VacCAP

Improving Fruit Quality

Issue 4 | April 2022

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.

Breaking Down Fruit Quality - Texture and Its Role in Blueberry

We often talk about how fruit quality is key when developing better blueberries. But what does fruit quality actually mean, and how is the Vaccinium *Agricultural Coordinated Project (VacCAP) team working to improve those characteristics?*

A new blueberry release, Creativa, from Fondazione Edmund Mach. Photo contributed by Giongo.

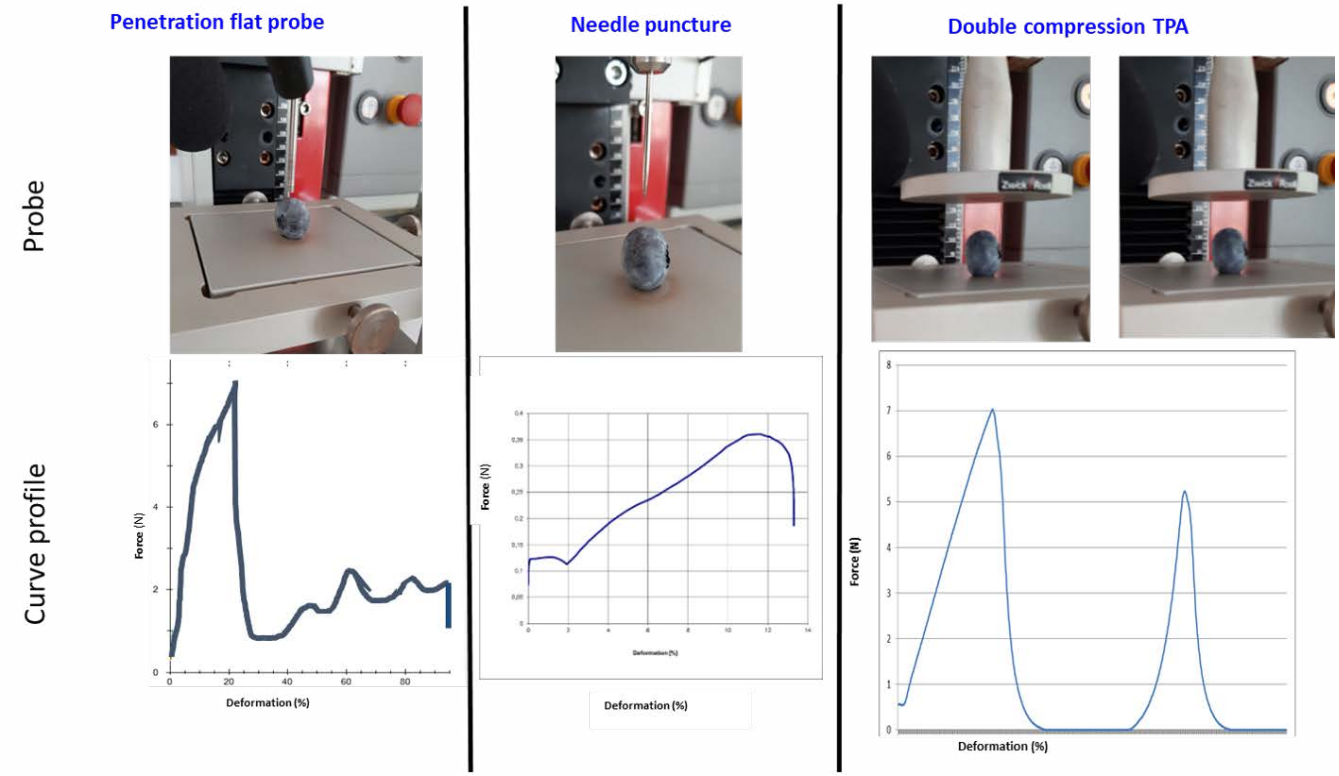
"We know that fruit quality is a complex and often elusive concept—which includes characteristics that are not only sensory related—in which the consumer is satisfied with the product they will eat." Dr. Lara Giongo, Co-PI and head of the Berries Genetics and Breeding Unit at Fondazione Edmund Mach (taly), said.

