Gradziel, T., M. Thorpe, R. Bostock, and S. Wilcox. 1997. Breeding for brown rot (*Monilinia fructicola*) resistance in clingstone peach with emphasis on the role of fruit phenolics. Acta Hort.. 465: 161- 170.

Luo, C. and G. Schnabel. 2008. Adaptation to fungicides in *Monilinia fructicola* isolates with different fungicide resistance phenotypes. Phytopathol. 98: 230-238.

Martínez-García, P.J., D.E. Parfitt, R.M. Bostock, J. Fresnedo-Ramírez, A. Vazquez-Lobo, E.A. Ogundiwin, T.M. Gradziel, and C.H. Crisosto. 2013. Application of genomic and quantitative genetic tools to identify candidate resistance genes for brown rot resistance in peach. PloS One 8: e78634.

Pacheco, I., D. Bassi, I. Eduardo, A. Ciacciulli, R. Pirona, L. Rossini, and A. Vecchietti. 2014. QTL mapping for brown rot (*Monilinia fructigena*) resistance in an intraspecific peach (*Prunus persica* L. Batsch) F1 progeny. Tree Genetics and Genomes 10: 1223-1242.

Rungjindamai, N., P. Jeffries, and X. Xu. 2014. Epidemiology and management of brown rot on stone fruit caused by *Monilinia laxa*. Eur. J. Plant Pathol. 140: 1-17.

Sharma, R.L. 2005. Management of brown rot (Monilinia laxa) in peaches in warmer arears. Acta Hort.. 696: 359-362.

Tate, K. and P. Wood. 2000. Potential ascospore production and resulting blossom blight by *Monilinia fructicola* in unsprayed peach trees. New Zealand J. Crop and Hort. Sci. 28: 219-224.

Verde, I., N. Bassil, S. Scalabrin, B. Gilmore, C.T. Lawley, K. Gasic, D. Micheletti, U.R. Rosyara, F. Cattonaro, and E. Vendramin. 2012. Development and evaluation of a 9K SNP array for peach by internationally coordinated SNP detection and validation in breeding germplasm. PLOS ONE 7(6), 10.

Zehr, E.I. 1982. Control of brown rot in peach orchards. Plant Dis. 66: 1101-1105.

Ziosi, V., M. Noferini, G. Fiori, A. Tadiello, L. Trainotti, G. Casadoro, and G. Costa. 2008. A new index based on vis spectroscopy to characterize the progression of ripening in peach fruit. Postharvest Biol. Technol. 49: 319-329.

Journal of the American Pomological Society 72(2): 101-112 2018

Root Distribution of ‘Brightwell’ and ‘Premier’ Rabbiteye Blueberries as Influenced by Pecan Shell Mulch

Ashley A. Hoppers[1,2], James D. Spiers[2], J. Raymond Kessler, Jr.[2], Amy N. Wright[2], and Arlie A. Powell[2]

Additional index words: agricultural byproduct, *Carya illinoinensis,* root growth, *Vaccinium virgatum*

Abstract

Pecan (*Carya illinoinensis* [Wangenh.] K. Koch) shell waste lacks effectual, economic disposal. If shells could be repurposed as mulch, then growers may be able to treat shell byproduct as a resource. In 2016, root distribution and growth of ‘Brightwell’ and ‘Premier’ rabbiteye blueberries (*Vaccinium virgatum* Aiton syn. *V. ashei* Reade) was examined using the Horhizotron™. Each Horhizotron™ had four wedge-shaped quadrants filled with 10 cm of an amended 80% pine bark and 20% sand (by volume) substrate, then 7.6 cm of “fresh” pecan shells (FPS), “aged” pecan shells (APS), pine bark nuggets (PB), or an unamended 80% pine bark and 20% sand substrate (PBS). Growth was determined weekly by measuring the horizontal root length (HRL) and root depth (RD) of the five longest roots on either side of a quadrant. Roots that grew into the substrate and mulch treatment layers were not measured separately. ‘Premier’ HRL showed roots in FPS grew a shorter distance across the quadrant profile than roots in PBS, but had similar HRL with APS and PB. In ‘Brightwell’, both shell treatments had shorter HRL across the quadrant than the roots in PB and PBS. RD measurements for ‘Premier’ showed roots generally initiated at the same depth for FPS, APS and PB, though the roots in PBS had shallower growth than the roots in PB and FPS. ‘Brightwell’ RD showed roots initiated more into the upper portions of the quadrant profile in APS and PBS than in FPS or PB. Root system architecture was reflected in root dry weight (RDW). For both cultivars, substrate layer RDW was similar across all treatments, but mulch layer RDW varied. Though APS had a higher mulch layer RDW than the PB treatment in ‘Premier’, differences in RDW within the mulch layer did not impact total root dry weight (mulch layer RDW + substrate layer RDW). In ‘Brightwell’, APS had a higher RDW than FPS and PB, though PBS was similar to both APS and FPS. Unlike ‘Premier’, total RDW in ‘Brightwell’ was impacted by differences in mulch layer RDW, as the quadrants that contained FPS and PB had a lower total root dry weight than the quadrants containing APS and PBS. These results indicated that root growth in pecan shells, as compared with root growth within and below pine bark, was not hindered.

The success of a blueberry planting is linked to site physical, chemical, and meteorological conditions. Though rabbiteye blueberries sometimes prosper in nutrient-poor mineral soils throughout the southeastern United States, they are best grown in sands and loams high in organic matter (Braswell et al., 2015). Compared with taproot systems, plant species with fibrous roots are often considered less problematic to transplant; however, this generalization has exceptions. For example, while the native ericaceous species mountain laurel (*Kalmia latifolia* L.) produces a fibrous root system, it periodically does not survive transplanting into the landscape (Wright et al., 2004a). Similarly, transplant survival of ericaceous members of the *Vaccinium* genus, such as the blueberry, can also be challenging.

Generally, transplant growth is most commonly limited by water stress (Price et al., 2011). By nature, blueberries possess a fibrous, shallow root system devoid of root hairs (Eck, 1988), which may predispose

[1] Corresponding author: aahoppers@uga.edu

[2] Department of Horticulture, 101 Funchess Hall, Auburn University, Auburn, AL 36849-7350

This research was supported by the Alabama Nut, Fruit, and Vegetable Industry, Alabama Agricultural Experiment Station, and the Hatch program of the National Institute of Food and Agriculture, U.S. Department of Agriculture.

them to water stress (Lyrene, 1997). Thus, the establishment of a healthy root system in mineral soils with depleted organic matter is critical for the survival of newly set blueberry transplants. The root system of the highbush blueberry was described as predominantly composed of fine roots that were concentrated at a 12–25 cm depth within the drip line (Gough, 1980). While the rabbiteye blueberry's root system penetrated more easily and deeply into the soil profile than the highbush blueberry (Himelrick et al., 2002), the rabbiteye blueberry root distribution is nonetheless shallow with roots rarely growing deeper than 40 cm into the soil profile (Patten et al., 1988; Spiers, 1998). Most roots develop within the top 20–30 cm in the soil, of which approximately 90% were located within the blueberry canopy's dripline (Gough, 1980; Sánchez and Demchak, 2003).

Results of several studies support the use of organic materials in blueberry production. Pine bark, peat, and sawdust were commonly used as soil amendments in conventional highbush blueberry culture (Burkhard et al., 2009). Such amendments promoted uniform root development (Spiers, 1986), and enhanced soil aeration and water-holding capacity (Haynes and Swift, 1986). In addition to organic soil amendments, thickly applied organic surface mulches (7–12 cm) after planting are commonly used, as they are ideal for regulating soil temperature (Burkhard et al., 2009; Spiers, 1995) and moisture extremes (Spiers, 1986). Mulches also improved blueberry transplant root development (Hicklenton et al., 2000), a key factor in transplant success.

Rapid initiation of new roots (Wright et al., 2004a) and resistance to water stress (Hicklenton et al., 2000) were critical factors in transplanting success. Yet, despite the influence of root growth on plant survival, data on root growth and root system architecture are often not collected because most methods are time consuming, destructive, or expensive (Wright and Wright, 2004b). Temperature, shoot growth, and seasonality influenced root growth in raspberry plants (*Rubrus idaeus* L.) (Atkinson, 1973) and plum (*Prunus salicina* Lindl.) (Bhar et al., 1970); however, studies focused on the nature of bush fruit root systems were scarce. This is particularly true for the cultivated blueberry. While it is known that the blueberry root system is shallow and fibrous (Austin, 1982; Braswell et al., 2015; Himelrick et al., 2002; Spiers, 1995), and many studies showed that blueberries benefit from surface mulch (Burkhard et al., 2009; Clark and Moore, 1991; Fonsah et al., 2008; Julian et al., 2012; NeSmith, 2003); few studies have investigated blueberry root system architecture within and below alternatives to the industry mulching standards, such as bark and sawdust.

When plants are transplanted into the landscape, uninterrupted plant growth depends on the formation of new roots outside of the original root ball (Wright et al., 2004a). Observation and measurement of roots as they grow is useful in determining root growth preferences, as is studying the location and depth of root formation (Jackson et al., 2005). Thus, understanding root system growth and architecture are important factors that influence transplant survival and production success (Wright and Wright, 2004a). Several instruments were used in the past to study root growth, including the rhizotron (Bohm, 1979; Huck and Taylor, 1982), portable rhizotron (Pan et al., 1998), and the rhizobox (Wenzel et al., 2001); however, these instruments are relatively expensive and limited in their ability to provide information. Other methods of measuring root growth were generally restricted to observation via subjective visual rating scales or by dry weight analysis, with both methods being destructive (Jackson et al., 2005).

The Horhizotron™, a horizontal root growth measurement instrument developed cooperatively between Auburn University and Virginia Tech, is newer and relatively inexpensive. Wright and Wright (2004b) reported that all materials used in the design were available at building supply stores, and the cost was less than $50.00 per unit. A key factor that makes the Horhizotron™ desirable is that it provides a simple, non-destructive means of measuring root growth under a variety of rhizosphere conditions. Unlike other container-type rhizotrons where roots are not visible until they reach the edge of the container, the Horhizotron™ is constructed of glass, which allows observation of the rate and direction of root growth into the surrounding landscape (Wright and Wright, 2004b). The design also allows the effect of multiple substrates to be evaluated on an individual plant simultaneously.

Pine bark is one of the most commonly used mulches and substrate amendments in the horticulture industry; however, concern regarding cost, supply, and consistency has motivated the search for suitable alternatives in crop production (Jackson et al., 2005; Lu et al., 2006). Amongst the potential organic mulch alternatives to pine bark is pecan shell waste. In 2015, the United States produced approximately 115 million kg of pecans (National Agricultural Statistics Service, 2016). Of that total production, 17% (19 million kg) was sold in-shell, while the remaining 83% (96 million kg) was sold shelled (National Agricultural Statistics Service, 2016). Of the 83% of production that was shelled prior to retail, 41% (39 million kg) was nutmeat and 59% (57 million kg) was shell waste. Most pecan production is located in the southern United States. Georgia has been the leading pecan producing state for the past 3 years, and was also a leading producer of blueberries (National Agricultural Statistics Service, 2016).

Ideal mulches are sourced from materials that are abundant, self-sustaining, and efficient in weed suppression. This category includes commercial standards like pine bark, but it may also encompass new, innovative materials. Because shell waste is a natural byproduct of the commercial pecan industry, the supply is annually renewed. Shell waste may be used in the horticulture industry either as a mulch or container substrate component. While phytotoxic substances and inadequate available water in shell-based substrates were suspected of stunting the growth of tomato plants (*Lycopersicon esultentum* Mill. 'Rutgers') (Wang and Pokorny, 1989), pecan shells as a mulch under peach trees (*Prunus persica* L. 'Loring') provided acceptable weed suppression (Stafne et al., 2009). The objective of this research was to investigate the effects of pecan shell mulch on rabbiteye blueberry root system architecture compared to pine bark using the Horhizotron™.

Materials and Methods

The Horhizotron™ is a non-destructive root measurement instrument that allows a container-grown plant to be fitted within four quadrants around a container plant's original root ball (Wright and Wright, 2004b). The Horhizotrons™ used in this research had four quadrants constructed from two 3.2 mm thick glass panes (20.3 × 26.7 cm) that were held together on the top and bottom with vinyl j-channels, and sealed with waterproof caulk (Wright and Wright, 2004b).

Figure 1. Horhizotron™ has four wedge-shaped quadrants that extend out from the root ball. Quadrants are constructed of glass panes connected by vinyl j-channels. The aluminum base onto which the glass panes are attached is fastened to a treated wood frame.

Figure 2. To exclude light and protect the root system from temperature extremes, exterior walls were constructed from foam insulation board and placed around each Horhizotron™.

Each Horhizotron™ had an aluminum base (0.6 m × 0.6 m × 0.3 cm) that was attached to a wooden frame (5.1 × 5.1 cm) constructed from treated lumber. An overhead view of the Horhizotron™ (Fig. 1) depicts the four quadrants extending outward from the original root ball in a star-like configuration. Drainage holes were made where the root ball sat, and within each quadrant to ensure proper drainage.

To exclude light and protect the root system from temperature extremes, exterior walls were placed around each Horhizotron™ (Fig. 2). The walls were made of foam insulation board 1.9 cm with an aluminum foil exterior and plastic interior (Wright and Wright, 2004b). Walls were assembled into one unit by connecting them with top and bottom j-channels, and then fastened into place by fitting them into a 2.5 cm rim around the perimeter of the aluminum base. Upper lids for each Horhizotron™ were made from two sections of foam insulation board (Fig. 3) with a portion cut out to expose the substrate surface immediately around the plant stem, which allowed for easy removal of the lids.

The experiment was arranged in a randomized complete block design. Each Horhizotron™ represented an individual block, and there were six blocks per cultivar. The rabbiteye blueberry cultivars 'Brightwell' and 'Premier' were evaluated because they are two widely grown cultivars in Alabama and the southeastern United States. Two different ages of pecan shells were evaluated: fresh pecan shells that were less than one-year-old (2015 harvest season) and aged pecan shells that were over one-year-old (2014 harvest season) (Whaley Pecan Company Inc., Troy, AL). The shells were milled, finely textured, and mostly free of residual nut meat. The shells were stored outdoors in uncovered piles. Pine bark mini-nuggets (West Fraser Mills, Opelika, AL) were also selected for a standard cultural practice. There were four treatments randomly distributed among each Horhizotron™ unit's four quadrants. The

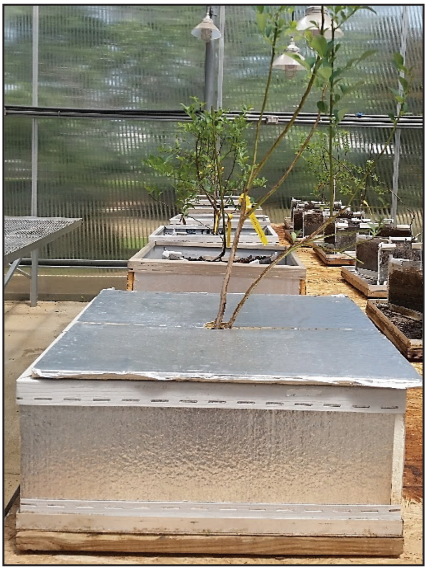


Figure 3. Upper lids for each Horhizotron™ were made from foam insulation board with a portion cut out around the plant stem.

treatments consisted of the three mulches: "fresh" pecan shells (FPS), "aged" pecan shells (APS), and pine bark mini-nuggets (PB). An unamended 80% pine bark and 20% sand (by volume) (PBS) substrate treatment was included with the purpose of adding a "no mulch" treatment.

On 26 Apr. 2016, six mature 11.4 L container plants each of 'Brightwell' and 'Premier' rabbiteye blueberry were removed from their containers and placed into the center of separate Horhizotrons™ (volume of each Horhizotron was 3.7 L) on a greenhouse bench at the Paterson Greenhouse Complex at Auburn University, Auburn, AL. Roots had established throughout the plant's original container profile and touched the edge of the substrate-container interface, but were not circling. When placed into Horhizotrons™, root balls of all plants were undisturbed and positioned snugly against the inner point of each wedge-shaped quadrant composed of two glass panes (20.3 × 26.67 cm) (Wright and Wright, 2004b).

Each of the four quadrants surrounding the root ball were then filled with 10 cm of an 80% pine bark and 20% sand substrate (by volume) amended per 0.76 cubic meter with 2.3 kg of Peafowl® 25N-1.76P-6.64K (Piedmont Fertilizer Company, Inc., Opelika, AL) and 0.7 kg Micromax® (Scotts Co., Marysville, Ohio). No lime was added to the substrate to maintain the acidic soil conditions required by *V. virgatum*. Once each of the four quadrants was filled with the appropriate amount of substrate, each quadrant was gently hand-watered to allow for substrate settling. The remaining space in the Horhizotron™ quadrants was then filled with 7.6 cm of one of the randomly assigned four treatments.

Though the technique used to apply the mulch treatments left the plants at-grade in the Horhizotrons™, layering the treatments on top of the substrate was intended to simulate the modified above-soil grade mulching practice used in conventional commercial blueberry operations, wherein the root ball is fully in the soil profile, and the organic mulch layer is applied above-grade. The unamended PBS substrate (no mulch) treatment was intended to represent traditional at-grade planting without an organic mulch layer. After planting, each plant's root ball and quadrants were hand-watered as needed with tap water to keep roots moist.

Measuring shoot growth was unnecessary due to the design of the Horhizotron™ (each individual plant grew in all four mulch treatments simultaneously); however, initial size indices of plant canopies ([height + widest width + width perpendicular to widest width]/3) were measured to document a baseline for plant size (Price et al., 2009). To measure total length, rather than new length, as roots grew out of the original root ball and along the glass panes of each quadrant profile, the horizontal root lengths (parallel to the base of the Horhizotron™) of the five longest roots visible along each glass pane of a quadrant were measured weekly. A transparent 1 cm × 1 cm grid was placed on the surface of the glass panes to assist with observation and measurement of the five longest roots on either side of a quadrant. Horizontal root length (HRL) measurements represented lateral root penetration into the substrate and mulch treatments after transplanting (Price et al., 2009). The same five roots used for the HRL measurements were used for root depth (RD) measurements, which represented root penetration vertical to the base of the Horhizotron™ and was also documented using the transparent grid. Roots growing into the substrate layer and the mulch treatment layer were not measured separately.

HRL measurements of 'Brightwell' and 'Premier' began 45 days after transplanting (DAP), and were repeated weekly thereafter until roots in one substrate reached the end of the Horhizotron™ quadrant (26 cm). When HRL measurements ceased for 'Brightwell' on 5 Aug. 2016 (101 DAP) and 'Premier' on 12 Aug. 2016 (108 DAP), final size indices of the canopies were measured, which was determined by measuring plant height from the crown to the top of the main shoot, and by taking cross sectional diameters parallel and perpendicular to the row ([height + widest width + width perpendicular to widest width]/3). Plants of 'Brightwell' were removed from Horhizotrons™ for root harvest on 7 Sept. 2016 (132 DAP) and 'Premier' on 12 Sept. 2016 (137 DAP).

