RosBREED

Combining Disease Resistance with Horticultural Quality in New Rosaceous Cultivars

V9.2 WINTER 2019

In This Issue

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Advisory Panel Meeting

Jim McFerson, Extension Team Leader, Washington State University

If RosBREED's Advisory Panel (AP) members at our final meeting thought they were going to have an easy time of it, Project Director Amy Iezzoni let them know immediately at 8:00am on Jan 10 it was business as usual. In the grand tradition of RosBREED, Amy and the project team had put together a typical jam-packed day, with overviews, updates on project objectives, crop-specific work sessions, report-outs, and a final roundtable.

As usual, the final roundtable was a vigorous exercise, with all 18 AP members in attendance sharing their constructively critical thoughts about project progress, project deliverables, and challenges needing attention. While comments were overwhelmingly positive and reinforced exactly how impactful RosBREED has been for stakeholders and the Rosaceae scientific community alike, it was hard to escape a wistful sensation – this really was the last time we would be meeting as a group. Adding a further note of melancholy, several project Team Leaders were unable to join us in San Diego, due to the U.S. federal government shutdown.

RosBREED 1 had been immediately followed by RosBREED 2, building on a core team, but adding members and objectives appropriate to address stakeholder priorities for new rosaceous crop cultivars combining disease resistances with superior horticultural quality. What were the possibilities of a RosBREED 3? After all, both earlier projects were judged "amazingly successful" by more than one of our AP members. In our last couple of AP meetings, we had been urged to come up with the next iteration, addressing new challenges for which genomic discovery, genetic analysis and cultivar development could be applied.

Acknowledging that call and the team's own desire to maintain research momentum, Amy described the considerable efforts made by team members and other scientists to develop another such large, comprehensive, ambitious and compelling proposal – a RosBREED 3.

Fig. 1. Amy Iezzoni gets excited about the international collaboration RosBREED has enhanced.

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In those efforts, it became clear that there was not sufficient commonality in need and opportunities across all the rosaceous crops to build a strong proposal. It did not meet the RosBREED criteria of "better together" due to the different trait priorities and widely divergent genomic/genetic needs in the community.

These divergent needs did not mean that the individual pieces did not have merit, it just meant that an overarching proposal did not seem the appropriate way to address these needs. Building on this, Amy emphasized the excellent possibilities for spin-off projects intended for SCRI or other competitive funding programs. Such projects might not be the scale of RosBREED, but could build on its outcomes to focus on more crop- or geographically-specific issues.

One of the proudest achievements of RosBREED has been the students and post-docs involved directly or indirectly. That next generation is now more than ready with cutting-edge genomic, genetic and breeding tools. Perhaps RosBREED 3 does indeed lie ahead, but even if not, the national and international Rosaceae scientific community is better-equipped to tackle stakeholder challenges.

Fig. 2. Cameron Peace reflects on the development of useful tools for rosaceous crops breeders.

Fig. 3. Vance Whitaker shares some of the exciting advances in strawberry breeding enabled by RosBREED.

Fig. 4. The final group picture from our annual Advisory Panel meeting in what was by far the brightest meeting day ever. Note the project scientists on the upper end of the stairway eager to take in the sunshine with Advisory Panel members.

International Partner Meeting

Audrey Sebolt, Jim McFerson

Since its inception, RosBREED has benefitted immensely from active collaboration with international scientific partners. By working collectively to leverage resources, share techniques and report on recent scientific advances, we have all helped to enhance progress for researchers and expedite outcomes for stakeholders. Along the way, we have also contributed to the development of a much more networked and interactive Rosaceae community. As Project Director Amy Iezzoni likes to point out, scientists and students who have been part of RosBREED are working on rosaceous crops in every continent except Antarctica.

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While our interactions have occurred throughout the year, a high point has always been a one-day meeting in Diego held immediately after RosBREED's annual project meeting with Advisory Panel members. The specific venue has shifted among various hotels, but the get-together was always scheduled prior to the Plant and Animal Genome Conference, allowing attendees to stretch their travel budgets and benefit from multiple professional interactions.