When discussing fruit quality in blueberry, that mostly refers to texture and flavor. These are the two main traits that Giongo's team is developing methodologies to evaluate, but they are also looking at variability of the gene pool that they have available.

"Texture and flavor are connected," Giongo said. "In blueberries, these two parameters are responsible for fruit quality, but they are quite difficult to assess objectively. We have to view sensory analysis from the perspective of understanding the consumers' expectations of the food."

However, objective assessment and standardizing methods of evaluating components like texture can enable downstream advances in breeding.

Giongo and her team have started to exchange their knowledge in this area—



Different methododologies for texture analysis in blueberry developed by Giongo's team, details about the probe and related texture profile (published in Postharvest Biology and Technology). Photo contributed by Giongo.

with a focus on blueberry firmness—with Dr. Penelope Perkins-Veazie, Co-PD, professor at North Carolina State University, and team members from the USDA. The methodology they have published on how to analyze texture in blueberry, as well as standardization methods that were tailored for blueberry, are at the heart of this exchange.

While recognizing the subjective nature of the term fruit quality, Giongo maintains that best horticultural practices throughout the blueberry production pipeline—from preharvest all the way down through postharvest—will promote quality.

"During preharvest we need to have good control of the propagation system," Giongo said. "We know that in blueberry, having certified, tissue culture plants is absolutely key to further production. Then there are a lot of environmental factors during harvest that contribute to the postharvest quality of the fruit that essentially are [related to] temperature and light."

At Fondazione Edmund Mach, Giongo and her team conduct experiments on these factors in order to advance their plant selections. They look at growing plants in an open field versus soilless production, as well as winter production in the greenhouses or indoors. This allows them to control the light, temperature, and relative humidity fruit are exposed to during their development.

At postharvest, there are other factors that can contribute to quality and higher value blueberries in the market—sorting, sanitizing, metered supply, and the type of storage are all critical. Giongo's different selections are tested in cold storage, in normal atmosphere, but also in modified atmosphere to separate the effects of storage.

"We do a lot of experiments in modified atmosphere," Giongo said. "There are different [storage] recipes that are tailored for the different genotypes. Different packaging technologies—like modified atmosphere packaging (MAP bags)—have an influence on postharvest maintenance of blueberry quality."

While all these factors are important, Giongo emphasizes that the most significant factor for postharvest quality is the genotype. Genotype makes, "the most impact and difference in the blueberry industry."

"We started this work before VacCAP, but the project helped us a lot in analyzing these differences. We see that the dynamics of the fruit after storage changes a lot and changes are not in a singular direction, but can change according to the genotype," Giongo said. "We can have an improved texture for some genotypes, or a detrimental texture for another genotype. So, getting this kind of high throughput phenotyping helps us clean [out unsuitable genotypes] before we get it to the growers."

Giongo notes that the process of determining fruit quality occurs throughout the entire growing process. The team uses texture for essentially all of their screening of seedlings and the new materials that they introduce into evaluations. It is a very quick analysis in terms of operation, and they can process a high number of samples during a single day over the production season. This helps them get objective measurements that then assists in selecting proper material to move forward or sideline.

Texture analysis is also part of storage studies. The team makes an evaluation at harvest of all the fruit and then splits the samples so fruits are placed in either normal atmosphere or modified atmosphere storage depending on where they see the

opportunity for a particular genotype. Then they analyze data and have a look at where the quality is in terms of texture.

"This means that we get segmentation of the quality and we address those that have a short shelf life in terms of our evaluated traits. Those that have a longer shelf life have a different destiny, they could be a bit more suitable for shipping or for longer periods in cold storage," Giongo said. "We focus on the best selections in every category and we further phenotype for the other traits, which means flavor or other fruit quality characteristics."

Giongo's team has also developed different methodologies and techniques to help distinguish the genotypes' subcomponents in order to understand why they fit into their respective segments.

