VacCAP

Improving Fruit Quality

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Issue 3 | August 2021

VacCAP Objective

The Vaccinium Coordinated Agricultural Project (VacCAP) is a nationwide project aimed at developing new genetic tools to enhance breeding for improved fruit quality of cranberries and blueberries.

VacCAP is a nationwide coordinated transdisciplinary project focused on addressing major bottlenecks limiting the growth of the U.S. *Vaccinium* industry by developing and implementing marker assisted selection (MAS) capacity in breeding programs.

This will enable breeders to select and pyramid fruit characteristics that positively contribute to fruit quality and market value.

Long term, the scientific resources developed will increase production of fruit with improved characteristics that meet ever-changing industry, market, and consumer preferences.

Fighting Fruit Rot: Dr. Nicholi Vorsa's Breeding Program Focuses on Developing Resistant Cranberry Cultivars

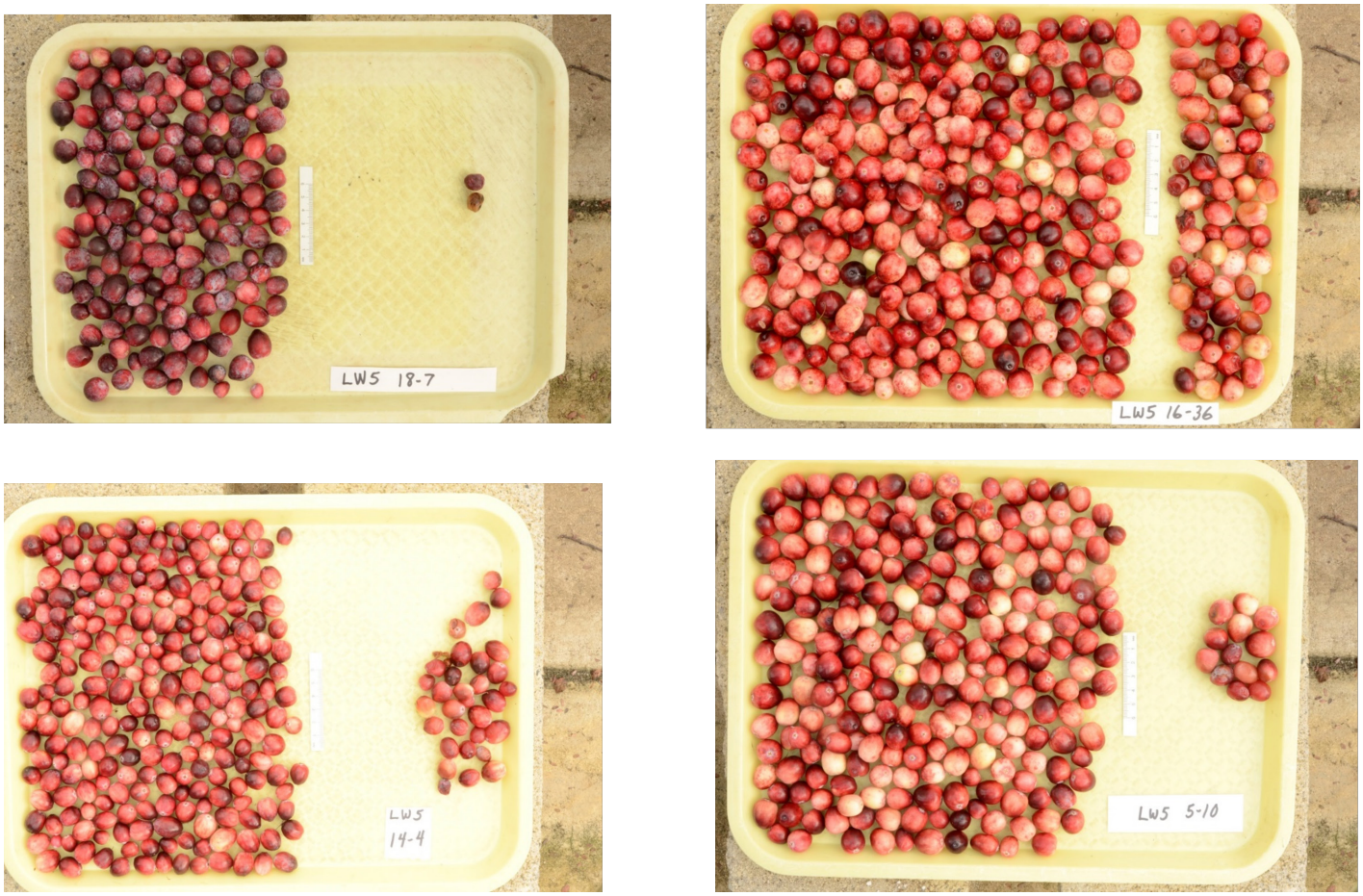
New Jersey is well known for many things: Bruce Springsteen, boardwalks, diners. But it also known as a hot spot for cranberry fruit rot.

