DEVELOPMENT OF PREDICTIVE TOOLS FOR BROWN AND SOUR ROT RESISTANCE IN PEACH AND NECTARINES

PROJECT LEADER: Dr. Carlos Crisosto

COOPERATORS: Ebenezer Ogundiwin, Richard Bostock, David Slaughter, Tom Gradziel, Dr. Themis Michailides

SUMMARY

Postharvest brown rot (BR) caused by *Monilinia fructicola* and sour rot (SR) caused by *Geotrichum candidum* are serious diseases of peach and nectarine in California. Current disease management is primarily by pre- and postharvest application of fungicides. Resistance to both fungi was surveyed among 81 (for BR) and 34 (for SR) commercial peach and nectarine cultivars as well as a few old cultivars, landraces, and closely related accessions. Of these, 22 cultivars were tested with both fungi. A total of 204 individuals of two peach segregating populations ('Loadel' × 'UCD96,4-55' and 'Dr. Davis' × 'F8,1-42') were evaluated for genetic analysis of resistance to BR. Two methods – wounded vs. nonwounded fruit - were used for inoculations with *M. fructicola*. All SR inoculations involved wounded fruit. BR lesion size varied among cultivars and among the progeny of both populations. SR lesion size also varied among cultivars tested. Yellow fleshed cultivars were significantly less susceptible than white cultivars to BR nonwounded inoculation ($P < 0.05$) but no significant difference was observed between both colors for wound inoculation. Nectarines were significantly less susceptible to BR wound inoculation than peaches ($P < 0.01$) but no significant difference was observed between the two fruit types for nonwounded inoculation. Lesion size was determined to be under genetic control from analysis of cultivar differences. A weak but significant linear relationship was observed between wounded and nonwounded BR inoculation methods (R^2 = 6-27%; P <0.01). However, several cultivars and progeny that displayed resistance to nonwounded inoculation were susceptible to wound inoculation. This indicated that similar as well as different resistance mechanisms may be present for wounded vs. nonwounded fruit. Host resistance also varied between SR and BR as t-test analysis showed significant differences between the reactions of the cultivars to both fungi. A number of wild peach accessions and old cultivars showed a high level of resistance to BR suggesting that these may be untapped sources of resistance to the fungus. DNA has been isolated from parents and progeny of the two mapping populations. A set of *Prunus* candidate genes in the cutin, lignin, chlorogenate, and caffeic acid biosynthesis pathways as well as resistance gene analogs has been assembled. Linkage mapping and QTL analyses for BR resistance is underway.

INTRODUCTION

Two major postharvest diseases of stone fruits are brown and sour rot caused by *Monilinia fructicola* (G. Wint.) Honey, and *Geotrichum candidum* Link (Adaskaveg et al., 2005; Biggs and Northover, 1985; Byrde and Willetts, 1977; Michailides et al., 2004). Effective control of these pathogens and other postharvest diseases is by routine application of chemical fungicides (Adaskaveg et al., 2005; Margosan et al., 1997) particularly if fruit is to be stored and/or shipped long distances. However, there is increasing concern about the environmental effects and safety of chemical fungicides, and the development of fungicide-resistant postharvest fungal pathogens has been reported (Hong et al., 1998). Regulatory agencies have reacted to public pressure and introduced comprehensive legislation to reduce pesticide use (Irtwange, 2006; Karabulut and Baykal, 2003).