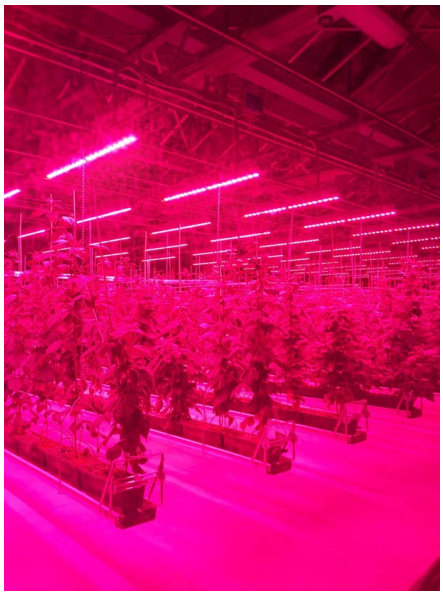
"The [tools] are definitely more complex than the easy-to-use tools that growers may use, but they have helped us in understanding all the textural profiles in this fruit," Giongo said. "All these evaluations that we made, and these methodologies that we have developed and published, we have started to correlate these data with simple and low-cost tools that might be much more accessible to growers looking to narrow down on fruit quality in a certain way."

With all this work dedicated towards developing methods and facilitating breeding efforts to improve fruit quality traits, Giongo and her team also have to look at what outside factors could impact the future of blueberries and how their work will be influenced.

"Climate change is a huge part of the future and where blueberry will go, but differently than other crops. I think that in blueberry, we are in a privileged position because we are developing research at the same time as the industry," Giongo said. "I think that the VacCAP project is an example of this, where the industry is going up very quickly, but also the research [community] is getting knowledge very quickly, having already known what was wrong in other crops. We are already doing positive things in terms of genetics and pre-breeding."

Giongo notes that new technologies across the production pipeline are improving. Storage and energy-saving shipping methods are working towards a point where it can guarantee the consumer receives good product with less waste.

"All these different components of the blueberry production pipeline have a role," Giongo said. "The key for the future is to get all this knowledge connected and spread."



(Top) Blueberry selections expressing variation in fruit quality and texture obtained at Fondazione Edmund Mach by Giongo. (Bottom Left and Middle) Indoor evaluation of berries at Fondazione Edmund Mach. (Bottom Right) New blueberry release, Perla, from Fondazione Edmund Mach. Photos contributed by Giongo.

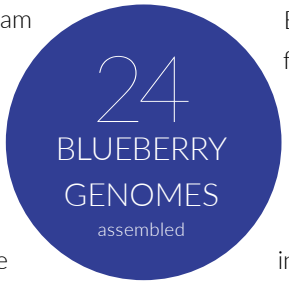
Some Assembly Required: How the VacCAP Team Is Developing the First *Vaccinium* Pangenome

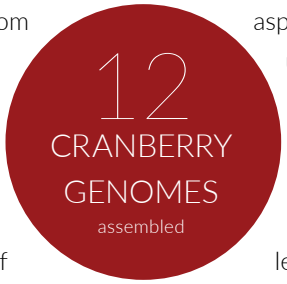
A cornerstone of VacCAP is to establish genomic resources and significantly expand the community's ability to identify and validate DNA markers associated with economically important traits in blueberry and cranberry. That objective is coming closer to fruition thanks to Dr. Patrick Edger, Co-PD and Associate Professor at Michigan State University, and his team. The Edger lab's role is to develop the first pangenome for Vaccinium*—specifically for blueberry and cranberry.*

"What we're doing here is identifying the unique genes that are only present in some cultivars that are associated with superior fruit quality," Edger said. "By making direct comparisons among different genomes, as well as layering on all the metabolite variation that's been profiled, we're going to make direct [fruit quality] associations with these novel genes that consumers ultimately will appreciate."

Edger defines the pangenome as a collection of genomes from multiple individuals of a species or group. His team is trying to quantify gene content differences within the genomes in addition to the "classical mutations".

"When we [used to] talk about genetic differences, we typically thought of it as in the context of very specific types of mutations, like single nucleotide polymorphisms, where there's base pair substitutions," Edger said. "Or insertions or deletions, where DNA sequences are either added or missing from a particular gene. That's the way we traditionally thought of things: that phenotypic variation—or the way that organisms look—was largely caused by these sorts of mutational differences."





