Development of Predictive Tools for Brown and Sour Rot Resistance in Peach and Nectarines

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ABSTRACT

Progress continues to be made in our efforts to understand the genetics of resistance to brown rot (BR) caused by *Monilinia fructicola* and sour rot (SR) caused by *Geotrichum candidum*, serious diseases of peach and nectarine in California. In the first year of this research (2007), analysis of cultivars' reaction to wounded and non-wounded fruit inoculations showed that lesion size of BR and SR was under genetic control. Results showed that similar as well as different resistance mechanisms may be present for wounded vs. nonwounded fruit inoculations. 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. A second year (2008) data collection on resistance to BR was conducted on the two peach segregating populations ('Loadel' × 'UCD96,4-55' and 'Dr. Davis' × 'F8,1-42'). Sixteen cultivars were also selected from last year's data and re-evaluated for resistance to BR. Data were collected from these 16 cultivars for fruit quality traits such as skin color, flesh color, firmness, SSC and TA. There was no significance correlation between fruit quality traits and lesion diameter indicating that these characteristics are genetically independent. Molecular marker data (candidate genes and SSRs) have been collected for the 'Dr. Davis' × 'F8,1-42' progeny population (Pop-BR1) and a scaffold linkage map has been constructed for Pop-BR1. Preliminary data analysis showed potential genomic regions conferring resistance to both wound and nonwounded BR inoculations. Association between resistance and molecular markers will be elucidated and informative markers converted to predictive tools that are applicable in marker-assisted breeding of superior peach and nectarine cultivars.

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 included canning peach and fresh market cultivars, peach and nectarine cultivars, canning peach breeding lines, segregating progeny of two mapping populations – Pop-BR1 ('Dr. Davis' × F8,1-42) and Pop-BR2 ('Loadel' × UCD96,4-55), 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 Bostock Lab, Plant Pathology Department, UC Davis and all sour rot inoculations were conducted at the Michailides lab, 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 to 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 - 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 between parents of Pop-BR1 was

conducted among publicly available *Prunus* SSR markers and EST SSR markers from our Chillpeach database (http://bioinfo.ibmcp.upv.es/genomics/ChillPeachDB/login.php). Most of these SSRs have been mapped to the reference T×E and Pop-DG linkage maps. Resistance gene analog degenerate primers developed for Rosaceae (Samuelian et al., 2008) were also tested on the parents and progeny of Pop-BR1. A scaffold map was developed for Pop-BR1 and used for preliminary quantitative trait analysis (QTL) of resistance to BR. Survey of polymorphism was also conducted for candidate genes. Bin mapping of candidate genes to the Prunus T×E reference map was attempted. Further molecular marker and QTL analyses are ongoing.

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 were assessed by linear correlations. Linkage mapping was conducted with the use of statistical software JoinMap® 4 (Van Ooijen 2006) and QTL analysis was accomplished with the use of non-parametric Kruskal-Wallis test and interval mapping procedure of MapQTL® 5.0 software (Van Ooijen 2005).

RESULTS

A total of 81 and 34 cultivars were surveyed for resistance to brown rot and sour rot in 2007, 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. BR inoculations were repeated in 2008 for a subset of 16 cultivars selected to represent the range of cultivar reactions to inoculations in 2007. Also for BR, 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 in both years, 2007 and 2008, 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 varieties 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 nonwounded.

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. However, some individuals that showed resistance to wounded inoculations expressed susceptibility to non-wounded inoculations and vice versa. This indicates that common as well as different genetic factors may control resistance to BR with or without epidermal barrier. 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 in both years 2007 and 2008. These distributions indicated that both populations are segregating for resistance to the fungus. Few peaks observable in some of the distributions indicate control by a few major loci.

Table 2 shows the lesion size averages of 16 peach varieties tested in both years 2007 and 2008. Four cultivars showed medium to very high resistance to both methods of brown rot inoculations in both years 2007 and 2008. These were ‘August Red’, ‘Country Sweet’, ‘September Bright’ and ‘Zee Lady’. Five cultivars showed high susceptibility to the two inoculation methods in both years – ‘Elegant lady’, Extra Late #3, ‘Fire Pearl’, ‘September Red’, and ‘Summer Sweet’. Ultra Early #1 showed consistent resistance to unwounded inoculation but consistent susceptibility to wounded inoculation in both years. The performances of the remaining six cultivars to both inoculation methods were inconsistent between the years.

