



# RosBREED

COMBINING DISEASE RESISTANCE WITH HORTICULTURAL QUALITY IN NEW ROSACEOUS CULTIVARS



## RosBREED: Enabling marker assisted breeding for brown rot (*Monilinia* spp.) resistance in peach

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### Introduction

Brown rot, caused by *Monilinia* spp., is one of the most important diseases of stone fruits. The fungus mainly infects blossoms and fruits, and causes significant yield losses. Estimated yearly cost to the U.S. stakeholders for chemical protection against bloom blight and pre- and post-harvest fruit decay is \$170M. In commercial peach production, the two phases of the disease (blossom and fruit infection) can only be controlled with routine fungicide applications, which is not only costly but also causes environment and fungicide resistance concerns. Although some degree of tolerance has been found in the Brazilian landrace 'Bolinha' and some cultivars and advanced selections developed in the UC Davis and USDA breeding programs, genetic resistance to brown rot in peaches is still lacking. The RosBREED project, is combining disease resistance with horticultural quality by evaluating sources of brown rot resistance/high tolerance in peach germplasm and enabling pyramiding and combining resistant alleles in fresh market and processing peach. The next generation of peach cultivars with superior horticultural quality and disease resistance will ensure sustainability of peach industry and reduce pesticides load in the agro-ecosystem. Phenotyping method and preliminary results from the first year of this study are presented and discussed.

### Materials and Method

- Pedigree connected peach germplasm from two peach breeding programs, Clemson University (SC) and University of California, Davis (CA), was used for the evaluation of the tolerance to brown rot susceptibility.
- From each available accession in the pedigree, 40 unblemished fruits at similar maturity were randomly selected and stored at 4°C for 2-4 days. Stored fruits were warmed up to room temperature for 24h prior to inoculation, and surface sterilized for 30 sec by immersion in 10% bleach (0.6% NaOCl), rinsed in deionized water, and dried (Fig. 1).
- Inoculum was prepared using spores from 7 to 10 day-old cultures, diluted with 0.01% Tween80 at the concentration of  $2.5 \times 10^4$  spores per ml (Martinez-Garcia et al., 2013) (Fig. 2).
- Inoculation was performed by applying 10  $\mu$ L of inoculum to surface of an unwounded fruits (both CA and SC). Fruit wounding was done by breaching the cuticle with a 22 gauge needle through the inoculum droplet to generate a small, 2 mm depth hole (SC only). Inoculated peaches were incubated in the humidified containers at room temperature ( $22 \pm 1^\circ\text{C}$ ) for 72 hours (Fig. 3).
- At the end of incubation lesion diameter (mm) was recorded. Disease severity for each genotype was calculated as the product of the average lesion diameter x proportion of fruit with lesions greater than 3 mm (Fig 4.& Fig 5.).