Another recurring feature of what have simply called the "International Partners Meeting" has been the energetic and thorough organization provided by RosBREED DNA Testing Team Leader Nahla Bassil. Regrettably, due to the most recent U.S. federal government shutdown, Nahla was unable to travel to San Diego and manage in person the final meeting, held 11 January 2019 at the Harbor Island Hilton Hotel and including 32 participants from 10 countries.

Despite her unforeseen absence, Nahla had developed a typically ambitious agenda and was able to contribute via a speakerphone, so we were able to enjoy her wonderful laughter and technical insights. We were also able to enjoy vigorous discussions of new genotyping tools, high-throughput DNA testing approaches, and genotyping needs.

Because this is the final year of funding for RosBREED and thus the final International Partners Meeting, a significant amount of time was spent on identifying crop-specific scientific knowledge gaps. In *Prunus*, the group suggested dissecting and elucidating an important locus-trait cluster on chromosome 4 via a community-wide search of their populations for recombinant individuals. Similarly, the strawberry group prioritized the need for gene discovery in a globally collaborative effort. Finally, in pome fruit, the group recommended a coordinated emphasis on genome-scanning.

This sort of widespread impetus to continue collaborative and complementary efforts in Rosaceae provides optimism our network of international partners will flourish well beyond RosBREED. In fact, this expanded network and the conclusion that "we are better together" ia a major project deliverable.

Fig. 5. The Prunus *working group discusses research needs. Shown here: (from l-r) Pere Arus, Fred Bliss, Amy Iezzoni, Jose Quero Garcia, Herman Silva, and Kathleen Rhoades.*

Fig. 6. Steve Knapp provides an update on strawbery advances.

Certainly, RosBREED's many technical deliverables would not have been possible without the ongoing contributions of time, insights and cost-sharing from our international partners and support from their home institutions.

Fig. 7. Lailiang Cheng, Cornell University.

The RosBREED team appreciates each and every one of you who made our International Partners meeting a much-anticipated event, with outputs that made our project more impactful, our programs more effective and the after-meeting parties more fun!

Featured Advisory Panel Member

Lailiang Cheng, Cornell University, Ithaca, NY

What is your job description?

I'm a tree fruit physiologist at Cornell University. The primary goal of my research is to develop a better understanding of nutrition (both carbon and minerals) physiology and stress physiology of apple trees to improve orchard productivity and fruit quality. I also have extension responsibilities that are closely tied with my research.

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Why are you involved with RosBREED?

RosBREED represents the largest effort by the Rosaceae genetics/breeding community in the U.S. to develop genetic markers for economically important traits such as fruit quality and disease resistance for improving breeding efficiency and accelerating cultivar development. This provides a great opportunity for physiologists to develop collaborations with the RosBREED team to better understand the physiology of these crops, which will benefit the U.S. Rosaceae crop industries and consumers.

How do you feel you can contribute to RosBREED?

I feel serving on the Advisory Panel for RosBREED has brought mutual benefits. The tremendous gains the RosBREED team has made in identifying QTLs and developing genetic markers for important traits have allowed physiologists like me to better understand the molecular physiology of apple by building on these gains. At the same time, my physiology expertise might help the RosBREED team to narrow down the list of candidate genes underlying certain traits and interpret things in a physiological context. More importantly, I feel this collaborative relationship developed during RosBREED will benefit our research programs for many years to come.

Fig. 8. Lailiang Cheng speaking during a field day event. Photo: Fruit Grower News.

Fig. 9. Carolyn Ross overseeing a sensory analysis of a non-rosaceous crop product.

Featured Team Member

Carolyn Ross, Washington State Univ, Pullman, WA

What is your job description?

I'm a Professor in the School of Food Scienece and Director of the WSU Sensory Science Program. I maintain both a research and teaching program at WSU. I teach Food Chemistry and Sensory Evaluation of Food and Wine. In the area of research, the overall objective of my research and graduate education program is to understand the theoretical basis underpinning the sensory perception (aural, oral, and tactile) of foods and wines and to correlate these psychophysical attributes with quantifiable characteristics.