A total of 230 SSRs and 37 candidate genes primer pairs were screened for polymorphism using the parents and progeny subsets of Pop-BR1 out of which 52 SSR and two CGs were polymorphic. The polymorphic SSRs generated 59 SSR markers. In addition, eight RGA markers were generated. The total number of markers available for linkage analysis was 69 (59 SSRs, 8 RGAs and 2 CGs). A scaffold linkage map was constructed from this data consisting of 31 markers spread over 12 linkage groups of two to five markers each. These were organized into 7 linkage groups corresponding to the T×E reference map using common SSR markers (Figure 3). The locations of putative QTLs conferring resistance to BR are also indicated in Figure 3.

Putative QTLs detected by non-parametric Kruskal-Wallis (KW) test and interval mapping are shown in Table 3 and Table 4, respectively. KW test detected three QTLs for nonwounded inoculation in 2007, two for wound inoculation in 2008 and one each for wound inoculation in 2007 and nonwounded inoculation in 2008. One QTL was stable for each inoculation method across the two years. Interval mapping analysis detected one QTL for wounded inoculation in 2007 on linkage group G1 controlling up to 52% of observed variation. One QTL was detected on the same linkage group for wounded inoculation in 2008 controlling about 24% of observed variation. The proximity of these two QTLs suggests that they may be controlled by the same gene. Marker saturation of this region will aid QTL position refinement. Two candidate genes in the cutin and lignin biosynthesis pathway mapped to regions on the T×E *Prunus* reference map corresponding to locations of two putative QTLs detected for BR resistance on po-BR1 (result not shown). Further work is needed to map these genes directly to Pop-BR1 and validate their relationship with the QTLs.

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.

A few putative QTLs have been detected for resistance. The knowledge of inheritance of resistance will lay the groundwork for molecular analysis of the resistance factors which in turn will translate to the discovery of molecular markers that can be used in marker-assisted selections to fast-track the development of brown rot-resistant peach and nectarine cultivars.

The results of this study have been presented as posters at two international conferences; the Plant and Animal Genome XVI Conference in San Diego, Jan 12 – 16, 2008, and the 4th International Rosaceae Genomics Conference in Pucon, Chile, March 16 – 19, 2008, and as an invited oral presentation at the Volcani Center, ARO, Bet Dagan, Israel on November 12, 2008.

FUTURE PLANS

We will continue with the molecular marker analysis of resistance to both fungi. The scaffold linkage map developed for Pop-BR1 will be expanded and a similar map will be constructed for Pop-BR2. Detailed QTL analyses will be conducted on the expanded maps to validate putative QTLs discovered so far and to detect additional QTLs. Markers closely linked to the resistance QTLs will be identified for use in breeding programs. With availability of funds, we will conduct a third 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 of SR (20 varieties) and challenge them with the fungus as we did for BR. This will validate their 2007

resistance/susceptibility groupings. Candidate genes and markers that are linked to BR resistance QTLs will be used to assess the subset.

PRESENTATIONS FROM THIS STUDY

Ogundiwin, E.A., Bostock, R., Gradziel, T., Michailides, T., Yaghmour, M., Parfitt, D. and Crisosto, C. 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.

