California Cling Peach Advisory Board

2011 Final Report

Project Title:	Brown rot resistance assessment in advanced peach selections/ Fruit surface factors affecting susceptibility of stone fruits to pathogens
Project Leaders:	Richard Bostock & Tom Gradziel
Cooperating Personnel:	Mohammad Yaghmour, Tatiana Roubtsova, Dan Parfitt, Carlos Crisosto, Pedro Martinez Garcia, Mary Ann Thorpe
Location:	Departments of Plant Pathology and of Plant Sciences, University of California, Davis

Summary:

A collection of 172 peach genotypes were evaluated for resistance to brown rot disease caused by *Monilinia fructicola* in laboratory assays of harvest mature fruit. Mean lesion diameters and incidence (proportion of infected fruit) were determined in inoculated fruit for each genotype, and from these values disease severity values were calculated. Fruit color, an indicator of quality and approximate maturity, also was estimated by color image analysis. We continue to include in these ongoing evaluations new genotypes and advanced lines with heritage from peach x almond hybrids, cv. Bolinha and USDA lines carried forward from previous years. Several QTLs associated with fruit brown rot resistance were identified by preliminary bioinformatic analyses that integrate the disease assay results from 2007-2009 of Pop-DF, a 'Dr. Davis' × 'F8,1-42' population segregating for brown rot resistance, with a SNP linkage map to the peach (cv. Loadel) haploid reference genome. In addition, the epiphytic bacterium *Pantoea agglomerans*, engineered to express a reporter gene in the presence of an appropriate chemical stimulus, called a bioreporter, was used to monitor surface sugars on peach and nectarine fruit following different treatments. We observed that fructose, the major fruit sugar, is present in a heterogeneous pattern on fruit surfaces. Also, comparison of brown rot resistant and susceptible genotypes at two maturity stages indicates significant differences in fructose availability among genotypes, as well as between intact fruit and fruit with micro-wounds. Bioreporters will provide an important adjunct to traditional chemical methods to detect differences in surface fructose and other chemistries that could influence fungal infection and fruit susceptibility, and thus improve our understanding of factors that could be targeted to improve resistance or to enhance the efficacy of biopesticides. Collectively, these findings are advancing our ongoing efforts to develop cultivars with improved fruit chemistry and disease resistance.

Objectives:

The primary objective of this research is to support the UC Davis cling peach breeding program by helping identify the most promising experimental selections that possess the desired characteristics of disease resistance and horticultural traits for subsequent multiplication and distribution in test orchards. A second objective is to identify genetic markers for brown rot resistance that can be used to facilitate the rapid selection of promising genotypes and to monitor for the presence of those markers as

genotypes progress through the breeding program. Related to these efforts are studies of the infection biology of the brown rot pathogen, *Monilinia fructicola*, with a current focus on fructoplane chemistry. We are using and developing bioreporters to prospect the fruit surface to understand the chemical factors that may influence the pathogen during the very earliest stages of infection.

Overview of 2011 Research

Evaluation of new genotypes and breeding selections. One hundred and seventy-two genotypes were evaluated for the period beginning 12 July to 20 September 2011. As in previous years, fruit of similar maturity were selected based on visual inspection of size and color from among the experimental lines. These were compared with fruit of similar maturity from commercial susceptible or moderately resistant clingstone peach cultivars. This year only the non-wounded inoculation format was used, which entails applying a droplet containing conidia (spores) of *M. fructicola* directly on the intact peach surface with a pipette. This provides an assessment of the epidermal and cuticular resistance of the fruit to direct penetration by the pathogen. Most of our previous work has focused on the epidermal resistance, in part because of the heritage (i.e., from Bolinha which has a strong epidermal resistance) and heritability of this trait in the breeding program.

Figure 1 graphically represents the disease severity rankings of the genotypes evaluated during 2011, in order from the most resistant (lowest disease severity) to the most susceptible (highest disease severity). The genotypes evaluated this season were new lines or materials brought forward from the previous seasons, but also included susceptible commercial standards for comparison (e.g., Dr. Davis, Ross, Carson). These data are also presented in a more detailed fashion in **Table 2, Appendix**. Of these, 102 genotypes, or 59%, had average lesion sizes less than or equal to 3 mm, which we consider to be resistant. **Figure 2** shows a highly resistant genotype and a susceptible genotype 72 hr after inoculation.

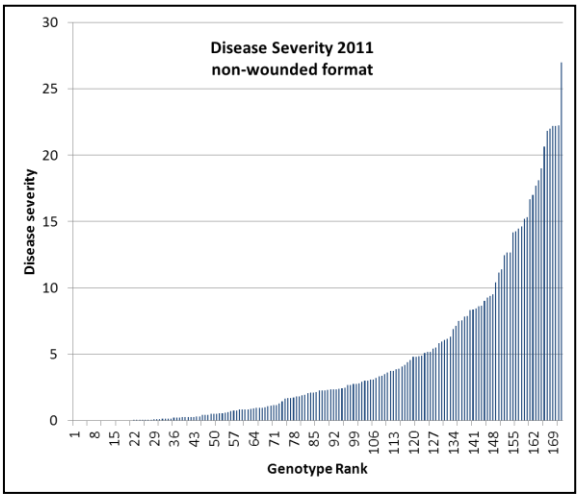


Fig. 1. Disease severity values of new genotypes evaluated in 2011, from the most resistant to most susceptible. Rankings for 172 individual genotypes are presented.