But Edger says that in the last five to 10 years, there's been a discovery that other types of mutations—including ones that duplicate entire genes—actually cause considerable amounts of phenotypic variation, and have been shown to be quite prevalent in various animal and plant systems.

"We are trying to quantify the copy number variation of every gene in the genome, within blueberry or cranberry, and between both crops," Edger said. "In addition to that there's something called presence absence variation, where some genes are uniquely present in only a subset of individuals within the species. We are currently quantifying both presence-absence and copy-number variation in both crops."

This is important because all of this collectively contributes to the variation of important traits—certain mutations may cause some genes not to work as well, but in certain instances better. With all this new information, the ultimate goal is to understand the influence of genetic variation and mutations on fruit quality characteristics.

"We are trying to understand how all this genetic variation influences phenotypic variation, particularly on fruit quality traits," Edger said. "We've known for the past 10-20 years that fruit quality traits are influenced by gene duplications. We have already uncovered a lot of different gene content variation between different lines."

Edger's team is currently assembling 24 genomes for blueberry, and 12 genomes for cranberry. They are then making direct comparisons between all of them—including between blueberry and cranberry genomes—to try to quantify what genes are uniquely present in individual crop types, as well as individual genomes.

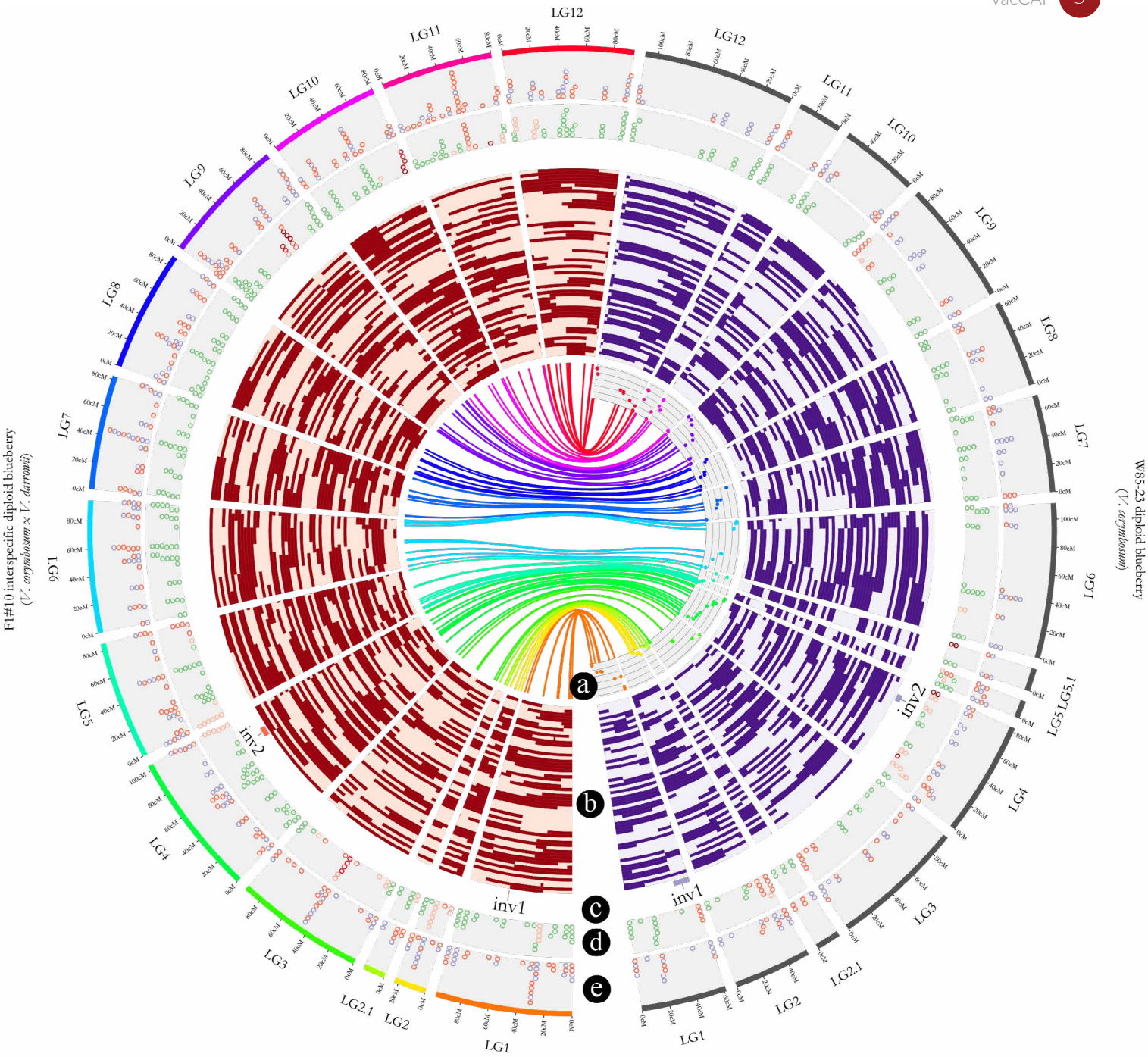
According to Edger, there's a number of challenging aspects to assembling a pangenome and people can underestimate the difficulty of annotating various features in the genome.