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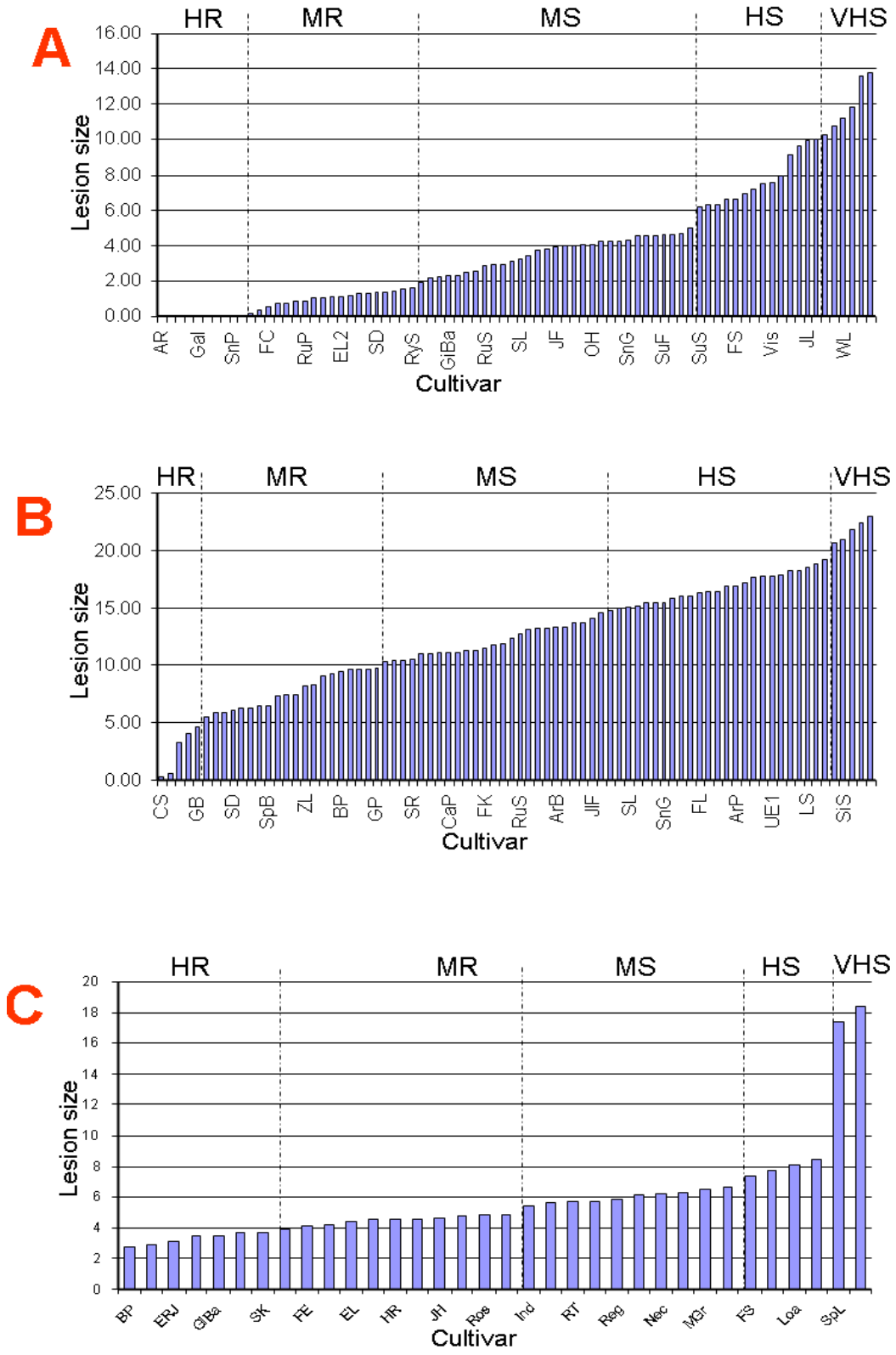

Figure 1: Reactions of peach and nectarine varieties to brown rot and sour rot inoculations. A = Nonwounded brown rot, B = wounded brown rot inoculation, and C = sour rot wounded inoculation

Table 1: Comparison between brown rot and sour rot wounded and nonwounded inoculations

Disease	**Genotypes**	**Year**	**Stat[a]**	***P***
Brown Rot	Varieties	2007	27.16	<0.001
		2008	39.7	<0.01
	Pop-BR1	2007	6.01	<0.01
		2008	30.75	<0.001
	Pop-BR2	2007	11.01	<0.01
		2008	17.0	<0.001
Sour Rot	Varieties	2007	6.83	<0.001