Fig. 2. Representative reactions of highly resistant (left, 08,13-154) and susceptible (right, Ross) genotypes from 2011 analyses. Photographs taken 72 hr after inoculation. Brown rot lesions (discolored fruit tissue) radiate out from the point of inoculation and are evident in the fruit pictured on the right.

08,13-154 Ross

***Analysis of the Pop-DF population for genetic markers*.** As part of a program to develop predictive tools for brown rot resistance in peach and nectarines, we previously had evaluated progeny lines from two mapping populations (Ogundiwin et al., 2008). One of these populations for which we have the most complete data, Pop-DF (aka 'Pop-BR1'), was derived from the cross 'Dr. Davis' × 'F8,1-42', the latter having disease resistance heritage from almond. The disease assay results from these evaluations are consistent with quantitative (polygenic) inheritance of the fruit resistance phenotype. In 2009, a series of genotypes were identified after 3 consecutive seasons of evaluation as showing consistently either high resistance or high susceptibility. Because of interruption in staffing, we temporarily stopped the analysis of DNA polymorphisms associated with brown rot resistance at that time. However, recently with the assistance of Dr. Pedro Martinez Garcia, we were able to conduct a preliminary analysis of the data, with very encouraging results. An excellent SNP (single nucleotide polymorphism) linkage map between Pop-DF and the peach (cv. Loadel) haploid reference genome has been constructed (Parfitt, Crisosto and Gradziel). From the data collected in the Pop-DF analyses, there appear to be two quantitative trait loci (QTLs) associated with the brown rot resistance phenotype on linkage group 1 (**Fig. 3**) and one QTL on linkage group 3 (not shown). The linkage map and genome resources will enable us to further assess, identify and refine any associations between SNP markers and disease resistance based on the Pop-DF disease assay results. We anticipate that the bioinformatic analyses will also enable us to identify additional QTL's if present, resistance gene analogs and, possibly, candidate genes with ascribed functions for linkage analysis. We anticipate we will have a clearer assessment of the strength of the molecular data in early 2012.

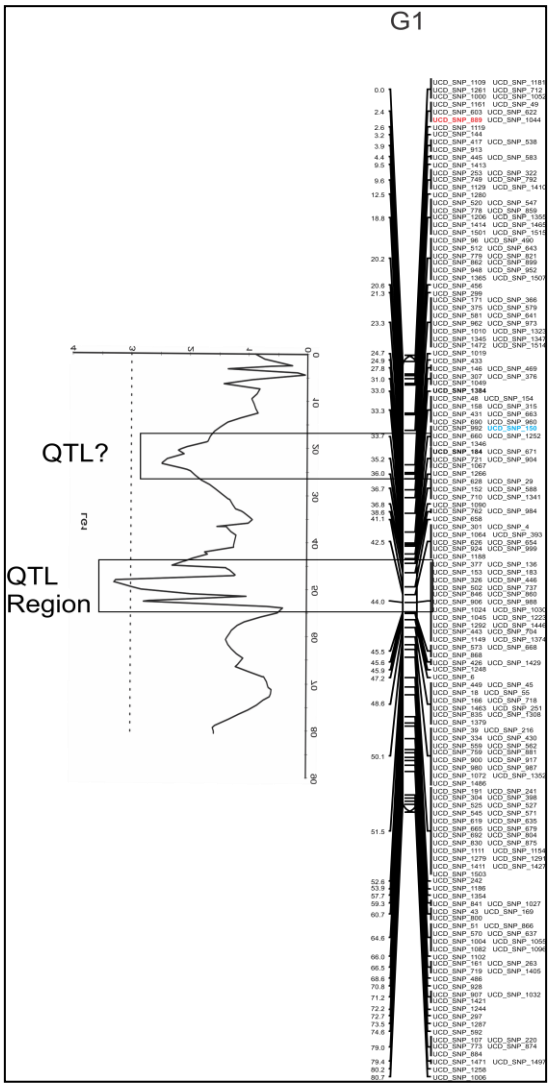


Fig. 3. Preliminary associations of QTL regions within linkage group 1 of the Loadel haploid reference genome based on the Pop-DF brown rot evaluations.

***Chemical prospecting of the fruit surface with bioreporters*.** Stone fruits, such as peaches and nectarines, become increasingly susceptible to pathogens as they mature and ripen. Associated with this increased susceptibility are structural changes in the fruit surface, which includes thinning of the cuticle, as well as changes in fruit surface chemistry, such as production of sugars and a decline of certain

phenolic compounds. Also, there are significant differences among different varieties in their susceptibility to postharvest diseases. For example, a white flesh cultivar, 'Bright Pearl', develops smaller lesions and is considered more resistant to sour rot (*Geotrichum candidum*) than the yellow flesh cultivar, 'May Grand'. Gil et al. (2002) compared fruit peel and flesh chemistry in yellow and white flesh cultivars and found significantly higher levels of phenolic compounds in some cultivars, such as 'Snow King' and 'Bright Pearl'. These cultivars were tested for their susceptibility to sour rot and found to be more resistant than Spring Lady, which had significantly lower amounts of phenols. In addition, titratable acidity was higher in yellow flesh than in white flesh peach cultivars. We have observed similar trends in fruit peel phenols among processing peach genotypes that differ in their resistance to the brown rot pathogen (Lee and Bostock, 2007). Prusky (1996) and Prusky and Lichter (2007) have reviewed pathogen quiescence in post-harvest diseases and discuss how fruit factors such as high acidity and phenols in unripe fruits can contribute to disease resistance.