Roots in each quadrant were cut from the original root ball where the substrate and treatment met the root ball. To observe the difference in root growth within the mulch treatments versus the substrate portions of the quadrants, roots that grew in the mulch layers were separated from the roots that grew in the substrate layers. Roots from the substrate and mulch layers were then separately washed and dried for 48 h at 66 °C, and weighed to determine root dry weight (RDW) in substrate and mulch treatment portions separately.

An analysis of variance was performed on all response variables using PROC GLIMMIX in SAS version 9.4 (SAS Institute, Cary, NC). Blueberry cultivars were analyzed as separate experiments. Root length and depth were analyzed as a randomized complete blocks design with repeated measures on dates, and root number as sub-samples. Blocks and the Horhizotron™ face were random variables in the model. Least squares means comparisons among mulches were determined using the simulate adjustment in the LSMEANS STATEMENT. Linear, quadratic, or cubic trends over dates were determined using qualitative-quantitative model regressions. All significances were at $\alpha = 0.05$ unless otherwise indicated.

Results and Discussion

As observed in a previous study using the

Table 1. Effect of mulch type on horizontal root length (HRL[z]) of *Vaccinium virgatum* 'Premier' and 'Brightwell' growing in Horhizotron™ in a greenhouse in Auburn AL.

	Premier HRL[z] (mm)										
Treatment[y]	45[x]	52	59	66	73	80	87	94	101	108	Sign.[v]
FPS	19.9 ns[w]	40.1 b	65.6 b	88.3 b	109.7 ab	120.2 b	141.0 b	154.5 b	170.2 b	185.2 b	Q***
APS	36.7	62.9 ab	84.7 ab	104.8 ab	127.9 ab	146.1 a	155.2 ab	175.4 ab	193.8 ab	209.0 ab	Q***
PB	26.2	57.6 ab	69.1 b	91.9 ab	108.2 b	124.5 ab	143.4 b	158.5 b	177.7 b	192.7 ab	Q**
PBS	43.7	65.1 a	97.3 a	114.6 a	132.2 a	156.3 a	176.1 a	190.3 a	207.7 a	213.8 a	Q***
	Brightwell HRL[z] (mm)										
Treatment[y]	45[x]	52	59	66	73	80	87	94	101		Sign.[v]
FPS	35.1 b[w]	73.4 ns	98.1 ns	119.4 ns	136.2 ns	151.8 b	163.0 c	172.1 c	181.5 b		Q***
APS	50.3 ab	75.4	99.1	118.1	138.9	158.7 b	175.2 abc	181.7 bc	194.4 b		Q***
PB	40.9 b	64.7	97.0	121.5	150.7	167.0 ab	182.6 ab	196.6 ab	212.9 a		Q***
PBS	63.1 a	78.3	97.4	122.1	151.6	177.1 a	190.0 a	208.7 a	218.6 a		C***

[z] HRL = root length measured parallel to the ground.

[y] Treatments were 7.6 cm of fresh pecan shells (FPS), aged pecan shells (APS), pine bark (PB), or unamended 80% pine bark and 20% sand (by volume) substrate applied on top of 10 cm of amended 80% pine bark and 20% sand (by volume) substrate in Horhizotron™ quadrants.

[x] Days after planting (DAP) in Horhizotron™ (Wright and Wright, 2004).

[w] LSmeans within columns and cultivars followed by common letters do not differ at the 5% level of significance, by the simulate adjustment.

[v] The mulch treatment by DAP interaction was significant. HRL was analyzed with repeated measures on 7 day intervals that began 45 DAP for both cultivars and concluded at 108 DAP for Premier and 101 DAP for Brightwell. Significant quadratic (Q) or cubic (C) trends using regression models at $\alpha = 0.01$ (**), and 0.001 (***).

Table 2. Effect of mulch type on root depth (RD[z]) measured from the surface of the soil profile of *Vaccinium virgatum* 'Premier' and 'Brightwell' growing in Horhizotrons™ in a greenhouse in Auburn AL.

	Premier RD[z] (mm)										
Treatment[y]	45[x]	52	59	66	73	80	87	94	101	108	Sign.[v]
FPS	59.3 ns[w]	90.7 ns	103.8 ab	114.0 ab	122.2 a	111.5 ab	113.3 ab	113.8 ab	122.0 ab	127.0 ab	C***
APS	69.2	93.8	110.3 a	116.0 ab	103.5 bc	102.3 abc	110.7 ab	106.8 b	105.8 b	110.2 abc	C***
PB	62.5	88.0	106.2 ab	117.5 a	119.8 ab	119.0 a	126.5 a	129.5 a	130.7 a	127.7 a	C**
PBS	65.0	81.7	85.3 c	84.0 c	87.0 c	93.0 c	99.0 b	104.7 b	105.0 b	100.2 c	Q*
	Brightwell RD[z] (mm)										
Treatment[y]	45[x]	52	59	66	73	80	87	94	101		Sign.[v]
FPS	87.6 ns[w]	115.2 a	116.0 a	118.8 ab	122.8 a	123.4 a	125.2 ab	123.4 c	122.6 b		C*
APS	94.0	104.4 ab	110.8 a	104.2 bc	99.4 b	104.2 b	110.8 b	116.0 ab	116.8 ab		C*
PB	90.4	99.8 bc	119.0 a	122.8 a	130.0 a	125.0 a	126.2 a	131.0 a	138.4 a		C***
PBS	83.4	92.4 c	93.2 b	100.4 c	95.2 b	92.6 c	96.2 c	106.4 bc	115.0 b		C***

[z] RD = root length measured perpendicular to the ground.
[y] Treatments were 7.6 cm of fresh pecan shells (FPS), aged pecan shells (APS), pine bark (PB), or unamended 80% pine bark and 20% sand (by volume) substrate applied on top of 10 cm of amended 80% pine bark and 20% sand (by volume) substrate in Horhizotron™ quadrants.
[x] Days after planting (DAP) in Horhizotron™ (Wright and Wright, 2004).
[w] LSmeans within columns and cultivars followed by common letters do not differ at the 5% level of significance, by the simulate adjustment.
[v] The mulch treatment by DAP interaction was significant. RD was analyzed with repeated measures on 7 day intervals that began 45 DAP for both cultivars and concluded at 108 DAP for Premier and 101 DAP for Brightwell. Significant quadratic (Q) or cubic

Horhizotron™, small spaces between the substrate and glass panes at the end of each quadrant air-pruned roots as they grew into them, ceasing growth at that point (Wright et al., 2007). For both cultivars, roots generally initiated further away from the original root ball towards the quadrant profile's end (26 cm) in the PBS and pine bark treatments (Table 1). This trend supported previous observations where roots may have proliferated into a smaller portion of the quadrant profile in those treatments (Wright et al., 2007). RDW was also greatest in the mulch layer for pine bark and aged shells. When compared with aged pecan shells, pine bark had a lower mulch layer RDW for both cultivars (Fig. 4, Fig. 5).

Roots of 'Brightwell' grew more deeply in quadrants with pine bark and fresh pecan shells, whereas the quadrants that contained aged pecan shell mulch and PBS had a shallower RD (Table 2). RD in 'Premier' began to separate between treatments at 66 DAP. By 73 DAP, trends in RD between each treatment were distinctive, and root growth was maintained at those respective depths for the remainder of the study. For 'Brightwell', RD differentiated between treatments by 52 DAP. Treatments remained at those respective depths throughout the remainder of the experiment; however, the RD trend observed with 'Premier' was more pronounced in 'Brightwell.'

RDW in the substrate layer was similar across all treatments, regardless of cultivar. This pattern of root distribution supports previous findings (Haynes and Swift, 1986; Hicklenton et al., 2000) where well-drained substrates composed of organic (bark) and inorganic (sand) materials effectively promoted blueberry root growth. Conversely, root growth within the mulch layer varied. In general, the differences observed between mulch layer RDW for 'Premier' were not pronounced. Pine bark mulch had a lower RDW than the other treatments (Fig. 4). While differences in root distribution amongst the mulch layers based on RDW for 'Premier' was quantifiable, those differences did not impact total RDW, which, like the substrate root layer, was similar across all treatments (Fig. 4). 'Premier' plants were uniform in size throughout the experiment, with an average initial growth index of 48 cm, and final growth index of 110 cm (data not shown).

Consequently, the main difference between cultivars was the variances in root distribution within the mulch layer. Treatment differences between mulch layer RDW were more pronounced for 'Brightwell' than for 'Premier'. Mulch layer RDW for 'Brightwell' was distinctively higher in aged pecan shells than in the fresh pecan shells and pine bark (Fig. 5). While mulch layer RDW did not influence total RDW for 'Premier' those differences did impact total RDW for 'Brightwell.' The same trends for RD and mulch layer RDW for 'Brightwell' were reflected in total RDW. Quadrants containing aged pecan shell mulch and PBS had a higher total RDW than quadrants with fresh pecan shell and pine bark mulches (Fig. 5). 'Bright-

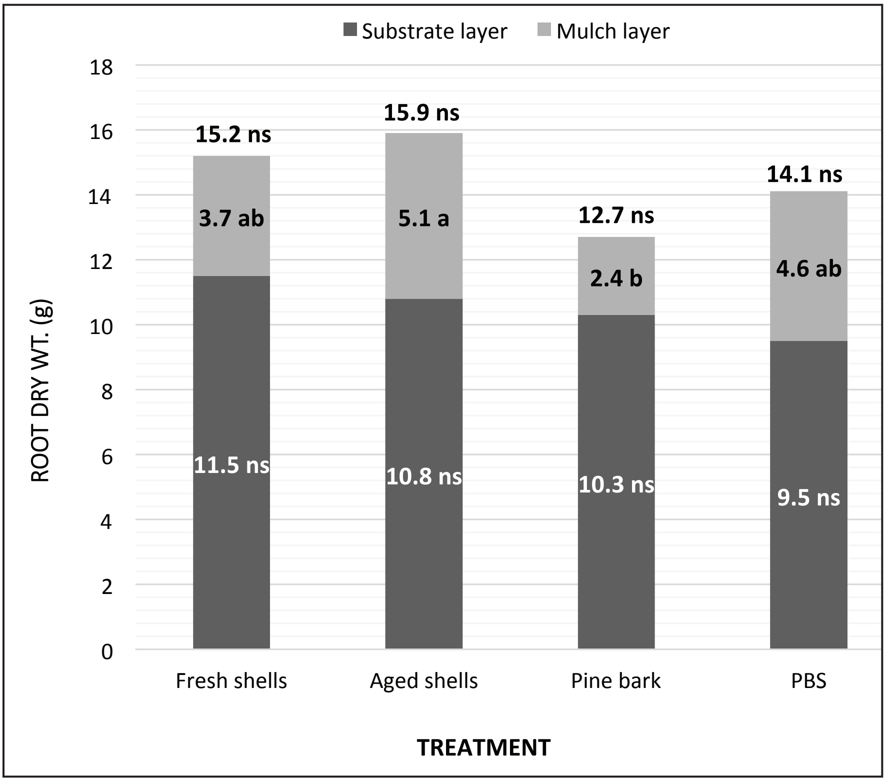


Figure 4. Root dry weight (RDW) of *Vaccinium virgatum* 'Premier'. Roots were divided into mulch (fresh shells, aged shells, pine bark, and unamended 80% pine bark and 20% sand substrate [PBS]) and substrate layers, then washed separately to determine mulch layer RDW and substrate layer RDW. Total RDW = mulch layer RDW + substrate layer RDW. Least squares means comparisons among mulch treatments and substrate layers using the Shaffer-simulated method at $\alpha = 0.05$. ns = not significant. All plants were grown in Horhizotrons™ in a greenhouse in Auburn, AL.

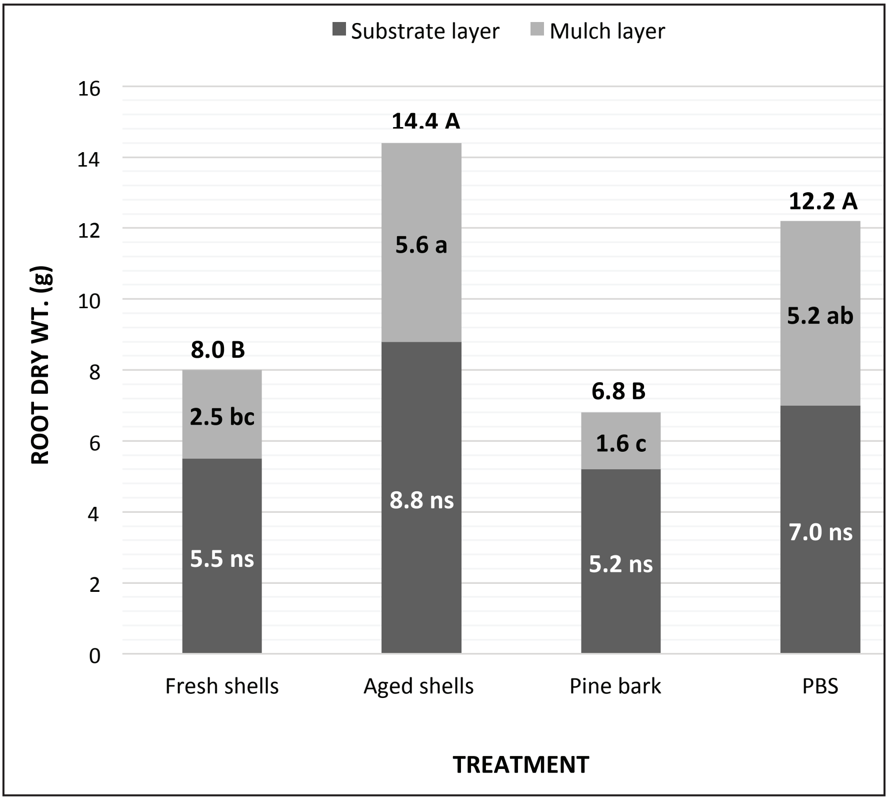


Figure 5. Root dry weight (RDW) of *Vaccinium virgatum* 'Brightwell'. Roots were divided into mulch (fresh shells, aged shells, pine bark, and unamended 80% pine bark and 20% sand substrate [PBS]) and substrate layers, then washed separately to determine mulch layer RDW and substrate layer RDW. Total RDW = mulch layer RDW + substrate layer RDW. Least squares means comparisons among mulch treatments and substrate layers using the Shaffer-simulated method at $\alpha = 0.05$. ns = not significant. All plants were grown in Horhizotrons™ in a greenhouse in Auburn, AL.

well' plants were uniform in size throughout the experiment, with average initial growth index of 47 cm, and final growth index of 113 cm (data not shown).

When organic mulches were tested as a cultural practice with blueberry transplants, they had a higher water stress tolerance (Hicklenton et al., 2000), and a more even root distribution extending from the plant crown (Spiers, 1986). Another blueberry root distribution study estimated that soil moisture and temperature were major limiting factors in blueberry root growth, and when mulches were used, most roots were concentrated under the mulched areas where soil moisture was prevalent and soil temperature reduced (Spiers, 1998). These findings were consistent with the results derived from the RDW of the substrate layers (below all mulch treatments), regardless of cultivar (Fig. 4, Fig. 5). Though the RDW was similar in the quadrants with PBS and aged pecan shell mulch for both cultivars, we hypothesize that had the PBS treatment been a true bare-ground treatment imposed in a field-production setting, the RDW would have likely been lower. Plant height, shoot growth, and root growth were greater for blueberry plants that were mulched than for those that were grown without mulch (Clark and Moore, 1991; Gough, 1980, Patten et al., 1988, and Spiers, 1995).

Another observation derived from the root distribution in this study was the general lack of roots that grew into the pine bark mulch layer as compared to the aged pecan shell mulch layer in both cultivars. This trend in root growth was similar to results of previous studies that evaluated blueberry root distribution under sawdust mulch (Gough, 1980; Shutak and Christopher, 1952). No roots were found growing in the undecomposed layers of sawdust mulch, which was approximately 10 cm thick (Gough, 1980). Rather, greater amounts of feeder roots were found growing below the mulch, beginning at a depth of 11 cm and increasing in density to a depth of 13 cm. These findings indicated that the depths at which the roots were found corresponded with the lower layers of undecomposed mulch and the upper layers of partially decomposed mulch. Similarly, Shutak and Christopher (1952) found limited blueberry root growth within the sawdust mulch layer itself; rather most roots were found growing in the lower, decomposed layers of the mulch closest to the soil surface.

Root distribution trends in this study showed that for 'Brightwell', root development within the aged shell mulch resulted in a higher RDW than that achieved

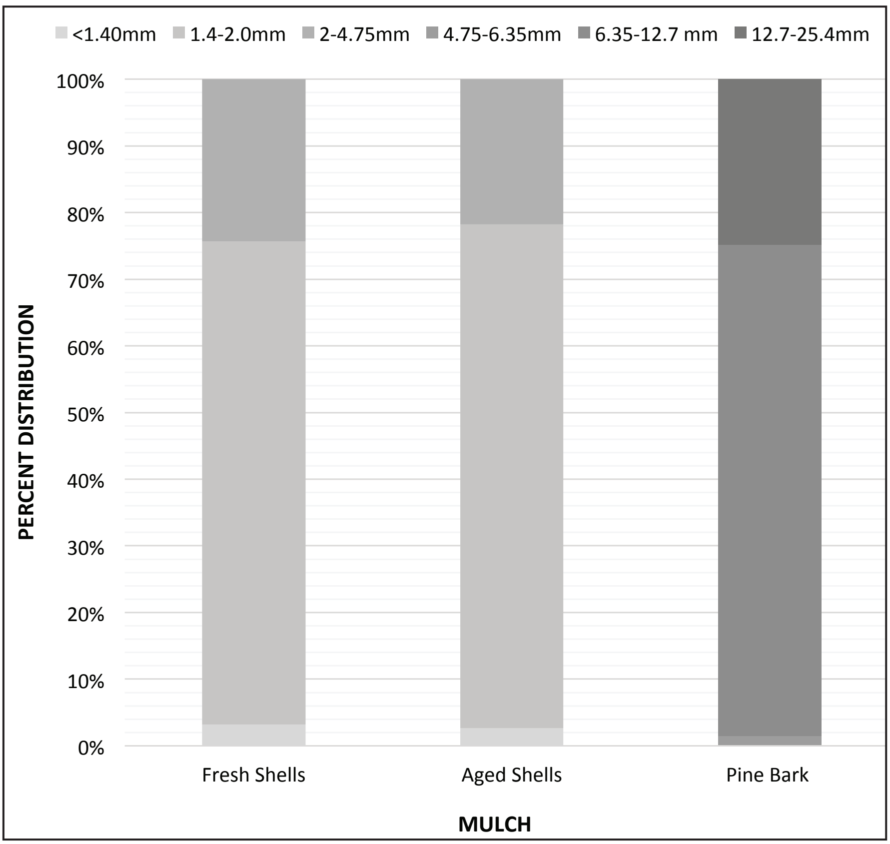


Figure 6. Particle size distribution by mulch type.

in the fresh shell and pine bark mulches. While differences in RDW in ‘Premier’ were not as prominent as those for ‘Brightwell’, more roots established within the aged pecan shell mulch layer than in the pine bark mulch layer. Considering the aged pecan shells used in this study were partially decomposed, it is hypothesized that the smaller particle size (Fig. 6) of the aged shell mulch, coupled with the level of decomposition, created a more hospitable environment for roots to develop than did the pine bark mulch.