Why are you involved with RosBREED?

RosBREED is a unique research program in that it has pulled together such a strong team of researchers and with these researchers, is pursuing the big questions in fruit breeding. It's very encouraging to see such a group working together.

How do you feel you can contribute to RosBREED?

As a food scientist, my research area encompasses the application of both sensory methods and instrumental measures to evaluate foods and specifically, fruit quality. Over the years, some of my research has focused on fruits, including apples, sweet cherries and strawberries and how growing conditions or processing results in changes in these products. I feel that I can contribute expertise in the area of fruit quality, as well as consumer behavior.

Fig 10. Rose infected with black spot.

Jewel in the Genome

Rose Black Spot

Amy Iezzoni, Project Director, Michigan State University

Black spot of rose, caused by the fungus *Diplocarpon rosae*, is a major disease of garden roses across the U.S. As the name implies, in susceptible cultivars infected leaves show black spots that become

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Fig. 11. Brite Eyes™. Photo: Stan Hokanson, UMinn.

surrounded by yellowing of the entire leaf. Eventually the infected leaves drops off the plant and a loss of vigor that reduces flower bloom and increases susceptibility to winter injury (Fig. 10). Black spot can be controlled by frequent fungicide applications; however, home gardeners and public garden managers resist using chemical control due to risks of exposure and environmental and cost concerns. Therefore, developing garden rose cultivars with resistance to black spot is a high priority for rose breeding programs.

Fortunately, some current rose cultivars show genetic resistance to this disease. One of these is the climbing rose Brite Eyes™ ('RADbrite') (Fig. 11). A genetic study led by the RosBREED rose team members at the University of Minnesota and USDA-ARS Corvallis, Oregon, determined that Brite Eyes™ has a resistance allele at a locus they named *Rdr*4, on the long arm of chromosome 5 homoeolog 4, that acts as a single Mendelian trait locus conditioning resistance to 12 of the 13 black spot races characterized to date (Fig. 12). Due to its broad range of resistance to black spot, and its dominant gene action, *Rdr*4 is an excellent locus to involve in breeding black spot resistant cultivars.

Fig. 12. Rating scale fro black spot evaluation (rating scale and image provided by Stan Hokason, UMinn.).

A DNA test for routine screening for *Rdr*4 resistance to rose black spot is currently being developed by the USDA-ARS group. Therefore, because knowledge of this locus will lead to more effective breeding of garden rose cultivars resistant to black spot, it is featured as a RosBREED "Jewel in the Genome".

Reference

Zurn JD, Zlesak DC, Holen M, Bradeen JM, Hokanson SC, Bassil NV (2018). Mapping a novel black spot resistance locus in the climbing rose Brite Eyes™ ('RADbrite'). Frontiers in Plant Science 9:1730.

Where Are the RosBREEDlings Now?

Jonathan Fresnedo-Ramirez, The Ohio State University

Where are you now and what are you doing?

I'm an assistant professor at Ohio State University in the Department of Horticulture and Crop Science. My position's primary focus is on research (80%) and graduate education (20%) regarding plant domestication and germplasm improvement of outcrossing species. Having such a broad position allows me to keep doing research on Rosaceae. I have projects ongoing in almond, apple, and black raspberry (representing the three Rosaceae subfamilies!), grapevine, and an undomesticated plant species (*Taraxacum*).

How were you involved with RosBREED?

Between 2010 and 2014, I was a breeding trainee in the demonstration breeding program for processing peach at UC Davis. Most of my interaction was with the peach group. Nowadays, I parasitize RosBREED in order to get access to resources such as genotyping, but overall to pick the brains of the community.

Did it prepare you for your current position?

