Journal of the American Pomological Society 69(1): 4-10 2015

Floral Bud Chill Requirement of Low-Chill Southern Highbush Blueberry Germplasm

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Additional index words: blueberry breeding, evergreen production, hydrogen cyanamide, *Vaccinium corymbosum*

Abstract

The University of Florida southern highbush blueberry (SHB) (*Vaccinium corymbosum* L. hybrids) breeding program has traditionally focused selection efforts in an area that annually receives ≥ 300 h of winter chilling at 0 to 7°C. Thus, many of the cultivars released from the breeding program are not adapted to regions in central and south Florida that receive less annual chilling unless dormancy-breaking agents such as hydrogen cyanamide (HC) are used. To assess the potential for expansion into very low-chill production areas, floral bud chill requirements for 23 advanced selections and two cultivars planted at two locations in Florida were estimated using an excised shoot forcing method. The majority (72%) of the genotypes assessed had at least 70% floral budbreak after accumulation of 200 chill-hours at 0 to 7°C. When HC application was compared with untreated plants of the same genotype, the dormancy breaking compound either hastened floral budbreak or increased the percentage of floral budbreak of insufficiently chilled plant material.

The predominant cultural management system used for SHB production in Florida allows the plants to go dormant for a short period of time before the onset of reproductive and vegetative growth in the spring. The onset of dormancy can vary due to local weather patterns, but typically occurs in December to January in most production areas of Florida. Once a blueberry plant enters dormancy, a period of low temperatures (e.g., chilling) is required for normal reproductive and vegetative development to occur. Insufficient chilling of blueberry plants results in reduced, delayed, and erratic reproductive and vegetative budbreak (Darnell and Davies, 1990). Although the optimum temperature for chilling accumulation is not known for all SHB genotypes, the model proposed by Chandler et al. (1937) with one unit of chill accumulation for every hour at 0 to 7°C is commonly used by producers to estimate chill accumulation. The chilling requirement varies by species and cultivar, and can range from greater than 1000 h for northern blueberry cultivars grown in temperate locations to 200 h for some SHB cultivars developed in Florida (Norvell and Moore, 1982; Williamson et al., 2002). For many potential blueberry production areas of Florida, the yearly accumulation of chill-hours in this range is less than necessary for most blueberry cultivars.

Both breeding and cultural practices have been used to address the potential for insufficient chilling in Florida. The University of Florida (UF) SHB breeding program utilized *Vaccinium darrowii* Camp, a related evergreen blueberry species native to Florida, which does not require chilling, in crosses with northern-adapted blueberry cultivars.

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This peer-reviewed paper was presented at the "Unique Blueberry Production Practices for Subtropical and Tropical Climates" workshop sponsored by the American Pomological Society at the American Society for Horticultural Sciences Conference, Orlando, FL on 30 Jul. 2014.

Plants selected from these crosses had commercial fruit quality and required ≤ 800 h of chilling (Lyrene, 2008). Because all early stages of selection occur within 40 km (25 miles) of Gainesville, FL (an area with a winter average of ≥ 300 chill-hours) many of the cultivars released from the program are presumed to have a chilling requirement near 300 h. Early on, it was recognized that the potential for selecting cultivars adapted to much lower chilling ranges would be possible, but the distances involved would prevent maintaining and evaluating sizable populations at remote locations (Lyrene and Sherman, 1977). However, at least two attempts have been made to enrich the pool of germplasm with lower chilling requirements either by evaluation in suitable locations (Obreza et al., 2000) or selecting for very early bloom in Gainesville (Lyrene, 1987).

Alternatively, a cultural method utilizing HC as a dormancy-breaking compound has been adopted by many Florida growers (Williamson et al., 2001; 2002). Under ideal situations, the use of HC results in greater vegetative budbreak, shorter fruit development period, increased yield, and improved plant health. However, response to HC is cultivar specific with respect to proper rates and timings, phytotoxicity can result in severely reduced yield, and the product has high human toxicity. Additionally, efficacy of HC application is highly dependent on the amount of chilling accumulated before application (Williamson et al., 2001). With current Florida blueberry production trends indicating increased plantings in central and south Florida locations (primarily Citrus, Sumter, Hernando, Lake, Orange, Pasco, Polk, Hardee, and Desoto counties), there is a need for development of SHB cultivars adapted to grow in areas receiving ≤ 300 chill-hours annually.