Conclusions

Pecan shells are an underutilized waste product of the pecan industry, and much of the pecan production in the United States is in relatively close proximity to regions growing blueberries. An objective of this research was to ascertain the potential for pecan shells to be used as mulch for rabbiteye blueberry production, or more specifically, to determine whether pecan shells negatively affected rabbiteye blueberry root growth. Horhizotrons™ were chosen for this experiment because they provided a nondestructive means for examining how blueberry root growth was influenced by treatments, and because each individual plant grew into the separate treatments simultaneously. This experiment indicated that the growth and development of the rabbiteye blueberry root system is not hindered by fresh pecan shell mulch or aged pecan shell mulch as compared with milled pine bark.

Literature Cited

Atkinson, D. 1973. Seasonal changes in the length of white unsuberized root on raspberry plants grown under irrigated conditions. J. Hort. Sci. 48:413–419.

Austin, M.E. 1982. An observation on rooting depth of rabbiteye blueberry plants. Univ. Georgia Agr. Expt. Sta. Res. Rpt. 394.

Bhar, D.S., G.F. Mason, and R.J. Hilton. 1970. In situ observations on plum root growth. J. Amer. Soc. Hort. Sci. 95:237–239.

Bohm, W. 1979. Methods for studying root systems. Springer-Verlag, Berlin.

Braswell, J.H., S.J. Stringer, B.J. Sampson, and D. Ingram. 2015. Establishment and maintenance of blueberries. Mississippi State Univ. Ext. Serv. Publ. 1758.

Burkhard, N., D. Lynch, D. Percival, and M. Sharifi. 2009. Organic mulch impact on vegetation dynamics and productivity of highbush blueberry under organic production. HortScience 44:688–696.

Clark, J.R. and J.N. Moore. 1991. Southern highbush blueberry response to mulch. HortTechnology 1:52–54.

Eck, P. 1988. Blueberry science. Rutgers Univ. Press, New Brunswick, N.J.

Fonsah, E.G., G.W. Krewer, K. Harrison, and D. Stanaland. 2008. Economic returns using risk-rated budget analysis for rabbiteye blueberry in Georgia. HortTechnology 18:506–515.

Gough, R.E. 1980. Root distribution of ‘Coville’ and ‘Lateblue’ highbush blueberry under sawdust mulch. J. Amer. Soc. Hort. Sci. 105:576–578.

Haynes, R.J. and R.S. Swift. 1986. Effect of soil amendments and sawdust mulching on growth, yield and leaf nutrient content of highbush blueberry plants. Scientia Hort. 29:229–238.

Hicklenton, P.R., J.Y. Reekie, and R.J. Gordon. 2000. Physiological and morphological traits of lowbush blueberry (*Vaccinium angustifolium* Ait.) plants in relation to post-transplant conditions and water availability. Can. J. Plant Sci. 80:861–867.

Himelrick, D.G., A.A. Powell, and W.A. Dozier. 2002. Commercial blueberry production guide for Alabama. Alabama Coop. Ext. Serv. Auburn and Alabama A&M Universities. Cir. ANR-904. 16 March 2016. <http://www.aces.edu/pubs/docs/A/ANR-0904/index2.tmpl>.

Huck, M.G. and H.M. Taylor. 1982. The rhizotron as a tool for root research. Adv. Agron. 35:1–35.

Jackson, B.E., A.N. Wright, J.L. Sibley, and J.M. Kemble. 2005. Root growth of three horticultural crops grown in pine bark amended cotton gin compost. J. Environ. Hort. 23:133–137.

Julian, J.W., B.C. Strik, H.O. Larco, D.R. Bryla, and D.M. Sullivan. 2012. Costs of establishing organic northern highbush blueberry: Impacts of planting method, fertilization, and mulch type. HortScience47:866–873.

Lu, W., J.L. Sibley, C.H. Gilliam, J.S. Bannon, and Y. Zhang. 2006. Estimation of U.S. bark generation and implications for horticultural industries. J. Environ. Hort. 24:29–34.

Lyrene, P.M. 1997. Value of various taxa in breeding tetraploid blueberries in Florida. Euphytica 94:15–22.

National Agricultural Statistics Service. 2016. Noncitrus fruits and nuts: 2015 summary. ISSN:

1948-2698. U.S. Dept. Agr. and the Agr. Stat. Board. 18 January 2017. <http://www.nass.usda.gov/Publications/Todays_Reports/reports/crop1015.pdf>.

NeSmith, D.S. 2003. Survival and vigor of southern highbush blueberry genotypes with and without pine bark mulch. Small Fruits Rev. 2:81–86.

Pan, W.L., R.P. Bolton, E.J. Lundquist, and L.K. Hiller. 1998. Portable rhizotron and color scanner system for monitoring root development. Plant and Soil 200:107–112.

Patten, K.D., E.W. Neuendorff, and S.C. Peters. 1988. Root distribution of 'Climax' rabbiteye blueberry as affected by mulch and irrigation geometry. J. Amer. Soc. Hort. Sci. 113:657–661.

Price, J.G., A.N. Wright, K.M. Tilt, and R.S. Boyd. 2009. Organic matter application improves posttransplant root growth of three native woody shrubs. HortScience 44:377–383.

Price, J.G., A.N. Wright, R.S. Boyd, and K.M. Tilt. 2011. Above-grade planting with organic matter improves post-transplant growth of two native shrub species. HortTechnology 21:520–524.

Sánchez, E. and K. Demchak. 2003. Determining when to irrigate highbush blueberry. Fruit Times. 22:11.

Shutak, V. G. and E.P. Christopher. 1952. Sawdust mulch for blueberries. Univ. Rhode Island Agric. Exp. Stat. Bul. 312:18.

Spiers, J.M. 1995. Substrate temperatures influence root and shoot growth of southern highbush and rabbiteye blueberries. HortScience 30:1029–1030.

Spiers, J.M. 1986. Root distribution of 'Tifblue' rabbiteye blueberry as influenced by irrigation, incorporated peatmoss, and mulch. J. Amer. Soc. Hort. Sci. 111:877–880.

Spiers, J.M. 1998. Influence of cultural practices on root distribution of 'Gulfcoast' blueberry. Acta Hort. 512:247–254.

Stafne, E.T., C.T. Rohla, and B.L. Carroll. 2009. Pecan shell mulch impact on 'Loring' peach tree establishment and first harvest. HortTechnology 19:775–780.

Wang, T.Y. and F.A. Pokorny. 1989. Pecan shells as an organic component of container potting media. HortScience 24:75–78.

Wenzel, W.W., G. Weishammer, W.J. Fitz, and M. Puschenreiter. 2001. Novel rhizobox design to assess rhizosphere characteristics at high spatial resolution. Plant and Soil 237:37–45.

Wright, A.N., S.L. Warren, F.A. Blazich, and U. Blum. 2004a. Root and shoot growth periodicity of *Kalmia latifolia* 'Sarah' and *Ilex crenata* 'Compacta'. HortScience 39:243–247.

Wright, A.N. and R.D. Wright. 2004b. The Horhizotron™: a new instrument for measuring root growth. HortTechnology 14:560–563.

Wright, A.N., R.D. Wright, J.F. Browder, and B.E. Jackson. 2007. Effect of backfill composition on post-transplant root growth of *Kalmia latifolia*. J. Environ. Hort. 25:145–149.

Journal of the American Pomological Society 72(2): 113-121 2018

A Review of *Neofabraea malicorticis* Biology and Management of Anthracnose Canker in Apple Orchards in the Maritime Pacific Northwest

Whitney J.Garton[1], Lisa W. DeVetter[1], Mark Mazzola[2], and Carol A. Miles[1,3]

Additional index words: *Malus ×domestica,* tree canker, *Neofabraea malicorticis,* cultural control, chemical control

Abstract

Cider apple (*Malus ×domestica* Borkh.) is an emerging crop in western Washington and the maritime Pacific Northwest (PNW) region in general, but the planting of new orchards and orchard productivity are limited by the widespread occurrence of anthracnose canker, caused by the fungal pathogen *Neofabraea malicorticis* (H.S. Jacks). In the maritime PNW region, the pathogen induces tree cankers that can kill newly planted trees and structurally weaken established trees. Current management practices include excising cankers during the dormant season and applying fungicides prior to autumn rains. Yet these management practices have not provided adequate disease control in the region, as new *N. malicorticis* infections of susceptible hosts occur even after applying the recommended controls. Poor management of anthracnose canker in the region is likely due to the lack of effective treatments, treatments being applied at the wrong time, or treatments not being applied over an adequate period of time. High inoculum levels and favorable environmental conditions for pathogen infection in the region also contribute to disease severity. Research on disease development and the management of *N. malicorticis* in an orchard environment is limited to dessert apples and is contradicting, which further exacerbates the difficulty in developing an effective disease management plan for cider apples. If cider apple production is to be successful in the maritime PNW, it is necessary to have a more comprehensive understanding of the pathogen, and to incorporate this knowledge into the development of an effective plan to manage anthracnose canker on apple. The objective of this review is to provide an overview of the existing literature on *Neofabraea* spp. in apple orchards, address factors that may explain why managing anthracnose canker has been difficult, and to identify topics for future research that will lead to more effective disease management.

Washington State is the leading producer of apple (*Malus ×domestica* Borkh.) in the U.S. and also is playing a leading national role in the expansion of cider apple production (Miles et al., 2017). In Washington, the cider apple industry was first established in the western half of the state where the climate is similar to regions of Europe in which cider apple trees have thrived for centuries. Production of cider apples is increasing in western Washington, where yield from a cider apple orchard is about 40,350 kg·ha^{-1} with a net value of $35,508 per ha ($0.88 per kg) (Galinato et al., 2014). As the production of cider continues to expand, the demand for specialty cider apples will increase, and already the demand greatly exceeds the supply (Galinato et al., 2014). In western Washington, the widespread occurrence of anthracnose canker, a fungal disease caused by *Neofabraea* species, is the major limitation to planting new cider apple orchards and is a constraint to long-term orchard productivity. In other apple production regions, *Neofabraea* species also induce a postharvest rot of pome fruit (known as bull's-eye rot); while bull's

[1] Department of Horticulture, Washington State University Northwestern Washington Research and Extension Center, 16650 State Route 536, Mount Vernon, WA 98273
[2] U.S. Department of Agriculture-Agricultural Research Service, Physiology and Pathology of Tree Fruits Research Laboratory, 1104 N. Western Avenue, Wenatchee, WA 98801
[3] Corresponding author: milesc@wsu.edu

eye rot can reduce marketable yield, it does not kill apple trees like anthracnose canker can do in western Washington. Although several species can incite anthracnose canker on apple, including *Phlyctema vagabunda* (Desm.) [synonym *N. alba* (E.J. Guthrie) Verkley] and *N. kienholzii* (Seifert, Spotts, & Lévesque, sp. nov.), *N. malicorticis* (synonyms *Cryptosporiopsis curvispora, Cryptosporiopsis malicorticis, Pezicula malicorticis, Gloeosporium malicorticis, Macrophoma curvispora*) is the primary causal agent of this disease (Zang et al., 2011). Developing a more thorough understanding of the biology of *N. malicorticis* and current management practices for anthracnose canker will help improve management strategies and protect cider apple production in western Washington.

Life Cycle of Neofabraea malicorticis. *Neofabraea malicorticis* is considered an aggressive fungal plant pathogen that is able to infect intact bark tissue, with most infections occurring through the lenticels (Cordley, 1900; Kienholz, 1939). Stem and trunk infections by *N. malicorticis* appear to occur primarily in the autumn but can take place throughout the winter and early spring during mild, moist weather (Davidson and Byther, 1992; Rahe, 2010). Infections first appear on the bark surface as small, circular spots that are red or purple when moist (Fig. 1). Mycelial growth occurs in the cambium beneath the bark for a period of time before killing the bark itself to form a visible canker. In inoculation studies, visible canker symptoms developed two to six months after inoculation (Dugan et al., 1993; Rahe, 1997a; Zang et al., 2011); however, the period of time required for symptom development to occur in response to natural infections is unknown. During the winter months,

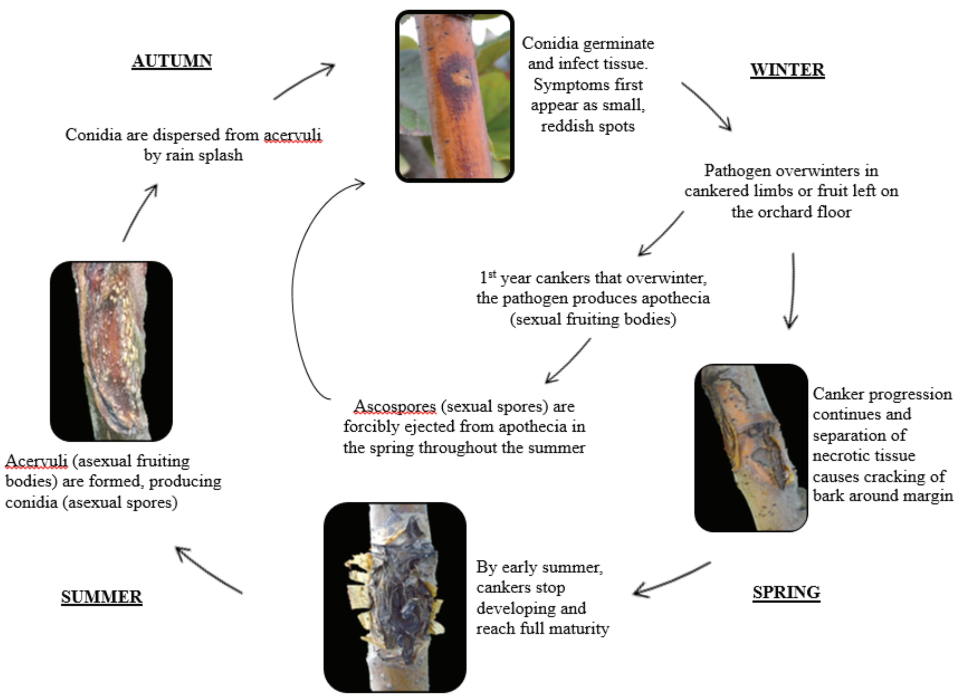


Figure 1. Disease cycle of anthracnose canker caused by *Neofabraea malicorticis.*

canker progression ceases but rapidly resumes development upon spring sap flow (Creemers, 2014). As the canker enlarges, infected bark tissues begin to peel away and the canker becomes elongated, sunken, and turns orange to brown. Following this, a distinct margin develops between healthy and necrotic tissue causing the bark to crack around the infected area. The necrotic bark tissue over the canker separates into small pieces and curls upwards from the lesion, and eventually sloughs off leaving bast fibers behind, giving the appearance often referred to as “fiddle-string” (Turechek, 2004). Larger cankers on main branches or trunks may not display the “fiddle-string” appearance. Cankers become fully developed by early summer, attaining full size ranging from 30 to 250 mm in length (Davidson and Byther, 1992).

By midsummer to late-autumn, acervuli (asexual fruiting bodies) form on mature cankers, producing conidia (asexual spores) that are disseminated by rain and wind to other parts of the tree, as well as surrounding trees and fruit, causing new infections (Creemers, 2014). The acervuli first appear as cream-colored pustules on the center of the canker surface, and later on the canker margin. As acervuli age, they become dark in color (Turechek, 2004). On cankers that are allowed to overwinter, apothecia (sexual fruiting bodies) may develop in the old acervuli and forcibly discharge ascospores (sexual spores) in the spring (Powell et al., 1970; Rahe, 1997a). The capacity of ascospores to incite infection in western Washington is uncertain and previous reports are conflicting. Creemers (2014) indicated that the sexual stage is insignificant in the disease epidemiology. In contrast, the British Columbia Ministry of Agriculture (2016) states that ascospores are responsible for inciting new infections on surrounding trees, while dispersal of conidia is responsible for localized intensification of the disease in infected trees. In an *in-vitro* study, ascospores were discharged from mature ascocarps onto cankered bark tissue from late March through Sept. under high humidity and mild temperature (4 – 13 °C) conditions (Jurkemikova and Rahe, 1998). The pathogen survives as mycelium in cankered limbs or in fruit left lying on the orchard floor, and can produce spores that incite new infections during cool, moist weather at almost any time of the year (Ogawa and English, 1991; Turechek, 2004).

Based on *in-vitro* inoculation studies, additional hosts of *N. malicorticis* include native PNW crab apple (*Malus fusca* Raf.), quince (*Cydonia oblonga* Mill.), flowering quince (*Chaenomeles japonica* Thunb.), peach (*Prunus persica* L.), serviceberry (*Amelanchier pallida* Greene), apricot (*P. armeniaca* L.), plum (*Prunus salicina* Lindel.), sweet cherry (*P. avium* L.), hawthorn (*Crataegus* spp.), and mountain ash (*Sorbus* spp.) (Kienholz, 1939). However, symptoms produced on stone fruit trees were not similar to those produced on pome fruit trees. Additionally, the pathogen did not produce fruiting bodies or spores on stone fruit trees, and it is presumed that these pathogens are not capable of inciting infection naturally on stone fruit trees (Kienholz, 1939).

Distribution and Impact of Anthracnose Canker. Populations of *N. malicorticis* have been reported throughout North America including British Columbia, California, Idaho, Illinois, Maine, Massachusetts, Michigan, and Nebraska, as well as in Africa (Zimbabwe), Oceania (Australia and New Zealand), and Europe (Denmark, Netherlands, and Portugal) (EPPO Global Database, 2017; Turechek, 2004). Looking at the historical reports of anthracnose canker on apple, Heald (1926) found a single occurrence of anthracnose canker in Nebraska. In southwestern and central Maine, anthracnose canker was reported on more than one thousand ‘McIntosh’ apple trees (Hilborn, 1938). In Massachusetts, anthracnose canker was first observed on one tree each of ‘McIntosh’ and ‘Cortland’, but very few new cankers were observed the following year (Boyd, 1939). In Santa Cruz County, California, several anthracnose cankers were detected in three apple orchards that were eight to 10 years old (Barnett, 1944; Kienholz, 1939). In the Fraser Valley of British Columbia, five out of six apple orchards that were surveyed were heavily infested with anthracnose canker, and disease incidence was 50% to 80% on a per tree basis (Rahe, 1997a). There have been recent reports of anthracnose canker killing 'McIntosh' apple trees in Michigan, though specific incidence and severity reports were not provided (Rahe, 2010).

A recent informal survey of growers in western Washington by Garton et al. (2016) found that a grower in Vashon Island removed 2% to 5% of their cider apple trees each year due to anthracnose canker. Additionally, a cider apple grower on San Juan Island reported that 80% of the trees in a 1 ha orchard were infected with anthracnose cankers. In Bellingham, a grower reported that 100% of the trees in his 4 ha apple orchard possessed anthracnose cankers, while a grower in Port Angeles reported that 66% of trees in a 1 ha established cider apple orchard and 16% of trees in a newly planted 1 ha cider apple orchard exhibited anthracnose cankers. In Everson, a grower reported removing 5 out of 6 ha of apple trees due to anthracnose canker.