Absolutely! Being part of RosBREED opened to me a myriad of opportunities to interact with a team of people convinced that a difference can be done on how to improve Rosaceae specialty crops. I consider that the comprehensiveness of the group, the clear vision and mission of the project, the holistic approach and the composition of RosBREED shaped my mindset in a way that I do not see myself pursuing breeding as an isolated entity but as part of a community or pursue the formation of communities. This approach allows to enhance and consolidate opportunities in our crops to respond to demands of growers, retailers, consumers and stakeholders. This mindset is not easy to develop while pursuing a graduate degree in an individual breeding program. In addition, RosBREED gave me the opportunity to interact with people with distinct roles in policy (e.g.,

Fig. 13. Jonathan Fresnedo-Ramirez .

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Fig. 14. Jonathan Fresnedo-Ramirez and his lab group at The Ohio State University: (from l-r) Matthew Willman, Cheri Nemes, Debbie Zaborski, Fresnedo-Ramirez, Katie D'Amico-Willman.

commodity groups) and approaching private industry to enhance opportunities. Again, that is not easy while working in an isolated breeding programs with reduced interactions.

What was your most memorable experience(s) with RosBREED?

I guess for me the most memorable experiences come from the Participant Meetings in MSU. It was so nice to see everybody willing to spend the few days with the focus in achieve something: the presentation the pedigrees in PediMap, the mapping of the QTLs with FlexQTL, the calculation the IBD of the relevant QTLs in the breeding program, the design of the markers to select for them. That feeling of pursuit and achievement developed during those days was really nice and motivating. Along that, having the opportunity to interact with the other breeding trainees and learn about their concerns and their frustrations with their crops, with the analyses, with their results, it was fascinating. Having the chance to have a nice informal conversation with very interesting and recognized people like Amy Iezzoni, Cameron Peace, John Clark, Jim Luby, all the co-PIs, is a really valuable and memorable experience.

Any advice for RosBREED's current participants?

Please, keep the hunger for contribution and make a difference going forward. Keep that persistence and drive that is so RosBREED-minded, whether it is in getting a new QTL, correcting a pedigree, caring for the education of the breeding trainees, enhancing the expertise of the postdocs, facing the closing of breeding programs, struggling to make sense in the last questionnaire to stakeholders, or simply to face the current and future challenges in these specialty crops. Please, keep alive your hunger for satisfying discoveries, and provide solutions!

Terrence Frett, Sun World Inovations

Where are you now and what are you doing?

I live in Bakersfield, CA and work for Sun World Innovations (SWI) (https://sunworldinnovations.com/) as an assistant breeder for table grape and stone fruit (peaches, nectarines, cherries, plums, apricots, and *Prunus* hybrids). Terry Bacon, Vice President of Variety Development, and I cover all tasks related to traditional stone fruit and table grape breeding and commercial testing. We also work with the Tech Support Team to develop "best practices" support for SWI's grape and stone fruit varieties worldwide.

Furthermore, I serve as SWI's molecular program specialist. I constructed the high-throughput and cost-efficient SWI Molecular Lab and developed the logistics for Marker-Assisted Seedling Selection (MASS) tissue collection and sample tracking. In the SWI Molecular Lab we identify and validate relevant fruit breeding loci across the program's material. Once validated, the information is used in Marker-Assisted Parent Selection (MAPS) to determine which parents to cross, and MASS, to determine which inferior seedlings to throw away in the greenhouse. MAPS and MASS are enhancing the efficiency of SWI to improve fruit flavor, color, post-harvest, seasonal availability, and disease resistance, and will continually expand and evolve. These molecular tools substantially reduce breeding operational costs, increase selection efficiency and genetic gain per breeding cycle, and thereby accelerate the development of new cultivars spanning the season.

How were you involved with RosBREED? Did it prepare you for your current position?

I was blessed as a graduate student to be a part of RosBREED in both my MS and PhD. I completed my MS at Clemson University in Dr. Ksenjia Gasic's molecular fruit breeding lab. During this time, I was one of the four RosBREED peach breeding students working together to

Fig. 15. Terrence Frett measuring stone fruit while working on his PhD at the University of Arkansas.

