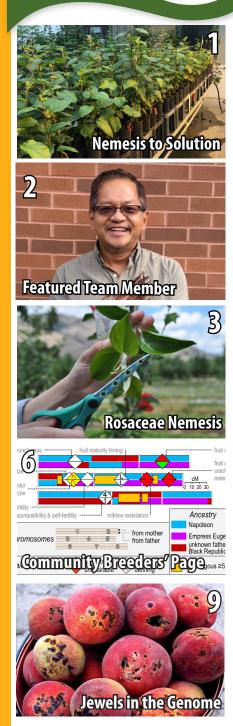
RosBREED

Combining Disease Resistance with Horticultural Quality in New Rosaceous Cultivars

In This Issue



Every Nemesis Needs a Solution

Jim McFerson, Extension Team Leader, Washington State University

Some perspicacious readers may notice in this issue a subtle shift in a regular feature of the newsletter. We are now highlighting not just the dreaded disease nemeses that threaten rosaceous crops, but are including potential breeding solutions to them.

As RosBREED scientists and Advisory Panel members have learned, Project Director Amy lezzoni is adamant that we never lose sight of our commitment to provide deliverables based on stakeholder priorities. An essential outcome of RosBREED includes superior new cultivars that help solve problematic disease issues among our eight different crops and 22 breeding programs.

Other outcomes are also important: New disease screening protocols, new DNA information, new software, new socioeconomic insights, new graduate students and postdocs, and new publications. Certainly, these deliverables all directly contribute to RosBREED's overall goal of combining disease resistance with horticultural quality.

Many have been highlighted in our newsletters, on our web site, and at our regular project meetings. However, as important as they are individually, these must lead to superior new cultivars for RosBREED to be considered fully successful. This goal requires individual breeding programs to become more efficient and effective, so the selections they release both satisfy customers and contribute to more profitable and sustainable industries.

Prior to developing the full RosBREED SCRI proposal, breeding teams worked closely with stakeholders to identify diseases that posed the most critical threats to their industries. These diseases, termed "Rosaceae Nemeses", have been the targets for every RosBREED Team. Inevitably, significant differences exist among crops regarding the quality and quantity of germplasm available to achieve the long-term goal. In some, discovery of resistance alleles was required; in others, sufficient knowledge already existed to begin the combining of disease resistance with horticultural quality in selections with commercial potential.

Now, as we pass the project's halfway mark, energized by exciting new discoveries and in many cases advancing selections with improved confidence and increased use of DNA information, Amy felt we needed to more explicitly and specifically document progress across crops, across nemeses and across the country.

In this issue, we focus on fire blight of apple (Fig. 1). Highlighted on page 3 is a sustained multidisciplinary, multi-institutional approach to identify and utilize promising germplasm. Within RosBREED 2, >500 germplasm accessions have been phenotyped using a protocol developed by the Pathology Team, led by Jay Norelli.

Building on previous results and this new information, RosBREED breeding teams are already developing new families to provide apple selections with durable multigene



Fig. 1. Susceptible reaction to apple fire blight. Photo credit: Sarah Kostick.

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Fig. 2. Jay Norelli and fire blight resistant apple germplasm under development at his USDA-ARS lab, Kearneysville WV. Photo credit: Jim McFerson.

resistance to fire blight, combined with superior horticultural quality (Fig. 2). Furthermore, development of those selections will be more efficient and effective, thanks to the DNA-informed strategies also being developed and implemented by the DNAinformed Breeding Team (see page 6)

Is the solution in hand right now? No, but the stage is set for U.S. apple growers to add a very powerful weapon in their battle with this Rosaceae Nemesis.



Rex Bernardo, Professor and Endowed Chair in Corn Breeding and Genetics, University of Minnesota, member of the RosBREED Statistical Genetics Team, and part-time acoustic guitar player.