Although multiple *Neofabraea* spp. may coexist with each other, the geographical distribution and relative importance of any single species may vary at each location (Gariépy et al., 2003; Henriquez et al., 2004; Kienholz, 1939). For example, Kienholz (1939) found that *N. perennans* [(Kienholz) Dugan, R.G. Roberts & G.G. Grove] was dominant in the Kootenay Valley and Okanagan Valley of British Columbia. In Nova Scotia, *P. vagabunda* and *N. malicorticis* were isolated from anthracnose cankers on the apple cultivars 'Cortland', 'McIntosh', 'Russett', and 'Spy' (Lockhart and Ross, 1961). In Australia, *P. vagabunda* and *N. perennans* were found to be the causal species of tree cankers and fruit rot, while *N. malicorticis* was reported as an exotic (rare) species (Cunnington, 2004). In contrast, Verkley (1999) reported populations of *N. malicorticis* in New Zealand. Verkley (1999) also reported *N. malicorticis* in parts of Europe (Denmark, Netherlands, and Portugal). In past studies in Europe, *N. malicorticis* and *N. perennans* were considered to be a single species (Boerema and Gremmen 1959; Sutton, 1980; von Arx, 1970), but this has since been resolved by molecular evidence demonstrating they are genetically distinct, although closely related (de Jong et al., 2001).

Environmental Conditions and Pathogen Virulence. While *N. malicorticis* occurs worldwide (EPPO Global Database, 2017; Turechek, 2004), the disease appears to be most damaging in areas where the climatic conditions include mild year-round temperatures, cool-humid summers, and abundant winter rains. In areas of cider apple production in western Washington where anthracnose canker is most prevalent, the temperature averages approximately 14 °C during the growing season (April–Oct.) and 6 °C during the dormant season (Nov. – Mar.) (WSU AgWeatherNet, 2017). The average relative humidity ranges from 73% to 82% during the growing season and 82% to 91% during the dormant season. The average amount of precipitation received is 76 mm during the growing season on average, and 101 mm during the dormant season. The amount of solar radiation received during the growing season is 506 MJ/m^2 on average and 157 MJ/m^2 during the dormant season.

The influence of temperature and moisture on *Neofabraea* spp. mycelial growth, sporulation, and germination has been evaluated mainly *in-vitro* or in studies addressing the development of bull's-eye rot on pome fruit. Mycelial growth of *Neofabraea* spp. in culture was observed in the range of 0 to 22 °C with optimal growth around 15 °C (Hortová et al., 2014; Kienholz, 1939; Miller, 1932; Senula, 1985). Kienholz (1939) observed an increase in mycelial growth by *N. malicorticis* in the range of 0 to 20 °C. Similarly, Miller (1932) found mycelial growth by *N. malicorticis* was greater at 15 °C than at 20 °C but noted that the pathogen was capable of growth at 0 °C. Senula (1985) found the optimum temperature range for *N. malicorticis* was 18 to 22 °C, while Hortová et al. (2014) found the optimum temperature range was 18 and 20 °C. Aguilar et al. (2017) indicated that growth of *Neofabraea* spp. may be inhibited when temperature approaches or exceeds 30 °C.

The climate conditions in the maritime PNW are conducive for *N. malicorticis* spore germination and growth all year long. Cordley (1900) reported that conidia of *N. malicorticis* germinated at 22 °C within 12 h, and germination was slowed at 29 °C. Spotts and Peters (1982) reported conidial germination of *N. malicorticis* at 10 and 20 °C but was greater at -1.1 °C with a relative humidity of 97% to 100%. Spotts (1985) also reported the viability of *N. malicorticis* conidia was greater at 10 and 20°C than at 30 °C when relative humidity ranged between 40% and 90%. In studies with the closely related pathogen *N. perennans*, Henriquez et al. (2008) found that fruit infection occurred when the temperature was between 10 and 30 °C and the period of wetness was ≥0.5 h, and suggested that moisture may have a greater impact on conidial dispersal than on infection itself.

Cultivar Susceptibility and Resistance. Information on cultivar susceptibility and resistance toward anthracnose canker is limited and contradictory. Braun (1997) surveyed 25 apple orchards throughout Nova Scotia for the presence of anthracnose canker, and found a greater incidence of anthracnose canker on 'McIntosh', 'Idared', and 'Golden Russet' than on 'Northern Spy', 'Gloster', or 'Red Delicious'. In two of the orchards where 'McIntosh' and 'Idared' were planted on M.26 and M.111 rootstocks, the authors found no difference in canker incidence due to rootstock but suggested that 'McIntosh' was less susceptible to anthracnose canker than 'Idared'. In addition, the authors observed that on 'Idared' and 'Golden Russett' the anthracnose cankers occurred on small twigs and spurs, whereas on 'McIntosh' the cankers appeared on the trunk, central axis, and scaffold limbs. These results regarding cultivar susceptibility contradict those of Borecki and Czynczyk (1985) in Poland, where the authors inoculated 26 cultivars with *N. malicorticis*; 'McIntosh', 'Melrose', 'Delikates', and 'Spartan' were rated highly susceptible, and 'Idared', 'NY 58-553-1', and 'Golden Delicious' as least susceptible. The authors further noted that none of the cultivars evaluated in the study were completely resistant to *N. malicorticis*. Currently, all apple cultivars, including cider cultivars, are considered to be susceptible to *N. malicorticis*, and 'Akane', 'Baldwin', 'Chehalis', 'Elstar', 'Empire', 'Gala', 'Gravenstein', 'Melrose', 'Spartan', and 'Sinta' were reported to be very susceptible (British Columbia Ministry of Agriculture, 2016; Creemers, 2014; Pscheidt and Ocamb, 2017).

Management Strategies for Anthracnose Canker. *Neofabraea malicorticis* can induce cankers that girdle young wood and structurally weaken established trees, resulting in severe damage or tree death (Davidson and Byther, 1992). Because these cankers serve as a source of inoculum capable of infecting adjacent trees and fruit, disease management relies heavily on excising cankers from infected trees in dry weather to minimize disease spread, and also application of fungicides prior to autumn rains (Pscheidt and Ocamb, 2017). Canker excision is a common cultural practice that is effective at managing canker diseases on fruit trees (Horner et al., 2015; Pscheidt and Ocamb, 2017). Excision of anthracnose cankers from apple trees (cultivars not specified) reduced the occurrence of new cankers by 45% (Byther, 1986); however, Rahe (2010) did not observe a reduction in the number of new canker infections on apple trees (cultivars nor specified) the year following canker removal. Excision of cankers or pruning creates wounds that can provide both entry points for pathogen colonization and leakage of contents from disrupted cells that can provide nutrients for pathogens (Bostock and Stermer, 1989). Thus, fungicide applications to wounded areas are recommended to prevent infection (Davidson and Byther, 1992; Zeller and Childs, 1925).

Research on managing anthracnose canker with fungicide applications on apple trees is limited and also contradictory. Current recommendations in Washington and Oregon include the application of captan, zinc, or copper-based products prior to autumn rains (Pscheidt and Ocamb, 2017). Creemers (2014) reported that anthracnose canker control was possible using fungicides, but listed chemistries (e.g., quinone outside inhibitors and fluodioxinil) that were ineffective in controlling diseases incited by *Neofabraea* spp. (Aguilar et al., 2015). In an *in-vitro* study, captan was moderately toxic to *N. malicorticis*, and copper-based fungicides were non-toxic (Rahe, 1997a). Spotts et al. (2009) investigated fungicide efficacy for controlling bull's-eye rot on apple fruit, and found that thiabendazole, thiophanate-methyl, pyrimethanil, and pyraclostrobin + boscalid controlled all *Neofabraea* spp., whereas zinc did not control *N. malicorticis* but basic copper sulfate did. In an orchard study, Byther (1986) found that zinc and basic copper sulfate reduced the number of new cankers on apple trees by 50% when applications were made in mid-Oct. and again in mid-Feb. Rahe (1997b) found that thiophanate-methyl and thiram were ineffective against anthracnose canker when applications were made every two weeks from midsummer through autumn (Aug. – Oct.) (British Columbia Government, 2016). Bordeaux mixture (basic copper sulfate and calcium hydroxide) is a traditional copper-based fungicide that has been recommended to manage anthracnose canker (Barss and Mote, 1931; Childs, 1927; Cordley, 1900; Heald, 1920). Copper-based products are the only materials recommended for control of anthracnose canker that are allowable in organic production, but only certain formulations of copper are registered and the material must be used in a manner that minimizes accumulation in the soil (OMRI, 2017; USDA-AMS, 2011).

It appears that *Neofabraea* spp. is sensitive to individual fungicides. These fungi may develop resistance to particular active ingredients or modes of action, further contributing to the difficulty in controlling anthracnose canker (Spotts et al., 2009). Furthermore, failure of fungicides to control anthracnose canker in the orchard may be due in part to the frequent rains in the region, which limits drying of the spray-applied materials on the bark surfaces (Rahe, 1997b).

Conclusions

Current anthracnose canker management recommendations (canker excision and the application of fungicides during late autumn) have not provided adequate disease control in the maritime PNW region, including western Washington. The lack of effective anthracnose canker control may be due to the application of fungicides at inappropriate times or inadequate duration of treatment application. Currently, fungicide applications are recommended before initiation of autumn rains and one month later (Pscheidt and Ocamb, 2017). Additionally, fungicide applications in late autumn through winter are recommended to prevent the germination of conidia. However, there are no recommendations on how often the fungicides should be applied. Information is needed on disease development and the key vulnerable stages of the pathogen in order to refine selection of effective chemistries, and target timing of fungicide applications to enhance management of *N. malicorticis* in an orchard environment.

The maritime climate of western Washington is conducive to disease development and dissemination of *N. malicorticis* all year long, suggesting the need for a year-round management plan to obtain effective control. Furthermore, the capacity of ascospores to infect trees in the maritime PNW has not been evaluated. Therefore, further investigations examining the disease cycle should be conducted to determine the timing of infections, and the capability of ascospore infection. This information may influence the timing and frequency of treatment applications, which could potentially improve management of this disease in the region.

If cider apple production is to be successful in the maritime PNW, an effective management program for anthracnose canker is required, as currently this disease is killing newly planted trees and limiting productivity of established orchards. While susceptibility of specialty cider apple cultivars to *N. malicorticis* has not been reported, observations at the Washington State University Northwestern Washington Research and Extension Center in Mount Vernon indicate that of the 70 specialty cider apple cultivars planted at this location, all are susceptible to the disease. Future work should investigate the level of cultivar susceptibility to anthracnose canker. An integrated management program for anthracnose canker should include removal of infected host tissues to reduce inoculum sources in the orchard. Such an approach may help minimize the over-reliance on chemical controls and potential development of fungicide resistance that has already been detected in certain populations of *Neofabraea* spp. (Weber and Palm, 2010). A better understanding of anthracnose canker management will also have positive implications on managing this disease outside of the maritime PNW in case future changes in climate lead to greater disease incidence in other regions.

Literature Cited

Aguilar, C., C. Xiao, and M. Mazzola. 2015. Management of bull's-eye rot of apple using pre-and postharvest fungicides. Phytopathology 105:S4.4 (abstr.).

Aguilar C.G., M. Mazzola, and C.L. Xiao. 2017. Timing of apple fruit infection by *Neofabraea perennans* and *Neofabraea kienholzii* in relation to bull's-eye rot development in stored apple fruit. Plant Dis. 101:800-806.

Anderson, H.W. 1940. Apple tree anthracnose in Illinois. Plant Dis. Rptr. 24:475.

Barnett, H.L. 1944. Anthracnose and other diseases in California apple-growing regions. Plant Dis. Rptr. 28:717-718.

Barss, H.P. and D.C. Mote. 1931. Orchard protection program for Oregon. Oregon State Agr. College Ext. Serv. Bul. 431:8-9.

Boerema, G.H. and J. Gremmen. 1959. Een oppervlakkige bastkanker bij appel en peer veroorzaakt door *Pezicula corticola*. European J. Plant Pathol. 65:165-176.

Borecki, Z. and A. Czynczyk. 1985. Susceptibility of apple cultivars to bark canker diseases. Acta Agrobotanica 38:49-59.

Bostock, R.M. and B.A. Stermer. 1989. Perspectives on wound healing in resistance to pathogens. Annu. Rev. Phytopathol. 27:343-371.

Boyd, O.C. 1939. Northwestern Anthracnose of apple reported from Massachusetts. Plant Dis. Rptr. 28:125-126.

Braun, P.G. 1997. Distribution and severity of anthracnose canker and European canker of apple in Kings County, NS. Can. J. Plant Pathol. 78:78-82.

British Columbia Ministry of Agriculture. 2016. Anthracnose and perennial canker of apple. 27 March 2017. <http://www2.gov.bc.ca/assets/gov/farming-natural-resources-and-industry/agriculture-and-seafood/animal-and-crops/plant-health/phu-anthracnose-pc.pdf>.

Byther, R.S. 1986. Apple anthracnose. Western Cascade Tree Fruit newsletter, p. 6-8.

Cao, D., X. Li, J. Cao, and W. Wang. 2013. PCR detection of the three *Neofabraea* pathogenic spp. responsible for apple Bull's eye rot. Adv. Microbiology. 3:61-64.

Childs, L. 1927. Perennial canker of apples. Annu. Rpt. Oregon Hort. Soc. 19:108-117.

Cordley, A.B. 1900. Apple tree anthracnose: A new fungus disease. Oregon Agr. Expt. Sta. Bul. 60:1-8.

Creemers, P. 2014. Anthracnose canker and perennial cankers, p. 51-53. In: T. Sutton, H.

Aldwinckle, A. Agnello, and J. Walgenbach (eds.). Compendium of apple and pear diseases. 2nd ed. The Amer. Phytopathol. Soc. Press, St. Paul, Minn.

Cunnington, J.H. 2004. Three *Neofabraea* spp. on pome fruit in Australia. Austral. Plant Pathol.

33:453–454.

Davidson, R. and R.S. Byther. 1992. Apple anthracnose: Bulls eye rot. Washington State Univ. Ext. Bul. 0940.

de Jong, S., C. Levesque, G. Verkley, E. Abelin, J. Rahe, and G. Braun. 2001. Phylogenetic relationships among *Neofabraea* species causing tree cankers and bull's eye rot of apple based on DNA sequencing of ITS nuclear rDNA mitochondrial rDNA and the β-tubulin gene. Mycol. Res. 105:658-669.

Dugan, F.M., G.G.Grove, and J.D. Rogers. 1993. Comparative studies of *Cryptosporiopsis curvispora* and *C. perennans*. I. morphology and pathogenic behavior. Mycologia 85:551-564.

EPPO Global Database. 2017. *Neofabraea malicorticis* distribution. 27 March 2017. <https://gd.eppo.int/taxon/PEZIMA/distribution>.

Galinato, S., R. K. Gallardo, and C.A. Miles. 2014. 2013 Cost estimation of establishing a cider apple orchard in western Washington. Washington State Univ. Ext. Fact Sheet FS141E.

Gariépy, T.D, C.A. Lévesque, S.N. de Jong, and J.E. Rahe. 2003. Species specific identification of the *Neofabraea* pathogen complex associated with pome fruits using PCR and multiplex DNA amplification. Mycol. Res. 107(5):528-536.

Garton, W., M. Mazzola, and C. Miles. 2016. Management of apple anthracnose canker. HortSci. 51:S156 (abstr.).

Hawkins, J.E. 1976. A cauterization method for the control of cankers caused by *Pseudomonas syringae* bacterial blast in stone fruit trees apricot. Plant Dis. Rptr. 60:60-61.

Heald, F.D. 1920. Apple anthracnose or black spot canker. Washington State Univ. Ext. Bul. 64.

Heald, F.D. 1926. Manual of plant diseases. P. 891, McGraw-Hill, N.Y.

Henriquez, J.L., D. Sugar, and R.A. Spotts. 2004. Etiology of bull's eye rot of pear caused by *Neofabraea* spp. in Oregon, Washington, and California. Plant Dis. 88:1134-1138.

Henriquez, J. L., D. Sugar, and R.A. Spotts. 2008. Effects of environmental factors and cultural practices on bull's eye rot of pear. Plant Dis. 92:421-424.

Hilborn, M.T. 1938. New or unusual records of apple diseases. Plant Dis. Rptr. 22:354.

Horner, I., M. Manning, and S. Casonato. 2015. Cauterising or pruning to minimize spread of cankers caused by *Pseudomonas syringae* pv. *Actinidiae* in kiwifruit. Intl. Symp. on Bacterial Canker of Kiwifruit 1095:145-152.

Hortová, B., D. Novotny, and T. Erban. 2014. Physiological Characteristics and Pathogenicity of eight *Neofabraea* isolates from apples in Czechia. Europ. J. Hort. Sci. 79(6):327-334.

Jurkemikova, K. and J.E. Rahe. 1998. The sexual stage of *Pezicula malicorticis* on anthracnose cankers. Can. J. Plant Pathol. 20:211 (abstr.).

Kienholz, J.R. 1939. Comparative study of the apple anthracnose and perennial canker fungi. J. Agr. Res. 59:635-665.

Lockhart, C.L. and R.G. Ross. 1961. *Gloeosporium album* and *G. malicorticis* on apples in Nova Scotia. Can. Plant Dis. Survey 41:280-285.

Miles, C.A., J. King, T.R. Alexander, and E. Scheenstra. 2017. Evaluation of flower, fruit, and juice characteristics of a multinational collection of cider apple cultivars grown in the U.S. Pacific Northwest. HortTechnology 27:431-439.

Miller, E.V. 1932. Some physiological studies of *Gleosporium perennans* and *Neofabraea malicorticis*. J. Agr. Res. 45:65-77.

Moulton, G. and R. Byther. 2009. Anthracnose control in apple trees. Western Washington Fruit Res. Foundation.15 August 2017. <http://www.raintreenursery.com/Control-of-Apple-Anthracnose-DVD.html>.

Ogawa, J. and H. English. 1991. Diseases of temperate zone tree fruit and nut crops. Univ. California Div. Agr. Natural Resources Publ. 3345:461.

Organic Materials Review Institute (OMRI). 2017. Basic copper sulfate. 28 July 2017. <https://www.omri.org/>.

Powell, D., B. Johnson, and E.G. Sharvelle. 1970. Diseases of apples and pears in the Midwest. North Central Reg. Ext. Publ. 16:4.

Pscheidt, J.W. and C.M. Ocamb. 2017. Apple (*Malus* spp.) Anthracnose (Bull's-eye rot). PNW Dis. Mgt. Handbook. Oregon State Univ. 23 March 2017. <https://pnwhandbooks.org/plantdisease/host-disease/apple-malus-spp-anthracnose-bulls-eye-rot>.

