VacCAP
IMPROVING FRUIT QUALITY

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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.

The CRISPR Question: Would Consumers Accept CRISPR Cranberries with Reduced Added Sugar?

CRISPR is a powerful gene editing tool, but how do consumers view this technology if it were to deliver cranberry products with less added sugar and improved flavor? Would they "buy into" CRISPR cranberries?

These are just some of the questions that Dr. Karina Gallardo, Co-PD and Professor in the School of Economic Sciences at Washington State University, and her team are trying to answer in their latest survey looking at consumers' willingness to pay for certain traits in cranberry products.

In their upcoming paper, "Would Consumers Accept CRISPR Fruit Crops if the Benefit has Health Implications? An Application to Cranberry Products", the team outlines that, despite cranberry products being viewed as healthy, added sugar to cranberry products can deter consumers. Gene editing tools such as the Clustered Regularly Interspaced Palindromic Repeats (CRISPR) offer a way to breed cranberries with lower acidity or increased sweetness from natural sugars, cutting down the need for added sugars, but would consumers be open to buying products resulting from the use of this technology?

For their survey, Gallardo and her team questioned consumers on their willingness to pay for sugar content, CRISPR, and cranberry flavor intensity for two cranberry products under different health-related information treatments.

Respondents were divided into groups with different treatments, or information presented to them before answering the questions. Treatment 1 was the control group with no additional information presented to the respondents. Treatment 2 briefly described the health benefits of cranberries (positive). Treatment 3 focused on the benefit of limiting sugar consumption, specifically noting "added sugars" (negative). Treatment 4 showed respondents the scripts from both 2 and 3, with the team expecting the health benefit information will offset the negative "added sugars" information.

"We also explained what total sugar content is. We explained cranberry flavor intensity is the combination of sensations influenced by taste, aroma, look, and texture. Then we explained what conventional breeding and CRISPR is," Gallardo said.

Alternative possibilities available for each attribute in each survey version

Attributes	Dried Cranberry	Cranberry Juice
Total Sugars (per serving size 1 cup)	· 29 g · 14 g	· 25 g · 12 g
Cranberry Flavor	· Full/Intense · Bland/Weak	· Full/Intense · Bland/Weak
Cranberry Breeding Technology	· Conventional Breeding · Gene Editing	· Conventional Breeding · Gene Editing
Price	· $1.99/6-oz bag · $2.99/6-oz bag · $3.99/6-oz bag	· $2.49/64-fl oz bottle · $2.99/64-fl oz bottle · $3.49/64-fl oz bottle

Table 1. List of attributes and attribute levels for sets of discrete choice experiment scenarios for dried cranberries and cranberry juice.

This was a choice experiment, where respondents were asked to imagined themselves presented with two cranberry product options in a grocery store setting. Gallardo gave the example:

Option A is a bag of sweetened and dried cranberries with intense cranberry flavor, 14 grams of sugar, and genetically edited, selling at a price of $3.99. Option B is a bag of sweetened and dried cranberries with weak cranberry flavor, 20 grams of sugar, and bred through conventional methods at a price of $2.99. Which of these two would you choose?

"We repeat this and we present a random combination of these attribute levels. For example, question one can have [the above example] combination but question two can have 14 grams of sugar with a bland/weak flavor, conventional breeding, and a cost of $2.99. Or the 29 grams of sugar, with a full/intense flavor, gene editing and a $3.99 price," Gallardo said. "We randomly combined these four levels of attributes. That allows us to estimate the value that people have for each of these attributes."

According to responses, the team noticed people are willing to pay less if sugar content information is presented on the product label, if CRISPR has been used, and if cranberry flavor is weak. However, compensated valuation analysis of products with different attribute levels indicated that consumers were willing to pay a premium for cranberry products with reduced added sugar content through CRISPR breeding and full/intense cranberry flavor relative to products with added sugar content, conventionally bred, and weak/bland flavor. Respondents that received information treatments highlighting cranberries' health benefits and recommendations to limit sugar intake were willing to pay less for regular sugar content cranberries even when compared to CRISPR-bred cranberries

Gallardo has a couple possible explanations for this in terms of regular versus reduced sugar content. Some of this might have to do with consumer perception of a healthy products and what they should offer.

"We have other studies that show people are more demanding with products that have a 'halo effect' [of health], like if I expect this product is going to be healthy, why the heck does it have more sugar than I should have? They are stricter with that," Gallardo said. "But if this was ice cream, for example, they wouldn't care that it has regular sugars, because it's an indulgence. With a 'halo effect' it is like a regiment or lifestyle."

The other explanation is the impact of the negative and positive treatments. Gallardo says the team initially anticipated that when presenting both, the positive treatment would offset the negative, resulting in a smaller discount. But that wasn't the case.