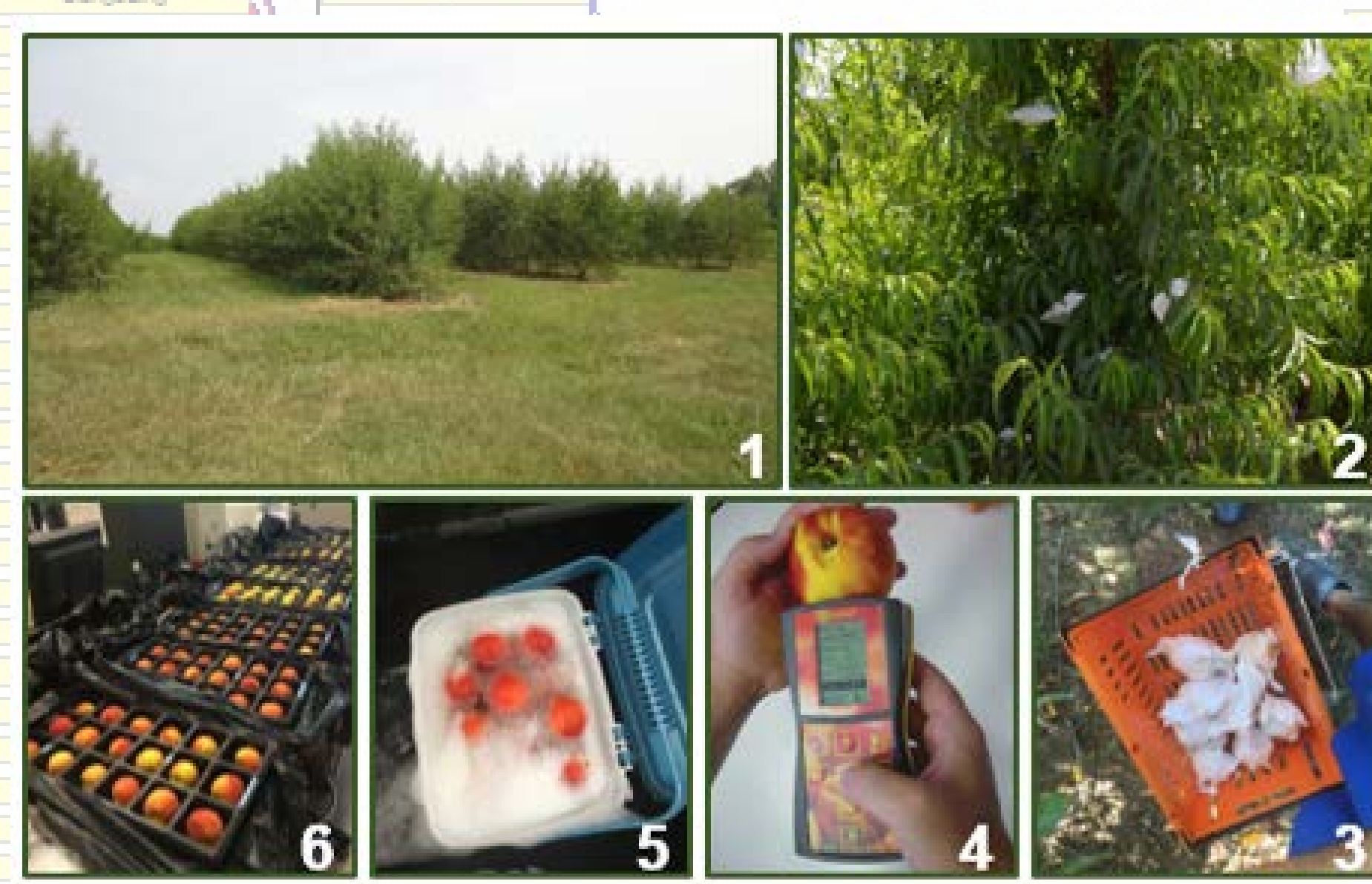


Fig. 1. Fruit sampling and sterilization 1) available accession; 2) bagged fruit to prevent pesticide deposit; 3) harvest; 4) maturity assessment (www.trturon.com); 5) surface sterilization; 6) ready for inoculation.