"We now have a high-quality annotation for each genome, leveraging a wide variety of different evidence," Edger said. "But we are also leveraging alternative approaches that don't require any annotation for constructing and evaluating the pangenome."

Edger's team is nearly done with assembling the pangenomes, with the final version anticipated within the next 1-2 months.

Developing these genomes is a key part of the VacCAP mission. Edger says there are two main reasons why the project needed to develop a pangenome. The first is, could VacCAP leverage the pangenome by identifying features or genes in the genome that were actually shared in common between blueberries and cranberries that were evenly spaced across all the chromosomes?

"Our goal is to develop a single genotyping platform that would work for not only breeders of southern highbush and northern highbush blueberry, but also cranberry and possibly other *Vaccinium* crops," Edger said. "Let's say there was a huckleberry breeding program that required molecular breeding



***Cranberry synteny**. Comparison of the LGs of the F1#10 and W85-23 parental maps. Linkage groups are numbered according to the homologous LGs in the Schlautman et al. 2015a) cranberry map, and links are drawn between common markers in each of the blueberry parental bin maps. (a) Scatterplots of the position of the common markers in each of the two maps are shown with intervals between lines representing 20 cM Kosambi map distance. (b) Bars show gametic recombination which occurred in both parents for a random subset of 60 progeny. (c) Regions of non-collinearity (Inv), and their relative size in cM, observed between the two parental bin maps. (d) Position of distored markers (cM) in both parental maps colored by the χ2 p-value obtained from the tests for distortion from expected Mendelian segregation ratios. Marker colors range from green for markers not showing distortion (χ2 p > 0.1) to dark red for markers showing highly significant segregation distortion (χ2 p < 0.0001). (e) Position of markers in both parental maps colored by the origin of the marker (red indicates markers previously developed in cranberry and blue indicates markers previously developed in blueberry). Graphic contributed by Dr. Juan Zalapa*

tools. They could leverage these VacCAP tools without having to come up with another CAP that was huckleberry specific. The idea is that those tools could be used by any *Vaccinium* breeder or geneticist and accelerate informed breeding efforts.

The second aspect is to identify unique cultivar specific genes, as well as identifying the underlying genetics that may encode favorable traits. Edger notes that a small subset of those genes would also be on the genotyping array.

"Over the next year, our focus will be to identify genetic variants associated with superior fruit quality," Edger said. "These genetic variants will serve as the foundation of a lot of the downstream analyses including as molecular markers to guide future breeding efforts."

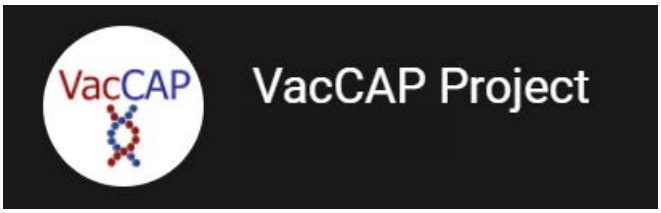
Watch: Exploring Frimness and Texture in Bluberry and Cranberry Webinar

On February 2, we presented a webinar titled "Exploring Firmness and Texture in Blueberry and Cranberry". The recording can be found on our VacCAP Project YouTube channel.