'Budd's Blues' (left), one of the four fruit rot resistant selections, compared to a susceptible cultivar (right). 'Budd's Blues' (US88-30, US88-126, US93-34, US94-176) from Pemberton, New Jersey are highly resistant to fruit rot, but have poor yield. Photo contributed by Nicholi Vorsa.

"Fruit rot is actually a series of diseases—Dr. Peter Oudemans calls it a disease complex—and there's five major organisms that cause fruit rot," Dr. Nicholi Vorsa, Co-PD and Professor at Rutgers University, said.

In New Jersey, it's a very serious problem because the climate is much more conducive to fruit rot.

"I've always said that New Jersey offers the best place for breeding for fruit rot resistance. However, for the growers, it's a problem," Vorsa said. "Every year, if we don't use fungicides, the New Jersey growers will experience basically 100%



Fruit rot resistant selections in the breeding program, harvested from 1ft² samples: sound fruit on left side of tray, and rotten fruit on right side of tray (2017, two fungicide application during bloom). Photos contributed by Nicholi Vorsa.

fruit rot. So, the crop, if not treated with fungicides, suffers great losses due to fruit rot."

Vorsa has spent the past 40 years breeding for cranberry cultivars more resistant to the disease complex. According to Vorsa, growers will try to reduce heat stress by irrigating during the day to cool the plants when it gets above 93 degrees Fahrenheit. But that adds moisture to the canopy, increasing the incidence of fruit rot.

"A lot of the fruit rot issues are exacerbated by the heat stress that we experience in New Jersey pretty much every year", Vorsa said.

According to Vorsa, it is critical for growers to start protecting their plants with fungicides early in the growing season.

"Research studies show that if plants are not sprayed with fungicides at about 10% into bloom that—regardless of whether you use spray afterwards or not—you experience high fruit rot," Vorsa said. "So, the notion is that fruit rot infection occurs during bloom."

Finding cultivars with natural resistance to fruit rot is an important step in battling the disease complex. Vorsa and his team are evaluating fruit rot in breeding plots. They're also using a process called genotyping-by-sequencing (GBS) to find quantitative trait loci (QTLs) associated with the fruit rot resistance. Vorsa and his team have identified four cultivars with sources of resistance: 'Budd's Blues', 'US89-3', 'Holliston', and 'Cumberland'. However the crop yields of these varieties are not high.

"We hope to find different QTLs in these populations and validate them in subsequent breeding and selection series where we can hopefully use molecular technology to enhance and pyramid these genes into cultivars highly resistant to fruit rot with desirable crop yield."

Vorsa's team came across these genotypes in their cranberry germplasm collection in 2001 and 2002 when working to eliminate the need for fungicide sprays. Out of more than 600 plants, they found these four cultivars that seem to have some level of resistance. However, these were not the most productive of genotypes, and the team hopes to develop fruit rot resistant cultivars with commercially acceptable yields through breeding.

"Back 30 years ago, I started crossing resistant cultivars such as 'Budd's Blues' with our most productive genotypes, such as 'Stevens', 'Pilgrim', and 'Ben Lear' that were generated from the first breeding cycle back in the 1920s for false blossom resistance," Vorsa said. "Unfortunately, it seemed that the yields were very, very poor, so I discontinued that approach. After 2002, cultivars such as 'Crimson Queen', 'Demoranville', and 'Mullica Queen' came on board. So, we attempted again to cross the resistance into the higher yielding genotypes. It seemed that these cultivars would perform better overall for yield. So, I got encouraged from that standpoint."

Something the team has discovered in crossing various genotypes has been the significance of the selection #35 parent.

Vorsa notes that three cultivars in particular—'Mullica Queen', 'Vasanna', and 'Haines'—have selection #35 as a parent, which seems to increase yield in the progeny.

"Our successful cultivars right now have the selection #35 in the background," Vorsa said. "Hopefully, we can amend the poor productivity of the resistant genotypes with the genetics of the better adaptive cultivars for yield. We have evidence that we should be able to select plants that have high yield and high fruit rot resistance."