The objective of this project was to estimate the floral bud chilling requirement of 23 advanced selections currently under evaluation in the UF SHB breeding program with and without HC application.

Materials and Methods

Plant material and trial locations. Twenty-three advanced selections and two standard cultivars ('Emerald' and 'Primadonna') from the UF SHB breeding program were evaluated for two consecutive seasons (2011-2012 and 2012-2013; Tables 1 and 2). Clonal replicates of each genotype were propagated in 2009 from softwood cuttings and were planted as 15 to 20 cm-tall liner plants at two trial locations in January 2010. The first trial site was the Plant Science Research and Education Unit near Citra, FL. No HC was applied at this trial site. The second trial site was at a grower farm near Windsor, FL. Hydrogen cyanamide was applied at 1.25% following commercial practices at this location on 19 Dec. 2011 and 21 Dec. 2012. At all trial sites, planting beds were incorporated with bark and were covered with woven fabric mulch for weed control. Drip irrigation with sulfuric acid to reduce soil pH and fertilizer injection was used according to current Florida blueberry production practices (Liu et al., 2012).

Estimation of floral bud chill requirement. An excised shoot system similar to that described by Spiers et al. (2006) was used to calculate floral bud chill requirement. Briefly, field plants at each location were subjected to natural chilling and lighting, and the environmental conditions at each location were recorded using data-logging weather stations (Hobo U30 GSM, Onset Corp., Bourne, MA). After the accumulation of 50, 100, 150, and 200 chill-hours (sum of the number of hours at 0 to 7°C), three random canes ≈ 50 to 60 cm-long from current season's growth, were sampled from each genotype at each location. Canes were placed in plastic bags, packed on ice in coolers, and transported to a greenhouse in Gainesville, FL. Canes were re-cut and placed in water in 4.7 L plastic buckets (Encore Plastics Corp., Sandusky, OH). Sponges (Identiplug, Fisher Scientific, Pittsburgh, PA) were used to hold and separate the canes in drilled holes in each

bucket lid. Canes were evaluated biweekly for floral bud development, and the percentage of total floral buds on a cane at or beyond floral budbreak (stage 5; Spiers, 1978) was recorded. The water was changed and canes were re-cut weekly.

Statistical analyses. The experiment was analyzed as a 2 x 4 x 25 factorial design (two locations, four chill hour sampling dates, and 25 genotypes) using JMP Pro version 11 software (SAS Institute, Cary, NC). Years were kept separate to avoid additional interaction terms. Percent budbreak percentages were arcsin-transformed for analysis and least square means are reported. Treatment means were separated using Tukey's HSD test $P \leq 0.05$) and the slice function was used to further explore interactions between main effects.

Results and Discussion

The overall floral budbreak percentage after three and five weeks of evaluation was used to determine the chilling requirement of each genotype (Tables 1 and 2). Three weeks was the evaluation period used by Spiers et al. (2006), to determine the chilling requirements of rabbiteye blueberries in a similar excised shoot assay, while five weeks is generally considered longer than optimal for evaluation (Krewer and NeSmith, 2006; Spiers, 2006). In the first season, the loca-

Table 1: Percentage of floral budbreak on excised southern highbush blueberry shoots after three and five weeks of greenhouse evaluation. Shoots were collected from two field locations during the 2011-2012 growing season after receiving 50, 100, 150, and 200 chill-hours (0 to 7ºC) and forced in a greenhouse.