Rahe, J.E. 1997a. Anthracnose canker of apples: Put away the sprayer and get out the knife and brush. Quarterly newsletter of the BC Fruit Testers Association. Cider Press 10:6-9.

Rahe, J.E. 1997b. Failure of fungicides to control anthracnose canker of apple. Can. J. Plant Pathol. 19:231-232 (abstr.).

Rahe, J.E. 2010. Anthracnose canker webinar. Fruit Growers News. 23 March 2017. <http://fruitgrowersnews.com/article/anthracnose-webinar-educates-growers/>.

Senula, A. 1985. Untersuchugen zur morphology und physiologie von *Cryptosporiopsis malicorticis* (Cordl). Nannf. und *Phyctaena vagabundha* Desm. Arch. Phytopathol. Plant Prot. 21:273-286.

Spotts, R.A. and B.B. Peters. 1982. The effect of relative humidity on spore germination of pear decay fungi and d'Anjou pear decay. Acta Hortic. 124:75-78.

Spotts, R.A. 1985. Environmental factors affecting conidial survival of pear decay fungi. Plant Dis. 69:391-392.

Spotts, R., K. Seifert, K. Wallis, D. Sugar, C. L. Xiao, M. Serdani, and J. Henriquez. 2009. Description of *Cryptosporiopsis kienholzii* and species profile of *Neofabraea* in major pome fruit growing districts in the Pacific Northwest USA. Mycol. Res. 113:1301-1311.

Sutton, B.C. 1980. The Coelomycetes. Fungi imperfecti with pycnidia, acervuli and stromata. Commonwealth Mycol. Inst., p. 696.

Turechek, W. 2004. Canker diseases. Diseases of fruits and vegetables 1:44-49.

U.S. Department of Agriculture-Agricultural Marketing Service (USDA-AMS). 2011. Copper sulfate and other copper products. Tech. Evaluation Rpt. 1-27.

Verkley, G.J.M. 1999. A monograph of the genus *Pezicula* and its anamorphs. Studies in Mycol. 44:1-180.

von Arx, J.A. 1970. A revision of the fungi classified as *Gloeosporium*. 2nd ed. J Cramer, Leher, N.Y.

Washington State University (WSU) AgWeatherNet. 2017. Washington State University Mount Vernon Station monthly data. 10 March 2017. <http://weather.wsu.edu/?p=93150>.

Weber, R.W.S. and G. Palm. 2010. Resistance of storage rot fungi *Neofabraea perennans*, *N. alba*, *Glomerella acutata* and *Neonectria galligena* against thiophanate-methyl in Northern German apple production. J. Plant Dis. Protection 117:185-191.

Zang, R., L. Huang, and C.L. Xiao. 2011. Species of *Neofabraea* responsible for anthracnose canker of apple trees in western Washington State. Phytopath. 101:S251-S255 (abstr.).

Zeller, S.M. and L. Childs. 1925. Perennial canker of apple trees. Oregon Agric. Expt. Sta. Bul. 217:1-17.

Journal of the American Pomological Society 72(2): 122-127 2018

Primocane-Fruiting Red Raspberry Cultivar Evaluation in High Tunnels

Eric Hanson[1], Brent Crain[1], and Diane Brown-Rytlewski[2]

Additional index words: *Rubus idaeus, Botrytis cinerea,* fruit rot, primocane, floricane

Abstract

Numerous primocane-fruiting red raspberry (*Rubus idaeus*) cultivars have been introduced recently. Evaluating many genotypes for productivity and fruit quality traits is expensive and time-consuming. Here we grew 11 cultivars in containers under a high tunnel to quickly compare their performance. 'Joan J', 'Caroline', 'Himbo Top', 'Anne' and 'Josephine' were the most productive, producing 400-500 and 1,400-1,600 g per plant during year 1 and years 2-3, respectively. The earliness of ripening (earliest to latest) was 'Autumn Britten' > 'Joan J' = 'Jaclyn' = 'Himbo Top' = 'Polka' > 'Caroline' = 'Anne' > 'Josephine' > 'Joan Irene' = 'Nantahala' = 'Crimson Giant'. Following a short storage period, berries of 'Polka', 'Caroline' and 'Himbo Top' rated high in appearance, and 'Caroline', 'Jaclyn', 'Joan Irene' and 'Josephine' had lower incidences of Botrytis gray mold.

Raspberry cultivars produce fruit on current season primocanes or second year floricanes. Most recently-released cultivars are primocane-fruiting because this trait allows growers to reduce pruning costs and potentially produce raspberries even where winters are too cold to over-winter floricanes.

Newer primocane-fruiting cultivars typically are evaluated in regional field trials to identify those with commercial potential (Hanson et al., 2005; Weber et al., 2005). Cultivars perform differently in different regions, but conducting regional field trials to compare cultivars is expensive and time-consuming. Field trials are particularly hard to justify where raspberries are a minor crop, such as in the Midwestern and Northeastern U.S. Potted growing systems are being researched for commercial raspberry production (Sonsteby et al., 2013; Svensson, 2016; Qiu et al., 2016) and may offer a more rapid and convenient way of comparing genotypes than in-ground culture (Andrianjaka-Camps et al., 2015). The purpose of this work was to compare the primocane productivity and fruit quality attributes of newer raspberry cultivars using a potted growing system.

Materials and Methods

The studies were conducted under a 7.3 x 61 x 4.3 m (W x L x H) high tunnel (Haygrove Tunnels, Inc., Redbank, Ledbury, UK) at the Southwest Michigan Research and Extension Center in Benton Harbor, MI (lat. 42.1 °N, long. 86.4 °W). The tunnel and the plant rows were oriented north to south. The tunnel was covered with Luminance THB polyethylene (BPI.Visqueen Horticultural Products, Stockton-on-Tees, UK) from late April or late May to late Oct. or early Nov. The tunnel sides and ends were left open except for approximately the last 3 weeks of the season when they were enclosed with plastic to retain heat. The experimental design was a randomized complete block design (RCBD), with eight replications (rows) in 2011 and four in 2012 and 2013, when the

[1] Department of Horticulture, Michigan State University, East Lansing, MI 48824.
[2] Michigan State University Extension, 121 East Maple St., P.O. Box 319, Mason, MI 48854
This work was sponsored in part by Michigan State University AgBioResearch, the Michigan State Horitculture Society, the North American Bramble Growers Association, and NIFA Hatch Project MICL02483
Corresponding author: Eric Hanson. hansone@msu.edu

trial area was reduced to the north end of the high tunnel. Within each row, each replicate consisted of 4 plants of the same cultivar.

Dormant rooted suckers were planted in 11.4 L white polyethylene growbags (Hydro-Gardens, Colorado Springs, Colo.) in a medium composed of 70% composted pine bark (1” minus) and 30% Canadian Sphagnum peat. The primocane-fruiting cultivars ‘Autumn Britten’, ‘Caroline’, ‘Crimson Giant’, ‘Himbo Top’, ‘Jaclyn’, ‘Joan J’, ‘Josephine’, ‘Nantahala’ and ‘Polka’ were planted in May, 2011, and the cultivars ‘Crimson Giant’, ‘Joan Irene’, and ‘Nantahala’ were planted in May, 2012. Canes were removed each Dec. so that only primocane fruit were produced.

Plants were spaced 0.4 m apart in four rows that were 2 m apart. To minimize border effects, plant rows began and ended at least 6 m from the ends of the tunnel. Each row also began and ended with at least three border plants. Plants were supported by installing metal posts every 2-3 m down each row. The tops of the posts were secured to a tensioned wire running the length of each row at a height of 1.8 m. Twine was installed on each side of the plants and secured to the posts to provide support for the plants. Additional twine was installed as the plants grew in height.

Plants were drip-irrigated with one 1.9 $L \cdot hr^{-1}$ emitter per pot (Netafim USA, Fresno, Calif.). Irrigation was applied once or twice per day for 20 min early in the season and up to eight times daily during warm weather later in the season when the plant canopy was large. Plants received 45 g of Osmocote 17-5-11 fertilizer (The Scotts Company, Marysville, OH) each April. Nutrition also was applied continually through the irrigation system, using a 21-7-7 soluble fertilizer with micronutrients (JR Peters Inc., Allentown, PA) to deliver N at 100 $mg \cdot L^{-1}$.
Insecticides were applied four to eight times annually between July and Oct. to control spotted wing drosophila (*Drosophila suzukii*). No fungicides were applied.
All canes were removed in late Nov. or early Dec. and the plants were stacked two or three high in long piles in the uncovered tunnel. Each pile was then covered with a 70 $g \cdot m^{-2}$ row cover material until growth began in early or mid-April, to protect plants from winter cold. The cover was vented occasionally during warm periods earlier in the spring to keep plants from beginning growth too early.

Ripe primocane fruit were harvested on a 2–5 day schedule (26 dates in 2011, 36 dates in 2012, and 33 dates in 2013). The total weight and number of fruit were recorded on each date and used to calculate average fruit weight. Relative maturity times were compared by determining the date on which the cumulative yield from a plot exceeded 10% of the seasonal total yield for that plot. Dates were expressed as the number of days after the earliest date recorded (e.g., July 30, day 1).

Berry quality and shelf-life were compared by collecting half-pint (0.24 L) samples on selected dates when sufficient fruit was available from at least one of the replicate plots of each cultivar. A replicate consisted of a set of samples collected from a plot of each cultivar in the tunnel. Shelf-life was evaluated on five dates in 2012 and 7 dates in 2013. The number of replicate samples varied on each date. Samples were placed in half-pint clamshell containers, enclosed in sealed black plastic bags, held for 1 – 2 d in 2°C cold storage, and then moved to 18°C for 24 – 36 h. Samples were opened and given appearance ratings of 1 (not salable), 2 (possibly salable but poor quality), 3 (salable but with significant defects), 4 (good quality, only minor/subtle defects) or 5 (excellent quality, no significant defects). Characteristics detracting from quality included mold, visible juice, small or variable size, variable color, and a dull rather than glossy surface. Total fruit and number of fruit with visible mold were then counted to determine the percent with mold.

Consumer preferences were evaluated by having volunteers at a local farmers market taste several berries of each cultivar and rate them for overall flavor, sweetness and perceived firmness on a scale of 1 (least) to 5 (most). Berries were assessed by 3 to 5 volunteers on each of six dates in 2011, by 6 to 12 volunteers on four dates in 2012, and by 2 to 11 volunteers on five dates in 2013.

Data were analyzed by year using SAS v9.4 (SAS Institute Inc., Cary, NC). The effects of treatments on yield, harvest date, consumer ratings (flavor, sweetness), and shelf-life (appearance, rot) were analyzed by analysis of variance (ANOVA) using PROC MIXED. The covariate *date* was added as a fixed effect in the model to control for the effect of date on consumer ratings and shelf-life. The response variable *rot* was log-transformed to normalize the distribution. Where variances were unequal (based on Levene's test and plots of residuals), a model with heterogenous variances was fit. Means separation was accomplished using Tukey's honestly significant differences (HSD). All statistical tests for significance were conducted at **α = 0.05.**

Results and Discussion

Fruit yields (Table 1) were much lower during the planting year (351 g average per plant across all cultivars) than in the second and third years after planting (1,050 and 1,190 g, respectively). Of the cultivars planted in 2011, 'Caroline', 'Himbo Top' 'Joan J' and 'Josephine' were the most productive in each of three years. 'Anne' and 'Polka' were ranked among the most productive in only one year, and 'Autumn Britten' and 'Jaclyn' were less productive in all three years. 'Joan J', 'Caroline' and 'Polka' also were the most productive of ten cultivars grown in an open field in Utah (Black et al., 2013). 'Nantahala' and 'Joan Irene' were the highest yielding of the 2012-planted cultivars, and 'Crimson Giant' was the lowest yielding cultivar. 'Crimson Giant' began fruiting too late to be productive at this site. Average yields were comparable to those of potted plants grown in a high tunnel in Switzerland (Andrianjaka-Camps et al., 2015), but about half of yields achieved in a similar system in Canada (Qiu et al., 2016).

Based on the date when 10% of the total yield was exceeded (Table 2), the earliest fruiting cultivar was 'Autumn Britten'. 'Himbo Top', 'Jaclyn', 'Joan J' and 'Polka'

Table 1. Primocane fruit yields of potted raspberry cultivars in a high tunnel in Benton Harbor, Mich., 2011-2013.

Cultivar	Yield ($g{\cdot}plant^{-1}$) 2011	2012	2013
		Planted in 2011	
Autumn Britten	348 b[z]	915 c	773 e
Anne	479 ab	1205 b	1212 bc
Caroline	410 ab	1363 a	1237 a-c
Himbo Top	413 ab	1200 a-c	1277 a-c
Jaclyn	345 b	876 c	945 d
Joan J	496 a	1468 ab	1404 ab
Josephine	353 ab	1115 a-c	1382 a
Polka	502 a	1171 b	994 c-e
		Planted in 2012	
Crimson Giant	†	248 a	531 b
Joan Irene	†	45 b	1016 a
Nantahala	†	220 a	1173 a

[z] Means within columns in the same section followed by common letters do not differ at α = 0.05 (Tukey's HSD).
† Planted in 2012; not included in 2011.

Table 2. Relative maturity times of primocane-fruiting raspberry cultivars expressed as the days relative to July 30 (day 1) when plots exceeded 10 % of their eventual total fruit yields.

Variety	2012		2013	
Autumn Britten	4	d[z]	3	f
Anne	20	ab	12	d
Caroline	17	b	14	cd
Crimson Giant	†		69	a
Himbo Top	9	cd	9	de
Jaclyn	5	d	9	de
Joan Irene	†		44	b
Joan J	7	cd	6	ef
Josephine	25	a	19	c
Nantahala	†		61	a
Polka	11	c	6	ef

[z] Means within columns followed by common letters do not differ at $\alpha = 0.05$ (Tukey's HSD).
† Not included in the trial in year indicated.

began ripening slightly later. 'Caroline' and 'Anne' began fruiting several days later, followed by 'Josephine'. 'Joan Irene', 'Nantahala' and 'Crimson Giant' began fruiting very late. Since these plants were in a high tunnel, which tends to promote earlier fruiting (Demchak, 2009; Hanson et al., 2011), plants grown without tunnels in a similar climate would likely fruit later. The order of harvest reported here is similar to that of trials containing some of the same cultivars (Hanson et al., 2011; Black et al., 2013), suggesting that the relative differences between cultivars in this potted system would be similar to that of field-grown plants. Earliness of harvest is an important trait since cultivars need to be chosen that meet desired marketing windows and do not begin fruiting too late in the fall to achieve profitable yields. The productivity of late maturing cultivars would be lower if they were grown in locations with shorter growing seasons or in open fields.

Consumer ratings of flavor were variable. The only statistically significant differences were that 'Jaclyn' rated higher in flavor than 'Autumn Britten' or 'Himbo Top' in 2011, and 'Jaclyn' and 'Josephine' were rated higher than several cultivars in 2013 (Table 3). In 2012, 'Jaclyn' also was perceived as sweeter than 'Anne', but there were no other differences between cultivars. There were no differences in consumer ratings of fruit firmness (data not shown).

To compare the shelf-life, cultivars were placed in early- or late-maturing groups so that adequate fruit were available for comparisons on common dates. The early cultivars that were rated high in appearance after a short storage period were 'Polka', 'Caroline' and 'Himbo Top' (Table 4). 'Polka' rated high because fruit had a uniform medium red color and glossy surface. The late-maturing cultivars (Table 5) had similar appearance ratings except that 'Anne' was rated higher than 'Crimson Giant' in 2013 only. The yellow-fruited cultivar 'Anne' also had a uniform size and color. 'Polka' and 'Anne' rated high in overall preference (flavor and appearance) in another study (Black et al., 2013).

Gray mold (*Botrytis cinerea*) was seldom visible on berries at harvest but developed on some fruit during storage. Early-maturing cultivars with lower incidences of mold were 'Caroline' and 'Jaclyn' (Table 4), and 'Joan Irene' and 'Josephine' had the least mold of the later maturing cultivars (Table 5).

Table 3. Consumer sensory appraisal of primocane fruit from raspberry cultivars in Benton Harbor, MI, 2011-2013; rating scale is 1 (least) to 5 (most).

	Flavor (1-5)			Sweetness (1-5)	
Variety	2011	2012	2013	2011	2012
Autumn Britten	2.7 b[z]	3.1	2.5 c	2.8	2.6 ab
Anne	2.9 ab	3.0	3.1 ac	2.7	2.4 b
Caroline	3.4 ab	3.0	3.0 ac	3.5	2.8 ab
Crimson Giant	†	†	1.8 c	†	†
Himbo Top	2.7 b	3.3	3.5 ac	2.7	3.0 ab
Jaclyn	3.7 a	3.5	3.9 a	3.2	3.2 a
Joan Irene	†	†	3.0 bc	†	†
Joan J	3.3 ab	3.0	3.3 ac	3.3	2.7 ab
Josephine	3.0 ab	3.1	3.8 ab	3.0	2.7 ab
Nantahala	†	†	2.4 c	†	†
Polka	3.4 ab	3.3	3.4 ac	3.3	3.0 ab

[z] Means within columns followed by common letters do not differ at $\alpha = 0.05$ (Tukey's HSD).
† Not included in trial in year indicated.

Table 4. Appearance rating (1=worst, 5=best) and percent rot of earlier-maturing primocane fruit from raspberry cultivars in Benton Harbor, MI, 2012 – 2013.

	Appearance (1-5)		Rotten berries (%)	
Variety	2012	2013	2012	2013
Autumn Britten	3.4 c[z]	3.6 b-d	4.8	6.8 a
Caroline	4.2 ab	3.7 a-c	1.1	0.7 b
Himbo Top	3.8 a-c	3.9 ab	4.0	2.7 ab
Jaclyn	3.6 bc	2.7d	1.3	2.7 ab
Joan J	3.7 a-c	3.1 cd	1.3	2.3 ab
Polka	4.4 a	4.5 a	2.8	2.6 ab

[z] Means within columns followed by common letters do not differ at $\alpha = 0.05$ (Tukey's HSD).

Table 5. Appearance rating (1=worst, 5=best) and percent rot of later-maturing primocane fruit from raspberry cultivars in Benton Harbor, MI, 2012-2013.

	Appearance (1-5)		Rotten berries (%)	
Variety	2012	2013	2012	2013
Anne	3.7	3.5 a	4.0 b	2.4 bc
Crimson Giant	3.7	2.6 b	10.0 a	14.1 a
Joan Irene	4.0	3.0 ab	1.3 ab	0.5 c
Josephine	3.7	3.3 ab	2.2 b	1.0 c
Nantahala	3.9	3.2 ab	11.0 ab	6.1 ab

[z] Means within columns followed by common letters do not differ at $\alpha = 0.05$ (Tukey's HSD).

‘Caroline’ and ‘Josephine’ previously have been described as having some tolerance to gray mold (Aprea et al., 2010; Hanson et al., 2011; Harshman et al., 2014). High tunnel environments reduce gray mold incidence (Hanson et al., 2011), at least partly because rain is excluded.