According to Vorsa, getting to this point in his research has taken quite a while—he started in 1985—and he feels fortunate to have found selection #35 through fingerprinting done on the early end. While these genotypes are promising, Vorsa does note that they don't have anything that's completely immune to fruit rot and thinks that a cultivar with yields like 'Mullica Queen' would be a long way off—if ever.

"I don't think we'll give up fungicides completely, but we can reduce the number of sprays and applications from five or six to one or two," Vorsa said.

Understanding fruit rot and its connections to heat stress is even more important when factoring in rising yearly temperatures and how climate change could impact places where fruit rot isn't currently as much of an issue.

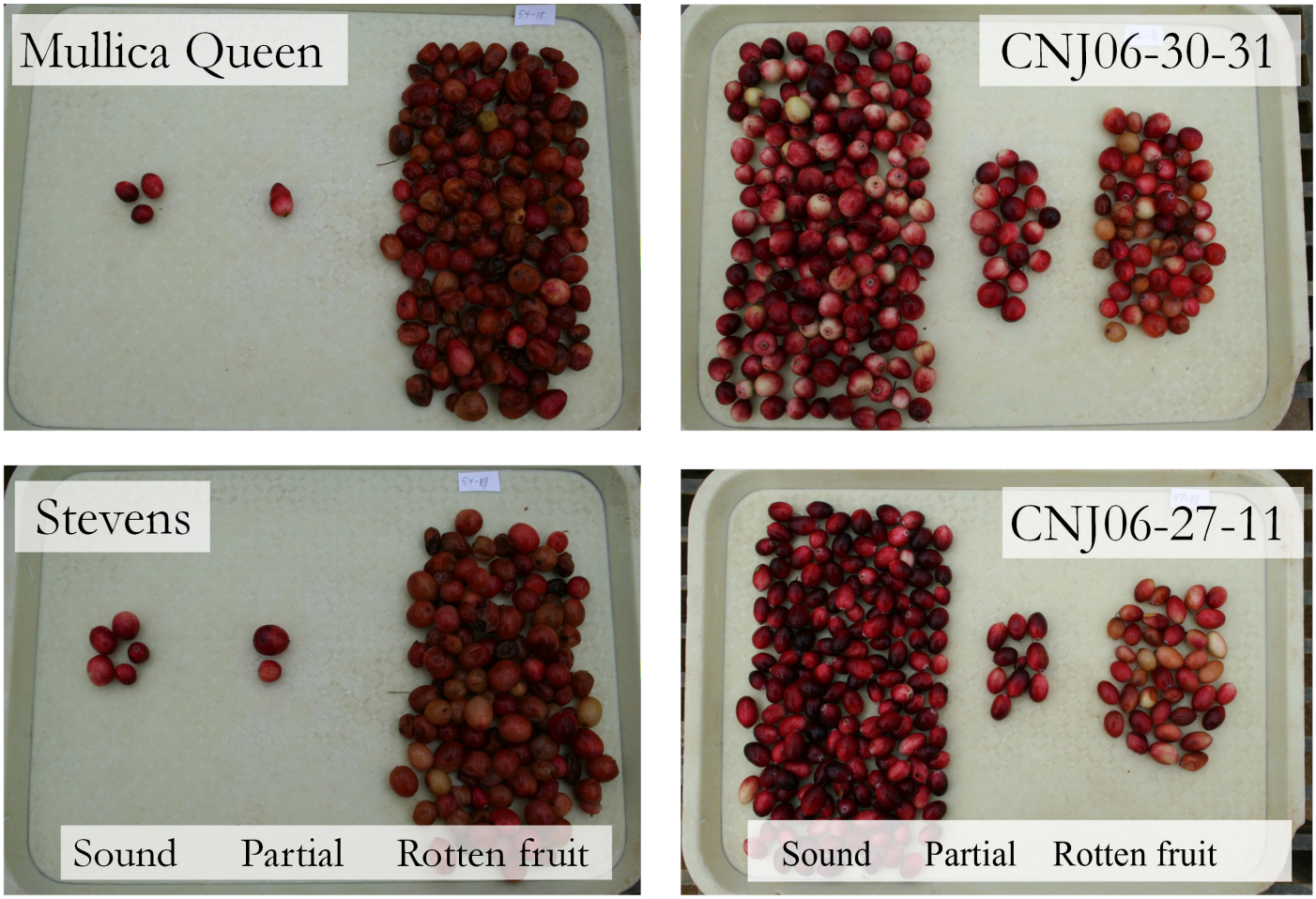
"If you have a sustained breeding program, you're basically breeding for the climate that exists today," Vorsa said. "I think the cultivars we selected here in New Jersey are doing fairly well. The last two decades, the temperatures have been higher. So, we're essentially selecting for adaptation for the current conditions."