	Floral budbreak after 3 weeks (%)					Floral budbreak after 5 weeks (%)				
	Chill-hour accumulation before sampling					Chill-hour accumulation before sampling				
Genotype	50	100	150	200	*Sig.*[Z]	50	100	150	200	*Sig.*
FL05-96	52.0	73.5	75.5	77.3	NS	64.2	80.2	84.4	77.3	NS
FL05-619	70.0	98.5	94.8	88.4	NS	84.8	100.0	98.3	93.0	NS
FL06-02	60.3	70.2	68.6	68.7	NS	74.3	82.9	71.0	77.5	NS
FL06-35	74.1	80.6	76.2	56.6	NS	83.4	87.9	85.6	68.3	NS
FL06-77	0.0	38.8	5.4	7.7	**	6.7	56.4	31.4	35.6	****
FL06-96	62.1	63.0	49.4	35.3	NS	75.4	74.1	69.6	66.7	NS
FL06-203	80.7	96.1	66.5	81.3	NS	86.3	96.1	80.8	85.6	NS
FL06-205	25.5	76.4	33.4	60.0	****	50.7	86.4	73.6	70.0	**
FL06-244	32.2	63.4	63.4	56.6	*	58.1	77.2	72.9	59.5	NS
FL06-245	76.7	92.2	78.5	93.8	NS	83.8	95.8	84.5	100.0	NS
FL06-316	50.2	64.2	62.0	71.0	NS	68.6	77.6	69.5	76.8	NS
FL06-354	32.8	73.2	61.8	52.6	**	41.4	82.8	70.9	52.6	***
FL06-362	57.3	88.9	75.8	78.6	NS	73.5	92.2	87.9	84.2	NS
FL06-377	74.4	82.4	80.2	70.5	NS	80.1	85.8	84.6	72.4	NS
FL06-435	37.1	77.8	53.0	74.7	**	55.8	87.5	76.8	82.8	*
FL06-457	30.0	76.1	54.2	38.0	***	62.9	84.5	86.5	54.3	**
FL06-518	71.3	83.0	75.0	62.0	NS	94.3	87.2	90.1	65.7	*
FL06-540	12.4	64.7	54.2	75.9	****	26.0	81.8	72.4	77.3	****
FL06-542	62.8	66.3	64.5	57.9	NS	79.2	78.6	85.8	65.2	NS
FL06-545	80.3	90.2	87.1	52.8	**	86.9	98.5	89.2	61.8	**
FL06-561	63.3	54.5	82.1	73.8	NS	72.7	59.1	85.4	73.8	NS
FL06-562	15.6	72.4	64.6	77.1	****	61.8	81.2	68.4	78.3	NS
FL06-571	81.8	89.9	69.6	76.0	NS	87.3	97.4	85.2	95.3	NS
Emerald	57.3	64.8	66.7	62.4	NS	65.0	76.2	71.8	65.8	NS
Primadonna	69.5	79.9	71.5	51.8	NS	84.1	93.2	75.1	55.4	**

[z] NS,*,**,***,**** Non-significant or significant at $P \leq 0.05$, 0.01, 0.001, or 0.0001 between chilling hour sampling within a genotype, according to *P*-values from sliced ANOVA.

Table 2: Percentage of floral budbreak on excised southern highbush blueberry shoots after three and five weeks of greenhouse evaluation. Shoots were collected from two field locations during the 2012-2013 growing season after receiving 50, 100, 150, and 200 chill-hours (0 to 7°C) and forced in a greenhouse.

	Floral budbreak after 3 weeks (%)					Floral budbreak after 5 weeks (%)				
	Chill-hour accumulation before sampling					Chill-hour accumulation before sampling				
Genotype	50	100	150	200	*Sig.*[z]	50	100	150	200	*Sig.*
FL05-96	29.2	20.1	77.8	92.2	****	34.0	40.7	79.4	92.2	****
FL05-619	25.4	88.5	89.1	100.0	****	81.7	98.1	94.0	100.0	NS
FL06-02	11.1	47.5	76.9	100.0	****	53.7	76.6	86.4	100.0	***
FL06-35	5.6	58.8	65.2	89.7	****	70.6	95.2	80.6	95.8	NS
FL06-77	0.0	12.5	8.3	49.1	***	0.0	25.7	20.0	70.9	****
FL06-96	2.4	39.7	32.6	47.3	**	57.9	68.3	79.8	80.2	NS
FL06-203	38.3	89.1	74.0	100.0	****	89.8	100.0	88.1	100.0	NS
FL06-205	2.6	24.2	34.1	73.1	****	14.4	45.9	64.7	83.0	****
FL06-244	10.3	34.7	71.8	82.4	****	39.0	57.9	84.4	87.5	****
FL06-245	10.0	33.4	55.6	89.0	****	32.0	51.7	79.0	95.2	****
FL06-316	0.0	21.8	40.5	63.5	****	24.6	70.6	67.7	69.9	****
FL06-354	0.0	17.7	28.7	66.8	****	13.9	30.7	48.3	71.3	****
FL06-362	10.6	77.8	85.4	95.8	****	40.3	100.0	93.8	100.0	****
FL06-377	24.5	66.6	88.2	87.9	****	66.0	91.7	94.8	87.9	NS
FL06-435	2.1	40.4	67.0	82.5	****	38.1	73.0	72.0	82.5	***
FL06-457	2.8	0.0	35.0	85.8	****	9.7	26.5	67.0	89.2	****
FL06-518	0.0	45.2	83.7	85.8	****	62.1	80.1	90.3	89.2	NS
FL06-540	4.8	27.3	58.9	68.3	****	19.7	46.5	74.9	71.6	****
FL06-542	5.4	41.0	60.0	29.2	****	43.8	67.8	75.5	38.8	**
FL06-545	21.0	45.3	55.2	92.5	****	64.9	73.0	65.5	92.5	NS
FL06-561	0.0	25.7	74.5	78.6	****	32.0	73.1	78.7	91.9	****
FL06-562	0.0	11.7	64.0	83.4	****	47.5	47.8	77.8	89.2	***
FL06-571	24.2	68.4	73.4	100.0	****	45.0	84.7	81.7	100.0	****
Emerald	0.9	30.6	40.7	75.4	****	37.7	68.9	61.3	85.9	***
Primadonna	16.3	16.5	59.0	69.4	****	60.7	86.1	81.3	76.1	NS