Conclusions

Raspberry cultivars were compared in a potted growing system. ‘Autumn Britten’ began fruiting the earliest, followed by ‘Joan J’ and ‘Polka’, ‘Himbo Top’ and ‘Jaclyn’, then ‘Caroline’, ‘Anne’, and ‘Josephine’. ‘Joan Irene’, ‘Nantahala’ and ‘Crimson Giant’ were the latest to begin fruiting. The cultivars ‘Joan J’, ‘Caroline’, ‘Himbo Top’, ‘Anne’, ‘Josephine’, ‘Nantahala’ and ‘Joan Irene’ produced the highest yields. Berries of ‘Polka’, ‘Caroline’, ‘Himbo Top’ and ‘Anne’ rated highest in appearance after a short storage. ‘Caroline’, ‘Joan Irene and ‘Josephine’ had lower incidences of Botrytis gray mold after storage. Results indicate a potted growing system can be used to readily compare numerous raspberry cultivars for regional performance regarding important production and quality traits. Although characteristics such as yield potential and harvest times were similar to those reported for some of the same cultivars when tested under field conditions, cultivars may respond differently under different production practices or conditions.

Literature cited

Andrianjaka-Camps, Z.N., M.S. Wittemann, A. Ançay and C. Carlen. 2015. New cultivars for quality production of primocane fruiting raspberries enriched in healthy compounds. Acta Hort. 1133:345-351.

Aprea, E., S. Carlin, L. Giongo, M. Grisenti and F. Gasperi. 2010. Characterization of 14 raspberry cultivars by solid-phase microextraction and relationship with gray mold susceptibility. J. Agric. Food Chem. 58:1100–1105.

Black, B.L., T. Lindstrom, R. Heflebower, B. Hunter, S. Olsen and D.G. Alston. 2013. Adaptability of primocane-fruiting raspberry cultivars to a high-elevation arid climate. J. Amer. Pomol. Soc. 67:47-56.

Demchak, K. 2009. Small fruit production in high tunnels. HortTechnology 19:44-49.

Hanson, E., S. Berkheimer, A. Schilder, R. Isaacs, and S. Kravchenko. 2005. Raspberry cultivar performance in southern Michigan. HortTechnology 15:716-721.

Hanson, E., M. Von Weihe, A. C. Schilder, A. M. Chanon and J. Scheerens. 2011. High tunnel production of floricane- and primocane-fruiting raspberries. HortTechnology 21:412-418.

Harshman, J. M., W. M. Jurick, K.S. Lewers, S.Y. Wang and C.S. Walsh. 2014. Resistance to Botrytis cinerea and quality characteristics during storage of raspberry genotypes. HortScience 49:311-319.

Qiu CP, Q. Xu, L. Gaudreau, A. Gosselin, L. Gauthier, A. Van Sterthem and Y. Desjardins 2016. Yield improvement of red raspberry by soilless cultivation with two propagation methods under northern Canadian climate. Acta Hort. 1133:195-200.

Sonsteby A, J.A. Stavang and O.M. Heide. 2013. Production of high-yielding raspberry long canes: The way to 3 kg of fruit per cane. J. Hort. Sci. Biotech. 88:591-9.

Svensson, B. 2016. Organic production of raspberries in high tunnels in Sweden, 2008-2014. Acta Hort. 1133:211-216.

Weber, C.A., K.E. Maloney and J.C. Sanford. 2005. Performance of eight primocane fruiting red raspberry cultivars in New York. Small Fruits Rev. 4:41-47.

Journal of the American Pomological Society 72(2): 128-134 2018

Matted-Row Strawberry Cultivar Productivity in Missouri, 2013-14

Martin L. Kaps[1]

Additional index words: total yield, marketable yield, berry size, harvest dates

Abstract

Ten short-day strawberry cultivars were evaluated for productivity in southcentral Missouri. The production system was matted rows on a planting ridge formed along the row center, 20 cm high (8 in). The planting was established in 2012, cropped in 2013, renovated and cropped again in 2014. Rows were spaced at 1.2 meter (4 ft). Yearly means of all cultivars for total yield were 3.4 and 2.7 kg per linear m row in 2013 and 2014, respectively. The cultivars 'AC Wendy', AC Valley Sunset', 'Allstar', 'Annapolis'. 'Brunswick', 'Galleta' and 'Jewel' were most consistent and can be recommended to Missouri growers. Marketable yields of all cultivars were 74% and 67% in 2013 and 2014, respectively. Weighted averages for fruit of all cultivars were 11.9 g and 11.0 g in 2013 and 2014, respectively. Harvest season length of all cultivars averaged 21 days in 2013 and 16 days in 2014.

Introduction

Evaluating strawberry cultivars is an ongoing research project at the State Fruit Experiment Station of Missouri State University. Local growers are interested in the productivity of newer cultivars in comparison to older standards. The continental climate of southcentral Missouri is rated 6a in the USDA Plant Hardiness Zones with an average annual minimum of -23.3 to -20.6 °C (-10 to -5 °F). Average yearly rainfall is 102 to 114 cm (40 to 45 in). The trial location is at 37° 9' N latitude, 92° 16' longitude, with an elevation of 442 m (1,450 ft). Typical seasonal weather patterns are a wet spring, warm to hot summer temperatures with high humidity, and fluctuating winter temperatures with little or no snow cover. Matted-row strawberry production is adapted to zones 5 and lower using cultivars with good runner production (Hancock et al., 1997, Masiunas et al., 1991). Missouri growers have long used this system although the annual hill or 'plasticulture' system is becoming more accepted. Use of plastic mulch on raised beds in the annual hill system allows for good fruit appearance and ease of picking (Stevens et al., 2007). Nursery availability of runner tips in Sept. has made the annual hill system possible in Missouri although it can be unpredictable due to variability in fall and winter temperatures from year to year (Kaps et al., 2005). Matted-row strawberries have a lower investment and can be very productive if good weed control is practiced. This is accomplished using herbicides, mechanical tillage, and hand hoeing or weeding (Pritts, 2003; Pritts and Handley, 1998). Use of killed cover crop residue is another means of suppressing weeds in a modified (advanced) matted-row system (Black et al., 2002). Raised beds as used in the advanced matted-row and annual hill systems are preferred by harvest labor and pick-your-own (PYO) customers (Stevens et al., 2007). Past reports from the station summarized the productivity of older strawberry cultivars; three of which, 'Allstar', 'Annapolis' and 'Brunswick', were included in this trial (Kaps et al., 2003; Kaps et al., 1990; Kaps et al., 1987). Seven additional short-day strawberry cultivars were evaluated in this trial (Table 1).

[1] Research Professor. State Fruit Experiment Station of Missouri State University, Mountain Grove, MO 65711
Email: martinkaps@missouristate.edu

Table 1. Strawberry cultivars, origin, and bearing season planted at Mountain Grove, MO 2013-2014.

Cultivar	Year Introduced	Origin	Bearing Season
AC Wendy	2005	Nova Scotia, Canada	early
AC Valley Sunset	2006	Nova Scotia, Canada	late
Allstar	1981	Maryland, USDA	late
Annapolis	1984	Nova Scotia, Canada	early
Brunswick	2002	Nova Scotia, Canada	mid
Daroyal	2006	France	mid
Donna	2007	France	late
Galletta	2008	North Carolina	early
Jewel	1985	New York	mid
Record	2007	Italy	late

Materials and Methods

This strawberry cultivar trial was conducted at the State Fruit Experiment Station of Missouri State University at Mountain Grove, MO. The soil is a Wilderness series, gravelly silt loam soil with 3 to 8 percent slope. There is a fragipan at 40 to 70 cm (15 to 30 in) depth. While this layer can limit rooting depth for some tree fruit, it probably did not limit strawberry rooting. It can slow internal drainage during high rainfall periods. Permeability is moderate above the fragipan and very low in the pan. Water holding capacity of the soil is low because of the shallow depth to the fragipan. Soil reaction varies with depth from 6.5 to 4.5. Organic matter content also varies from 3.0 to 0.5%.

Tall fescue (*Festuca arundinacea* Shreb.) grass was the permanent ground cover in the years prior to planting establishment. A year prior to planting, a rotation of summer and winter cover crops was used to build soil organic matter and suppress weeds. The site was plowed and disced, and then summer and winter cover cropped with buckwheat (*Fagopyrum esculentum* Moench) and cereal rye (*Secale cereal* L.), respectively. The site was not fumigated because the ground had been out of strawberry production for six years. New ground not previously planted to strawberries or fallow and cover cropped ground usually does not require soil fumigation (Pritts, 2003; Pritts and Handley, 1998).

Soil was sampled from the planting sites and tested for nutrients. Nitrogen, phosphorus, potassium, and boron were applied at 90, 110, 105, and 4.5 kg per ha (80, 100, 95, and 4 lb per A), respectively. These were broadcast as dry fertilizer (boron sprayed as Solubor) and incorporated into the soil prior to planting. Rows were spaced at 1.2 meter (4 ft) with a planting ridge formed along the row center with a tractor drawn cultivator, 20 cm high (8 in).

The strawberry cultivars, year introduced, origin, and bearing season are listed in Table 1. The experiment was a randomized complete block with four replications. Each replicate consisted of twelve plants per 4 m (13 ft) of row. Dormant crowns for all the cultivars were obtained from Nourse Farms (South Deerfield, MA 01373). Crowns were planted on 11 April 2012 at 30.5 cm (12 in) spacing within the row. Following planting, Dacthal W-75 (DCPA) pre-emergent herbicide (AMVAC, Los Angeles, CA 90023) at 13.5 kg/ha (12 lb/A) was applied over the plant rows and middles with a boom sprayer. Straw mulch was spread between rows. Hand weeding and hoeing were done through the summer. Blossoms were removed the first growing season. Plants runnered to form a 60 cm (24 in) wide matted row for fruiting. In the fall season, Devrinol 50-DF (Napropamide) pre-emergent herbicide (United Phosphorus Inc., King of Prussia, PA 19406) at 9 kg/ha (8 lb/A) was applied in mid-Nov. in a similar manner as the spring Dacthal application. Straw mulch was spread over the plant rows in early Dec. for winter protection. It was raked between the plant rows the following April. Drip and sprinkler irrigation were used to supplement rainfall during the growing season. Sprinkler irrigation was also used for spring frost control. Following the 2013 harvest, rows were renovated on 10 July for a second year of production. This involved mowing off the foliage above the plant crown and narrowing the plant rows to 30.5 cm (12 in) with a dual-head rotovator. This retained mother plants and some adjacent daughter plants. An application of Dacthal W-75 (DCPA) pre-emergent herbicide at 13.5 kg/ha (12 lb/A) and sprinkler irrigation followed two days later. Nitrogen was applied two weeks later at 45 kg per ha (40 lb per A). New runners were allowed to peg and root at random.

Plantings were harvested approximately two times per week over four weeks. Marketable and cull yields per plot were recorded in 2013 and 2014. Cull yield included fruit that were small, damaged by pests, or overripe. Small fruit were in the 5-6 g range or less. Total yield in kg per linear m row (Table 2) and percent marketable yield (Table 3) were reported. Percent marketable yield was based on the formula: [(total yield - cull fruit weight) / (total yield)] X 100. Fruit weight (g) was determined from a random 25-fruit sample taken at every picking. Weighted averages were calculated using the formula: summation of picking 1 through N [(fruit sample weight on day N / 25 fruit sample) X (yield on day N / total yield)] (Table 4). Dates for first, peak, last harvest, and season length (days) for each cultivar are reported (Table 5). A one-way analysis of variance was performed on the raw data by year using SPSS Statistics (IBM Corp., Armonk, NY 10504) and means separated by Tukey-Kramer HSD (*P*=0.05).

Results and Discussion

Strawberry bloom occurred from 24 April to 10 May in 2013. A low temperature of

Table 2. Total Yield of Strawberry Cultivars at Mountain Grove, MO 2013-2014.

Cultivar	Total Yield (kg / linear m row)[z] 2013		2014		2 Year Means
AC Wendy	3.3	ab[y]	2.5	bcd	2.9
AC Valley Sunset	3.4	ab	2.6	abcd	3.0
Allstar	3.9	ab	3.1	ab	3.5
Annapolis	3.3	ab	2.7	abc	3.0
Brunswick	3.6	ab	3.6	a	3.6
Daroyal	4.2	a	1.6	d	2.9
Donna	2.0	c	1.8	cd	1.9
Galletta	3.8	ab	2.7	abc	3.3
Jewel	3.0	bc	3.6	a	3.3
Record	3.9	ab	2.4	bcd	3.1
Yearly Means	3.4		2.7		3.1

[z] To convert to MT/ha, multiply by 5.55; to convert to T/A multiply by 2.45.
[y] Means in a column not followed by a common letter are significantly different by Tukey-Kramer HSD, P ≤ 0.05.

0 ºC (32 ºF) was recorded on 2 and 3 May, but no blossom damage was noted. Yearly mean total yield of all cultivars was 3.4 kg per linear m row [8.3 tons per acre (T/A)] in 2013 (Table 2). This was comparable to previous cultivar trials in Missouri (Kaps and Byers, 2008; Kaps et al., 2003; Kaps et al., 1990). Matted-row strawberry cultivar trials in other states have shown comparable (Swartz et al., 1985; Stevens et al., 2007) or lower yields (Dozier et al., 1992; Handley and Dill, 2002). Yields were determined from 4 m (13 ft) research plots. Thus, it is risky to extrapolate from these small plots to larger plantings and assume that total yield will increase proportionally. A conservative estimate of what commercial strawberry growers might obtain is about one-half to two-thirds of cultivar trial yields because small and late fruit would not be harvested (Hancock et al., 1997).

In the second production year, blossoming occurred 22 April through 5 May 2014. There were no spring frosts during bloom. Yearly mean total yield of all cultivars was 2.7 kg per linear m row (6.6 T/A) row in 2014 (Table 2). This was a decrease from the first to the second production year of 21%. Previous trials have shown more of a decrease, ranging from 27 to 48% (Kaps and Byers, 2008; Kaps et al., 2003; Kaps et al., 1990). Missouri growers typically crop matted-row strawberries for several years. A progressive decline in yield will occur over several years due to competition between plant crowns, weed infestation, and foliar disease. Eventually matted-row strawberries become unprofitable after a number of bearing seasons and must be renewed (Hancock et al., 1997; Pritts, 2003; Pritts and Handley, 1998).

Most cultivars in the trial had yields suitable for commercial production in 2013. There were few significant differences across the cultivars (Table 2). 'Donna' had the lowest yield in 2014. A decline in yield occurred in 2014 for most cultivars but not all of them. 'Brunswick' and 'Jewel' either maintained or increased in yield in the second year. 'Brunswick' has shown consistency in yield and fruit size over four bearing seasons (Jamieson and Nickerson, 2004). 'Jewel' is a standard for the northeast US with good fruit size (Nourse, 2009; Pritts, 2003; Pritts and Handley, 1998; Weber, 2005). 'Daroyal and 'Donna' had the lowest yield in 2014. Reasonably consistent yielding cultivars were 'AC Wendy', 'AC Valley Sunset', 'Allstar', 'Annapolis', 'Galletta', and 'Record'.

'Allstar', 'Annapolis', and 'Brunswick' have yielded well in previous trials and performed well in the present trial (Kaps and Byers, 2008; Kaps et al., 2003; Kaps et al., 1990). 'Allstar' continues to be a popular offering by strawberry nurseries (Galletta et al., 1981; Hokanson and Finn, 2000; Nourse, 2009). 'Annapolis' is broadly adapted, productive, and early maturing (Estabrooks et al., 1989; Jamieson, 2003a; Jamieson, 2003b; Nourse, 2009). 'Brunswick' is a good high yielding, midseason cultivar that is an alternative to 'Honeoye' (Jamieson and Nickerson, 2004; Nourse, 2009).

Marketable yield percent averaged 74% in 2013 and 67% in 2014 (Table 3). These are lower than in previous trials when they exceeded 90% (Kaps and Byers, 2008; Kaps et al., 2003; Kaps et al., 1990). The highest marketable yields varied by year and cultivar with few significant differences across the cultivars. 'AC Wendy', 'Brunswick', 'Daroyal' had the highest marketable yield in 2013 and 'Jewel' highest in 2014 (Table 3). The previously stated consistent yielding cultivars had acceptable marketable yields, except for 'Record'.

Fruit weight is a weighted average of 25 fruit taken at each harvest. Thus, fruit weight is emphasized more on high yield dates. This is probably more representative of what a grower might obtain, since large and small fruit measured at lower yield dates have less emphasis. A fruit weight above 10 g was considered good in previous cultivar trials in Missouri (Kaps and Byers, 2008; Kaps et al., 2003; Kaps et al. 1990). The yearly mean fruit

Table 3. Marketable Yield of Strawberry Cultivars at Mountain Grove, MO 2013-2014.

Cultivar	Marketable Yield (%) 2013		2014		2 Year Means
AC Wendy	83.3	a[z]	73.3	ab	78.3
AC Valley Sunset	61.3	cd	66.3	ab	63.8
Allstar	75.8	abc	68.3	ab	72.1
Annapolis	77.3	abc	74.8	ab	76.1
Brunswick	84.5	a	62.0	b	73.3
Daroyal	82.0	a	70.5	ab	76.3
Donna	63.0	bcd	65.5	ab	64.3
Galletta	78.3	ab	69.3	ab	73.8
Jewel	75.3	abc	77.0	a	76.2
Record	55.0	d	45.3	c	50.2
Yearly Means	73.6		67.2		70.4

[z]Means in a column not followed by a common letter are significantly different by Tukey-Kramer HSD, $P \leq 0.05$.

weight for all cultivars was 11.9 and 11.0 g in the years 2013 and 2014, respectively (Table 4). 'AC Valley Sunset' had significantly larger fruit in both years along with 'Record' in 2014. 'Daroyal' had smaller fruit in 2013 and again in 2014 along with 'Donna'; however, they were not always significantly different from other cultivars. Yearly mean fruit weight dropped about 8% from 2013 to 2014 which is consistent with previous trials (Kaps and Byers, 2008; Kaps et al., 2003). Research has not shown a compensation in fruit size at lower yield in strawberry. Rather, fruit weight was negatively correlated to number of crowns per plant, plants per meter, and fruit set (Swartz et al., 1985). Plant and crown numbers would be expected to be higher in the second bearing year, although these were not determined in the trial.

Strawberry blossoming usually starts in mid to late April and extends into May in southern Missouri (Kaps et al., 2005; Kaps et al., 2003; Kaps et al., 1990). The blossoming period lasts about ten days depending on spring temperatures. The first harvest date is usually in late May. First harvest started on 24 May 2013 for the cultivars 'AC Wendy', 'Annapolis', and 'Daroyal' (Table 5). For

Table 4. Fruit Weight of Strawberry Cultivars at Mountain Grove, MO 2013-2014.