Texture and firmness are important traits in blueberry and cranberry. These traits affect the ability of fruits to withstand the stresses of harvest, handling, and postharvest quality. They also influence consumer preference, as consumers prefer firmer and crispier fruits. Breeding efforts have focused on producing firmer fruits to meet these needs.

Our speakers reviewed the methods for quantifying and assessing texture and firmness in blueberry and cranberry fruits using the most current phenotyping techniques.

Speakers included Dr. Penelope Perkins-Veazie, North Carolina State University, Dr. Lara Giongo, Fondazione Edmund Mach, and Juan Zalapa, USDA-ARS, University of Wisconsin-Madison.



Student Spotlight: Heeduk Oh

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 this segment, get to know ***Heeduk Oh****, a PhD student at North Carolina State University with advisors Dr. Massimo Iorizzo and Dr. Penelope Perkins-Veazie.*

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

My project aims to understand the physiological and molecular mechanisms controlling fruit shelf-life in blueberry. For now, I am focusing on finding the link between fruit characteristics and fruit quality. More specifically, I am trying to answer how fruit characteristics like texture (e.g., firmness) and appearance (e.g., size) of the berry contribute to quality in terms of shelf-life or bruising from mechanical harvest.

We hope that our research will inform breeders about which characteristics to select for in order to develop new cultivars with prolonged storability/shelf-life or improved suitability for mechanical harvest.

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

Working with the most competent researchers in the field and learning from them through collaboration is always exciting and inspiring. I think this is the best part of being involved in such an extensive research project.

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

I plan to interweave my knowledge in postharvest fruit physiology and my skills in molecular biology and bioinformatics to develop a research program that aims to improve fruit quality such as shelf-life by utilizing genetics/genomics and computational methods.

Anything else you would like to add?

I want to thank my advisors, Dr. Penelope Perkins-Veazie and Dr. Massimo Iorizzo, for their mentorship and for guiding me through my research.

Breeder Spotlight: Ed Grygleski

In our Breeder Spotlight Series, we 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. In this spotlight, we spoke to ***Ed Grygleski****, President of Valley Corporation.*

Please describe your role in the cranberry industry.

Valley Corporation is a fourth generation cranberry farm in Tomah, WI. Our farm started commercially growing cranberries in the 1880s and was originally owned by one of the LaCrosse Footwear founders. We farm 305 acres of cranberries and are family owned with Ed A. and Ann Grygleski and Mary and Bill Klouda families. Recently the fourth generation of Nathan and Megan Grygleski have started a farming life here as well.

In 1974, Ed J. Grygleski started breeding native varieties in search of a better fresh fruit berry. Since that time we have evaluated thousands of seedlings and we have expanded over 20 proprietary varieties through many generations of cross breeding. It takes 12-15 years to grow, evaluate, and expand a variety into a commercial size field.

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

Some of our varieties we have recently expanded are:

- Crimson King – Early Season, large fruit and very high yields
- Midnights – Early, high yielding, great re-bud
- Pilgrim King – Midseason, largest fruit, very firm, high yields
- Badgers – Midseason, low canopy, very high yields, oblong shape
- BG – Midseason, high yield
- SuperStar – Midseason, high yield
- Granite Red – Midseason, fresh fruit variety with extreme firmness
- Goldski Crosses – Unique recessive white fruit with a beautiful golden color

We have a family tree map of these varieties and pictures of our farm at our website www.cranberryvine.com

What are some challenges in the breeding program?

Being a commercial grower while breeding new varieties does present different challenges and benefits. All of our new hybrids are planted in fully renovated beds. Maintaining vine purity in the plots is always a challenge while the bed establishes. We have learned a few techniques to maintaining the plots throughout the years including metal walkways to keep vines from crossing over. Disease pressure after roundup sprays can be intense and destroy growing berries if we aren't very careful on the timing and rate. Fertilizer application timing

and rates are hard to optimize in a checker board bed of new varieties. Making selections to expand always has risks but the longer we can evaluate new cultivars the more certain we become of the best selections. Being a grower allows us to evaluate the varieties under commercial growing conditions. Also, Wisconsin has a much lower incidence of virus and phytoplasma as opposed to some other growing regions. The risk of spreading disease is much lower from vines grown away from these problems.