[z] NS,*,**,***,**** Non-significant or significant at $P \leq 0.05$, 0.01, 0.001, or 0.0001 between chilling hour sampling within a genotype, according to *P*-values from sliced ANOVA.

tion, genotype and chill hour sampling date main effects and two-way interactions were all significant ($P \leq 0.05$). However, in the second season the location main effect was not significant after either three or five weeks of evaluation, while all other main effects and interactions were significant ($P \leq 0.05$).

In general, floral budbreak was higher in the first season than the second season. For example, 36% of the genotypes evaluated had over 70% floral budbreak within three weeks after only receiving 50 h of chilling in the field, while no genotypes evaluated in the second season reached that level of budbreak after accumulation of only 50 chill-hours. A likely explanation for this observation is that the plants were only in their second growing season after rooting during the first data collection year. We have often observed a tendency toward more evergreen characteristics in young plants, and the second evaluation season in this study (third growing season) is likely more representative of mature plant response to chilling accumulation. However, most growers in Florida and other subtropical regions expect to harvest some fruit from SHB plants in the second growing season.

When the interaction between location and chill-hour sampling date was examined (Fig. 1), the second season showed a clear progression in floral budbreak percentage in response to chill-hour accumulation, with

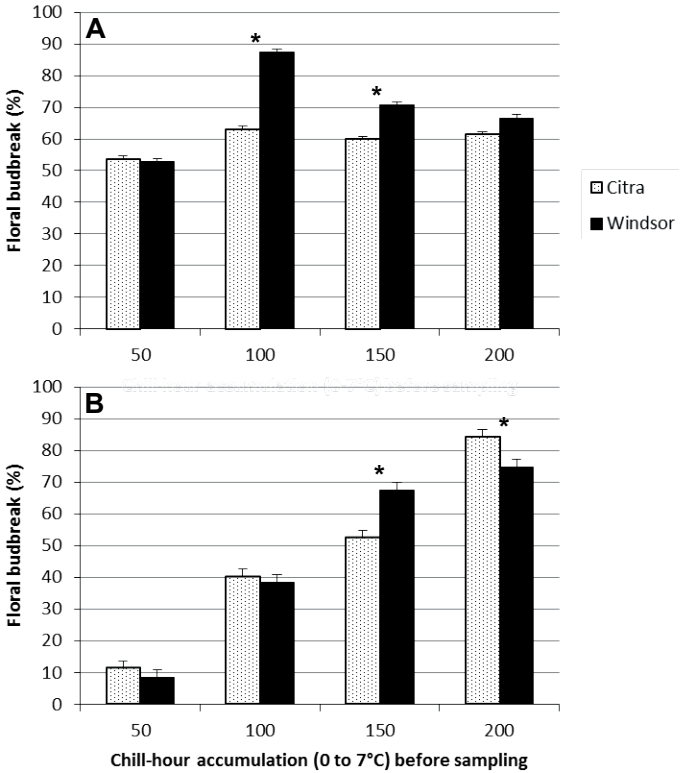

Fig. 1: Percent of floral budbreak on excised southern highbush blueberry shoots during the (A) 2011-2012 (B) and 2012-2013 growing seasons. Assessment was after three weeks of forcing in a greenhouse. Asterisks (*) indicate significant differences ($P \leq 0.05$) between locations within a chill-hour sampling date, according to *P*-values from sliced ANOVA.