Cultivar	Weighted Average (g) 2013		2014		2 Year Means
AC Wendy	10.7	cd[z]	9.8	bc	10.3
AC Valley Sunset	17.5	a	13.9	a	15.7
Allstar	12.4	bc	11.0	b	11.7
Annapolis	10.7	cd	9.7	bc	10.2
Brunswick	11.4	c	11.2	b	11.3
Daroyal	8.2	d	8.9	c	8.6
Donna	10.1	cd	8.8	c	9.5
Galletta	11.2	c	9.8	bc	10.5
Jewel	12.6	bc	11.3	b	12.0
Record	14.2	b	15.1	a	14.7
Yearly Means	11.9		11.0		11.5

[z] Means in a column not followed by a common letter are significantly different by Tukey-Kramer HSD, $P \leq 0.05$.

Table 5. First, Peak and Last Harvest Dates of Strawberry Cultivars at Mountain Grove, MO 2013-2014.

	First Harvest		Peak Harvest		Last Harvest		Season Length (days)	
Cultivar	2013	2014	2013	2014	2013	2014	2013	2014
AC Wendy	24-May	22-May	29-May	27-May	20-Jun	9-Jun	28	19
AC Valley Sunset	6-Jun	30-May	13-Jun	2-Jun	24-Jun	13-Jun	19	15
Allstar	29-May	27-May	10-Jun	27-May	20-Jun	9-Jun	23	14
Annapolis	24-May	22-May	29-May	27-May	17-Jun	9-Jun	25	19
Brunswick	29-May	22-May	6-Jun	27-May	17-Jun	9-Jun	20	19
Daroyal	24-May	22-May	29-May	27-May	13-Jun	6-Jun	21	16
Donna	29-May	27-May	6-Jun	27-May	17-Jun	9-Jun	20	14
Galletta	29-May	22-May	31-May	27-May	17-Jun	9-Jun	20	19
Jewel	3-Jun	27-May	13-Jun	27-May	20-Jun	9-Jun	18	14
Record	10-Jun	30-May	13-Jun	6-Jun	24-Jun	13-Jun	15	15

these same cultivars, first harvest started on 22 May 2014 along with 'Brunswick' and 'Galletta'. The late cultivars 'AC Valley Sunset' and 'Record' had first harvest on 6 to 10 June 2013, and 30 May 2014. Peak harvest date occurred anywhere from the first picking date to twelve days later depending on cultivar. Season length averaged twenty-one days in 2013 and sixteen days in 2014. The latest harvest date occurred on 24 June 2013 and 13 June 2014 for 'AC Valley Sunset' and 'Record'. The short harvest seasons made it difficult to classify cultivars into early, mid, and late season categories for southern Missouri. There was considerable overlap in harvests for the cultivars.

Conclusion

Based on total and marketable yields, and fruit weight in 2013 and 2014, 'Daroyal' and 'Donna' are not recommended. 'Donna' had good total yield and berry size in both years, but marketable yield was lower and thus, it is not recommended. These cultivars are from French and Italian breeding programs (Table 1) and maybe less adapted to southern Missouri. Even so, other growers may find these cultivars to be productive and they should not necessarily be avoided. The remaining cultivars can be recommended to Missouri growers. 'Allstar' and 'Jewel' are already accepted by the strawberry industry as productive cultivars (Weber, 2005). The Maryland USDA cultivar 'Allstar' is well adapted to Missouri as it has performed well over many years and is a favored cultivar by growers (Kaps and Byers, 2008). The New York cultivar 'Jewel' has a long record of productivity and is also a favored cultivar by growers (Pritts, 2003; Pritts and Handley, 1998). The Canadian cultivars 'Annapolis' and 'Brunswick' did well in this trial and in previous trials and we continue to recommend them (Kaps and Byers, 2008; Kaps et al., 2003; Kaps et al., 1990). The two Canadian cultivars 'AC Wendy' and 'AC Valley Sunset' can be added to those recommended. 'Galletta' from North Carolina yielded well in both years and can also be recommended.

Literature Cited

Black, B.L., J.M. Enns and S.C. Hokanson. 2002. A comparison of temperate-climate strawberry production systems using eastern genotypes. HortTechnology 12:670-675.

Dozier, W.A., D.G. Himelrick, A.W. Caylor, J.A. Pitts, M.H. Hollingsworth and W.B. Webster. 1992. Performance of strawberry cultivars in central and north Alabama grown on the matted row system. Alabama Agric. Exp. Sta. Bull. 619. 32p.

Estabrooks, E.N., M. Luffman and S. Michaud. 1989.

Strawberry cultivar evaluation in New Brunswick, Canada. Adv. Straw. Prod. 8:58-61.
Galletta, G.J., A.D. Draper and H.J. Swartz. 1981. Allstar strawberry. HortScience 16:792-794.
Hancock, J.F., B.L. Goulart, J.J. Luby and M.P. Pritts. 1997. The strawberry matted row: Practical cropping system or dated anachronism? Adv. Strawberry Res. 16:1-4.
Handley, D.T. and J.F. Dill. 2002. Performance of strawberry cultivars grown in a matted-row system in Maine, p. 32-33. In: S.C. Hokanson and A.R. Jamieson (eds.). Strawberry Research to 2001. ASHS Press, Alexandria, VA.
Hokanson, S.C. and C.E. Finn. 2000. Strawberry cultivar use in North America. HortTechnology 10:94-106.
Jamieson, A.R. 2003. Strawberry cultivars for perennial production, p. 167-170. In: N.F. Childers (ed.). The Strawberry, A Book for Growers, Others. Dr. Norman F. Childers Publications, Gainesville, FL.
Jamieson, A.R. 2003. ‘Annapolis’ strawberry. J. Amer. Pomol. Soc. 57:98-99.
Jamieson, A.R. and N.L. Nickerson. 2004. ‘Brunswick’ strawberry. HortScience 39:1781-1782.
Kaps, M.L. and P.L. Byers. 2008. Matted-row strawberry cultivar productivity in Missouri, 2005-2006. J. Amer. Pomol. Soc. 62:70-76.
Kaps, M.L., P.L. Byers, and M.B. Odneal. 2005. Annual strawberry systems in Missouri. J. Amer. Pomol. Soc. 59:175-181.
Kaps, M.L., M.B. Odneal, and P.L. Byers. 2003. Strawberry cultivar performance in Missouri, 1997-1999. J. Amer. Pomol. Soc. 57:19-25.
Kaps, M.L., M.B. Odneal, J.F. Moore, Jr. and R.E. Carter. 1990. Strawberry cultivar evaluation in Missouri. Fruit Var. J. 44:158-164.
Kaps, M.L., M.B. Odneal and J.F. Moore, Jr. 1987. Strawberry cultivar evaluation in the Missouri Ozark region. Adv. Straw. Prod. 6:36-40.
Masiunas, J.B., S.C. Weller, R.A. Hayden and J. Janick. 1991. Effect of plant spacing on strawberry yield in two cultural systems. Fruit Var. J. 45:146-151.
Nourse, N. 2009. Strawberry Varieties for Today and Tomorrow (PDF file). Proc. New Eng. Veg. and Fruit Conf. Available from https://newenglandvfc.org/sites/newenglandvfc.org/files/content/proceedings2009/SVforTT.pdf.
Pritts, M.P. 2003. Perennial strawberry production and marketing, p. 140-166. In: N.F. Childers (ed.). The Strawberry, A Book for Growers, Others. Dr. Norman F. Childers Publications, Gainesville, FL.
Pritts, M. and D. Handley (eds). 1998. Strawberry production guide for the Northeast, Midwest, and eastern Canada. NRAES-88. Northeast Regional Agricultural Engineering Service. Ithaca, NY. 162p.
Swartz, H.J., J. Popenoe and J.A. Fiola. 1985. Yield component analysis of the 1984 Maryland-USDA replicated trials. Adv. Straw. Prod. 4:45-52.
Weber, C. 2005. Geneva strawberry variety trial results 2004. New York Berry News. 4(1):6-9.
Stevens, M.D., J.D. Lea-Cox, B.L. Black and J.A. Abbott. 2007. A comparison of fruit quality and consumer preferences among three cold-climate strawberry production systems. HortTechnology 17:586-591.

About The Cover:

‘Romang’ is a new mid-season sweet persimmon cultivar from the National Institute of Horticultural and Herbal Science, Rural Development Administration in Korea.

Journal of the American Pomological Society 72(2): 135-143 2018

GA_{4+7} Soak Before Cold Stratification Enhances *Juglans nigra* Seedling Production

Michelle R. Warmund[1], and J. W. Van Sambeek[2]

Additional index words: black walnut, hormones, plant growth regulators, ProGibb, Promalin, Provide

Abstract

Eastern black walnut (*Juglans nigra* L.) seeds typically require a long period of stratification and often have low germination. A study was conducted to evaluate the effect of three formulations of gibberellic acid at 250 $mg \cdot L^{-1}$ or tap water as a soaking treatment for 'Thomas' black walnut seeds for 24 h before stratification for 30, 45, 60, 75, or 90 d. Gibberellic acid treatments included 1) GA_3 (ProGibb®); 2) GA_{4+7} (Provide®); and 3) 6-benzyladenine (BA) + GA_{4+7} (Promalin®). Percent walnut shoot emergence 60 days after planting, days to 20% and 80% shoot emergence (E20 and E80), and early seedling growth from black walnut seeds were evaluated. Percent shoot emergence was always higher for seeds soaked in GA_{4+7} or BA + GA_{4+7} when compared with other treatments. Shoot emergence for some seeds soaked in GA_{4+7} and BA + GA_{4+7} occurred with 30 d stratification and percent emergence increased with longer stratification periods. Seeds soaked in GA_3 had higher percent shoot emergence than those soaked in tap water only. Also, seeds soaked in GA_3 had fewer days to 20% shoot emergence when stratified for 45 or 60 d than those soaked in tap water and stratified for the same period of time. Addition of BA at 250 $mg \cdot L^{-1}$ apparently did not enhance percent shoot emergence, E20, E80, or seedling height or weight. With timely harvest, hulling, seed selection, and soaking walnuts with 250 $mg \cdot L^{-1}$ GA_{4+7} followed by 90 d stratification, 82% shoot emergence (i.e., germination) was attained.

Eastern black walnuts are recalcitrant and often have a low germination percentage (Dorn and Mudge, 1985; Flores et al., 2016). Immediately after harvest, intact black walnut seeds generally require stratification for 90 to 120 d, resulting in only about 50% germination (Brinkman, 1974). Early workers recommended immediate hulling after harvest, air-drying, and storage in moist peat at 1 to 3 °C for five to six months to promote seed germination (Muenscher and Brown, 1943). Later propagation methods included selecting large black walnut seeds for stratification and floating hulled nuts in water to remove small walnuts with shriveled (i.e., stenospermocarpic) kernels (Brinkman, 1974; Chase, 1947; Warmund and Van Sambeek, 2014). Cracking hulled black walnuts before stratification slightly improved germination percentage compared with the untreated controls (64% vs. 54%) when evaluated 270 days after planting, but cracking sometimes damaged kernels or increased kernel susceptibility to pathogenic microorganisms (Gaur, 1980). *Penicillium*, *Mucor*, *Phompsis*, *Fusarium*, and *Papulaspora* spp. were isolated from cotyledonary surfaces of kernels when walnuts were cracked (Kessler, 1978). Stratification and soaking intact walnuts in 10 or 20% sulfuric acid solutions for 30 min also reduced germination compared with untreated stratified controls (Gaur, 1980).

Exogenous application of gibberellic acid to recalcitrant seeds promotes germination (Frankland, 1961). For black walnut, stratification and GA_3 treatments at 125 or 250 $mg \cdot L^{-1}$ enhanced germination after plant-

[1] Professor of Horticulture and Extension Fruit Specialist, Division of Plant Sciences, University of Missouri, Columbia, MO 65211 (WarmundM@missouri.edu)

[2] Emeritus Research Plant Physiologist, USDA Forest Service, Northern Research Station, Columbia, MO 65211
The use of trade or firm names in this publication is for reader information and does not imply endorsement by the U.S. Department of Agriculture of any product or service.

ing in outdoor nursery beds in Butan (Gaur, 1980). Dorn and Mudge (1985) reported conflicting results for enhanced black walnut germination when shells were notched, seeds were subsequently vacuum-infiltrated with GA_3 and placed in a greenhouse at 21°C under mist. Soaking intact black walnuts in GA_3 at 400 mg·L^{-1} and stratification for two months resulted in 69% germination in a shaded greenhouse in Iran (Parvin et al., 2015).

The use of other gibberellins and cytokinins to promote germination of seeds, including *Juglans microcarpa* (C.A. Leslie, personal communication) and *Corylus avellana* (Frankland, 1961), has been studied. However, results of early experiments investigating the use of growth regulators to replace or reduce stratification are unclear due to the paucity of information regarding time of fruit harvest and hulling, as well as storage of nuts before stratification was initiated. Thus, the objective of this study was to evaluate percent shoot emergence, days to 20 and 80% shoot emergence (E20 and E80), and early seedling growth of hulled 'Thomas' black walnuts soaked in either of two forms of gibberellins alone (GA_3, GA_{4+7}), BA + GA_{4+7}, or tap water for 24 h before stratification for 30, 45, 60, 75, or 90 d.

Materials and Methods

Seeds from seven 'Thomas' black walnut trees grafted to seedling Thomas rootstock and planted in the clonal repositories at the Horticulture and Agroforestry Research Center, New Franklin, MO, were used for this study. Trees were selected based on age (20 years-old) and their genetic identities confirmed by DNA fingerprinting, using a series of ten single sequence repeat microsatellite markers (Warmund and Coggeshall, 2010). Trees were spaced 12.1 x 12.1 m apart and were growing without irrigation or pesticides in a Menfro silt loam (fine-silty, mixed, superactive, mesic typic hapludalfs). Pelletized ammonium nitrate (34N-0P-0K) was applied annually with 67 kg·ha^{-1} and 45 kg·ha^{-1} in late April and late October, respectively. Ground cover was mowed as needed. When ~ 20% of the walnuts were on the ground, those remaining on trees were harvested with a tree shaker (Model 2138, Savage Equipment, Madill, OK) and a collection device (Nut Wizard, Louisville, KY) was used to gather fruits from the ground on 11 Oct. 2016. Immediately after harvest, fruits from each tree were hulled with a locally produced machine (Lane, 2000). The following day each nut was weighed and those > 31 g were soaked in growth regulator solutions (250 mg·L^{-1} gibberellic acid) or water. Soaking treatments included GA_3 (ProGibb®; Valent BioSciences, Walnut Creek, CA), GA_{4+7} (Provide®; Valent BioSciences, Walnut Creek, CA), BA + GA_{4+7} (Promalin®; Valent BioSciences, Walnut Creek, CA), and tap water. For the five replications of each treatment, 125 walnuts were soaked in 19 L-plastic containers using a 5 L solution. After walnuts were soaked for 24 h, they were air-dried at 21 °C for 15 min. Next, 25 of the 125 walnuts were placed in 3.8 L polyethylene bags (Pactiv Corp., Lake Forest, IL) and stored at 5 °C for 30, 45, 60, 75, or 90 d for stratification. After each stratification period, five replications of 25 walnuts of each treatment were planted in 40 x 40 x 15-cm (depth) polyethylene flats (Stuewe & Sons, Tangent, OR), using potting medium (ProMix; Premier Tech Horticulture, Québec, Canada) moistened with 1 L tap water. For each stratification period, flats were arranged in randomized complete block design in the greenhouse maintained at 26 °C under natural light and uniformly irrigated as needed. Shoot emergence (i.e., seed germination) was recorded every other day for 60 d. For germinants, the mean number of days to 20% (E20) and 80% (E80) shoot emergence during the 60-day greenhouse period was calculated. Germinants were then harvested, roots were washed free of potting media, and plant tissue (excluding nut shells) was oven-dried at 65 °C for 48 h to determine seedling dry weights. Non-germinated seeds were cut transversely on a bandsaw to assess the cotyledons. Stenospermocarpic seeds and those with decayed cotyledons were omitted from statistical analyses.

Because no water-soaked control seeds germinated with 30 d of stratification, these data were omitted from statistical analyses. For all other stratification periods (45, 60, 75, and 90), the odds (i.e., probability) of shoot emergence of each growth regulator treatment were calculated, using the GLMMIX procedure of the SAS statistical analysis software (SAS Institute, Cary, NC) with a link = logit function for a binomial distribution due to the non-normal distribution of data. Odds were calculated from the antilog of the logit value and back-transformed [% shoot emergence = odds / (1 + odds)] for reporting shoot emergence percentage by stratification time for each growth regulator treatment. Mean differences among odds were determined using the LSMEANS statement ($P \leq 0.05$). Days to E20 and E80, seedling height, and dry weight were subjected to analysis of variance (ANOVA) using the PROC GLMMIX procedure of SAS and means were separated by Fisher's protected LSD test ($P \leq 0.05$). Orthogonal contrasts were performed to evaluate linear, quadratic, and cubic responses to varying stratification times, using the PROC GLM procedure of SAS.

Results

Percent shoot emergence varied among growth regulator treatments and the stratification times, but there was no interaction between these variables (Table 1). After 60 d in the greenhouse, the average percent shoot emergence for BA + GA_{4+7}, GA_{4+7}, and GA_3 seeds stratified for 30 d was 20, 18, and 6, respectively, whereas none of the water-soaked control seeds germinated at this time (data not shown). For all other stratification periods, seeds were more likely to emerge when soaked in BA + GA_{4+7} (62% emergence) or GA_{4+7} (60% emergence) than when soaked in GA_3 (34% emergence) (Table 1). Walnuts soaked in water alone were the least likely to germinate with only 27% shoot emergence across all stratification periods. For 45, 60, 75 and 90 d of stratification,

Table 1. Percent shoot emergence of *Juglans nigra* seed treated with selected growth regulators and stratification periods.[z]

	Stratification time (d)[y]				
Treatment	45	60	75	90	Mean (treatment)
BA + GA_{4+7}	34	52	73	87	62 a
GA_{4+7}	33	47	78	82	60 a
GA_3	10	23	42	60	34 b
Water only	4	18	33	52	27 c
Mean (stratification time)	20 d	35 c	57 b	70 a	
Significance					
Treatment (T)	<0.0001				
Stratification time (ST)	<0.0001				
T x ST	0.3840				

[z] Walnut seeds were soaked in solutions of each growth regulator (250 $mg \cdot L^{-1}$ gibberellic acid) before stratification and were subsequently grown in a greenhouse for 60 d.

[y] Values represent 5 replications per treatment with 25 seeds sown per replication. PROC GLIMMIX using a logit link for binomial distributions was used to analyze germination data. Back transformed data [% shoot emergence = odds (1+odds)] are presented. Mean differences among odds were determined using the LSMEANS statement. Within a column or row, means followed by the same letter are not significantly different, according to Fisher's protected LSD test ($P \leq 0.05$).

percent shoot emergence across all treatments increased significantly for each period of time (20, 35, 57, and 70%, respectively).