Pathogens such as *M. fructicola* respond to these changes by expressing genes and proteins that are important for the pathogen to successfully infect the fruit (Lee et al., 2010). We are interested in ripening-associated changes in fruit surface chemistry that may contribute to this increased susceptibility. Of particular interest are changes in sugars, pH and redox chemicals (antioxidants and pro-oxidants), as these are known factors that can influence the expression of pathogenicity factors by fungi such as plant cell wall degrading enzymes. There is a strong desire within the industry and among consumers to reduce the use of chemical fungicides, as well as concern about the development of fungicide-resistance in pathogen populations (Adaskaveg et al., 2005; Ma and Michailides, 2005). Due to this perception, alternative methods such as biocontrol or biopesticides could be useful. Competitive exclusion is one mechanism of action for biocontrol, whereby the biocontrol agent competes with the pathogen for nutrients and space (Janisiewicz and Korsten, 2002; Janisiewicz and Buyer, 2010). However, providing evidence for this and other postulated mechanisms for biocontrol has been difficult due to the absence of experimental methods with sufficient resolution to study the microbial ecology and chemical dynamics of the fruit surface.

Although previous studies in our lab have examined some of these changes by analysis of fruit tissue extracts, bioreporters have the potential to visualize chemical changes on the fruit surface with unprecedented spatial clarity. Bioreporters are epiphytic bacteria that have been engineered to express a reporter gene in the presence of an appropriate chemical stimulus (Mercier and Lindow, 2000; Miller et al., 2001). During the summer of 2010 we conducted a proof-of-concept study to see whether bioreporters can be used to sense and visualize changes in sugars on the surface of peach and nectarine fruit following different treatments. The epiphytic bioreporter *EH299R-fruB* (*Pantoea agglomerans*) with *gfp* (encoding green fluorescence protein) under control of the *E. coli fruB* promoter (Leveau and Lindow, 2001) was used to evaluate fructose. The bioreporter *EH299R-nptII* with *gfp* under the control of the promoter *nptII* was used as a fructose nonresponsive, GFP-positive control. These preliminary experiments demonstrated that the *EH299R* strains correctly respond to the presence or absence of fructose, and that fructose is, in fact, present in a heterogeneous pattern on the surfaces of peach and nectarine fruit. In addition, these results indicated that *EH299R-fruB* may be useful for obtaining quantitative data on chemical differences in surface chemistry that can influence disease development and for studying competitive interactions between microbes by providing a sensitive means to determine which nutrients are available, and when and where they become available.

EH299R-fruB detected differences in surface fructose among genotypes (**Fig. 4, Fig. 5**). When *EH*299R-*fruB* is incubated in the absence of fructose, the bacteria barely express GFP (baseline expression). GFP is expressed by this strain in response to fructose in the medium, in a concentration dependent manner (data not shown). Likewise, in the presence of fruit surface washes, GFP is strongly induced in *EH299R-fruB*, indicating the presence of fructose in the washings. **Figure 4** illustrates how the bacteria detected more fructose on fruit of the brown rot susceptible 'Dr. Davis' relative to the brown rot resistant 'F8, 1-42'. Fructose was detected on the fruit surface of all genotypes, regardless of whether fruit were wounded or non-wounded. There was significant effect of genotype, ripeness, and wounding on the availability of fructose on fruit surfaces (**Table 1**). In addition, there was an interaction between fruit genotype and ripeness, and significant interaction between ripeness and wounding.

In general, when comparing the four peach genotypes, fructose availability was significantly higher on the surface of susceptible genotypes relative to a highly resistant genotype (cv. 'Bolinha'; **Fig. 5**). Although fructose content on the fruit surface of 'F8, 1-42' trended lower than susceptible genotypes and higher than 'Bolinha', the 'F8,1-42' differences were not statistically significant from the other genotypes. The role of cuticle architecture and other factors that may account for genotype and maturity differences in fruit surface chemistry will require further investigation. These experiments will be repeated in 2012 and the data from two years should help resolve these relationships.

Nectarine fruit preconditioned for 24 h at 22°C and 99% humidity, a practice used by the industry to promote ripening and minimize internal breakdown, revealed the presence of fructose in the washings of the fruit surface after the treatment (**Fig. 6**). When the same fruits were washed at the same location at 48 h after the start of preconditioning, fructose levels in the washings as detected by the bioreporter were comparable to the first wash. This suggests that fructose continues to be exuded to the fruit surface during this incubation. The experiment was designed to sample more time points during a five-day period; however, latent brown rot infections that emerged among these fungicide-free experimental fruit precluded extending the evaluations beyond the first 48 h.

Our results indicate the impact of several factors on the availability of fructose on peach and nectarine fruit. The next step is to translate the fluorescence data gathered by flow cytometry into a more quantitative measure of surface fructose concentrations and relate that information to fruit susceptibility or resistance to *M. fructicola*.

Future plans

An ongoing goal of the program is to identify the most promising early and late maturing genotypes, since these are often the most vulnerable to brown rot disease and can present a difficult challenge for disease management. A few of these are now in regional trials and coming into fruit-bearing maturity, thus an assessment of their performance in the field is now possible. An immediate goal for next year is to complete the molecular marker analysis for brown rot resistance, and to further develop the bioreporter system for studying fruit surface chemistry in relation to disease phenotypes.