Host resistance to plant pathogens is perhaps the most cost effective and environmentally safe strategy for disease management. Although commercial cultivars are generally susceptible to brown rot (Ogawa et al, 1985; Cantoni et al., 1996), improved levels of resistance have been identified in some cultivars such as 'Bolinha', (Feliciano et al., 1987; Bostock et al., 1994; Gradziel et al, 2003), and two breeding lines in the breeding program of Dr. S. P. Gonzalez, Universidad Autonoma de Queretaro, Mexico. Research efforts are ongoing to breed peach cultivars resistant to brown rot. The cling-peach breeding program of Dr. Tom Gradziel (UC Davis) has incorporated sources of resistance from almond into several breeding lines (Gradziel, 2002, Gradziel et al., 2003). Bostock et al. (1999) reported that chlorogenic and caffeic acids are major phenolic acids in the epidermis and subtending cell layers of peach fruit and that their concentrations are especially high in immature fruit with a high level of resistance to brown rot and decline as fruit mature with a corresponding increase in disease susceptibility (also see Lee and Bostock, 2006). The processing canning peach breeding program of UC Davis is incorporating the epidermis-based resistance to brown rot into improved cultivars through a recurrent selection program (Gradziel et al., 2003). Augmenting traditional breeding practices with more modern molecular mapping technologies will better equip the breeder to meet the challenge of breeding sustainable resistance.

The detection of sour rot caused by *G. candidum* in peach and nectarine is relatively new (Michailides et al., 2004). In the program of Drs. Michailides and Bostock, several peach and nectarine cultivars have been observed to possess high levels of resistance to this pathogen.

The main goal of our group is to develop predictive molecular tools that peach and nectarine breeders can use to quickly develop disease resistant superior cultivars such that there will be less reliance on chemical fungicide usage. The specific objectives of this research are: 1. Determine the genetic control of resistance to brown and sour rot in peach cultivars and two cling peach progeny populations, and 2. Develop scaffold linkage maps with these populations and localize genomic regions controlling resistance with tightly linked molecular markers.

MATERIALS AND METHODS

Plant Material

Fruit were collected at commercial maturity from fields at the Kearney Agricultural Center (KAC), Parlier, UC Davis/USDA Germplasm Repository, and from organic growers. Fruit were either transported from KAC to Davis for brown rot inoculations or from Davis to KAC for sour rot inoculations. Materials sampled include canning peach and fresh market cultivars, peach and nectarine cultivars, canning peach breeding lines, segregating progeny of two mapping populations, old peach cultivars and related wild accessions. Many cultivars were obtained from multiple sources.

Inoculations and lesion size measurements

All brown rot inoculations were conducted at the Plant Pathology Department, UC Davis and all sour rot inoculations were conducted at KAC, Parlier. Prior to inoculation, fruit flesh color was measured with the nondestructive impact firmness sensor as an indicator of maturity (Slaughter et al., 2006). Fruit were surface sterilized by allowing them to sit for 30 seconds in a 10% bleach solution. They were rinsed twice by dipping them in separate buckets of clean water, and then allowed to dry on paper towels. Crispers were prepared by washing with hot soapy water and rinsing with 95% ethanol, and air drying. The bottom of the crisper was covered with 1/8 to 1/4 of an inch of water, and lined with a crisper liner. Fruit were placed in crispers with the smooth, flat side up. Inoculum of *Monilinia fructicola* (brown rot) and *Geotrichum candidum* (sour rot) spore was prepared with 25,000 spores/ml concentration. Inoculation was done by pipetting a 10 µl drop of spores onto the fruit. Controls are prepared in the same way, except sterile water was used instead of spores. Wounded inoculation was achieved by wounding the peach fruit surface with a flamed metal tool with a sharp point, and inoculating with the spores. Only wounded inoculation was carried out for sour rot. After inoculation closed crispers were covered with 2 layers of damp cheesecloth and allowed sit for 15 hours. The inoculum drops were then removed by wicking away with a Kimwipe, and the crisper lids were replaced. Three days after inoculation the lesion diameters were measured with a ruler.