When days to E20 were calculated for 30 day stratification periods, seeds soaked in BA + GA_{4+7} averaged 46 d and those in GA_{4+7} averaged 50 d (data not shown). Since only 6% of the shoots of seeds in GA_3 treatments stratified for 30 d emerged, E20 (and E80) values were not calculated. For all other stratification periods, there was a significant interaction of treatment and stratification time for days to E20, and a linear response to stratification time (Table 2). Walnuts soaked in water only and stratified for 45 or 60 d had greater E20 values than all other treatments and stratification times. When the stratification period was 75 days, seeds soaked in GA_3 and water required 34 and 37 d for 20% germination, respectively, whereas those soaked in BA + GA_{4+7} or GA_{4+7} required ~ 29 to 30 d. E20 values for walnuts soaked in BA + GA_{4+7} or GA_{4+7} were also lower than that of water–soaked seeds after 90 d of stratification, but E20 values were similar for walnuts soaked in BA + GA_{4+7} or GA_3.

E80 values also differed among treatments (Table 3). Walnuts soaked in BA + GA_{4+7} or GA_{4+7} had lower mean E80 values (49 d) than seeds treated with GA_3 (51 d) or soaked in water alone (53 d). E80 values exhibited a quadratic response to stratification time. For 45, 60, 75, and 90 d of stratification, E80 values for walnuts were 58, 56, 50, and 38 d, respectively. There was no interaction of treatment and stratification time for E80 values.

Seedling heights of walnuts soaked in BA + GA_{4+7} or GA_{4+7} and stratified for 30 days averaged 10.8 and 13.6 cm, respectively, after 60 d in the greenhouse. Seedling dry weight for BA + GA_{4+7}-soaked walnuts stratified for 30 d was 1 g and that for GA_{4+7} was 1.6 g. For all other stratification times, seedling heights and dry weights for seeds soaked in BA + GA_{4+7} or GA_{4+7} were greater than those receiving other treatments (Table 4). However, seedling dry weight of GA_3-

Table 2. Days to 20% (E20) shoot emergence of *Juglans nigra* seed treated with selected growth regulators and stratification periods.[z]

	Stratification time (d)[y]				
Treatment	45	60	75	90	Mean (treatment)
BA + GA_{4+7}	41.4	33.4	29.6	24.0	32.1 c
GA_{4+7}	38.8	32.8	29.2	21.8	30.7 c
GA_3	42.0	41.8	34.4	26.4	36.2 b
Water only	52.0	47.4	37.4	28.4	41.3 a
Mean (stratification time)	43.6 a	38.9 b	32.7 c	25.2 d	
Significance[y]					
Treatment (T)	<0.0001				
Stratification time $(ST)_L$	<0.0001				
ST_Q	0.0682				
T x ST_L	0.0147				
T x ST_Q	0.1279				

[z] Walnut seeds were soaked in solutions of each growth regulator (250 $mg \cdot L^{-1}$ gibberellic acid) before stratification and were subsequently grown in a greenhouse for 60 d. Means represent 5 replications per treatment with 25 seeds sown per replication. For germinants, the mean number of days to 20% (E20) shoot emergence was calculated. Within a column or row, means followed by the same letter are not significantly different, according to Fisher's protected LSD test ($P \leq 0.05$).

[y] Linear (L), quadratic (Q), and cubic orthogonal contrasts were performed to test the trend of different stratification times for E20.

Table 3. Days to 80% (E80) shoot emergence of *Juglans nigra* seed treated with selected growth regulators and stratification periods.[z]

Treatment	Stratification time (d) 45	60	75	90	Mean (treatment)
BA + GA_{4+7}	57.6	53.4	48.2	34.8	48.5 c
GA_{4+7}	57.6	54.6	47.8	35.2	48.8 c
GA_3	58.0	57.4	50.6	38.6	51.2 b
Water only	59.0	58.2	53.6	41.2	53.0 a
Mean (stratification time)	51.8 a	55.9 b	50.1 c	37.5 d	
Significance[y]					
Treatment (T)	<0.0001				
Stratification time $(ST)_L$	<0.0001				
ST_Q	0.0001				
T x ST_L	0.0913				
T x ST_Q	0.8109				

[z] Walnut seeds were soaked in solutions of each growth regulator (250 $mg \cdot L^{-1}$ gibberellic acid) before stratification and were subsequently grown in a greenhouse for 60 d. Means represent 5 replications per treatment with 25 seeds sown per replication. For germinants, the mean number of days to 80% (E80) shoot emergence was calculated. Within a column or row, means followed by the same letter are not significantly different, according to Fisher's protected LSD test ($P \leq 0.05$).

[y] Linear (L), quadratic (Q), and cubic orthogonal contrasts were performed to test the trend of different stratification times for E80.

soaked walnuts was greater than that of water-soaked controls. Seedling height and dry weight exhibited quadratic responses to stratification time (Table 4). For 45, 60, 75 and 90 d of stratification, seedling height increased at each period of time. However, walnuts receiving 90 d stratification had greater seedling dry weight than those receiving fewer days of stratification.

Discussion

Plant hormone concentrations fluctuate in walnut seed tissues during stratification and the period immediately afterwards when exposed to warm temperatures (Somers et al., 1989). The highest concentrations of GA_3 and GA_{4+7} were recovered from the embryonic axis of seeds during the first 60 d of stratification, decreased when sampled at 120 or 180 d of chilling, but increased when seeds were removed from cold storage and exposed to ambient temperatures. The highest concentrations of abscisic acid and cytokinins were recovered in the embryonic axes when analyzed at 180 d of stratification, but decreased when removed from cold storage. In seeds, gibberellins induce enzymatic activity which degrades cell walls in the endosperm and subsequently hydrolyzes starches and protein into compounds needed for cellular activity and embryonic growth (Somers and Van Sambeek, 2003; Weaver, 1972). Genetic control of gibberellin biosynthesis, metabolism, and signaling has been studied in *Arabidopsis* and cereal crops, but this has yet to be explored in woody plants (Hedden and Thomas, 2016).

Exogenous applications of gibberellin have been used successfully to break seed dormancy and enhance seed germination of many plant species (Krishnamoorthy, 1975; Weaver, 1972). In our study, soaking walnut seeds in GA_3, GA_{4+7}, or BA + GA_{4+7} before stratification enhanced shoot emergence and required fewer days to attain 80% shoot emergence when compared with a tap water only soak (Tables 1 and 3). GA_{4+7} treatments were more effective in promoting seed

Table 4. Height and dry weight of *Juglans nigra* seedlings after 60 d in a greenhouse.[z]

Main effect	Seedling ht. (cm)	Seedling dry wt. (g)
Treatment		
BA + GA_{4+7}	24.9 a	1.96 a
GA_{4+7}	23.9 a	1.82 a
GA_3	20.0 b	1.47 b
Water only	19.3 b	1.13 c
Stratification time (d)		
45	31.3 d	1.27 b
60	50.0 c	1.35 b
75	75.3 b	1.48 b
90	94.3 a	2.28 a
Significance[y]		
Treatment (T)	<0.0001	<0.0001
Stratification time $(ST)_L$	<0.0001	<0.0001
ST_Q	<0.0001	<0.0001
T x ST_L	0.5056	0.0696
T x ST_Q	0.8387	0.0247

[z] Walnut seeds were soaked in solutions of each growth regulator (250 mg·L^{-1} gibberellic acid) before stratification and were subsequently grown in a greenhouse for 60 d. Means represent height and weight of germinants from 5 replications per treatment with 25 seeds sown per replication. Within each column, means followed by the same letter are not significantly different, according to Fisher's protected LSD test ($P \leq 0.05$).

[y] Linear (L), quadratic (Q) orthogonal contrasts were performed to test the trend of different stratification times. *P* values for ANOVA.

germination than GA_3 (Table 1). These results agree with others where a GA_{4+7} treatment resulted in greater seed germination of *Galeopsis pyrenaica*, *Lycopus europaeus* (Thompson 1969) and cereals (Mayer and Poljakoff-Mayber, 1989) than a GA_3 treatment. Additionally, Thompson (1969) reported that GA_{4+7} promoted germination at lower concentrations than GA_3, which may explain the lower percent shoot emergence for GA_3-soaked seeds than GA_{4+7}-soaked seeds in our study using one concentration of gibberellin (250 mg·L^{-1}).

Although cytokinins are commonly associated with the promotion of cell division, there are reports of enhanced germination following their exogenous application to some seeds or their embryos after pericarp and testa tissues were removed (Frankland, 1961; Weaver, 1979). In experiments with *Juglans microcarpa*, intact seeds soaked in BA + GA_{4+7} (Promalin®) at 62.5, 125 or 250 mg·L^{-1} had 70% to 90% germination, but treatments without BA were not included in the study (Leslie et al., 2014). In our study, percent shoot emergence, days to shoot emergence, and seedling heights and dry weights were similar for GA_{4+7} soaking treatments with or without BA. These results indicate that BA at 250 mg·L^{-1} did not enhance black walnut germination and there would be no additional benefit derived from its use, especially due to the higher product cost of BA + GA_{4+7} relative to that of GA_{4+7}. Shoot emergence from BA + GA_{4+7} and GA_{4+7} –soaked seeds occurred with as little as 30 d stratification and percent emergence increased with longer stratification periods (Table 1). About 50% shoot emergence occurred in the present study when seeds were soaked in either GA_{4+7} treatment and stratified for 60 d, whereas those in tap water required an additional month of stratification to attain the same emergence percentage. After a 90 d stratification period, GA_{4+7}-treated black walnuts had over 80% germination (i.e., shoot emergence) while seed soaked in water had 52%, which is close to the typical 50% germination reported by Brinkman (1974). Days to 20% shoot emergence for seeds treated with GA_{4+7} were also reduced by 17 d and E80 values were reduced by 22 days when stratification time was increased from 45 to 90 d. The taller seedling heights and plant dry weights resulting from GA_{4+7} treatments compared with the tap water soak are likely due to the more rapid seed germination which allowed more time for growth in the greenhouse.

In conclusion, high percentages (73% to 87%) of shoot emergence (i.e., germination) were attained in this study, which may be attributed to timely harvest, immediate hulling of fruits, selection of sound walnuts with high fresh weight, immediate soaking in 250 $mg \cdot L^{-1}$ GA_{4+7} followed by 75 to 90 d stratification, and exposure to relatively high temperatures after cold storage. The increased seed germination derived from a GA_{4+7} soak and rapid early seedling growth provides nurserymen with a more efficient method for black walnut seedling production. This may be especially significant for nurserymen who start black walnut seeds in greenhouses for rapid germination and early seedling growth, resulting in larger plants than those grown in outdoor nursery beds.

Literature Cited

Brinkman, K.A. 1974. *Juglans* L. Walnut, p. 454-459. In: C.S. Schopmeyer (ed.). Seeds of woody plants in the United States. USDA Handbook No. 450. U.S. Govt. Printing Office, Washington, DC.

Chase, S.B. 1947. Eastern black walnut germination and seedbed studies. J. For. 45:661-668.

Dorn, C.M. and K.W. Mudge. 1985. Vacuum infiltration of gibberellic acid stimulates germination of dormant black walnut seeds. J. Environ. Hort. 3:172-175.

Flores, P., D. Poggi, S.M. Garcia, M. Catraro, and N. Gariglio. 2016. Effects of pre-stratification storage conditions on black walnut seed post-stratification germination capacity. Intl. J. Fruit Sci. 17:29-40.

Frankland, B. 1961. Effect of gibberellic acid, kinetin and other substances on seed dormancy. Nature 192:678-679.

Gaur, N.V.S. 1980. Effect of gibberellin, sulphuric acid, and hand cracking on the germination and growth of seedlings in walnut. Indian J. Hort. 37:16-19.

Hedden, P. and S.G. Thomas. 2016. The gibberellins. Wiley Blackwell, Hoboken, N.J.

Kessler Jr., K.J. 1978. Nursery practices and the germination performance of black walnut seed. Phytopathol. News 12:174.

Krishnamoorthy, H.N. 1975. Gibberellins and plant growth. Wiley & Sons, New York.

Lane, B. 2000. Notes from over a quarter-century of building black walnut processing equipment. The Nutshell. Northern Nut Growers Assn. 54:1-12.

Leslie, C., G. McGranahan, W. Hackett, L. Walawage, Q. Zhang, J. Grant, J. Caprile, B. Coates, R. Buchner, J. Hasey, D. Doll, E. Fichtner, K. Pope, A. Dandekar, M. Aradhya, D. Tricoli, and S. Mysore. 2014. Walnut improvement program. University of California. 20 Oct. 2017. < http://ucce.ucdavis.edu/files/repositoryfiles/2014_003_ocr-156298.pdf>.

Mayer, A.M. and A. Poljakoff-Mayber. 1989. The germination of seeds. 4th ed. Pergamon Press, New York.

Muenscher, W.C. and B.I. Brown. 1943. Storage and germination of nuts of several species of *Juglans*. Northern Nut Growers Assn. Annu. Rpt. 34:61-62.

Parvin, P., M. Khezri, I. Tavasolian, and H. Hosseini. 2015. The effect of gibberellic acid and chilling stratification on seed germination of eastern black walnut (*Juglans nigra* L.). J. Nuts 6:67-76.

Somers, P.W., and J.W. Van Sambeek. 2003. Physiological and ultra-structural changes in black walnut embryos during stratification and germination. Northern Nut Growers Assn. Annu. Rpt. 94:107-119.

Somers, P.W., J.W. Van Sambeek, and G.R. Gaffney. 1989. Hormone fluctuations during stratification and germination of black walnut seed. Proc. 7th Central Hardwood Forest Conf. USDA Forest Serv. Gen. Tech. Rpt. NC-132:175-180.

Thompson, P.A. 1969. Comparative effects of gibberellins A_3 and A_4 on the germination of seeds of several different species. Hort. Res. 9:130-138.

Warmund, M.R. and M.V. Coggeshall. 2010. Flowering and fruit characteristics of black walnut cultivars at the University of Missouri repository. Acta Hort. 861:177-182.

Warmund, M.R. and J.W. Van Sambeek. 2014. Ambered kernels in stenospermocarpic fruit of eastern black walnut. HortScience 49:1142-1147.

Weaver, R.J. 1972. Plant growth substances in agriculture. W.H. Freeman, San Francisco.

Book Review

Achieving Sustainable Cultivation of Apples

ROBERT M. CRASSWELLER[1]

Apples are considered the king of tree fruits with more literature and scientific research about this commodity than any other tree fruit crop. This is the first new comprehensive textbook in nearly 15 years on the latest scientific information on apple production. The book is edited by Dr. Kate Evans from Washington State University. The contributing authors are recognized leaders in their fields of expertise. The book is divided into four major parts; physiology and breeding; cultivation techniques; diseases and pests; and lastly as embedded in the title, sustainability.

The first part consists of eight chapters. The first two are devoted to information on the apple genome; what is known about the genome and potential new techniques impacting future cultivar development. The remaining six chapters present the information on changes that are occurring and have occurred in tree and fruit growth, flowering, fruit development, rootstocks and marker-assisted breeding. The chapter on flowering and pollination was exceptionally interesting.

Part 2 is devoted to innovations in cultivation techniques. There is a chapter on tree growth and influence of training systems including the importance of the use of plant growth regulators. Part of this chapter has a discussion on the growth habits of different cultivars and their impact on pruning techniques. Interestingly, the author mentions 'Cosmic Crisp' which is an apple cultivar that for the seeable future can only be produced by growers in the state of Washington (USA). Another chapter on plant nutrition stresses emerging issues affecting nutrient management. The tree fruit industry is currently struggling with labor availability and producers are moving toward increased mechanization techniques. Harvest assist mechanization is addressed specifically in the text. Postharvest handling and storage is an area that is rapidly changing and the extensive reference list at the end of the chapter is invaluable.

Part 3 is devoted to disease and pest problems in apple production including information on new disease and insect resistant apple cultivars. Chapters in this section specifically address fungal pathogens, virus and virus-like problems and bacterial diseases, especially fire blight. Arthropod management is broken down into primary and secondary pests. The chapter on development of pest and disease resistant apple "varieties" is a little too much European focused and would have benefited with a broader scope.

The final Part 4 covers the issue of sustainable apple production including economics, consumer trends and the impact on the environment. This latter part reflects the increasing trend in the industry to quantify environmental impacts and consequences of traditional orchard production practices. Although this book is part of the Sustainable Series by the publisher, this part of the book is the smallest section. It does however provide interesting chapters. One chapter in this part of which most production scientists may have little background covers consumer trends in apple sales. This is an area that many of us have little experience and can serve to put all the scientific break throughs in perspective.

Individual chapters in the textbook are

[1] Department of Plant Science, The Pennsylvania State University, University Park, PA 16802.Email: rmc7@psu.edu
Evans, K. (Editor) 2017. Burleigh Dodds Science Publishing in Agricultural Science Number 018. Print: (ISBN 987-1-78676-04-1). Online (ISBN 978-1-78676-042-5). 591pp. Hardcover $256.90. Kindle $159.99

uniformly laid out into similar sections beginning with an introduction that generally describes what attributes will be covered and their importance. As with any scientific text, the moment it is released changes have occurred in the field of study. However, *Achieving* makes up for this common problem by providing in each chapter extensive current references and more importantly a section titled "*Where to look for further information.*" The latter takes the form of internet links to organizations, conferences or scientific societies that specialize in the topic of the chapter. Another important feature of some chapters is the section devoted to "*Future trends and conclusions.*" Here the authors attempt to predict areas that researchers and educators will (or should) be working to develop.

I did find that in some instances the ordering of the chapters seemed a little misplaced. The chapter on sustainable approaches to postharvest diseases seemed out of place. It should have been either in Part 3, Diseases and Pests, or in Part 4, Sustainability. The chapter on growing organic apples in Europe may also have been better placed in Part 2 in cultivation techniques. One major item that would add great value to this text would be the inclusion of information on sustainable production practices in the People's Republic of China since that country is the leading producer of apples.

However, I think all the chapters did provide valuable information. United together they make this a good reference or college level text. This would be a good text to accompany a second level undergraduate or graduate level course in fruit production. Commercial apple growers and industry members would also benefit from this text to provide an interesting concise view of the current state of the industry and its future direction. This book represents the most recent source of the current status of apple production in the Western world. There is liberal use of color images and graphs throughout.

Correction:

In volume 71(4), in the article by Amanda J. Aance, Bernadine C. Strik, and John Clark "Table grape cultivar performance in Oregon's Willamette Valley", there has been a cultivar designation change for the new cultivar referred to in the paper as 'Passion'. It will not be called 'Compassion'.

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 included parenthetically when the organism is first mentioned. American spelling conventions and SI units should be used. 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, email adresses 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

Before 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 coauthors on the paper have had the opportunity to review it before 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 manuscripts electronically to the Editor: Dr. Richard Marini, 203 Tyson Building, Department of Plant Science, University Park, PA 16802-4200 USA; E-mail: richmarini1@gmail.com. Acceptable format is MSWord.

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 $50.00 per page for APS members (at least one author is a member) and $65.00 per page ($32.50 per half page) for nonmembers will be made to authors for those articles constituting publication of research. In addition to the page charge, there will be a charge of $40.00 per page for tables, figures and photographs.

American Pomological Society 2018 Annual Meeting

to be held in conjunction with

The Annual Conference of the American Society for Horticultural Science

31 July – 3 August • Washington, DC

The Washington Hilton Hotel

Call for Wilder Silver Medal Nominations

The Wilder Committee of the American Pomological Society (APS) invites nominations for the 2018 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 2018.