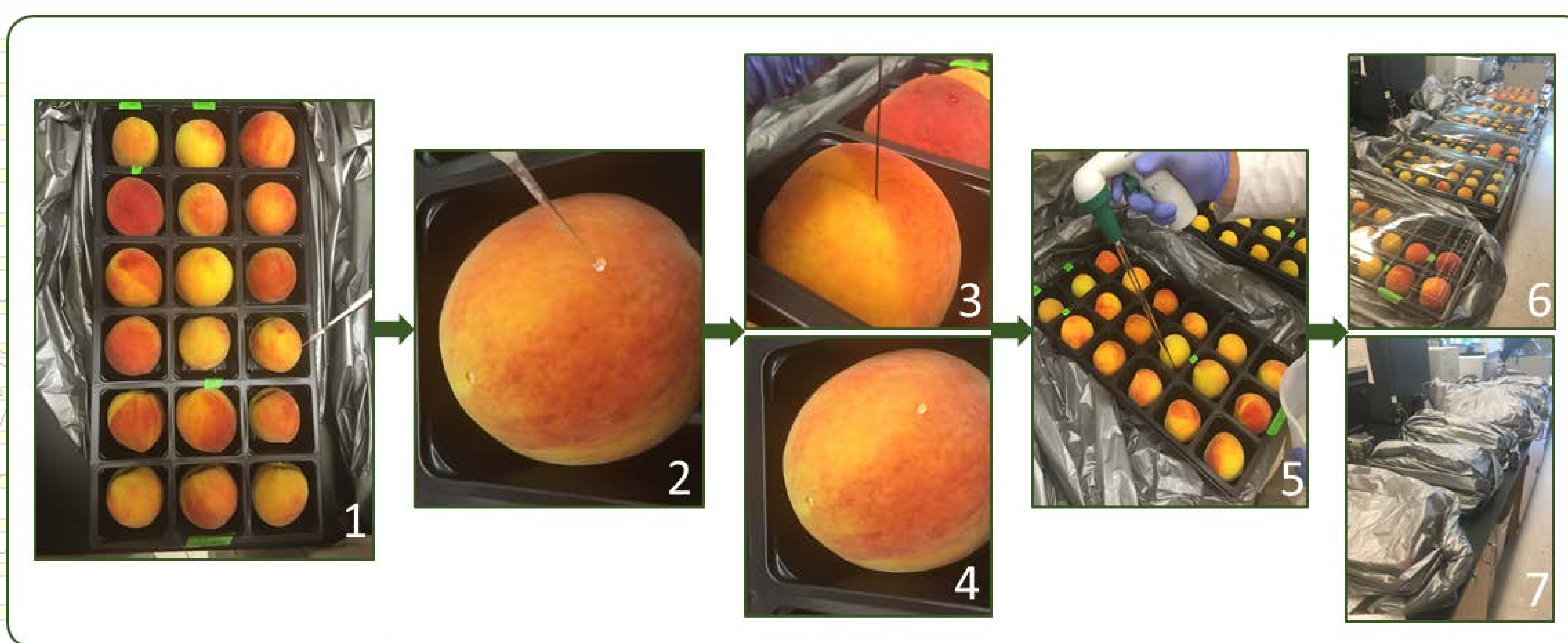


Fig. 3. Fruit inoculation and incubation. 1-4) parallel inoculation for wounded fruits (3) and non-wounded fruits (4); 5) Maintaining humidity; 6&7) 72h incubation