Molecular analysis (ongoing)

Leaf samples were collected from all the progeny and available parents of the two mapping populations at UC Davis and transported on ice to the Molecular Lab at KAC. DNA isolation from the leaf samples was achieved through the standard CTAB method. Candidate gene sequences in the cutin, lignin, chlorogenate, and caffeic acid biosynthesis pathways were obtained from public databases such as the NCBI (http://www.ncbi.nlm.nih.gov/) and GDR (http://www.bioinfo.wsu.edu/gdr/) as well as from our ChillPeach database (http://bioinfo.ibmcp.upv.es/genomics/ChillPeachDB/login.php). Primers were designed for these candidate genes using Primer3 (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3.cgi). Survey of polymorphism among these candidate genes as well as other available molecular markers (SSRs, SRAPs & RAFs) is underway. Linkage mapping and QTL analyses will follow.

Statistical Analysis

Analysis of variance (ANOVA) was conducted on the lesion size data using the GLM procedure of SAS. Relationships between resistances to brown rot wounded and nonwounded inoculations

and between resistances to sour rot and brown rot were assessed by linear correlations and t-test, respectively.

RESULTS

A total of 81 and 34 cultivars were surveyed for resistance to brown rot and sour rot, respectively. Out of these, 24 cultivars were challenged with both fungi. Fruit collection was made from fungicide-free sources and many cultivars inoculated with brown rot were obtained from two or more sources, making a total of 123 entries. Also for brown rot, a total of 204 progeny of 'Loadel' × 'UCD96,4-55' (82 progeny) and 'Dr. Davis' × 'F8,1-42' (122 progeny) cling peach populations were inoculated with brown rot to assess segregation for resistance to the fungus. In addition 12 old cultivars and wild accessions were inoculated with brown rot for discovery of new resistance sources.

The reactions of peach and nectarine cultivars to wounded and unwounded brown rot and wounded sour rot inoculations are presented in Figure 1. Based on the distributions, five groups were identified for each fungus/inoculation method as follows: HR = highly resistant, MR = medium resistance, MS = medium susceptible, HS = highly susceptible, VHS = very highly susceptible. Lesion size was generally larger for wounded inoculations compared to non-wounded.

Relationships between resistances to brown rot wounded and nonwounded inoculations and between resistances to sour rot and brown rot are indicated in Table 1. There were small but significant correlations between wounded and nonwounded inoculations among the cultivars as well as among progeny of the two mapping populations. Significant differences were observed between brown rot and sour rot resistance reactions among the 24 cultivars inoculated (wounded inoculation) with both fungi.

Figure 2 shows the frequency distributions of the reactions of the two cling peach progeny populations to both wounded and nonwounded inoculations. These distributions indicated that both populations are segregating for resistance to the fungus.

Table 2 is the summary of ANOVA of the different reaction groupings and fruit types. Yellow fleshed cultivars were significantly more resistant to nonwounded inoculation compared to their white fleshed counterparts ($P < 0.01$), however, no significant differences were observed between the two groups for wounded inoculation. Nectarines were significantly more resistant to brown rot wounded inoculation compared to peaches ($P < 0.05$), but both fruit types reacted similarly to nonwounded inoculation. Fresh market and canning peach cultivars reacted similarly to both wounded and nonwounded inoculations, although only about 7% of all cultivars tested were canning peaches.

DISCUSSION

The reactions of various genotypes of peach and nectarine to brown rot and sour rot inoculations indicated that there is genetic resistance to these postharvest fungi. Some established cultivars showed very good resistance to the fungi under the experimental conditions used in this study. This showed that perhaps postharvest fungicide applications can be reduced or cancelled for

these cultivars. Because lesion size were larger for wounded inoculations across the board compared to nonwounded inoculations, care should be taken during harvest to minimize physical injury to the fruit to avoid cracks on the skin through which the fungi can gain entrance. Organic growers may find the information generated in this study helpful in selecting cultivars for their production. A weak but significant linear relationship was observed between wounded and nonwounded BR inoculation methods. However, several cultivars and progeny that displayed resistance to nonwounded inoculation were susceptible to wound inoculation. This indicated that similar as well as different resistance mechanisms may be present for wounded vs. nonwounded fruit. Host resistance also varied between sour rot and brown rot as t-test analysis showed significant differences between the reactions of the cultivars to both fungi. A number of wild peach accessions and old cultivars showed a high level of resistance to brown rot (results not shown) suggesting that these may be untapped sources of resistance to the fungus.