Materials and Methods

***Disease Assays*.** Disease assays were performed as described in previous reports. Briefly, freshly harvested fruit, selected at random from trees at the UC Davis Pomology Orchards, were stored at 4°C, usually 4 days to as much as 2 weeks in a few cases, until the day of the assay. Stored fruit were

warmed to room temperature prior to inoculation. Fruit were surface sterilized for 30 sec by immersion in 10% bleach (0.6% NaOCl), rinsed, and dried.

Unblemished fruit of each genotype were placed in humidified plastic containers with fruit liners. Approximately 20-40 fruit per genotype were prepared, with the number varying depending upon the availability of fruit for that genotype and whether both inoculation formats were to be used. Each fruit was inoculated with a 10 μL droplet containing conidia of *Monilinia fructicola* at a concentration of 2.5 x 10^4 spores per mL from 7 to 10-day-old cultures maintained on V-8 juice agar. Controls included fruit treated with a droplet of water. Lesion diameter (mm) was recorded 3 days after inoculation and incubation of the peaches in the humidified containers at room temperature (22 ± 1°C). Disease severity for each genotype was calculated as the product of the average lesion diameter X proportion of symptomatic fruit (disease incidence). The data were collated and analyzed using Microsoft Excel.

Fruit color determinations. Fruit color determinations as a measure of peach maturity were made using a standard method we have used in the past, which utilizes a hand-held spectrophotometer (Minolta) that assays peel color as a measure of maturity. In addition, color photographs at the end of the disease assay were taken with a digital camera for each genotype evaluated.

Bioreporters and fruit surface chemistry - The recombinant bacterial bioreporter *EH*299R-*fruB* (*Pantoea agglomerans*) expressing the green fluorescent protein (GFP) under control of the fructose-responsive *fruB* promoter (Leveau and Lindow, 2001) was used to evaluate fructose availability in peach and nectarine fruit in the laboratory. *EH299R-nptII* with GFP constitutively expressed (i.e. always on) under the control of the promoter *nptII* was used as a fructose-nonresponsive, GFP-positive control. Bacteria were grown in LB broth with kanamycin (LB-Kan) for 18 hr. An aliquot of each strain was then transferred M9 liquid minimal media to make a dilution of 1:100, which was incubated for 4 hr. This step was then followed by incubating the bacteria in M9 liquid media containing different concentrations of fructose.

Bacteria also were incubated with fruit surface washings. Four peach genotypes were selected for these experiments: two brown rot susceptible cultivars ('Loadel' and 'Dr. Davis'), and two brown rot resistant genotypes (cv. 'Bolinha' and 'F8, 1-42'). Peach fruit were harvested at two stages, stage II (green) and stage III (ripe). Fruit were placed in humidified plastic crispers and incubated at 22°C and approximately 99% humidity. Two 1-cm square areas were designated on each fruit cheek. One square was wounded with a sterilized razor blade to create a wound that just breaks the skin to resemble a microwound (fissure). The other square was left unwounded. Each square was "washed" by applying a 100 μl droplet of water to the surface. Fruit washes were filter-sterilized by passage through 0.2 μm filters. The filters were washed with additional 900 μl sterile DI water. Fruit surface washes were stored at -20°C until processing. To assay for the presence of fructose in the fruit washings, *EH*299R-*fruB* cells were incubated with an aliquot of the fruit washings. After 4 hours of incubation, the bacterial cells were processed by flow cytometry to detect and quantify cells responding to fructose by the fluorescence of GFP. The machine measures the fluorescence of 50,000 bacteria, and thus each determination represents the average of 50,000 readings per fruit. Each fruit was treated as a replicate, with four replicates per experiment. Each experiment was performed twice.

To examine the effect of preconditioning and ripening on fructose availability the on fruit surface, nectarine fruit (cv. 'Late Le Grande') were collected at the maturity stages II and III. The fruit were incubated in humidified plastic containers and incubated at 22°C for 24 hours. Fruit then received the same treatments and procedures to measure fructose as described above. The same fruit surface was washed at two time points, after 24 and 48 hr of preconditioning.

Acknowledgments

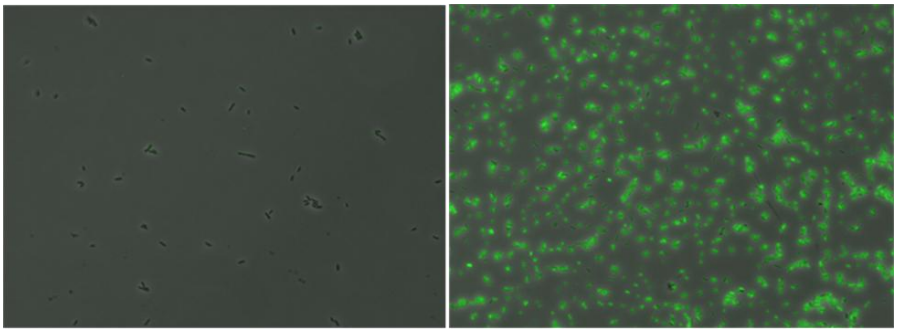
In addition to the cooperators indicated on page 1 of this report, we thank Ik Joon Jo and Tivonne Nguyen for their assistance in this study. Research supported by grants from the California Cling Peach Growers Advisory Board.