[a]: R^2 (%) for Brown Rot; t Stat for Sour Rot

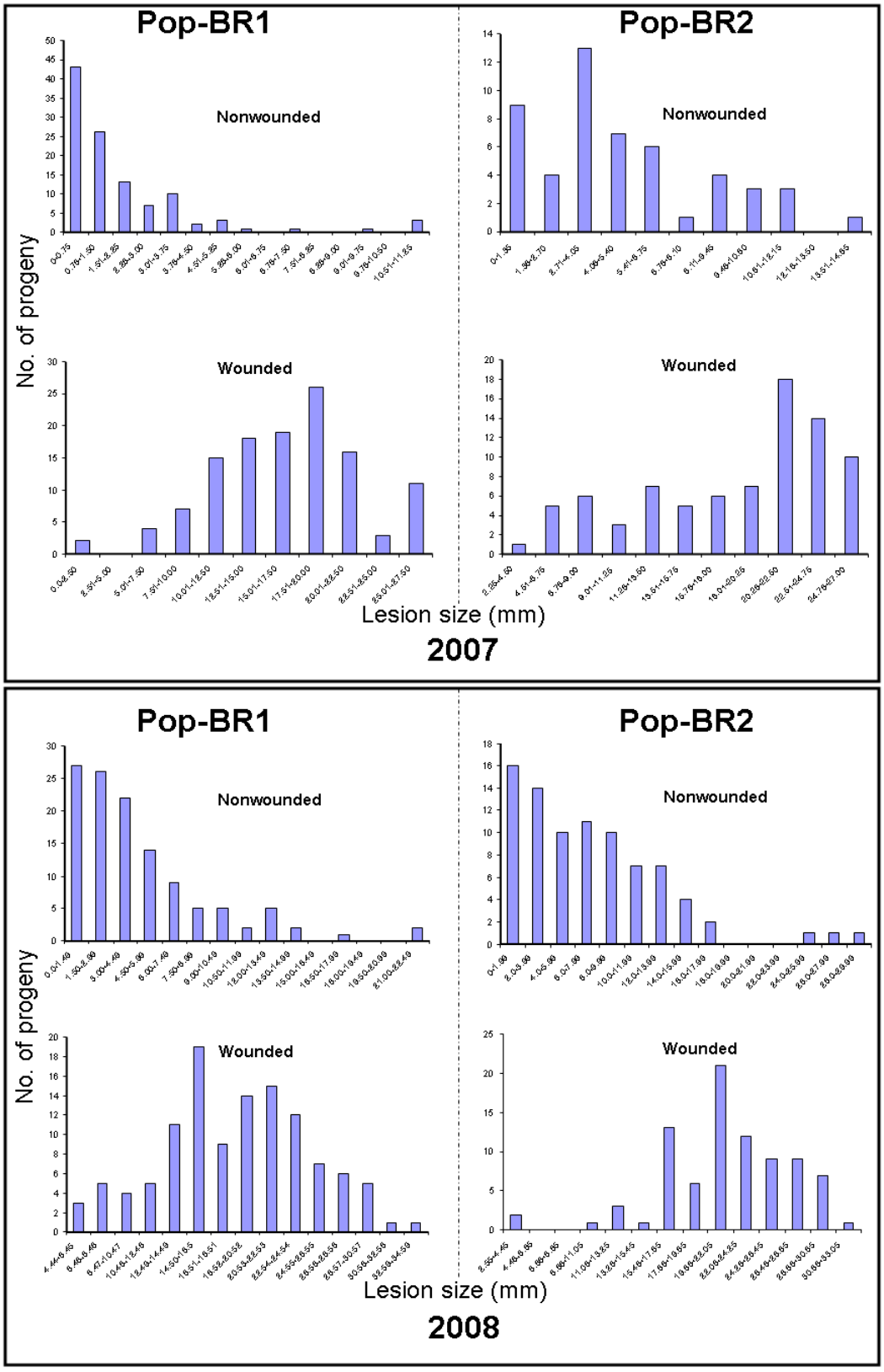

Figure 2: Frequency distributions of two cling peach progeny populations showing segregation of resistance to brown rot wounded and nonwounded inoculations

Table 2: Reaction of 16 selected varieties to wounded and unwounded inoculation with brown rot in 2007 and 2008.

	2007		2008		Average	
Variety	**Unwounded**	**Wounded**	**Unwounded**	**Wounded**	**Unwounded**	**Wounded**
Arctic Pride	10.30	16.93	0.3	0.0	5.30	8.46
August Red	0.00	3.28	0.0	0.0	0.00	1.64
Country Sweet	0.00	0.20	0.6	2.1	0.29	1.17
Elegant Lady	9.67	15.83	15.4	20.3	12.52	18.08
Extra Late # 3	7.45	17.75	18.5	13.4	12.98	15.58
Fire Pearl	6.25	11.85	13.3	15.8	9.76	13.84
Georgia Belle	0.00	4.60	9.3	33.4	4.63	19.00
September Bright	0.00	5.85	0.0	0.0	0.00	2.93
September Red	4.63	10.60	13.0	13.8	8.81	12.20
Snow Princess	0.00	0.60	7.3	11.8	3.63	6.18
Summer Bright	3.73	7.50	18.9	21.2	11.32	14.34
Summer Sweet	6.23	13.28	14.2	10.9	10.20	12.10
Summer Zee	5.00	11.83	0.0	0.0	2.50	5.91
Sweet Blaze	10.00	16.45	2.3	4.1	6.17	10.29
Ultra Early #1	3.10	17.75	0.0	20.6	1.55	19.17
Zee Lady	3.83	8.15	1.3	4.3	2.57	6.20