Featured Team Member

Rex Bernardo

Rex Bernardo's involvement in RosBREED 2 has been one of those serendipitous results of right place, right time. After all, why would Rex be involved in RosBREED 2 when his main research responsibilities are in maize breeding and quantitative genetics, and his University of Minnesota endowed chair mandates that he focus on maize? The right place was Miami and the right time was the American Society for Horticultural Science annual meeting in August 2012. A Solanaceae research group invited Rex to talk at the meetings about his research on genome-wide selection—the use of genomewide DNA markers to breed for quantitative traits. While waiting for his afternoon session, Rex attended an RosBREED 1 morning session where he first learned about the "Jewels in the Genome" work funded by SCRI. When Rex to spoke that afternoon, several RB 1 folks came to listen and learn about simulation and maize-centric studies that showed the effectiveness of genome-wide selection to improve maize grain yield and other complex traits.

Unknown to Rex, the RB 1 leaders had already been plotting what RB 2 might look like. A week later, Jim Luby, apple breeder and Rex's colleague at Minnesota, sent Rex an email to ask if he'd be interested in joining the RB 2 proposal. In Rex's words, thinking about bushels per acre of corn was getting boring, (but don't tell others, especially his corn-breeding friends ...) and apple and peach and cherry and strawberry seemed like very tasty and appealing research subjects. So Rex signed on as part of the RB 2 Statistical Genetics Team.

Genome-wide selection leverages cheaper and quicker genotyping against pricier and slower phenotyping. It involves the development of statistical models from a "training population" that has been genotyped and phenotyped, so that the performance of a "test population" that has been genotyped but not yet phenotyped, can be predicted. Genome-wide selection in maize is fairly straightforward, because the parental lines are homozygous and, other than the Bt and herbicide-resistance transgenes, there are no major genes routinely used in cultivar development. In contrast, apple and other rosaceous clones are highly heterozygous and several "Jewels in the Genome" are routinely used in breeding. These differences in population structure and in genetic architecture require maizecentric statistical models be modified for rosaceous species, which is the topic of Elizabeth Blissett's PhD research at Minnesota, where Liz is co-advised by Rex and Jim Luby.

Being a part of the RB 2 community has allowed Rex to expand his network of contacts to scientists who work on horticultural crops. RB 2 has given Rex a deeper appreciation of the difficulties of working in crops with long generation intervals and for which quality traits rather than yield are of prime interest. Rex says that his work on RB 2 has also brought back memories of his previous work on breeding sweet potato—a self-incompatible, heterozygous, asexually-propagated crop—in the Philippines, where he was born and raised. Rex obtained his B.S. degree in agriculture in the Philippines in 1983, then traded living by a beach to study maize breeding and quantitative genetics at the University of Illinois. After obtaining his PhD degree in 1988, Rex worked as a research scientist for Limagrain Genetics in Champaign, IL. Rex switched career paths and joined the agronomy faculty at Purdue University in 1997, then moved to his current position at Minnesota in 2000. Throughout his career in both the seed industry and academe, Rex has focused on improving breeding methods for quantitative traits in maize. Because maize has long served as a model species for developing breeding methods applicable to various other crops, Rex

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is hopeful and confident that the learnings in maize genome-wide selection can be translated to rosaceous species.

Plant breeding education is one of Rex's key interests, and he has written two textbooks: "Breeding for Quantitative Traits in Plants" (2002, 2010), and "Essentials of Plant Breeding" (2014). His latest contributions in plant breeding education were developing BreedingGames software for gamification of plant breeding (http://bernardo-group. org/books-and-software/) and developing a new course entitled Professional Skills for Plant Scientists. His latest research focuses on targeted recombination as a breedingby-design approach. Rex received the Young Crop Scientist Award from the Crop Science Society of America in 1999, was elected Fellow of the Crop Science Society of America and of the American Society of Agronomy in 2005, and received the Plant Breeding Impact Award from the National Association of Plant Breeders in 2015.

Rex's top five strengths from StengthsFinder are Strategic, Belief, Learner, Responsibility, and Ideation. In his leisure time, Rex enjoys playing the acoustic guitar, taking his wife, Chona, on dates (usually a restaurant, tandem biking, or swing dancing), and hanging out with their six grown children.