FUTURE PLANS

We will continue with the molecular marker analysis of resistance to both fungi. The scaffold linkage maps will be constructed for both populations and QTL analysis of resistance will be conducted. Markers closely linked to the resistance QTLs will be identified for use in breeding programs. With availability of funds, we will conduct a second year round of inoculation experiments on the progeny populations. This is very important for the reliability of QTL analysis because it will allow us account for non-genetic variation due to experimental errors and environmental factors. In addition, we will select representatives of each reaction groups for both wounded and nonwounded inoculations and challenge them with the fungi. A detailed quality assessment (soluble solids, TA, firmness, etc) will be carried out on this subset and related to resistance reaction.

PUBLICATION FROM THIS STUDY

Ebenezer Ogundiwin, Richard Bostock, Tom Gradziel, Themis Michailides, Mohammad Yaghmour, Dan Parfitt, and Carlos Crisosto (2007). Towards molecular genetic analysis of resistance to brown rot and sour rot in *Prunus persica*. Plant & Animal Genome Conference XVI, San Diego, 12-16 January 2007.

REFERENCES

Adaskaveg, J., Forster, H., Gubler, W.D., Teviotdale, B.L. and Thompson, D.F. 2005. Reduced-risk fungicides help manage brown rot and other fungal diseases of stone fruit. California Agriculture 59, 109-114.

Biggs, A.R., and Northover, J. 1985. Inoculum sources for Monilinia fructicola in Ontario peach orchards. Can. J. Plant Pathol. 7, 302-307.

Bostock, R.M., Adaskaveg,J. E., and Madden S. 1994. Role of cutinase in pathogenicity of the brown rot fungus, *Monilinia fructicola*. Central Valley Postharvest News 3, 16-17.

Bostock, R.M., Wilcox, S.M., Wang, G., and Adaskaveg, J. E. 1999. Suppression of *Monilinia fructicola* cutinase production by peach fruit surface phenolic acids. Physiological and Molecular Plant Pathology 54, 37-50.

Byrde, R.J.W., and Willetts, H.J. 1977. The brown rot fungi of fruit. Their biology and control. Pergamon Press, New York.

Cantoni, L., Bassi, D., and Tacconi, N. 1996. Brown rot in stone fruits, aspects of biology and techniques of selection for resistance. Riv. Frutticoltura Ortofloricoltura 58, 59-65.

Feliciano, A., Feliciano, A.J., and Ogawa, J.M. 1987. *Monolinia fructicola* resistance in peach cultivar Bolinha. Phytopathology 77, 776-780.

Gradziel, T.M. 2002. Almond species as sources of new genes for peach improvement. Acta Hort. 592, 81-88

Gradziel, T.M. 2003. Interspecific hybridizations and subsequent gene introgression within *Prunus* subgenus *Amygdalus*. Acta Hort. 622, 249-255.

Hong, C., Michailides, T.J., and Holz, B.A. 1998. Effects of wounding, inoculum density, and biological control agents on postharvest brown rot of stone fruits. Plant Disease 82, 1210-1216.

Horvat, R.J., Chapman, G.W., Jr., Robertson, J.A., Meredith, F.I., Scorza, R., Callahan, A.M., and Morgens, P. 1990. Comparison of the volatile compounds from several commercial peach cultivars. J. Agric. Food Chem. 38, 234-237.

Irtwange, S.V. 2006. Application of biological control agents in pre- and postharvest operations. Agricultural Engineering International: the CIGR Ejournal. Invited Overview No. 3. Vol. VIII, 12 p.

Karabulut, O.A., and Baykal, N. 2003. Biological control of postharvest diseases of peaches and nectarines by yeasts. J. Phytopathology 151, 130-134.