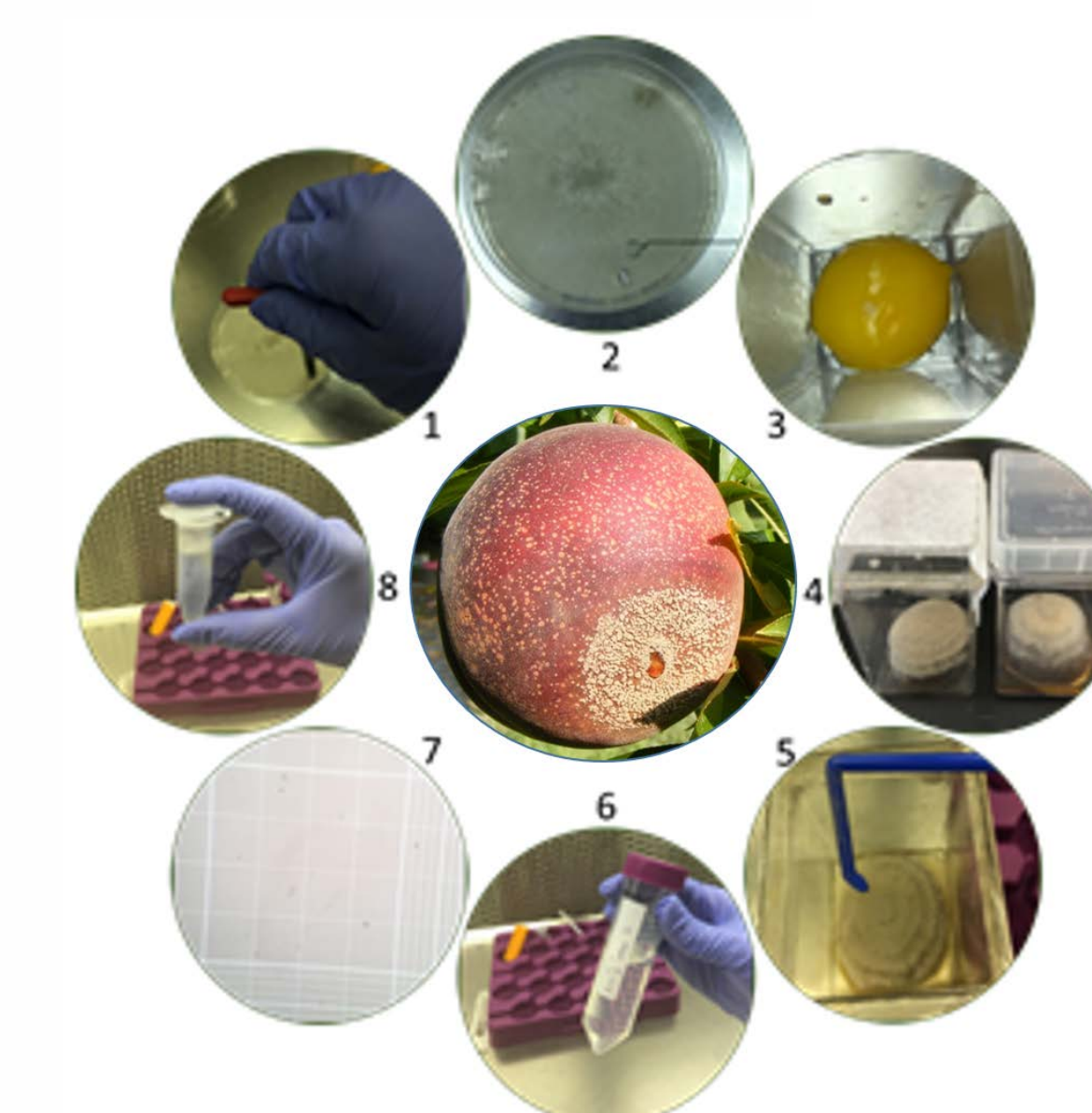


Fig. 2. Inoculum preparation. 1-4) stock multiplication; 5) spores collection; 6-7) spore counting and inoculum dilution; 8) inoculum prepared.