Thanks to Rex for providing his "Featured Team Member" summary.

Rosaceae Nemesis Finding the Solution to the Fire Blight Nemesis

Amy Iezzoni, Project Director, Michigan State University

Fire blight, caused by the bacterium *Erwinia amylovora*, is a devastating disease threatening U.S. apple growers. Older cultivars such as 'Delicious' are resistant, but many newer and widely-grown cultivars are susceptible, including 'Fuji', 'Gala', 'Cripps Pink', and 'RubyFrost*'. Under high disease pressure, entire orchard blocks can be completely wiped out, and even lighter attacks cause substantial economic losses. Early on in RosBREED, this disease was given priority status (see RosBREED Newsletter Dec 2014. Norelli JJ. Rosaceae Nemesis: Apple Fire Blight).



Fig. 3. Phil Forsline and Michael Wisniewski characterizing Malus sieversii accessions, USDA-ARS Geneva, NY. Photo credit: Jay Norelli.

Fortunately, sources of fire blight resistance exist and have been utilized, but most are wild species like *Malus floribunda*, with fruit that are small (crabapple size) and bitter, compromising eating quality. Apple breeders have successfully transferred fire blight resistance from *M. floribunda* accessions into commercially acceptable cultivars such as 'Enterprise', 'Freedom', 'Liberty', and 'Pristine[™]'; however, the eating quality and storability of these cultivars doesn't meet contemporary standards for consumers and industry stakeholders. Furthermore, additional sources of resistance could enhance the durability of resistance.

One approach to address this nemesis is to seek such additional sources of resistance in species other than the M. floribunda, ideally in germplasm with acceptable fruit qualities. Fortunately, more than 20 years ago, Phil Forsline, then curator of the USDA-ARS Malus collection housed in Geneva, NY, led various expeditions to acquire just that sort of material. Sponsored by the USDA-ARS National Plant Germplasm System, Forsline and colleagues made repeated visits to Kazakhstan, a country spanning a vast region from the Caspian Sea east to the western border of China where extensive forests of *M. sieversii*, a wild progenitor species of domestic apple, were found (Forsline et al. 2003).

They brought back germplasm, mostly seed, that subsequently has been maintained, characterized, and distributed (Fig. 3). Since their field observations in Kazakhstan suggested their collections might be a source of genetic resistance to apple diseases, including fire blight, they carefully gathered seed and propagation material from individuals exhibiting low to zero levels of disease. Just as exciting, they were able to collect samples from trees exhibiting larger fruit size and better eating quality than other wild *Malus* species (Figs. 4, 5).

Fast forward 20 years. Apple breeding and genetics have advanced to a stage where genetic factors underlying resistance can be identified and combined with alleles known to result in crunchy, juicy, apples with excellent storage qualities. However, apple breeders must have access not only to that promising germplasm, but genetic information to help select individuals most efficiently used as parents to combine disease resistance and fruit quality.



Fig. 4. Malus sieversii *Kazakhstan germplasm, USDA-ARS Geneva, NY. Photo credit: Jay Norelli.*



Fig. 5. Variability in Malus sieversii *fruit, USDA-ARS Geneva, NY. Photo credit: Jay Norelli.*

To that end, among the highly variable germplasm collected during the Kazakhstan expeditions, various research teams have over time identified 12 *M. sieversii* accessions with resistance equivalent to highly resistant controls (Harshman et al., 2017). Recently, using a standardized phenotyping protocol developed by Plant Pathology Team Leader Jay Norelli (Fig. 2), Washington State University (WSU) graduate student Sarah Kostick and colleagues screened 556 accessions from the RosBREED apple Crop Reference Set and identified additional promising individuals (Figs. 6, 7, 8).

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Fig. 6. Panoramic view of field inoculation in 2017 by the WSU apple breeding team, Columbia View Orchard, Orondo, WA. Photo credit: Sarah Kostick.