Table 1. Mixed model analysis of the effect of different factors on fructose availability on the peach fruit surface**.**

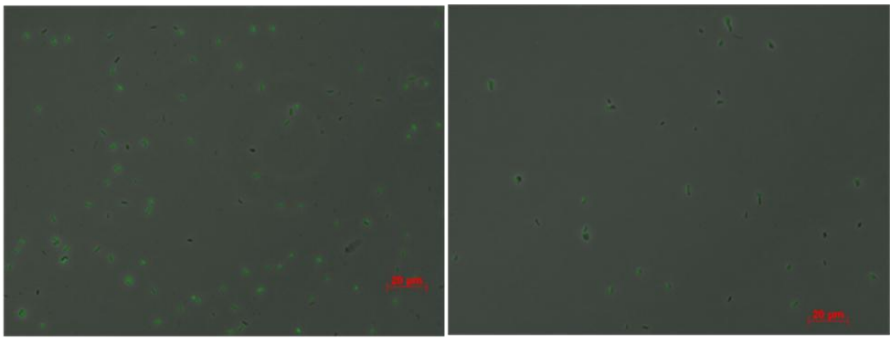
Source (Effect)	***P***
Genotype	0.01*
Ripeness	<0.0001*
Wounding	<0.0001*
Genotype × Ripeness	<0.0001*
Genotype × Wounding	0.22
Ripeness × Wounding	0.05*
Genotype ×Ripeness × Wounding	0.61

* Indicates level of significance

Fig. 4. Cells of *Pantoea agglomerans* strains (*EH299R*) visualized by fluorescence microscopy. When the reporter gene is under control of the *nptII* promoter (nptII), the cells express the green fluorescent protein (GFP) with or without fructose, which serves as a positive control. When the reporter gene is under control of the fructose-inducible promoter (fruB), the cells only express GFP in the presence of fructose. The figures indicate that fruit washings from the surfaces of 'Dr. Davis' and 'F8, 1-42' contain levels of fructose above the threshold to induce GFP in *fruB* bioreporter cells.

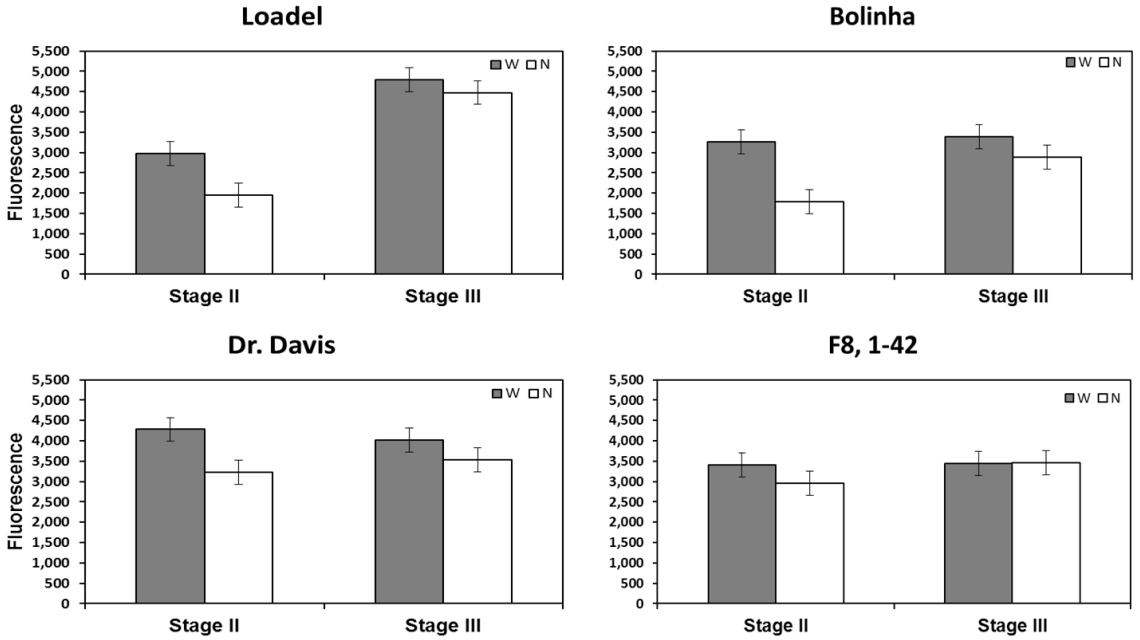


fruB- 0 nm Fructose nptII- 0 nm Fructose



Dr. Davis (Fruit surface wash-NW) F8, 1-42 (Fruit surface wash-NW)

Fig. 5. Fluorescence of *Pantoea agglomerans EH*299R-*fruB* cells by flow cytometry as a relative measure of fructose concentrations in fruit surface washes from wounded and non-wounded fruit as a function of host genotype and fruit maturity stage. Stage III fruit of 'Loadel' and 'Dr. Davis' are brown rot susceptible; 'Bolinha' and 'F8, 1-42' are brown rot resistant. Mean and SEM are indicated.