"In any of the cases when you present both sets of information, the discount is larger. This is telling us that the positive information about the health benefits of the product is not offsetting the negative information of the added sugar," Gallardo said. "The FDA said if you put on the front of the label the health claims of cranberries, it is going to offset the negative influence of the added sugar additional line in the nutrition facts panel. These [responses show] it doesn't offset. People are willing to discount more, even though they know cranberries have a [health benefit]. But knowing that there is added sugars, people don't care, they're willing to discount more than just cutting out the sugars alone."

When comparing CRISPR to conventional breeding, Gallardo notes that consumers view this gene-editing tool more favorably if it is able to reduce added sugars and enhance flavor. The team calculated the value of a cranberry product made with conventional breeding, that has added sugar content and weak/bland flavor compared to a product that is CRISPR-bred, with reduced added sugar and full/intense flavor.

"By kind of combining all the goods for CRISPR-bred and all the bads with conventional, people actually are willing to pay more for CRISPR," Gallardo said. "Our results showed people are willing to pay more for CRISPR cranberries with reduced added sugar because the reduction of added sugar is viewed positively. This benefit [of reduced added sugar] offsets any suspicion or negativity they have with CRISPR because the aversion to added sugar is larger than for CRISPR."

The team decided to delve further into the responses, with latent class analyses to identify variations in the acceptance/rejection of the different attributes of sweetened and dried cranberries and cranberry juice. They split the control group into three different classes: strong CRISPR rejection (class 1), mild CRISPR rejection (class 2), and the indifferent to CRISPR group (class 3). They looked at which groups were more willing to discount certain traits more than the others. The team wanted to identify what type of consumers were in these classes and how demographics like income or gender might vary among responses.

Respondents were recruited through Qualtrics' consumer research panels. Gallardo's team was given three demographics to control, including age, geographical representation, and income—which they wanted a close as possible to the United States breakdown of income. They also screen for shoppers who make the grocery purchasing decisions for their household.

"[The respondent] needs to make the decisions, even if they don't consume themselves and are buying for somebody else in the house. For example, I want to hear from the mother that buys cranberry juice for her kids but doesn't drink it herself." Gallardo said.

The team also screened for how frequently people consumed cranberries and their knowledge of cranberry products. For the sweetened and dried cranberry questions, respondents needed to have consumed them in the past year. Cranberry juice questions required people to know the difference between cranberry cocktail juice blends and 100% juice.

For the sweetened and dried cranberries, they wanted to hear from a mix of shoppers who eat cranberries occasionally and those who are loyal consumers of sweetened and dried cranberries that consume them frequently.

"[Respondents that consumed cranberry juice] were more connoisseurs than those that consumed sweetened and dried cranberries, because we were asking specific questions about the difference between 100% juice and cocktail, and not everybody knows that." Gallardo said. "Maybe that's why [we observed] the differences in our results, right? [Consumers that drink] juice are more like the loyal consumers because they needed to know about these differences. Whereas [those that consumed] the sweetened and dried cranberries were the occasional consumers. That is a caveat in our study, we cannot compare side by side between juice and dry cranberries because our consumers are different, with juice drinkers more knowledgeable about the cranberry product than consumers of sweetened and dried cranberries."

Gallardo says surveys like this show consumers are receptive of CRISPR methodologies if they are informed of the direct benefit to them and identify the main reason why they consume a product. This is good news for scientists working with CRISPR tools to expedite the creation of cultivars with enhanced characteristics such as improved flavor.

"For cranberries it is the health aspect. If you tell them, 'Look, your cranberries are going to have less added sugars, if we bred them using CRISPR,' I think they will be more acceptive to CRISP than if they didn't know this information." Gallardo said. "In the future, if you're going to have a label or a law that dictates that you need to label CRISPR like genetically modified organisms (GMO), you need to put next to it 'to allow the reduction of sugars' or something like that."

Mitigating Cranberry Fruit Rot Through Breeding for Resistance

Fresh ripe cranberries add a bright festive touch to holiday meals. Cranberries are also used to make sweetened dried cranberries (SDCs), juices, and other products. Unfortunately, various fungal pathogens can attack cranberries in the field or after harvest and cause the fruit to rot. In fact, crop loss to fruit rot is the number one problem cranberry growers face in the Northeast growing region of the U.S. The problem is increasing in other growing regions, presumably due to changing environmental conditions.

One way to reduce the incidence and negative impacts of cranberry fruit rot is to develop cultivars that can resist pathogen attack. This is a challenging task for two main reasons. First, the fungal pathogens that cause fruit rot are numerous and vary from year to year, bed to bed and region to region. Thus, resistance must be broad spectrum. Second, cranberry is a woody perennial that can require 15-20 years to develop a new variety.