Fortified with this phenotypic data, and utilizing new genotypic information, breeders have already made crosses to strategically develop new cultivars with durable, multigene resistance to fire blight. In the meantime, genetic studies are underway to more fully characterize the loci that providel the disease resistance exhibited by this novel germplasm so that it can be incorporated with maximum efficiency using a DNA-informed breeding strategy.

Twenty years is a long time to wait for Phil Forsline's pioneering germplasm



Fig. 7. A range of responses to fire blight infection is displayed among Malus sieversii accessions. Stem on the left shows crook-type damage; stem on the right shows recovery and healthy regrowth. Photo credit: Sarah Kostick.

Fig. 8. Standardized phenotyping for fire blight reaction in the field using scissors dipped in inoculum to infect the plant via cut leaves. Photo credit: Sarah Kostick.



collections to pay off with delivery of superior new cultivars, but we are getting closer, and the end is in sight!

References

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Harshman JM, Evans KM, Allen H, Potts R, Flamenco J, Aldwinckle HS, Wisniewski ME, Norelli JL. 2017. Fire blight resistance in wild accessions of Malus sieversii. Plant Disease doi.org/10.1094/PDIS-01-017-0077-RE

Community Events

RosBreed Advisory Panel Meeting 11 January 2018 San Diego, CA

RosEXEC 14 January 2018 San Diego, CA

Rosaceae Genomics Conference 9

26-30 June 2018 Nanjing Agricultural Univ Nanjing, China www.rgc9.org

ASHS

31 July-3 August 2018 Washington, DC

Meet our Advisory Panel Members

Our Advisory Panel members are a critical part of RosBREED, as they validate from their experience the impact of our research and guide our priorities. They dedicate not only a day (plus two for travel) to our Advisory Panel meetings in January, but they also are called upon during the year to provide feedback to questions our project members have.

Thanks so much to all our Advisory Panel members-see you next year in San Diego!

What is your job description?

I am a horticulturist employed by one of the larger groups of pear growers located in central Washington State. My duties cover IPM, harvest decisions, fertilizer recommendations, and general horticulture consulting. I also serve the Oregon and Washington pear industry as chairman of the Pear Research Sub-Committee. This committee reviews and recommends projects and funding in pear research.



Bob Gix Industry Advisory Panel Member

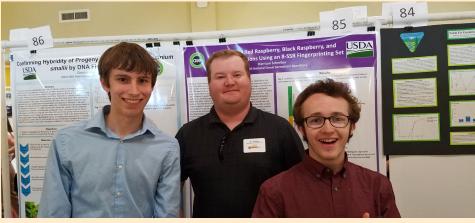
Why are you interested in RosBREED?

I was appointed to represent the PNW pear industry on the RosBREED's Advisory Panel. The pear cultivars we currently grow are heirlooms dating back centuries. Growers need the opportunity to grow smaller trees that come into production sooner (rootstock improvement). This will improve labor efficiency, facilitate easier IPM, and allow production of more uniform fruit quality. Pear growers have watched the success apple growers have had with new cultivar introduction in the marketplace. There is interest in new cultivars resistant to disease and insect pressure as well as providing the consumer a superior eating experience. The genetic tools developed through RosBREED will be used to reduce the development time for these changes.

How do you feel you can contribute to RosBREED?

My industry involvement has exposed me to many of the challenges growers face from planting the first trees to harvest many years later. I also work on the postharvest life of the fruit from harvest to the consumer. This experience combined with my involvement as chairman of the Pear Research Sub-Committee gives me a unique industry perspective. I certainly do not have a scientific understanding of genetics and genomics, but I do have a lot of experience, practiacl knowledge of producing quality fruit, and a passion for solving growers' problems. So, I contribute by helping the RosBREED team share information with stakeholders in a clear and understandable language and also by representing the pear industry in the project.

USDA-ARS Corvallis Student Interns Are Awesome



Jason Zurn (c.) RosBREED Crop Postdoc with his two Apprenticeship in Science and Engineering (ASE) students at poster presentations on 18 Aug 2017 in Portland, OR. Christopher Cebra (l.) and Harrison Schreiber (r.) are both high school students in Corvallis, OR.