As the climate gets warmer, Vorsa anticipates cultivars from New Jersey will even do better in Wisconsin.

"These genotypes require heat for fruit size," Vorsa said. "And New Jersey typically has the largest fruit of any growing area, followed by Wisconsin and then the West Coast. The temperatures that they are exposed to basically have an impact on yield, and if you have a berry size that increases by 10%, that's basically increasing yield by 10%."

Vorsa is hopeful their next generation breeding cycle and selection plants have cultivars that are more adapted to the warmer climate, while also having the yield and fruit rot resistance they're looking for. How long will growers have to wait for these new cultivars? Vorsa says it depends.

"We have a population that was replicated four times—it was a cross done in 2014. We have over 220 individuals, and I'm working with Juan Zalapa (USDA-ARS) and Massimo Iorizzo (University of North Carolina) on this to use as a mapping population," Vorsa said. "We are finding some progeny with, higher level of resistance and improved yields ."



Resistant selections have yields comparable to 'Mullica Queen' and 'Stevens', but much less rotten fruit. One bloom fungicide application was applied to these samples, whereas five to six are typically applied in in New Jersey. Photos show fruit harvested from 1 ft² samples from two susceptible cultivars and two resistant selections. Photos contributed by Nicholi Vorsa.

Sensory Tasting Survey Helps Connect Fruit Quality Traits and Consumer Preferences in Blueberry

Sensory testing surveys offer important consumer feedback and useful insights to researchers. Dr. Charles Sims, Co-PI and Professor at the University of Florida (UF), recently concluded a blueberry sensory study to get a better understanding of consumers' preferences when it comes to buying fresh blueberries.

Dr. Patricio Muñoz, Co-PD and blueberry breeder at UF, selected 20 blueberries cultivars from Florida that represented a wide variation in fruit quality. Sims then selected 90 participants to taste five of these cultivars at a time over four controlled tasting sessions.

"We do a lot of sensory testing with fresh fruits and vegetables, and I've worked with a lot of plant breeders in different crops," Sims said. "What we really want to know in this project is how much did the participants, who define themselves as blueberries consumers, like the different blueberry cultivars."

Questions in the survey focused on how much panelists liked various characteristics of the samples they tried, such as texture, flavor, and how sweet or sour the berries are.

"We were asking specific sensory questions. And the bottom line is, how much do you like it overall? And is that driven mainly by appearance or texture or some flavor characteristic?" Sims said.

Dr. Karina Gallardo, Co-PD and Professor at Washington State University, and Dr. Elizabeth Canales, Co-PI and Assistant Professor at Mississippi State University, also contributed some questions about consumption and preferences in connection with consumer willingness to pay for blueberries with superior fruit quality.

"[Gallardo and Canales] measure willingness to pay—an important criteria to determine how much people really liked a blueberry," Sims said. "It's just a different way to ask people the same question, in my opinion, how much do you like it overall? And then how much are you willing to pay for it? That's a little different approach to getting at the same thing, and they should correlate."

Canales and Gallardo are attempting to connect panelists' willingness to pay to their ratings of each sample in terms of taste, texture, and just general perceptions of fresh blueberry fruit quality. "Hopefully, we'll also be able to relate the willingness to pay to the actual physical measures of sweetness and acidity and texture" Canales said.

The team made sure to select a diverse group of participants in the survey, ranging from people that consume blueberries twice a year to some that consume blueberries more than once a month. "We tried to select participants that would be considered an average shopper of blueberries—not an expert blueberry tester by any means," Sims said. "And these people have not been trained in any way on what to look for in blueberries. Some people really value the appearance, some value the texture, and some people really want the good flavor, the sweetness, so each consumer may be looking for a different thing. But that's all part of the big picture."

20 BLUEBERRY CULTIVARS were sampled in the study

They believe the testing showcased a good separation of results. Sims said that when Muñoz reviewed the results, the cultivars that scored highest were the ones that he would expect, while the ones scored the lowest were also predictably the poorest.

Sims also noted that when breeders select what they think are the best blueberries, it's usually correct, but that it is good to have that substantiated by larger number of panelists who are average consumers.

