BROWN ROT DISEASE ASSAY AND PHENOTYPIC DATA COLLECTION (R. BOSTOCK)

FRUIT SELECTION

Fruit of similar size and maturity, based on visual assessment were selected at random from trees at the UC Davis Pomology Orchards.

- Fruit maturity is determined use of the DA-meter (www.trturoni.com).
- It may require several picks in order to get high numbers of mature fruit.

Unblemished fruit of different genotypes with similar maturity in the test population were collected each week over a 6 to 7 week period (July to August).

No fungicides were applied during the growing season.

Most of the genotypes (~80%) within the population were at harvest maturity during a three-week period in August and fruit from these genotypes were evaluated during this time.

FRUIT EVALUATION

Harvested fruit were stored at 4ºC, for 2-4 days, until the day of the assay.

1. Stored fruit were warmed to room temperature for 24 h prior to inoculation.
2. Fruit were surface sterilized for 30 sec by immersion in 10% bleach (0.6% NaOCl), rinsed in deionized water, and dried.
3. Approximately 20 unblemished fruit of each genotype were placed in humidified plastic containers (30.5 cm x 22.9 cm x 10.2 cm, Model 295C; Pioneer Plastics, Dixon, KY) with fruit tray liners (M-24B; FDS Manufacturing Co., Inc., Pomona, CA).
4. The number of fruit tested varied depending upon the availability of fruit for each genotype, but typically a minimum of 20 fruit per genotype were inoculated (without injury) and evaluated for each trial.

FRUIT INOCULATION

1. Each fruit was inoculated with a 10 µL droplet (non-wounded) containing conidia of *M. fructicola*

a. Mix of isolates including MUK-1; [12]) at a concentration of 2.5 x 10^4 spores per mL from 7 to 10-day-old cultures maintained for a maximum of 4 weeks on V-8 juice agar (revive new clones every 4 weeks from storage.

2. Controls were treated with a 10 µL droplet of water.
    a. All controls scored as 0 (no lesions).
    b. In addition to inoculations of non-wounded fruit (i.e., intact cuticle), parallel inoculations of wounded fruit were made by applying a 10 µL droplet of inoculum to a wound created by breaching the cuticle with a 22 gauge needle to generate a small hole to a depth of 2 mm (wounded).
    c. Controls were fruit with the wounds treated with a 10 µL droplet of water.

ASSESSING SUSCEPTIBILITY

1. Lesion diameter (mm) was recorded **3 days after inoculation** and incubation of the peaches in the humidified containers at room temperature (22±1°C).
    a. 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 (disease incidence).

2. Standard cultivars (e.g., 'Dr. Davis', 'Loadel', 'Ross', or 'Carson') that are susceptible to brown rot were included each week, depending on their maturity and availability, to insure the presence of a positive control for each week's disease assay.

3. Many genotypes within the population proved to be susceptible, especially after wound inoculation of the fruit, further affirming viability and pathogenicity of the inoculum. The data were collated and statistically analyzed using Microsoft Excel and JMP software version 7.0.

4. Fruit color determinations, as an estimate of peach maturity were made immediately prior to inoculation. The method used throughout this study is a standard method we have used within the breeding program, which utilizes a handheld spectrophotometer (Konica-Minolta CM700) to measure peel color (which is highly correlated with flesh color in processing peaches).

5. Transmittance values for the visible spectrum (400-700 nm) were collected for each fruit and recorded. In addition, color photographs were taken with a digital camera for each genotype evaluated at the end of the disease assay and catalogued, providing a record for each individual fruit.