Christopher Cebra's poster was entitled: *Confirming Hybridity of Progeny of 'Nocturne' x Vaccinium smallii by DNA Fingerprinting.*

Harrison Salisbury's poster was entitled: *Evaluation of Red Raspberry, Black Raspberry and Blackberry Accessions Using an 8-SSR Fingerprinting Set.*

So Is Wanfang!

Congratulations to **Wanfang Fu**, RosBREED M.S. student at Clemson University, for winning the 2017 American Pomological Society UP Hedrick Award for her paper titled *Enabling Breeding for Brown Rot* (Monilinia spp.) *Resistance in Clemson University Peach Breeding Program*. Wanfang's award certificate was presented at the American Pomological Society meeting in Hawaii, September 2017.



Community Breeders' Page Experiencing "the genotype" with haplotype mosaics - an Events article

Cameron Peace, DNA-Informed Breeding Team Leader, Washington State University

Haplotype mosaics are here! At last, genomics technology through a genetics lens is providing a complete picture of the breeding-relevant genome of each individual. This way of connecting breeders experientially with genomes was unveiled at the 2017 annual conference of the American Society for Horticultural Science (ASHS) at Waikoloa, Hawaii, 19–22 September 2017.

Haplotype mosaics visually display an individual's trait locus alleles, genetic variation by ancestry, and signatures of selection at any and every point in the genome (Fig. 9). Phenotypes provide a sensory connection for breeders to their plants. Now, haplotype mosaics open the door to experiencing the genomes of breeding material. By seeing the holistic "genotype" of their germplasm individuals, breeders can understand better what they have, then target development of what they want – informed from this genomic perspective.

In the first Fruit Breeding oral session at the ASHS conference, haplotype mosaics and examples of their breeding utility were demonstrated for sweet cherry and apple ("What you see is what you can improve: Breeding utility of genome-wide haplotype mosaics"). These two crops have the most advanced RosBREED datasets and subsequent genetic analyses.

Haplotype mosaics integrate three genetic vectors (Fig. 10). All it takes for any crop is:

- Germplasm sets: that are large, pedigree-connected, and representative of target uses;
- 2. Genome-spanning markers: at highenough density and repeatable across

further germplasm; RosBREED's apple, peach, and cherry SNP arrays are efficient for this purpose;

- Genotypic data curation: by skilled geneticists, to remove noise so that remaining genetic signals are bright, resulting in robust genome-wide haploblocks and their haplotypic variation;
- 4. Hypothesis-driven genetic analyses: in upstream research, to propose pedigree-filling candidates and to identify then characterize traitinfluencing alleles.

The synthesis of this genetic information of patterns of DNA sequence variation and recombination over generations enables **emergent applications** for breeding, including:

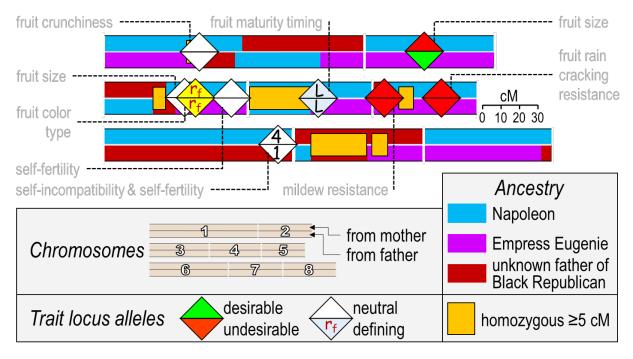


Fig. 9. Haplotype mosaic of 'Rainier', a classic Washington-bred sweet cherry cultivar. Segments that 'Rainier' inherited via its parents from its three specified ancestors are displayed across the eight chromosomes of sweet cherry. In some cases, these ancestral segments are homozygous, highlighting consequences of inbreeding as well as signifying recent common ancestry in generations behind known ancestors. Trait locus alleles are indicated with phenotypic effects and ancestral origins. Despite the commercial success of 'Rainier', there is still much to be improved.