Margosan, D.A., Smilanick, J.L., and Henson, D.J. 1997. Combination of hot water and ethanol to control postharvest decay of peach and nectarines. Plant Disease 81, 1405-1409.

Michailides, T.J., Morgan, D.P., and ,Day, K.R. 2004. First Report of Sour Rot of California Peaches and Nectarines Caused by Yeasts. Plant Dis. 88:222.

Ogawa, J.M., Manji, B.T., and Sonoda, R.M. 1985. Management of the brown rot disease on stone fruits and almonds in California. In: T.J. Burr (ed.), Proc. Brown rot of stone fruit workshop. New York Agricultural Experiment Station, Geneva, New York. p. 8-15.

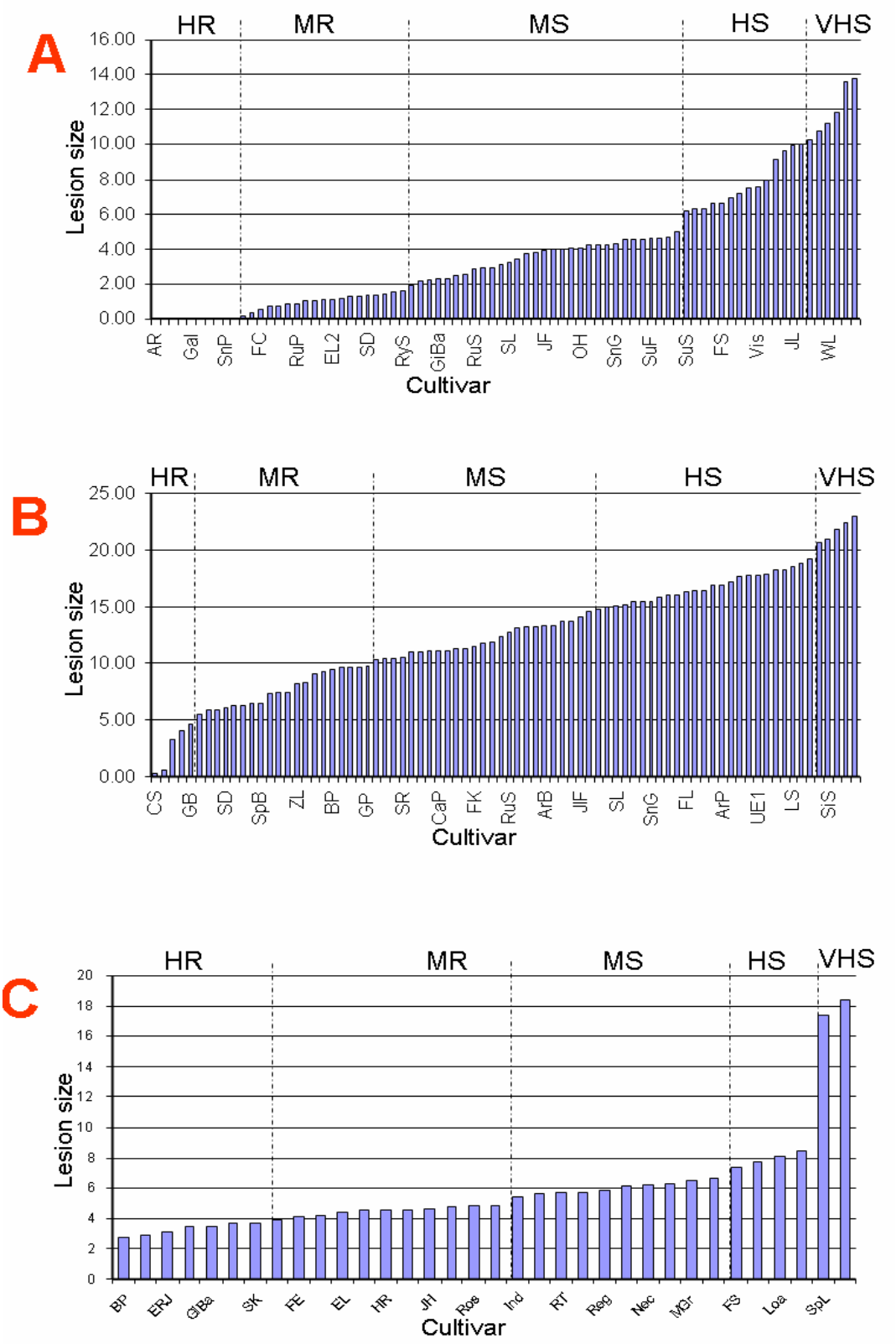

Figure 1: Reactions of peach and nectarine cultivars to brown rot and sour rot inoculations. A = Nonwounded brown rot, B = wounded brown rot inoculation, and C = sour rot wounded inoculation

Table 1: Comparison between brown rot (BR) and sour rot (SR) and between wounded and nonwounded inoculations

	Variables	Stat[a]	*P*
1	BR on nonwounded varieties vs. BR on wounded varieties	27.16	<0.001
2	BR on nonwounded Pop-BR1 progeny vs. BR on wounded Pop-BR1 progeny	6.01	<0.01