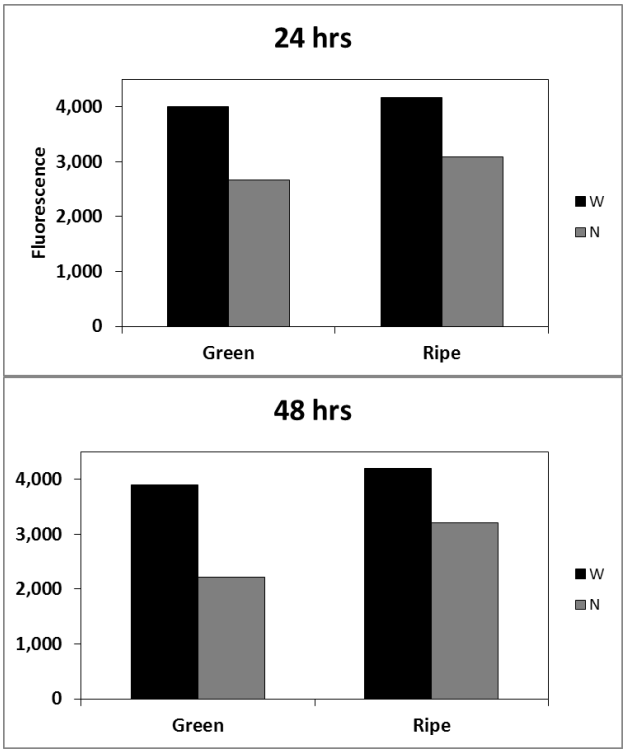


Fig. 6. Fluorescence of *Pantoea agglomerans EH*299R-*fruB* cells in the presence of fruit surface washes of nectarine (cv. Late Le Grande) after 24 and 48 hours incubation at 22°C and 99% humidity. Fruit washes were collected on the same fruit surface at the same sampling position at each time point.

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Appendix

Table 2 below contains a listing in order of the most brown rot resistant to most brown rot susceptible peach genotypes that were evaluated during 2011 in the nonwounded format for the new and carry forward selections. Mean lesion diameters and standard deviations (SD), disease incidence (proportion of fruit infected), and disease severity (lesion diameter x incidence) for each genotype are presented. Harvest dates are indicated. Peaches were evaluated for resistance soon after harvest, according to the following schedule: group A, July 12; B, July 19; C, July 26; D, Aug 2; E, Aug 9; F, Aug 16; G, Aug 23; H, Aug 30; I, Sept 6; J, Sept 13; K, Sept 20.

Appendix - Table 2

Genotype	Harvest Date	Mean lesion diameter (mm)	SD	Incidence (lesion>3mm)	Disease Severity
05, 17-89	18-Aug	0.0	0.0	0.00	0.0
Carolyn G/ PG 2-22+23	17-Aug	0.0	0.0	0.83	0.0
07, 11-249	25-Aug	0.0	0.0	0.00	0.0
05, 18-171	25-Aug	0.0	0.0	0.00	0.0
05,16-152	1-Sep	0.0	0.0	0.00	0.0
05,17-140	7-Sep	0.0	0.0	0.00	0.0
00,16-133	8-Sep	0.0	0.0	0.00	0.0
05,16-156	7-Sep	0.0	0.0	0.00	0.0
05,16-164	7-Sep	0.0	0.0	0.00	0.0
2007,12-225	15-Sep	0.0	0.0	0.00	0.0
2007,12-187	15-Sep	0.0	0.0	0.00	0.0
2007,12-175	15-Sep	0.0	0.0	0.00	0.0
2007,12-180	15-Sep	0.0	0.0	0.00	0.0
2007,12-227	15-Sep	0.0	0.0	0.00	0.0
2007,12-173	15-Sep	0.0	0.0	0.00	0.0
2007,12-206	15-Sep	0.0	0.0	0.00	0.0
2007,12-146	15-Sep	0.0	0.0	0.00	0.0
2007,12-134	15-Sep	0.0	0.0	0.00	0.0
2007,12-190	15-Sep	0.0	0.0	0.00	0.0
2007,12-164	15-Sep	0.0	0.0	0.00	0.0
99, 4-123/ NSW5-20+21	17-Aug	0.2	12.0	0.05	0.0
Carson/PG 1-20+21	13-Jul	0.3	1.2	0.05	0.0
LorriMag/PG 10-24+25	13-Jul	0.5	2.1	0.05	0.0
98, 4-177/ NSW5-16+17	17-Aug	0.8	0.0	0.07	0.1
05, 19-40	1-Sep	0.6	1.9	0.10	0.1
2007,12-191	15-Sep	0.6	1.7	0.11	0.1
2007,12-157	15-Sep	0.5	1.4	0.13	0.1
2007,12-231	15-Sep	1.5	6.4	0.06	0.1
89, 9-82/ PG 4-27a+28	17-Aug	0.9	9.3	0.10	0.1
2007,12-203	15-Sep	0.7	1.9	0.14	0.1
NJC 83/PG 5-15	13-Jul	1.1	3.7	0.11	0.1
99,15-99	14-Sep	1.0	2.5	0.15	0.2
08, 13-154	25-Aug	1.6	5.0	0.10	0.2
Lillard/EN6-27/PG 8-12+13	31-Aug	0.8	1.8	0.20	0.2
00,16-119	14-Sep	0.7	2.0	0.30	0.2
NSC 86/PG 5-16	7-Jul	1.4	3.6	0.15	0.2
Bol Q/ PG1-29	25-Aug	2.8	9.8	0.08	0.2
05, 16-192	1-Sep	1.2	2.7	0.20	0.2
2007,12-189	15-Sep	1.5	3.7	0.17	0.3
05, 17-96	18-Aug	1.7	4.0	0.15	0.3
2007,12-214	15-Sep	1.3	3.0	0.20	0.3
05,29-95	1-Sep	1.4	3.1	0.20	0.3
2007,12-234	15-Sep	2.3	6.4	0.13	0.3
97,3-16/NSW 4-11	7-Jul	2.0	5.5	0.15	0.3
2007,12-167	15-Sep	1.8	4.5	0.17	0.3
98, 2-161/PG 5-25	13-Jul	2.6	2.9	0.16	0.4
05, 17-69	1-Sep	1.3	2.2	0.33	0.4
D,62-193/PG 8-6+7	7-Jul	2.1	4.6	0.21	0.4
08,13-188	7-Sep	1.7	2.9	0.31	0.5
92, 13-67/ NSW 2-24	24-Aug	1.8	2.6	0.30	0.5
08, 13-178	1-Sep	3.2	7.8	0.17	0.5
2007,12-151	15-Sep	2.4	5.5	0.22	0.5
05, 17-36	18-Aug	2.0	3.8	0.29	0.6
05,18-100	7-Sep	2.5	4.6	0.25	0.6
H,6-55/Reigel/PG 2-10+11	31-Aug	3.6	8.1	0.20	0.7
92, 14-73/ NSW2-27	24-Aug	2.5	4.4	0.30	0.8
Diamante NSW 3-41+42	20-Jul	3.1	7.2	0.25	0.8