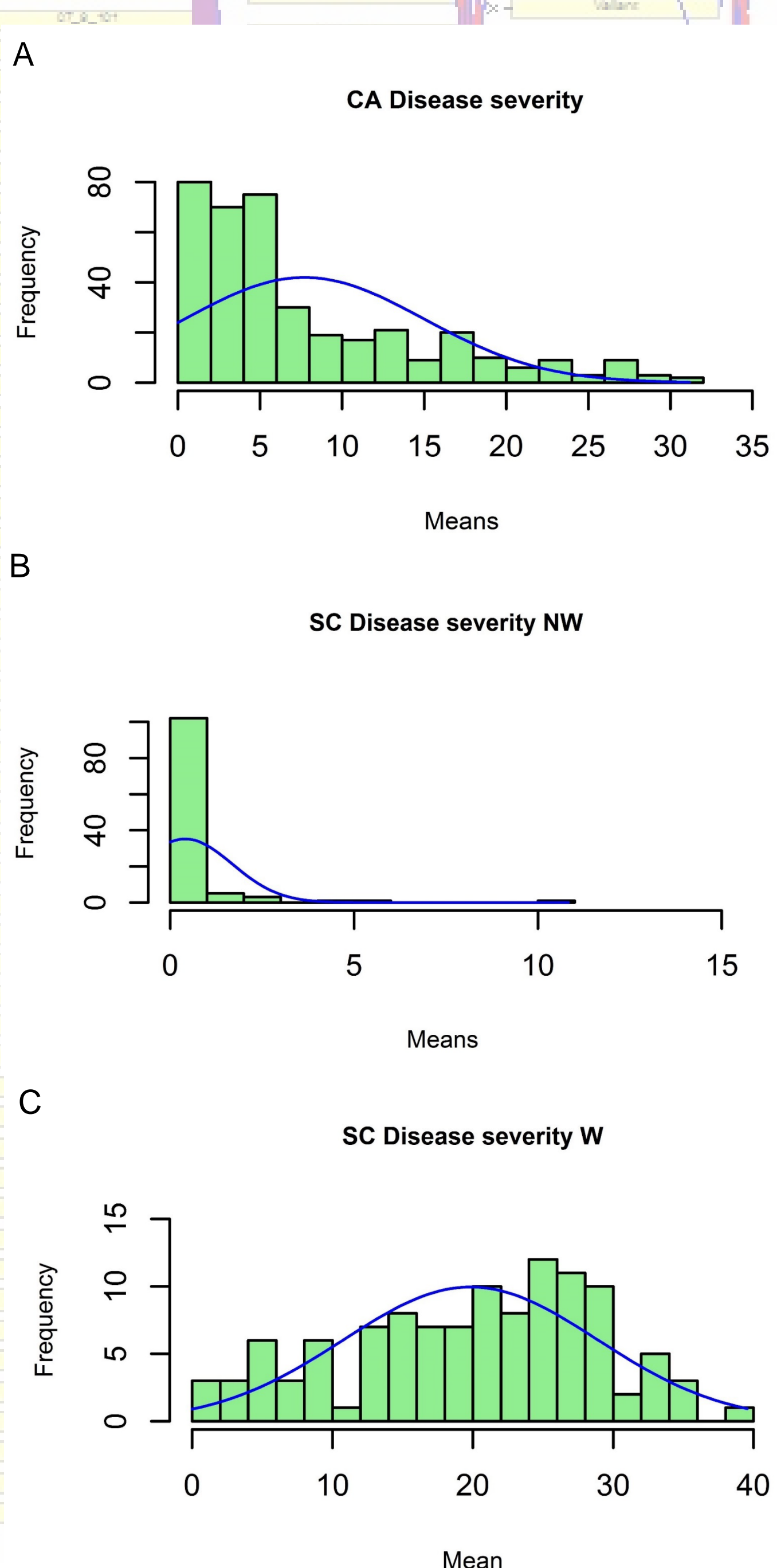


Fig. 5. Frequency of disease severity observed on unwounded (NW) (A & B) and wounded (W) (C) fruit in pedigree connected germplasm. CA germplasm was observed using unwounded protocol (A) and SC germplasm using both unwounded (B) and wounded (C) protocol.