Developing broad spectrum disease resistance will involve a variety of plant traits and the expression of many plant genes that control those traits. One trait the team has explored is the abundance of natural waxes that are present on the surface of cranberry. Our recently published research demonstrated some of the benefits of surface wax (also called epicuticular wax) in cranberry, such as reduced water loss and resistance to sunscald (fruit 'sunburn').

Our latest research suggests that the waxes can also act as a physical barrier to pathogen invasion. In addition, the wax layer is hydrophobic, so water runs off and fungal pathogens have difficulty adhering to the fruit and causing infection. Thus, cranberry fruit with high epicuticular wax is one of the many targets we are pursuing to develop more fruit rot resistant cranberries.

We have developed a genetic marker that can inform us as to whether a cranberry seedling might produce fruit with the desired amount of epicuticular wax. This will allow us to speed up the breeding process since we can select plants in the seedling stage rather than wait 3-5 years for them to produce fruit.

The USDA team performing this research includes Dr. James Polashock (Project Leader), Dr. Joseph Kawash (Bioinformaticist), Dr. Lindsay Erndwein (Research Associate), and Kristia Adams (Biological Sciences Technician). Collaboration with Rutgers University Scientists is critical to project success.

Cranberries with low wax (left; note the shiny fruit surface) and high wax (right). Photos by Lindsay Erndwein

Identification of Molecular and Genetic Markers Associated With Resistance to Anthracnose Fruit Rot

Anthracnose fruit rot is among the most destructive and widespread fruit disease of blueberries across the United States, impacting both yield and overall fruit quality. This is echoed by the results from a published VacCAP survey (Gallardo et al., 2018) from nearly 400 stakeholders across the blueberry industry, which revealed that anthracnose fruit rot, as well as other fruit quality traits, is among the top industry concerns. Thus, a goal from a collaborative project between Dr. Patrick Edger, VacCAP Co-PI, and collaborators, including Dr. Tim Miles, small fruit pathologist, and members of their labs at Michigan State University was to develop a more cost effective and environmentally conscious solution to combat anthracnose fruit rot.

"Anthracnose fruit rot, caused by the fungal pathogen *Colletotrichum fioriniae*, significantly impacts strawberry, grape, and blueberry production worldwide. In blueberries, anthracnose is highly prevalent and widespread and is the most important pathogen, affecting fruit at both pre- and post-harvest stages," Sam Thompson, PhD student in the Miles lab, said. "This infection ultimately results in considerable damage to fruit structure and quality, leading to significant losses in highbush blueberry production."

Fortunately, natural variation in resistance against anthracnose fruit rot was identified to be present among cultivars.

"This enabled us to truly investigate this important trait in cultivated blueberry, which not only permits us to gain valuable insight into the underlying genetics of resistance, but also to develop molecular markers that will enable breeding programs to more easily select and release superior cultivars in near future," Dr. Edger said.

"We sought to identify markers that breeders can use to predict resistance against anthracnose. We identified three genomic markers and one metabolite marker significantly associated with anthracnose resistance in blueberry," Mackenzie Jacobs, a PhD candidate in the Edger lab, said.

In summary, three loci on different chromosomes—17, 23, and 28—were found to be significantly associated with resistance to anthracnose in fruit. Metabolite profiling and analysis was also performed, and a metabolite with characteristics of a quercetin rhamnoside (flavonoid) was found to be significantly more abundant in resistant fruits although some variation was observed in both resistance and susceptible individuals. To gain further insight into the underlying resistance mechanism, gene expression analysis was performed on inoculated fruit across several days following infection. These analyses revealed significant associations between differentially expressed genes and some metabolic pathways including flavonol biosynthesis and genes previously associated with pathogen resistance.

Image of a blueberry fruit exhibiting symptoms and signs of anthracnose fruit rot in the field. Photo by Timothy Miles

This work has the potential to make a large impact on the blueberry industry.

"This is an early step in the journey of producing more resistant cultivars to anthracnose fruit rot," Jacobs said. "Giving breeders the ability to screen for resistance via genetic and metabolite markers can shave years off of the process of determining which lines to move forward with."

The original paper is available here: https://doi.org/10.1093/hr/uhad169

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Team Spotlight: Dr. Jenyne Loarca

In our Team Spotlight Series, we want to introduce you to the team members who help make VacCAP possible through their passion and hard work. In this segment, get to know ***Dr. Jenyne Loarca****, a Principal Investigator (AFRI NIFA Postdoctoral Fellowship) at the University of Wisconsin-Madison with advisors Dr. Juan Zalapa & Dr. Amaya Atucha.*

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

I wrote and was awarded an AFRI-NIFA fellowship grant to explore genetic and phenotypic diversity for fruit quality traits in a large cranberry diversity panel. After our diversity panel here in Wisconsin failed to produce fruit, I pivoted towards collaborating with our colleagues at the P.E. Marucci Center for Blueberry and Cranberry Research to evaluate their diversity panel. In addition to characterizing this germplasm for traits that are important for consumers and processors, including sugars, acids, firmness, texture, internal structure, and cell wall integrity, I plan to to perform a genome-wide association study (GWAS) to identify major QTL associated with these traits, with the long-term goal of identifying excellent breeding parents and facilitating marker-assisted breeding efforts. My project also involves travelling into the wild to collect cranberry propagules from remote bogs in the Appalachian Mountains and in Alaska. These wild cranberries are genetically diverse and can be used to study unique traits that evolved to promote their survival in these unique environments.