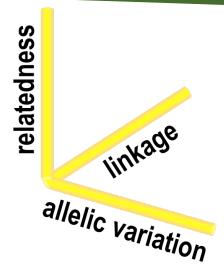


Fig. 10. Getting your bearings on the breedingrelevant genomic landscape. Haplotype mosaics integrate three genetic vectors – patterns of: similarity among alleles within each locus, linkage among loci within each individual, and relatedness among individuals within a population. Characterization (qualification of identity/relatedness)

- Pedigree reconstruction

 (actual relationships among individuals, such as grandparent-grandchild or first cousins once-removed on the maternal side)
- Ancestry composition (actual chromosomal segments inherited by an individual from ancestors and shared with other descendants)
- Tracked introgression

 (actual chromosomal segments inherited by an individual from exotic sources)

Evaluation (qualification of performance potential)

- Trait locus genotypic composition (genome-wide complement of trait locus alleles of an individual)
- Allele distribution
 (sources of an individual's alleles of interest and their sharing with other breeding
 individuals)

The pitch for software programming was made in a separate oral presentation (NRSP10 resources for translational tree fruit research) within the ASHS workshop of "NRSP10: Bioinformatic and Database Resources for Specialty Crops". Software solutions are needed to make haplotype mosaics readily accessible to Rosaceae crop breeders. With such software in hand (tablet, smart phone, laptop), breeders could readily view the genomes of their material and play with the possibilities of what could be crafted.

A central premise of the RosBREED approach, from a technical point of view, is that we must expand from locus-specific considerations to a genome-wide approach. Haplotype mosaics, a qualitative approach, is one way that we're going genome-wide. There is another major way too: genome-wide predictions of breeding value and performance, which is a quantitative approach (because the individual alleles, loci, and pedigree relationships among individuals are not explicit considerations). It's with such new ways of making genomic data accessible that we are aiming for a synthesis of germplasm, genomics, genetics, & superior new cultivars.

RosBREED by the Numbers

\$250M

Estimated economic loss to South Carolina peach due to severe cold in 2017.

13,158,563

Number of Affymetrix genotypic data points generated for rose. * 17

Number of RosBREED publications in peer reviewed journals 2017! **

* Genotypic data are of two populations (total of 191 individuals) segregating for black spot. ** www.rosbreed.org/publications2

Cultivar Corner

'MN55' APPLE

Inventors: David Bedford and James Luby University of Minnesota

When was 'MN55' released?

First trees were available to growers in MN and WA in 2014. First fruit were available in 2017 and will be increasingly available in later years. Fruit will be sold under the First Kiss trademark by Minnesota growers. The Rave trademark will be used for fruit produced by other licensed growers in the U.S.

What makes 'MN55' special?

MN 55 has the crisp texture of 'Honeycrisp', ripens one month earlier, and may be even juicier. The fruit have shown an exceptional ability to color and avoid sunburn for a cultivar that ripens in early to mid-August. The fruit maintain their texture for much longer than other earlyseason cultivars, up to four months in ambient atmosphere refrigerated storage. The flavor can have cherry notes and is tarter than 'Honeycrisp'.

What is the pedigree?

'Honeycrisp' × AA44. AA44 is a selection made at the University of Arkansas derived from germplasm developed by the Purdue-Rutgers-Illinois cooperative disease resistance breeding program begun in the mid-20th century. Although AA44 was never formally released, it has been available for years under the name 'MonArk'.



Fruit of 'MN55' grown in WA

When was the cross made?

1997

What is the size of the family from which 'MN55' was selected? Do any siblings from this cross the have commercial potential?

149 trees were planted to the stage 1 (seedling) orchard. Currently, no siblings are in advanced testing stages.

How has this cultivar been used in RosBREED?

'MN55' has been used in our crossing program and its offspring screened using markers developed through RosBREED. A few of its siblings were included in the RosBREED 1 apple Crop Reference germplasm set. We used tools developed in part via RosBREED — the International RosBREED Consortium 8K SNP array and FlexQTL[™] software — to discover that AA44 has a region of chromosome 3 that is unique in our germplasm in its contribution to early-season ripening.