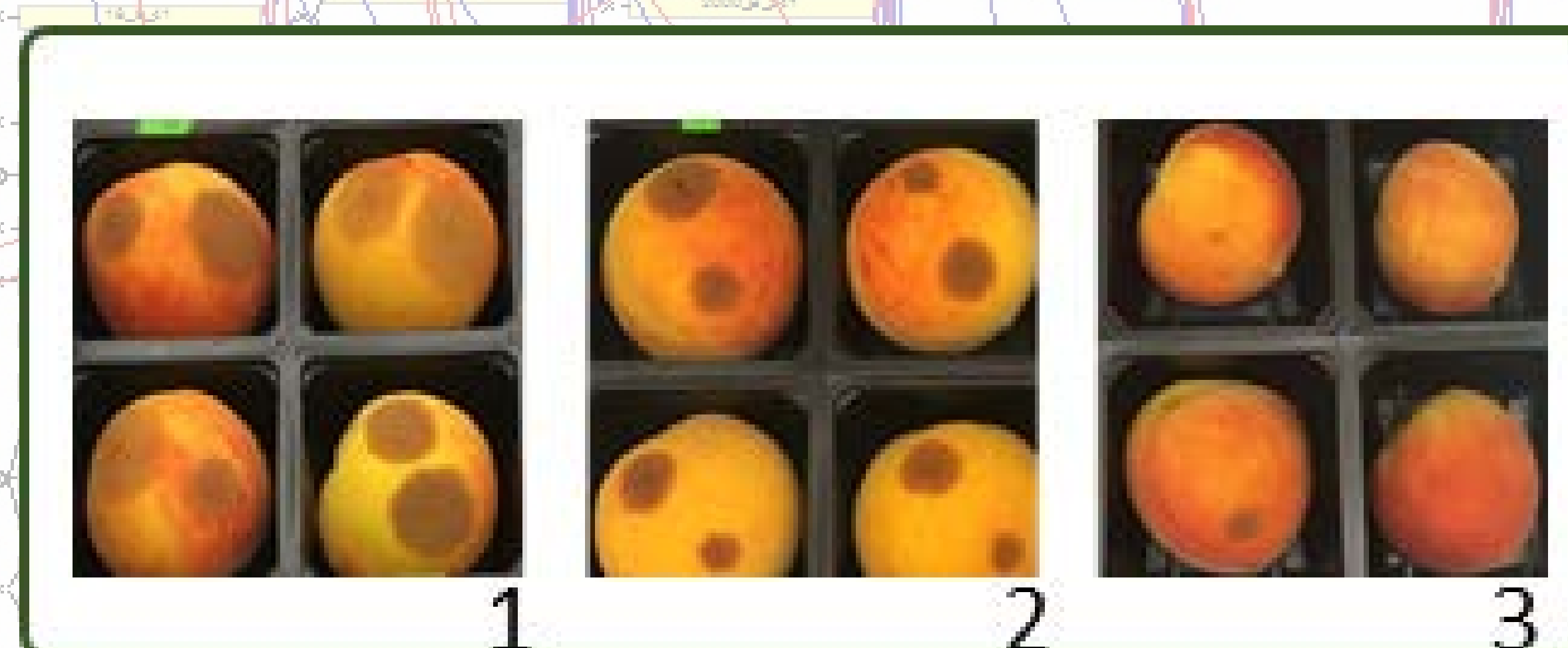


Fig. 5. Disease severity observed in pedigree germplasm. 1) highly severe 2) moderately severe 3) low severity.



Fig. 4. Disease severity assesment. Each lesion diameter was measured twice by rotating ruler for 90° and average diameter was used for disease severity calculation.

### Future plan and Potential benefits

- Preliminary data from disease evaluation in 2015 shows variability in responses to brown rot infection in unwounded (CA) and wounded fruit (Fig. 5 A-C).
- Pedigreed germplasm is suitable for QTL discovery using pedigree based analysis (PBA) (Bink et al., 2014).
- DNA tests for routine selection against brown rot susceptibility will be developed and validated in peach germplasm.
- Enabling marker-assisted breeding for brown rot tolerance/resistance will facilitate faster development of peach varieties with increased tolerance towards this devastating disease ensuring sustainability and profitability of peach industry.
- Consumers will benefit from greater access to a more stable supply of nutritious fruit that will translate into increased per capita consumption and improved human health and well-being.

### References

- Bink et al. 2014. Theor. Appl. Genet. doi:10.1007/s00122-014-2281-3  
 Martinez-Garcia et al. 2013. PLOS One 8(11) e78634. doi:10.1371/journal.pone.0078634.