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Fig. 16. Terrence Frett sampling seedlings from the Sun World Innovations breeding program.

enable MAB collectively across the four programs. I then headed to the University of Arkansas, to pursue a PhD under Distinguished Professor Dr. John Clark, and continue with the RosBREED peach team. Learning by doing under the guidance of RosBREED breeders and molecular scientists shaped me into a hybrid of a traditional and molecular fruit breeder, both of which are key to my current position and future.

What was your most memorable experience(s) with RosBREED?

During my time with the group I was able to connect with the most influential fruit breeders and molecular scientists in the USA and beyond as well as several fellow fruit breeding graduate students (future breeding colleagues!).

Any advice for RosBREED's current participants?

Continually expand your network. Connections are key to success.

Congratulations, Lichun!

Fig. 17. Lichun Cai celebrates his second place award for his presentation at the Ninth International Rosaceae Genomics Conference, Nanjing, China (June 2018).

Community Events

XV EUCARPIA
Fruit Breeding & Genetics Symposium
3-7 June 2019
Czech Republic
www.eucarpiafruit2019.org

ISHS *Rubus & Ribes* Symposium
25-28 June 2019
Zurich, Switzerland
www.ishs.org/symposium/614

ASHS Annual Conference
21-25 July 2019
Las Vegas, NV
www.ashs.org/page/GeneralConference

Community Breeders' Page

The results are in! DNA information use is commonplace in U.S. Rosaceae breeding

Cameron Peace, Michael Coe, Alexandra Johnson

DNA-informed breeding is increasingly routine in rosaceous crop improvement. Five years ago, as RosBREED 1 was ending, we concluded that DNA-informed breeding had become conventional in U.S. Rosaceae breeding. This statement meant that most (>50%) breeders were using DNA-based information in their breeding decisions in one way or another.

The estimated proportion was actually 59%, determined from survey responses by approximately 55% of known Rosaceae breeders in the country in 2014. The point was that practical application of DNA-based diagnostics of breeding germplasm was not just a fad. In past decades, it promised to be valuable for our breeding programs, and over time it has proven to be both doable and useful.

RosBREED 1 helped move the dial by targeting key challenges to routine DNA-informed breeding, which included development of DNA tests for traits of breeding value, provision or facilitation of genotyping services, and emphasis on valuable applications other than marker-assisted seedling selection. Over the past four and a half years of RosBREED 2, we have continued to identify and address challenges to routine application expressed by breeders. Research breakthroughs across the world have supported further innovations.

Correspondingly, the barriers to DNA-informed breeding continue to fall, the number of adopters of this approach are rising, and applications are increasing across the spectrum of breeding operations.

A recent online survey involved many of you. This survey, combined with interviews about some programs, has provided the most detailed and accurate understanding yet of the extent to which U.S. Rosaceae breeding programs (Figure 18) have access to DNA-informed breeding tools and information in addition to how such resources are used. The response rate was huge!

Thus, we now have robust knowledge of DNA information access and use for 90% of the 80 active community breeding programs, or 92% of all 100 U.S. Rosaceae breeding programs when we add the 20 demonstration breeding programs of RosBREED 2. The DNA-informed breeding situation for the remaining 8% of programs is unknown.

Our database revealed that each U.S. Rosaceae breeder runs an average of two Rosaceae crop breeding programs (53 breeders, 100 programs). Breeders with multiple programs were asked to respond separately for each crop; this new approach allows us to measure DNA information use at the level of breeding programs rather than focusing on breeders as individuals.