"And so that's where we come in. Instead of just depending on the breeder—who is normally right—we want to ask a broader pool of people," Sims said. "This work really helped validate what the breeder has thought about all these years. I think for the stakeholders, this really provides a vote of confidence that the cultivars being selected are actually the ones that people really like."

The next step is to replicate the survey with 20 cultivars from Oregon. The team notes allowing participants to taste the berries and then respond to questions can give more realistic and reliable answers since they're using experience to inform their preferences.

But there are obstacles to tasting surveys. This survey will have a smaller sample size with testing locations only in Florida and Oregon. There are other factors that could weigh on a tasting survey, like participant fatigue, limited product, and where the product is coming from.

"The caveat of this study is that it is not generalizable, because of the logistics it's impossible to have a participant pool of more than 100 or 200," Gallardo said. "Because we want the blueberries to come as fresh as possible, since they don't have

Participants sample different blueberry cultivars and record their impressions during a sensory study at the University of Florida. Photos contributed by Charles Sims.

much time off shelf life."

Once all the surveying is complete, the team will compile the information to see what qualities consumers preferred in their blueberries and what correlates best with characteristics such as volatiles, sugars, and texture.

These results will help inform other initiatives within VacCAP as they work to better understand what makes a better berry both genetically and for consumers.

"The only reason to grow blueberries is to sell them to consumers. And the bottom line is if a consumer doesn't like it, no matter what breeders do, nobody's going to buy the blueberry," Sims said.

Sims describes his team's initiative as where "the agriculture meets the consumer" as they learn what consumers want out of their berries. "If we know what the consumer likes, and we can start backing that up to the plant breeders, then they can use their genetic tools to really improve the cultivars more than just doing it haphazardly," Sims said. "We feel like this is a more strategic approach. You know, if they have the best information—like these are the volatiles that they really need to be concerned about, this is the range of sugar levels they need to have, this is the range of texture—it just helps the breeders develop better blueberry cultivars."

VacCAP Webinar Series Recordings Are Now Available on YouTube

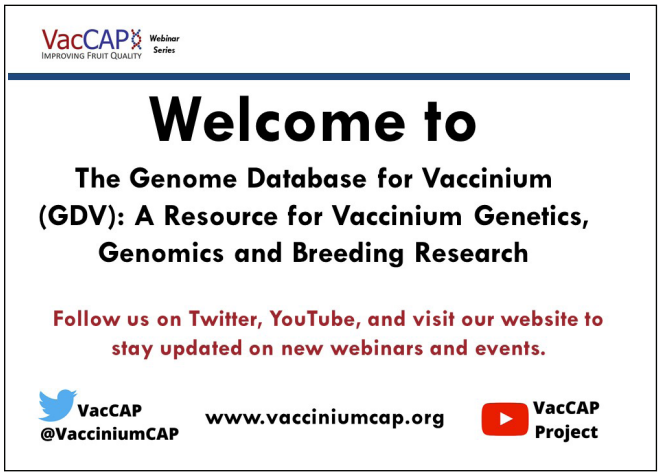
On August 19, we presented a webinar titled "The Genome Database for *Vaccinium* (GDV): A Resource for *Vaccinium* Genetics, Genomics and Breeding Research".

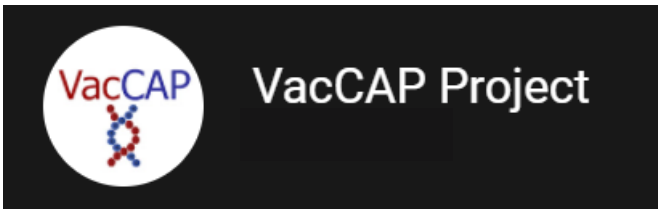
The recording can be found on our VacCAP Project YouTube channel.

The Genome Database for *Vaccinium* (GDV, www.vaccinium.org) is a community resource that integrates publicly available genetics, genomics, and breeding data for *Vaccinium* crops in a single online resource.

In this introductory online seminar, we provide an overview of GDV functionality and highlight how the data and tools can be used to enable basic, translational, and applied research in blueberry and cranberry.

This is the first in a series of webinars aimed at providing in-depth training on the use of GDV for the *Vaccinium* research community. Future webinars will provide more focused training on key aspects of the database.





Student Spotlight: Alan Yocca

In our Student Spotlight Series, we want to introduce you to the students who help make VacCAP possible through their passion and hard work. In our inaugural segment, get to know ***Alan Yocca****, a PhD Candidate in the Edger Lab at Michigan State University.*

What is the project you're working on for VacCAP about?