higher chill accumulation resulting in greater floral budbreak as expected. Although the location × genotype interaction was significant for both evaluations made at three and five weeks in both seasons, relatively few genotypes were significantly different when the interaction was sliced to determine differences among genotypes within a location. For example, only seven (28%) and five (20%) genotypes had significantly different floral budbreak between the two trial locations after three weeks evaluation in year one and two, respectively (data not shown). Interestingly, there was no overlap among genotypes between years. Again, this may be due to the immature plants evaluated in the first year. However, since the primary cultural difference related to floral budbreak between the two trial sites was HC application, it is possible these genotypes represent those that are particularly suited for use in this management system. The majority of these genotypes had higher floral budbreak percentages at the Windsor location where HC was applied after the 50 chill-hour accumulation sampling date (Table 3).

The genotypes that had higher floral budbreak on shoots collected at Citra than Windsor may be sensitive to the rate and/or timing of HC application. The cultivar 'Primadonna' that is included in this group is known to be susceptible to flower bud injury following HC treatment (Williamson et al., 2014).

From the data presented here, it remains

Table 3: Percentage of floral budbreak on excised southern highbush blueberry shoots after three weeks of greenhouse evaluation. Shoots were collected from two field locations during the 2011-2012 and 2012-2013 growing season after receiving 50, 100, 150, and 200 chill-hours (0 to 7°C) and forced in a greenhouse. Only genotypes that had a significant difference in floral budbreak between locations are presented.

	Location[z]		
	Windsor	Citra	
Genotype	Floral budbreak (%)	Floral budbreak (%)	*Sig.*[y]
---------- 2011-2012 Season ----------			
FL06-77	24.5	1.4	**
FL06-96	66.9	37.9	***
FL06-244	65.1	42.6	**
FL06-435	70.8	50.4	*
FL06-457	66.5	32.6	****
FL06-562	72.1	42.7	***
Emerald	76.4	49.1	**
--------- 2012-2013 Season ----------			
FL06-245	70.5	23.7	****
FL06-542	43.1	24.6	*
FL06-518	45.4	63.9	*
FL06-561	35.7	54.3	*
Primadonna	28.3	52.3	**

[z] Hydrogen cyanamide at 1.25% was applied to plants at Windsor on 19 Dec. 2011 and 21 Dec. 2012, whereas those at Citra were untreated.

[y] NS,*,**,***,**** Non-significant or significant at $P \leq 0.05$, 0.01, 0.001, or 0.0001 between genotype within a location, according to *P*-values from sliced ANOVA.

challenging to assign an absolute chilling requirement for each genotype. In the 2011-2012 season, the chill hour sampling date with the highest percentage of genotypes reaching ≥ 70% floral budbreak was the 100 chill-hour date. However, the percentage of genotypes reaching 70% floral budbreak was reduced in the later sampling dates following more chill accumulation. Data from the second season (2012-2013) when plants were older may be a more accurate estimate of the chilling requirements because percent floral budbreak for each genotype continued to increase with additional chilling after ≥70% budbreak had occurred (Table 2). Thus, no genotype assessed in this study had a chilling requirement of 50 hours, but three (12%), six (24%), and nine (36%) were confirmed to have chilling requirements of 100, 150, and 200 h, respectively.

These very low-chill genotypes will be important to further develop blueberry production regions in central and south-central Florida, and in similar climates worldwide. Additionally, genotypes with such low chilling requirements may be better suited to protected culture where an "evergreen" cultural management system is used to prevent southern highbush blueberry genotypes from going dormant. Although the report here only considers floral budbreak, it will also be critical to evaluate the chilling requirement of vegetative buds on these genotypes.

Acknowledgements

The authors thank Island Grove Agricultural Products, Mixon Family Farms, and Straughn Farms for plant material used in this study. This project was funded in part by the Florida Department of Agriculture and Consumer Services Specialty Crop Block Grant #018006, Expanding Blueberry Production in Florida by Selecting Germplasm Adapted to Low-Chill Environments.

Literature Cited

Chandler, W.H., M.H. Kimball, G.L. Philp, W.P. Tufts, and G.P. Weldon. 1937. Chilling requirements for opening of buds on deciduous orchard trees and some other plants in California. California Agric. Expt. Sta. Bul. 611.