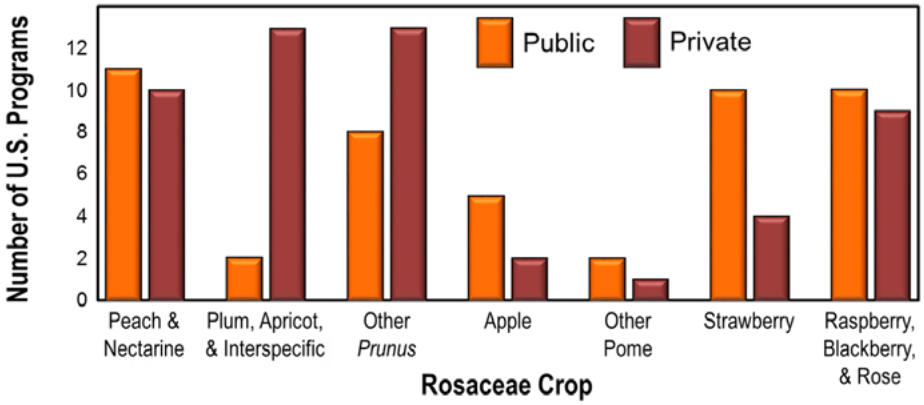

Fig. 18. The active 100 US Rosaceae crop breeding programs, half conducted aat public institutions and half private, are managed by 53 breeders.

In the survey, DNA-informed breeding was arranged in 3+1 categories: identity/relatedness, parental selection, and seedling screening, plus involvement in "upstream research" of direct relevance to the program, such as developing or validating DNA tests for breeding materials and targeting characteristics important for a particular program. Breeders were asked about their familiarity with these applications, their extent of use of DNA information for each, and their avenues and ease of access to DNA-based diagnostics services. Respondents were also asked about challenges, opportunities, and training needs for their programs to further benefit from DNA information.

The results are in! Some use of DNA information is now reported by an estimated 73% of U.S. rosaceous crop breeding programs, with 38% of responding programs reporting using DNA information on an ongoing, routine basis (Figure 19). Of the three types of direct applications to breeding tasks, the most common application is for identity verification or determination of relatedness; 63% of programs have done this, and 33% of programs report doing this on an ongoing, routine basis. Use of DNA information for parental selection is reported by 49% of programs, with 27% doing this on an ongoing, routine basis. Typically the most challenging application of DNA information is for seedling selection; this was reported by 38% of programs, with 21% doing this on an ongoing, routine basis. Almost half (48%) of programs are now involved in upstream research on DNA information of direct relevance to their work, with 27% doing this routinely.

Survey respondents unsurprisingly cited costs, in terms of both time and money, as a challenge for incorporating DNA information in their program operations and decision-making. Other challenges