With Dr. Edger, I am working to create a *Vaccinium* pangenome. A pangenome is a representation of all genes present within a group of organisms. There are many genes that are only present in a single genotype. We hope to identify those genes and characterize them. Previous pangenome studies taught us these "dispensable" genes are highly enriched in functions critical to crop improvement such as response to abiotic stress. We hope characterization of these genes will allow researchers to better understand *Vaccinium* diversity and specific genes that contribute to important traits.

What is something you like or find most interesting about your work?

Developing resources for *Vaccinium*, a high value crop, that have never been created before excites me. I am generating a resource I hope will allow other scientists to understand the function of the *Vaccinium* genome and improve blueberry and cranberry quality in the future.

What do you hope to do in the future after your work here?

I hope to continue working with plant genomes to understand their evolution and function.

Breeder Spotlight: Dr. Paul Sandefur

In our Breeder Spotlight Series, we'll interview blueberry and cranberry breeders to learn more about their roles, challenges in their breeding programs, and have them highlight some of their favorite new cultivars. For our first spotlight, we spoke to ***Dr. Paul Sandefur****, Blueberry Breeding Manager for Fall Creek Farm and Nursery.*

Please describe your role in the blueberry industry.

Blueberry Breeding Manager for Fall Creek Farm and Nursery - Coordination of a global team dedicated to development of blueberry cultivars for all chill levels and production systems.

My role is to support our nursery achieve its goal of providing growers with the plants they want to grow and thereby produce the berries consumers want to eat.

Cultivar Highlight - Please tell us about some top cultivars you're excited about and why you chose them.

Last spring we released three new high chill varieties that are just beginning to be planted at commercial volumes:

- ArabellaBlue™ 'FC14-062'– released for its early harvest window, large aromatic crisp fruit, and vigorous precocious production.
- LoretoBlue™ 'FC11-118'– released for its reliable production of ultra firm fruit in the mid-late harvest season that can maintain exceptional quality in long term cold storage.
- LunaBlue™ 'FC12-205'– released for its uniform late season fruit that in our trials has shown less fruit shrivel and sunscald than currently available cultivars.

What are some challenges in the breeding program?

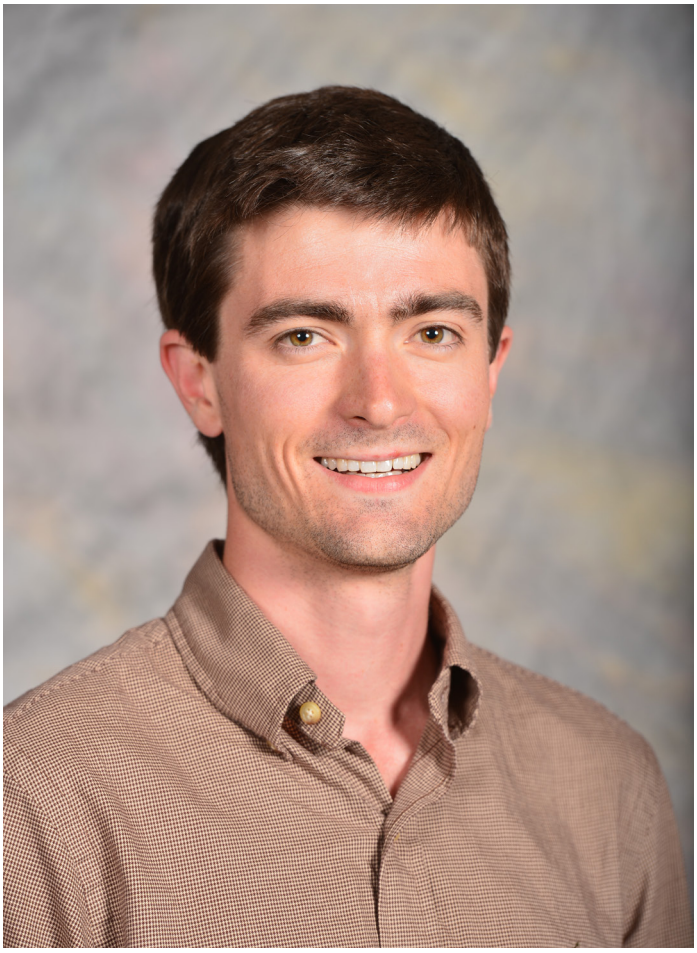
One of the biggest challenges we have in the program is effectively trialing advanced selections in enough diverse locations and production systems to confidently make release decisions and provide growers with accurate guidance on how best to manage new cultivars.