Appendix - Table 2

Genotype	Harvest Date	Mean lesion diameter (mm)	SD	Incidence (lesion>3mm)	Disease Severity
2007,12-170	15-Sep	2.5	4.5	0.33	0.8
05,19-139	7-Sep	2.5	4.2	0.33	0.8
90,10-162/NSW 2-2->5	7-Jul	2.7	4.3	0.32	0.8
Sherman/PG 1-26+269	7-Jul	2.9	5.1	0.30	0.9
99, 16-131/ NSW 5-32	24-Aug	2.6	4.1	0.35	0.9
2007,12-132	15-Sep	2.3	2.9	0.40	0.9
05, 16-160	1-Sep	4.8	10.7	0.20	1.0
94,7-172	14-Sep	2.4	4.4	0.40	1.0
97, 2-152	20-Jul	3.4	5.9	0.30	1.0
00, 8-164/NSW 6-30+31	24-Aug	3.2	5.8	0.33	1.1
Hesse/NSW4-26+27	31-Aug	5.6	12.5	0.20	1.1
08, 13-176	25-Aug	3.4	6.2	0.33	1.1
E, 217/PG 8-11	27-Jul	2.6	7.1	0.44	1.2
00,17-265	14-Sep	3.2	5.4	0.40	1.3
00,9-129	14-Sep	2.9	4.1	0.50	1.4
05,17-57	25-Aug	2.8	3.2	0.60	1.7
Kakamas	7-Sep	2.8	2.6	0.60	1.7
92, 11-57/Nsw 1-33 (compact)	10-Aug	5.1	9.3	0.33	1.7
97,4-32/NSW4-16+17	31-Aug	3.5	5.0	0.50	1.8
96, 8-192/ NSW 5-12+13	24-Aug	4.5	7.3	0.40	1.8
87,12-28	14-Sep	4.1	5.1	0.45	1.8
92, 7-25/NSW 1-31	10-Aug	4.1	5.6	0.47	1.9
97,7-79/NSW4-22+23	31-Aug	7.4	11.0	0.26	1.9
98,9-7/NSW5-18+19	31-Aug	4.1	5.0	0.50	2.1
2007,12-200	15-Sep	4.2	5.8	0.50	2.1
98,16-156/NSW 5-4	7-Jul	5.3	7.6	0.40	2.1
00, 2-18/NSW 6-22+23	10-Aug	4.5	5.9	0.47	2.1
05, 17-129	18-Aug	3.0	2.7	0.75	2.3
2007,12-140	15-Sep	4.5	5.0	0.50	2.3
91,17-195	14-Sep	4.1	5.7	0.55	2.3
NJC 123/PG 5-8	27-Jul	5.8	3.4	0.40	2.3
05, 16-147	18-Aug	7.0	12.1	0.33	2.3
Lovell/FPS 8+9	25-Aug	6.3	9.3	0.38	2.3
08, 13-190	1-Sep	5.3	4.5	0.44	2.4
F8,1-42	7-Sep	4.3	5.7	0.55	2.4
08, 3-168	25-Aug	7.3	12.4	0.33	2.4
F8,5-166	14-Sep	4.5	3.7	0.56	2.5
00, B-202/NSW 6-14+15	10-Aug	6.0	9.2	0.44	2.7
00,8-150/NSW6-26+27	31-Aug	6.1	7.7	0.44	2.7
92, 13-62/ NSW 1-35	24-Aug	4.9	5.8	0.56	2.7
F8,5-147	14-Sep	5.1	6.4	0.55	2.8
PP 19, 4-40	20-Jul	5.6	6.6	0.50	2.8
E,5-43/PG 8-8+9	13-Jul	4.3	4.7	0.69	2.9
Halford/ PG 2-14+15	31-Aug	5.0	5.8	0.60	3.0
E22-59	7-Sep	4.0	2.9	0.75	3.0
05, 17-216	18-Aug	6.2	8.3	0.50	3.1
2005,11-66	15-Sep	7.4	6.7	0.42	3.1
Carson PG 1-20+21	20-Jul	7.1	10.4	0.45	3.2
9-20 C	20-Jul	8.1	3.6	0.41	3.3
00,8-153/NSW6-28+29	31-Aug	6.8	7.8	0.50	3.4
91, 9-161/NSW 2-19-->23	10-Aug	7.5	9.5	0.47	3.5
Everts/ PG 2-16+17	31-Aug	9.0	12.5	0.40	3.6
91, 12-4/NSW 4-37	10-Aug	7.5	9.0	0.50	3.7
05, 16-170	25-Aug	9.4	13.5	0.40	3.8
91,17-262	7-Sep	6.5	6.9	0.60	3.9
05, 17-130	18-Aug	6.3	6.3	0.63	3.9