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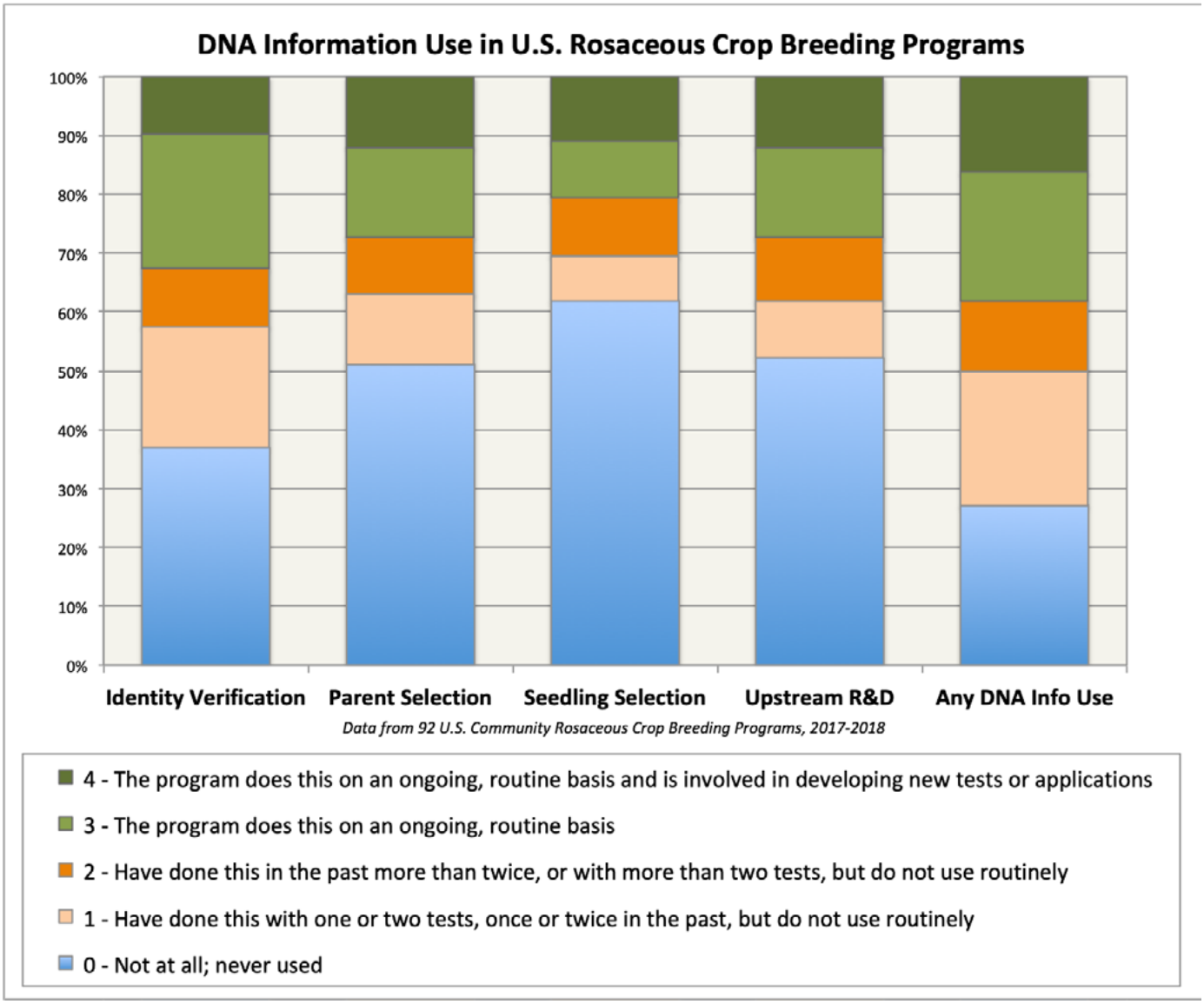

Fig. 19. Data for 92 of the 100 active U.S. rosaseous crop breeding programs come from self-report surveys or interviews conducted in 2017 or 2018.

noted included availability of accurate phenotype information for target traits; availability of technical expertise in managing, analyzing and interpreting large amounts of genotype and phenotype data; and lack of validated DNA markers and tests for certain traits in some breeding populations, especially in cases where wild or other new genetic material has been introduced.

Noted opportunities for new applications of DNA information included the rising importance of phenological traits (e.g., bloom and fruit/nut maturity timing) and improved vigor and abiotic stress resistance that have not yet been addressed with DNA marker and test development, in addition to further improvement in disease resistance or tolerance and fruit and nut quality.

We will be examining the results in greater detail, including comparisons between accessibility and use of resources, between community breeders and demonstration breeders, between private and public institutions, among types of applications, among crop groups, and over time.

These results are being used in RosBREED's multi-year endeavor to make DNA-informed breeding tools and information accessible and useful for breeders of rosaceous crops.

DNA-informed breeding in Rosaceae is not yet ubiquitous but it is commonplace. Many compelling opportunities exist for using tools and information already available as well as for targeted future research.

Thanks to all survey respondents who took the time to carefully answer the survey questions as well as meet with us during past years' in-person visits. Your communicated experiences, successes, concerns, and needs are bridging the chasm for DNA-informed breeding.

In the next issue

- **Rosaceae Nemesis & Solution**
- **Progress and Updates from our Annual Participant Meeting**
- **Where Are the RosBREEDlings Now?**
- **Featured Members**

Funding for *RosBREED: Combining disease resistance with horticultural quality in new rosaceous cultivars* is provided by the Specialty Crop Research Initiative Competitive Grant 2014-51181-22378 of the USDA National Institute of Food and Agriculture.

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