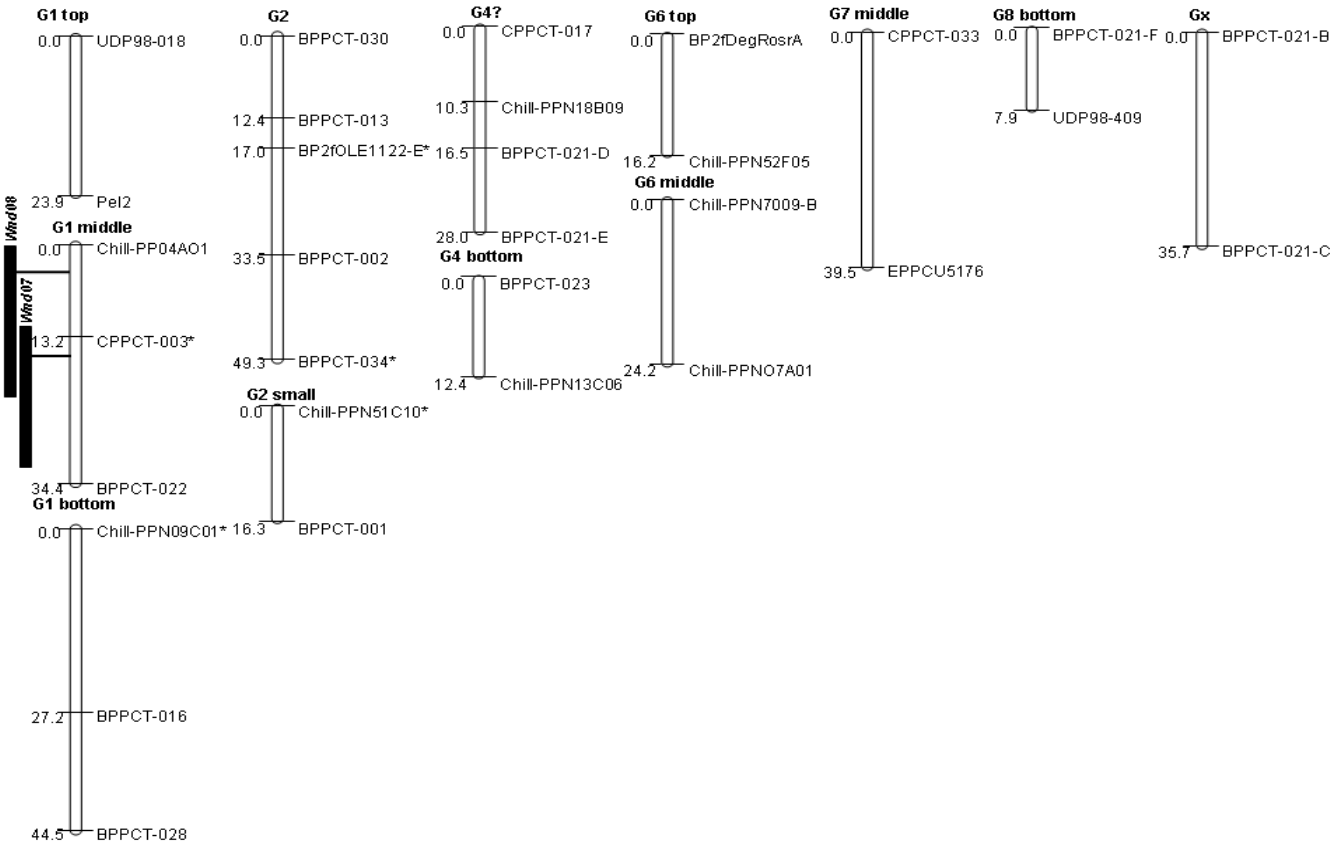

Figure 3; Partial linkage map of Pop-BR1; linkage group numbers and orientation derived from SSR markers in common with the *Prunus* reference T×E map; Group Gx could not be assigned a known number; BR resistance QTL markers detected by non-parametric Kruskal-Wallis test asterisked; black bars represents putative BR resistance QTLs located by interval mapping analysis.

Table 3: Putative QTLs for resistance to wounded and nonwounded BR inoculations detected by non-parametric Kruskal-Wallis test

Trait	Markers	LG	K*	*P*
Wound 07	BPPCT034	G2	10.14	0.05
Nonwound 07	CPPCT003	G1	6.85	0.01
	ChillPPN09C01	G1	7.10	0.01
	BP2fOLE1122-E	G2	7.57	0.01
Wound 08	CPPCT003	G1	9.46	0.005
	BPPCT034	G2	8.37	0.05
Nonwound 08	CPPCT003	G1	4.86	0.05

K* = the Kruskal-Wallis test statistic, LG = linkage group, *P* = significance level

Table 4: Putative QTLs for resistance to BR detected by interval mapping analysis of MapQTL®

Trait	Marker Interval	LG	Position (cM)	LOD	% explained
Wound 07	CPPCT003-BPPCT022	G1	28.18	3.74	52.0
Wound 08	ChillPPN04A01-CPPCT003	G1	2.0	3.39	24.0