Appendix - Table 2

Genotype	Harvest Date	Mean lesion diameter (mm)	SD	Incidence (lesion>3mm)	Disease Severity
2007,12-131	15-Sep	6.5	8.9	0.63	4.1
PP 97, 2-208	20-Jul	8.4	8.2	0.50	4.2
92,14-73	7-Sep	5.9	5.7	0.75	4.4
05, 10-236	25-Aug	6.5	6.1	0.70	4.6
96, 3-153/NSW4-4+5	27-Jul	8.7	9.0	0.55	4.8
01, 2-85/NSW 6-48+49	27-Jul	7.0	7.1	0.69	4.8
98,4-24/NSW5-ATB	31-Aug	9.1	9.4	0.53	4.8
05,18-148	7-Sep	7.3	6.4	0.67	4.9
08, 13-179	25-Aug	8.5	8.2	0.60	5.1
00,16-125	14-Sep	6.9	6.4	0.75	5.2
91, 12-54/ NSW 2-11-->18	3-Aug	8.7	8.4	0.60	5.2
F8,5-156	14-Sep	8.1	8.3	0.67	5.4
99,19-231	14-Sep	8.5	7.8	0.65	5.5
96,9-229	8-Sep	10.5	10.8	0.56	5.8
96,8-190/ NSW 5-10+11	3-Aug	10.0	10.4	0.60	6.0
08,29-138	7-Sep	10.4	11.9	0.58	6.1
F8,5-159	14-Sep	7.9	7.4	0.78	6.2
NJC 133/PG 5-9	13-Jul	9.8	9.7	0.65	6.3
92, 14-6/ NSW 2-25+26	3-Aug	8.2	6.4	0.84	6.9
99, 12-155/NSW 5-6+7	27-Jul	11.0	9.5	0.65	7.2
05, 17-54	18-Aug	10.0	8.7	0.75	7.5
00, 2-16/ NSW 6-20+21	31-Aug	8.9	7.7	0.85	7.6
90, 9-134/ NSW 2-7-->10	3-Aug	11.4	11.2	0.68	7.8
Carson/PG 1-20+21	27-Jul	10.5	6.1	0.75	7.9
F8,5-171	14-Sep	9.7	6.9	0.86	8.3
05,11-67	8-Jul	12.9	11.5	0.65	8.4
05, 11-74	15-Jul	11.3	9.2	0.75	8.4
05,16-168	7-Sep	14.3	4.9	0.60	8.6
08, 3-180	18-Aug	10.8	7.9	0.80	8.6
TX 86606/ PG 5-18	24-Aug	10.0	6.0	0.90	9.0
18, 8-11/ Sw 21- 1-->6	25-Aug	11.6	8.5	0.80	9.3
05, 11-69	18-Aug	12.5	10.1	0.75	9.4
05, 10-240	18-Aug	9.5	0.7	1.00	9.5
97, 7-75/NSW 4-20+21	27-Jul	12.5	8.7	0.83	10.4
EN,19-30	14-Sep	12.8	9.2	0.81	10.4
Carson/PG 1-20+21	3-Aug	14.9	11.8	0.75	11.2
Novolmade/PG 12-16+17	17-Aug	11.4	6.7	1.00	11.4
05, 10-79	20-Aug	14.8	9.3	0.84	12.5
18,8-23	7-Sep	12.7	5.7	1.00	12.7
97, 3-238/ NSW 4-12+13	3-Aug	16.9	12.1	0.75	12.7
08, 3-173	25-Aug	17.0	12.8	0.83	14.2
00,8-164	7-Sep	16.3	7.1	0.88	14.3
Carson/ PG 1-20+21	3-Aug	16.3	10.0	0.89	14.5
Carson/ PG 1-20+21	10-Aug	15.9	9.0	0.92	14.6
05, 11-76	25-Aug	17.1	9.4	0.89	15.2
05, 20-11	1-Sep	15.3	11.5	1.00	15.3
05, 16-101	25-Aug	16.7	12.5	1.00	16.7
Ross/PG1-2+3	24-Aug	22.7	4.3	0.75	17.0
05, 10-73	25-Aug	18.7	9.3	0.95	17.7
91,16-154	7-Sep	18.1	6.4	1.00	18.1
08, 13-181	25-Aug	19.0	3.0	1.00	19.0
ROSS/PG1-2+3	17-Aug	25.8	9.5	0.80	20.7
05, 11-63	15-Jul	21.8	7.3	1.00	21.8
96, 1-171/ NSW 4-2+3	3-Aug	23.2	11.6	0.95	22.0
05,11-65	7-Sep	24.2	5.0	0.92	22.2
Ross/PG 1-2+3	17-Aug	23.5	10.7	0.94	22.2
Ross	24-Aug	22.2	7.6	1.00	22.2

Appendix - Table 2

Genotype	Harvest Date	Mean lesion diameter (mm)	SD	Incidence (lesion>3mm)	Disease Severity
05, 11-81	25-Aug	22.2	6.4	1.00	22.2
05, 10-174	25-Aug	27.0	7.6	1.00	27.0