Any other interesting notes about 'MN55'?

Every new cultivar has its challenges. For 'MN55', premature fruit dropping is a challenge. Also, it unfortunately did not inherit scab resistance from the PRI germplasm or from 'Honeycrisp'. Using RosBREED analysis tools, we were also able to predict its susceptibility to soft scald, a fruit storage disorder. Prepared with this knowledge, growers can use higher temperature fruit conditioning regimes before fruit are placed into refrigerated storage.



Why should growers plant 'MN55'?

Of special note for an early-ripening cultivar, fruit has excellent crunch, flavor, juiciness, coloring and storability, making growers very happy. Need proof? Image shows a happy JP Jacobson, White Bear Lake, MN grower, with trees of 'MN55'.

Jewels in the Genome

Fruit Bacterial Spot Resistance in Peach

Amy Iezzoni, Project Director, Michigan State University; Ksenija Gasic, Prunus Team Leader, and Cassia DaSilva Linge, Prunus Post-Doctoral Associate, Clemson University

Bacterial spot of peach, caused by *Xanthomonas arboricola* pv. *pruni*, is a serious disease in humid peach production regions, where it can cause severe defoliation and such a reduction in fruit quality that the fruit is unmarketable. Infected fruits exhibit an array of blemishes, such as surface pitting and cracking. Antibacterial chemicals offer only limited control; therefore, developing cultivars with reduced susceptibility to bacterial spot is a high priority for the breeding programs in humid production areas, like the southeastern United States.

Fortunately, there is genetic variation in susceptibility for this disease within the elite germplasm of U.S. breeding programs. A genetic study led by the RosBREED Peach Team at Clemson University identified the presence of a locus named *Xap.Pp.OC-6.1*,

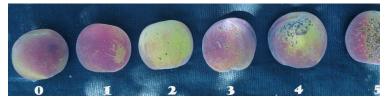
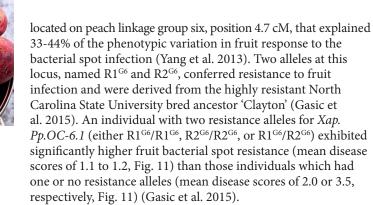


Fig. 11. Contrasting bacterial spot symptoms on peach fruits (0-5 scale). From PhD thesis of Dr. Nannan Yang, Clemson University, "Mapping Quantitative Trait Loci Associated with Resistance to Bacterial Spot (Xanthomonas arboricola pv. pruni) in Peach."

In the next issue

- Meet more members of our Advisory Panel
- Progress and updates from key teams from the third third of the year
- Our next "Jewel in the Genome" focuses on disease resistance
- What will be the next Rosaceae Nemesis? What will be the solution?



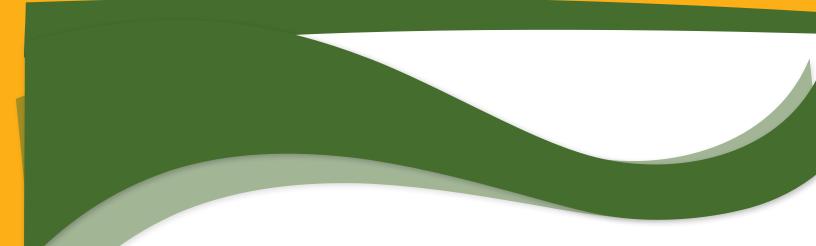
A DNA test for routine screening for alleles at *Xap.Pp.OC-6.1* conferring fruit resistance to bacterial spot was developed by the DNA-Informed Breeding Team. This SSR test, named Ppe-Xap, can distinguish among the resistance (R1^{G6}, R2^{G6}) and susceptibility (S^{G6}) alleles, but cannot distinguish an allele associated with intermediate disease reaction (I^{G6}). Therefore, because knowledge of this genetic region will lead to the more effective breeding of peach cultivars for fruit bacterial spot resistance, it is featured as a RosBREED "Jewel in the Genome".

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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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