Darnell, R.L. and F.S. Davies. 1990. Chilling accumulation, budbreak, and fruit set of young rabbiteye blueberry plants. HortScience 25:635-638.

Krewer, G. and D.S. NeSmith. 2006. Blueberry cultivars for Georgia. Univ. Georgia, Tifton. 4 Dec. 2014. <http://www.smallfruits.org/Blueberries/production/06bbcvproc_Nov0206.pdf>.

Liu, G., J. Williamson, G. England, and A. Whidden. 2012. How to calculate fertigation injection rates for commercial blueberry production. 12 Oct. 2014. <https://edis.ifas.ufl.edu/hs1197>.

Lyrene, P.M. 1987. Early-flowering, early-ripening

blueberry germplasm for central Florida. Proc. Fla. State Hort. Soc. 100:296-300.
Lyrene, P. 2008. Breeding southern highbush blueberries. Plant Breeding Rev. 30:353-414.
Lyrene, P.M. and W.B. Sherman. 1977. Breeding blueberries for Florida: accomplishments and goals. Proc. Fla. State Hort. Soc. 90:215-217.
Norvell, D.J. and J.N. Moore. 1982. An evaluation of chilling models for estimating rest requirements of highbush blueberry (*Vaccinium corymbosum* L.) J. Amer. Soc. Hort. Sci. 107:54-56.
Obreza, T.A., J.G. Williamson, R.L. Darnell, and P.M. Lyrene. 2000. Blueberry cultivar evaluation in southwest Florida. Proc. Fla. State Hort. Soc. 113:26-28.
Spiers, J.M. 1978. The effect of stage of bud development on cold injury in rabbiteye blueberry. J. Amer. Hort. Sci. 103:452-455.
Spiers, J.M., D.A. Marshall, and B.J. Smith. 2006. Method to determine chilling requirement in blueberries. Acta Hort. 715:105-109.
Williamson, J.G., G. Krewer, B.E. Maust, and E.P. Miller. 2002. Hydrogen cyanamide accelerates vegetative budbreak and shortens fruit development period of blueberry. HortScience 37:539-542.
Williamson, J.G., B.E. Maust, and D.S. NeSmith. 2001. Timing and concentration of hydrogen cyanamide affect blueberry bud development and flower mortality. HortScience 36:922-924.
Williamson, J.G., J.W. Olmstead, G.K. England, and P.M. Lyrene. 2014. Southern highbush blueberry cultivars from the University of Florida. 4 Dec. 2014. < https://edis.ifas.ufl.edu/hs1245>.

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About the cover: 'Kerman' Pistachio

Native to Asia Minor and Western Asia, pistachios (*Pistacia vera* L.) were introduced to California in 1854, but were not farmed commercially until the 1960s and 70s, after the release of a new, well-adapted variety, 'Kerman', and the mechanization of harvest. Pistachio cultivation really surged after 1979, when an embargo of nuts from Iran, then the world's dominant producer, provided a huge boost to the domestic industry. Demand from export markets continues to grow, and pistachio cultivation has been highly profitable and fast growing in recent years. California raises more than 98% of the domestic crop, and the United States now equals or exceeds Iranian production.

'Kerman', named after a province in eastern Iran where its seed parent originated, virtually monopolizes California pistachio cultivation, along with the distribution of individual male trees for pollination. 'Kerman' is dominant because it blooms late and bears heavy crops of large, attractive nuts, which split well and are easy to harvest. It's not perfect, however, because its trees are strongly alternate bearing, and their nuts' flavor is mild compared to the best European and Asian varieties. The danger of the 'Kerman' monoculture is that if new pests or diseases threaten pistachio orchards, the lack of genetic diversity will make it more difficult for the industry to find resistant varieties.

Nuts are harvested using mechanical shakers once they start opening on the tree. Just a few are sold as green nuts, which are highly perishable; the rest must be processed within a day, to avoid undesirable staining of the shells and kernels. Preparation of the nuts occurs in a plant that rubs the moist, fibrous hulls off the nuts and dries them down to about 8% moisture. These are "raw" pistachios, which account for a minor portion of sales. Roasting and flavoring then occurs throughout the year in batches. The kernels are often eaten whole, either fresh or roasted and salted. They are also used in a wide variety of products ranging from ice cream to various confectionary items, and other prepared foods.

Photograph taken at the Santa Barbara Pistachio Company in Cuyama (1 October 2010) © by David Karp.