Being able to accurately predict performance and thereby reduce the resources that must be dedicated to trialing would be a major win.

Where do you see the future of* Vaccinium *breeding going in the next 20 years?

Over the next 20 years I see a continued focus on the classic traits such as yield and fruit quality.

However, I believe future varieties must be suited for mechanical harvest and have lower input requirements.



Mastery of traditional breeding techniques will still be essential to develop superior cultivars, but deployment of a variety of molecular tools will be required to succeed in the increasingly competitive breeding arena.

New Imaging Methods Help Researchers Find Blueberry Cultivars Resistant To Bruising

Bruising is a persistent problem plaguing the blueberry industry. At this time, fruit damaged by mechanical harvesting should not be sold to the fresh market. This hinders export opportunities and forces the industry to rely on expensive labor for handpicking that can be difficult to access.

Dr. Changying "Charlie" Li, Co-PI and Professor at the University of Georgia, and his team are endeavoring to help solve this problem. They are developing methods to evaluate internal bruising of blueberries using imaging methods in an attempt to find genotypes and cultivars more resistant to bruising.

"We try to use imaging methods to quantitatively measure internal bruising. I think internal bruising for blueberries is a challenging problem. Blueberries are dark in color. And it's very difficult to visually assess bruised tissues through this dark-blue skin," Li said.

This internal bruising occurs because of the mechanical impacts sustained when being harvested by standard machine harvesters. According to Li, enzymatic reactions in the tissue cause the fruit to become discolored, but a human cannot see that damage readily through the skin of the blueberry.

"My group has developed one imaging method through a prior project," Li said. "We used hyperspectral imaging, either through reflectance or transmittance, to detect internal bruising, and we achieved a certain success."

Hyperspectral imaging (HIS) is a technique that utilizes a wide spectrum of light instead of the usual red, green, and blue in image pixels, allowing for an examination of an object in physical or chemical changes that cannot be seen by RGB images.

"The reason that this method works is, through mechanical impact, certain blueberry fruit cells will rupture, and free water will be available. And under free water we have different absorption [of the light] than intact cells," Li said. "And I think we will improve the visibility through reflectance and transmittance hyperspectral imaging, so we can spot and quantify the internal bruising."

In addition to this method Li and his team developed earlier, they are also trying to explore other new methods.

"The first one is called a 3D hyperspectral," Li said. "Basically, we shed structured light onto the fruit surface. Structured light means it has certain patterns, and then we use another camera to detect this pattern. And then from this we can derive the shape of the fruit. So basically, we can get a 3D shape of the fruit through a single camera whereas typically at least two cameras are needed to perceive the 3D shape of an object."

The hyperspectral data they collect will have three-dimensional spatial information that helps the team to correct for optical effects caused by the shape of the spherical fruit surface.

Another technique Li and his team are exploring is to quantify the discolored bruised area of a sliced fruit using artificial intelligence (AI). Currently, evaluators take RGB images and rank this bruising by observing the discolored area through a slice—but it is very subjective. Others use Photoshop or other imaging software where they can mark up the discolored area and then calculate the bruise ratio over the entire surface.

"But this is a very laborious and time-consuming process," Li said. "My lab is trying to use machine learning—a subset of artificial intelligence to analyze images very quickly and accurately to quantify internal bruising in a few seconds or a fraction of seconds, so we can get all these 50 samples in a batch to get analyzed."

Most blueberries are handpicked for the fresh market, but this is very expensive because of labor prices and limited availability. But standard mechanical harvesters that promote harvest efficiency create impact damage to fruit and fruit develops internal bruising. Li cites that as much as 78% of fruit harvested by mechanical harvesters have severe bruise damage, affecting growers' bottom line.

"This is an economic issue; this is also food quality issue. Consumers will not get very high-quality blueberries if they experience too much excessive internal bruising," Li said. "That's why I think through this current project, we try to develop better cultivars and genotypes that are more resistant to this internal bruising. And my technique is trying to quantify this internal bruising more accurately and more effectively."

Bruising is not just a static issue, according to Li. Internally bruised fruit has a shorter shelf life, and consumers are less likely to buy low quality food that is soft and visually unappealing. Li also notes that bruised fruit can favor certain fungi and other postharvest diseases, reducing the economic output. Dr. Lisa Wasko DeVetter, Co-PI and Associate Professor at Washington State University, says that bruising in the Pacific Northwest and elsewhere can limit export opportunities for fresh market fruit.