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

I have really enjoyed working with cranberry stakeholders in industry to learn about their needs. The more we communicate and learn, the more specific and relevant the research questions I can ask, and I can then design experiments that address those needs. For example, I visited the Ocean Spray HQ in March 2023 and learned about some important traits that would improve processing efficiency. Luckily, I saved remnant fruit from my Fall 2022 harvest, and I collaborated with the Atucha lab to design an experiment to assay the diversity panel for these traits and analyze the data as part of the planned pipeline.

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

I think often about the lack of biodiversity in the global food system. As climate change ramps up, crop scientists are asking whether the few crops that we grow will still be growable in the future. People in the Global South are particularly vulnerable and agricultural research plays an important role in reducing poverty and hunger. What solutions could there be if the crops we currently grow become ungrowable due to climate change? Should we study more diverse crops and attempt to improve them through plant breeding? What crops have the most potential for change and adaptability? Do we need to change the way we grow, store, and distribute our foods? Which issues can be solved with plant breeding and which require systemic change within a social system? These are the kinds of questions asked by scientists concerned about food security and sustainability.

Anything else you would like to add?

Here's a photo of me in October 2022 visiting Cranberry Creek – the largest producer and processor of cranberries in the U.S.!

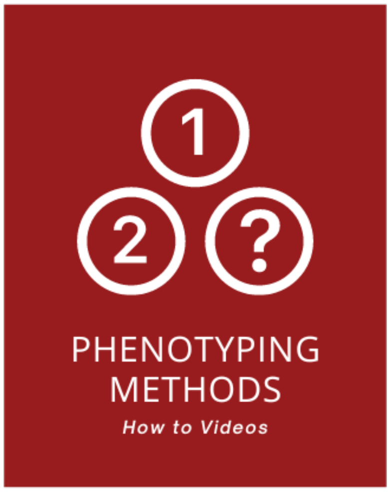
Check Out the Latest VacCAP Videos

We are thrilled to share with you the latest VacCAP feature videos, giving you a closer look at the research and projects being developed under VacCAP. These highlight Barcoding as a Breeding Tool and Breeding for Blueberry Flavor. Thank you to Dr. Lisa Wasko DeVetter, Co-PI at Washington State University, for leading this project and partnering labs (Dr. Patricio R. Muñoz and Dr. Luis Felipe Ferrao at the University of Florida Blueberry Breeding and Genomics Lab, and Dr. Charlie Sims at the University of Florida Food Science and Human Nutrition Department. These videos were made in collaboration with the team at the WSU College of Agricultural, Human, & Natural Resource Sciences.

Barcoding as a Breeding Tool: Learn how DNA barcoding can facilitate plant breeding and is used in VacCAP.

Breeding for Blueberry Flavor: Learn how sensory evaluation can facilitate breeding higher quality blueberries and is used in VacCAP.

Check Out These VacCAP Resources



Newsletter Staff & Contributors

Editors
- Massimo Iorizzo, Project Director, *North Carolina State University*
- Amaya Atucha, Co-PD, *University of Wisconsin-Madison*
- Lisa Wasko DeVetter, Co-PI, *Washington State University*
- Josie Russo, Communications Specialist, *University of Wisconsin-Madison*

Design
- Josie Russo, *University of Wisconsin-Madison*

Feature Article Authors
- "The CRISPR Question: Would Consumers Accept CRISPR Cranberries if the Benefit has Health Implications?" by Josie Russo (*Department of Plant and Agroecosystem Sciences, University of Wisconsin-Madison*) and Dr. Karina Gallardo (*School of Economic Sciences and the Research and Extension Center, Washington State University)*
- "Mitigating Cranberry Fruit Rot Through Breeding for Resistance" by Dr. James Polashock, *Philip E. Marucci Center for Blueberry and Cranberry Research and Extension, USDA-ARS*
- "Identification of Molecular and Genetic Markers Associated With Resistance to Anthracnose Fruit Rot" by Dr. Patrick Edger and Mackenzie Jacobs, *Department of Horticulture, Michigan State University*

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Partners: Do You Want to Contribute to the VacCAP Newsletter?

If you are interested in contributing or have announcements for events, publications, or another other initiative that you want to share, contact Josie Russo at jrusso2@wisc.edu with the subject line "VacCAP Newsletter Contribution"

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