3	BR on nonwounded Pop-BR2 progeny vs. BR on wounded Pop-BR2 progeny	11.01	<0.01
4	SR on nonwounded varieties vs. BR on nonwounded varieties	6.83	<0.001

[a]: R^2 (%) for 1, 2 & 3, t Stat for 4.

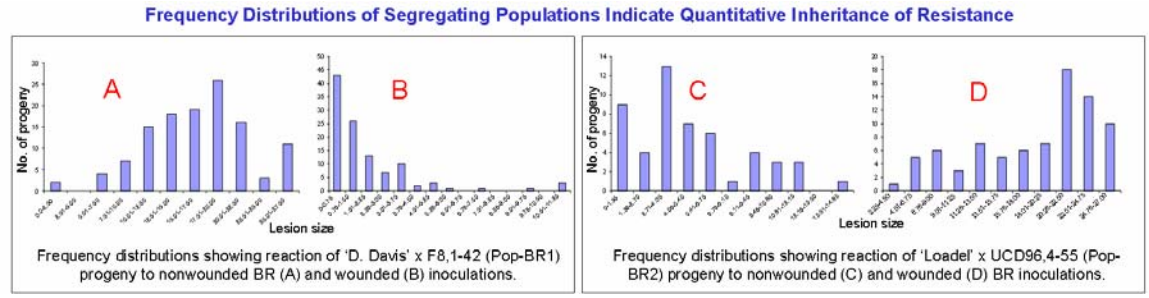

Figure 2: Frequency distributions of two cling peach progeny populations showing segregation of resistance to brown rot wounded and nonwounded inoculations

Table 2: ANOVA of the mean lesion size of reaction classes and fruit types among peach and nectarine cultivars inoculated with brown rot

Inoculation	Group*	No. of cultivars	Mean Lesion Size	*P*
Nonwounded	Yellow	56	3.29	
	White	25	5.32	0.01
	Peaches	49	4.35	
	Nectarines	32	3.26	NS
	Fresh Market	75	3.95	
	Processing	6	3.53	NS
	HR	12	0.04	
	MR	18	1.16	
	MS	30	3.65	
	HS	15	7.68	
	VHS	6	11.91	0.001
Wounded	Yellow	55	12.40	
	White	25	12.61	NS
	Peaches	48	13.48	
	Nectarines	32	10.94	0.03
	Fresh Market	75	12.32	
	Processing	5	14.72	NS
	HR	12	6.99	
	MR	18	11.82	
	MS	30	12.62	
	HS	14	15.13	
	VHS	6	18.39	0.001

***** HR = highly resistant, MR = medium resistance, MS = medium susceptible, HS = highly susceptible, VHS = very highly susceptible