"It can limit our opportunities for export markets where fruit will have to be in a shipping container for a longer period of time," DeVetter said. "We'd like to have high quality fruit with less bruising that can last for those long shipping routes and still maintain good quality for those customers."

Li is currently working with his collaborator, Dr. Penelope Perkins-Veazie, a postharvest physiologist at North Carolina State, who collected fruit samples for them to analyze as images. After quantifying the internal bruising, this data will be provided to geneticists to use in the selection of genotypes less prone to internal bruising.

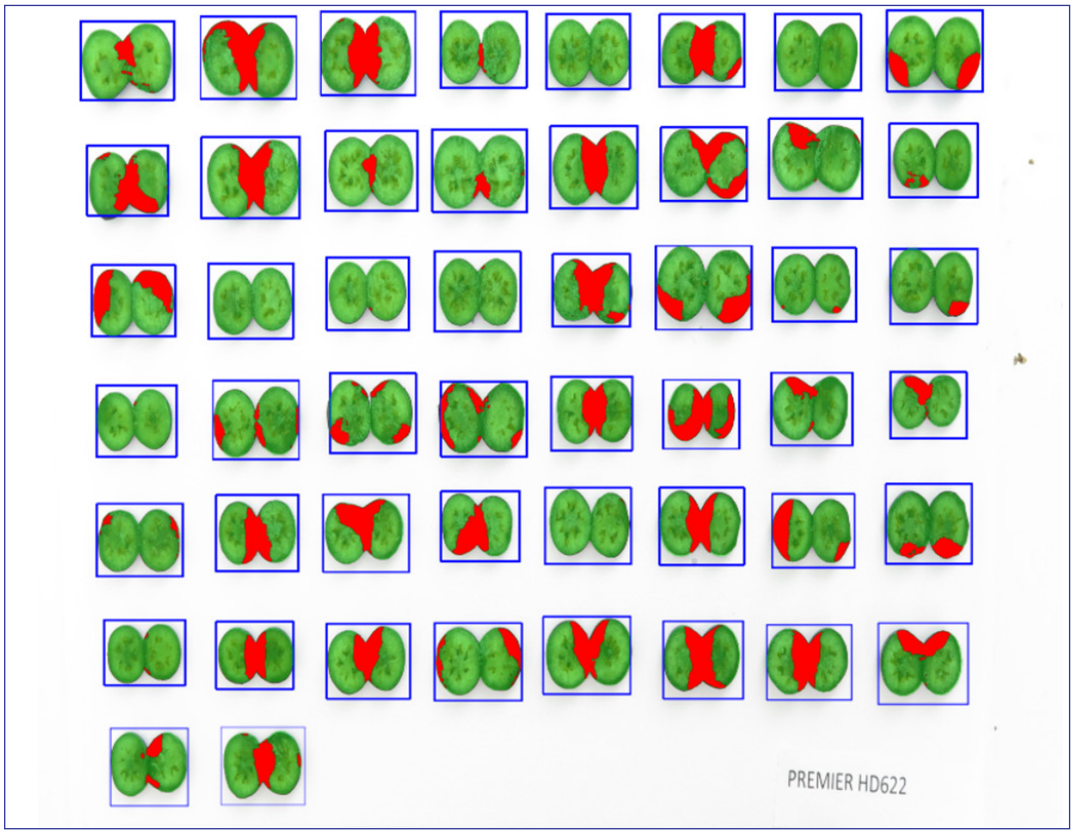
"I also think the data could be useful for machine designers or machine manufacturers to try to improve their machines

to reduce internal bruising. And if we can easily, very quickly identify and quantify those internal bruises, we can improve the machine design," Li said.

Working together as an interdisciplinary team is key to accomplishing this goal.

"We want to advance mechanical harvesting of fresh market blueberries to adjust for labor shortages and high costs of harvesting in the blueberry industry," Li said. "Current mechanical harvesting technologies create excessive internal bruising. So that's why I think through this interdisciplinary research project, we engineers work with horticulturists and other plant scientists together to rapidly quantify internal bruising. We can then select the best genotypes to complement new machine harvest technologies, provide advice for growers, and eventually increase the economic sustainability for the blueberry industry."

The image shows the segmented berry (in green) and bruised area (in red), from which the bruise ratio of each berry can be calculated. Original image provided by Dr. Penelope Perkins-Veazie



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