Although not any one variety is perfect, it is our viewpoint that farm diversity of high yielding varieties is important to reduce risks of yield fluctuation, disease pressure, and management styles. Yield is always the top consideration as we evaluate new varieties, but there are other important traits that we look for. Some of those include rebudding of fruiting uprights, firmness, disease resistance, and uniformity of color. Uniformity of vine canopy has recently become one of our more important observations. Some varieties can start out good, but as the beds age, the canopy can open up and become irregular which leads to yield decline.

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

As we continue to breed new varieties, the focus will continue to shift to fruit traits as the yields become maximized. As we watch our trays of vines grow from seed in the spring, we are always excited to see what the next generation of crosses bring.

Get To Know VacCAP Team Member Dr. Claire Luby

Dr. Claire Luby is the newest Co-PD to join the VacCAP team. She is a Plants Research Geneticist at the USDA-ARS Horticultural Crops Research Unit in Corvallis, Oregon, USA, where the lab focuses on blueberry breeding and genetics. We spoke with Luby about joining the blueberry industry, challenges, and the future of her breeding program.

What's it like coming into the blueberry industry and creating a breeding program?

The blueberry industry has been incredibly supportive since starting in this position in summer 2021. I can tell there is a lot of interest in making sure that the breeding program is successful. I've also been fortunate to step into a program that is already quite established. This has made the transition much smoother than if I were starting from scratch. There are so many great plants to work with that are already part of the program, and many projects that are already ongoing, and that has made the transition quite smooth.

What made you excited to get involved in the industry?

I'm excited by the energy surrounding the blueberry industry and the collaborative nature of the research in this area. I really enjoy working with the growers, nurseries, and other researchers and the scope of projects that we get to work on—everything from heat damage mitigation to breeding high quality cultivars.

What are you most excited about when it comes to working on VacCAP project?

I think this project has so much potential to help us support plant breeding efforts in blueberry. I'm impressed by the scope of the project and the potential to connect some of the important fruit quality traits in blueberry with both genetic markers and consumer sensory data.

What do you see the main challenge being? Challenges in general?

I think some of the primary challenges in blueberry breeding are that there are so many important traits to consider, from fruit quality to harvest and post-harvest to disease tolerance/resistance traits. In addition, production is expanding rapidly and there is desire for new cultivars, which puts some pressure on getting new selections out into trials quickly.

Where do you see your breeding program in the next 10-20 years?

We are hoping to release some new cultivars with exceptional fruit quality, firmness, that retain quality in storage, that are disease tolerant and that are met enthusiastically by growers and the industry. Also, I'm interested in research on how

(Top) Dr. Claire Luby, Co-PD and Plants Research Geneticist at USDA-ARS Horticultural Crops Research Unit in Corvallis, Oregon, USA. (Bottom) Luby discussing blueberry breeding and VacCAP with growers in OR, USA. Images contributed by Luby and Dr. Lisa Wasko-DeVetter.

breeding can contribute to more sustainable production systems and mitigate effects of climate change, like that of the heat dome event that we experienced last summer.

What's it like transitioning to work with perennial crops like blueberry? Please highlight a bit of your previous background.

Before accepting this position, I was working primarily on seed propagated crops, and had a lot of experience working

on carrot. So, in some ways, blueberries are quite a different crop and production system. However, the transition has been relatively smooth.

Breeding programs are set up in much the same way, even if the time scales, genetics, and propagation techniques are different. I've really enjoyed diving into learning about the specifics of blueberry genetics, breeding, and production systems.

Is there anything new and exciting coming out of the USDA-ARS breeding program that you'd like to share with the VacCAP community?

We have a lot of very promising selections in the pipeline in our breeding program. We're working on getting them out into larger scale grower trials to determine viability as cultivars. It has also been exciting to have some detailed data on theses selections from the VacCAP project.

Luby presenting to blueberry growers at a field day in Oregon, USA. Photo contributed by Wasko-DeVetter.

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