Journal of the American Pomological Society 72(2): 94-100 2018

Breeding for Brown Rot (*Monilinia* spp.) Tolerance in Clemson University Peach Breeding Program

Wanfang Fu[1], Ralph Burrell[1], Cassia Da Silva Linge[1], Guido Schnabel[1] and Ksenija Gasic[1]

Additional index words: disease, fruit, RosBREED, QTL, haploblock

Abstract

Brown rot, caused by *Monilinia* spp., is one of the most economically important diseases of stone fruits. The fungus mainly affects the blossoms and fruit, and the resulting disease can lead to significant pre- and postharvest yield losses. Estimated yearly cost to the U.S. stakeholders for chemical protection against the disease can reach $170M. Although some degree of resistance in peach landraces ('Bolinha') and interspecific material (almond × peach) has been reported, genetic resistance to brown rot in peaches is still lacking. In commercial peach production, the disease is managed by practicing sanitation and the application of fungicides. The Clemson University peach breeding program within the RosBREED project aims to understand the genetics behind the peach fruit response to brown rot with the ultimate goal of combining disease resistance with high fruit quality via DNA informed breeding. To this end, 26 cultivars /advanced selections and 138 progeny, representing 9 breeding families, with 'Bolinha' source of resistance have been phenotyped for fruit response to brown rot using wounded and non-wounded disease assays in 2015 and 2016. Previously obtained genotypic data, and reported QTLs associated with brown rot response in peach fruit, were used to obtain preliminary information on variability in brown rot associated genomic regions. Phenotypic performance or trait values of these alleles/ haplotypes were discussed. The data presented here provide a foundation for developing predictive DNA information that has potential for immediate application in U.S. peach breeding.

Brown rot, caused by *Monilinia* spp. is one of the most important diseases concerning stone fruits. As a polycyclic disease, brown rot may cause severe yield losses by affecting peaches in two phases: blossom and twig blight caused by ascospore infection in spring and pre- and postharvest fruit decay caused by conidia infection in summer (Zehr et al., 1982; Tate et al., 2000). Although some degree of resistance has been identified in the Brazilian cultivar 'Bolinha' (Feliciano et al., 1987) and some interspecific hybrids (almond × peach) developed in the peach breeding program in California (Gradziel et al., 2003), most of the commercial peach cultivars are susceptible to brown rot (Martinez-Garcia et al., 2013). The disease is still mainly controlled by routine fungicide applications in conventional production systems, which can cause environmental and health issues (Sharma, 2005; Rungjindama et al., 2014). Consumer demand for fewer chemical treatments in fruit production has been increasing over the last years. In addition, new *Monilinia* strains with fungicide resistance have been reported, suggesting that the chemical approach may become less efficient (Luo et al., 2008; Chen et al., 2013, 2015). Thus, the main goal for current peach breeding programs is to develop new cultivars with enhanced disease resistance/tolerance and high-quality fruit by enhancing appearance, along with improved flavor and aroma.

Previous studies have shown that brown rot resistance in peach is inherited as a polygenic and quantitative trait (Gradziel et al., 1993, 2002; Martinez-Garcia et al., 2013; Pacheco et al., 2014). QTLs associated with brown rot have been reported in peach × almond (Martinez-Garcia et al.,

[1] Department of Plant & Environmental Sciences, Clemson University, Clemson, South Carolina, USA
[*] Corresponding author: Ksenija Gasic, kgasic@clemson.edu

2013) and in peach (Pacheco et al., 2014). Martinez-Garcia et al. (2013) detected 2 QTLs (*QTL1.1* and *QTL1.2*) associated with brown rot resistance/tolerance in peach fruit on linkage group (LG) 1 by assessing an F_1 progeny from controlled cross between the peach cultivar Dr. Davis (female) and the almond × peach F_2BC_2 introgression line 'F8, 1–42'. Analyzing an F_1 progeny from the cross between the two commercial cultivars 'Contender' (moderate resistance) x 'Elegant Lady' (susceptible), Pacheco et al. (2014) mapped two QTLs, one for skin resistance, *SK-if_2009*, on LG2, and another for flesh resistance, *FL-rd_2009*, on LG3. Once the QTLs were mapped, the associated genomic regions could be further analyzed to detect haplotypes associated with brown rot resistance. Thus, the objective of this study was to evaluate the brown rot infection responses in peach fruits from the Clemson University breeding program, identify haploblocks/haplotypes in previously reported QTLs associated with brown rot and determine the phenotypic performance/trait values of detected haplotypes/alleles.

Materials and Methods

Phenotypic evaluations for fruit responses to infection with *Monilinia fructicola* were performed in two years (2015 and 2016) using 8 cultivars/advanced selections and 131 progeny from 8 crosses with 'Bolinha' as source of resistance to brown rot. Additional 18 cultivars and 7 progeny from 1 cross were evaluated only in 2016. For each genotype, 40 fruits were randomly selected and bagged at 'pit hardening', to prevent pesticide contact. The fruits were harvested at commercial maturity, stored at 4°C for 2-4 days until the day of the assay, and were allowed to warm to room temperature for 24h before inoculation. Fruit surface was sterilized by 30sec immersion in 10% bleach (0.6% NaOCl), rinsed in deionized water, and air dried. Out of 40 bagged fruits, 20 unblemished fruits of similar maturity determined by I_{AD} (Ziossi et al., 2008) were used for inoculations. Parallel inoculations, 10 fruits each, for both wounded and non-wounded treatment were performed following the protocol of Martinez-Garcia et al. (2013). Non-wounded fruits were inoculated by adding a 10µL droplet of inoculum with the concentration of 2.5×10^4 conidia per ml of *M. fructicola* isolate KH-13. This highly virulent single-spore isolate was obtained from nectarine trees in Seneca, SC in 2013. Wounded treatments were accomplished by applying a 10µL droplet of inoculum to an intact fruit surface and then breaching the cuticule through the droplet using a 22 gauge needle and creating an injury about 2 mm deep. Inoculated fruits were incubated in dark under humid condition at room temperature (22±1°C). Lesion diameters (mm) were recorded after 72h incubation and disease severity index (DSI) for each individual was calculated as the product of average lesion diameter × disease incidence (proportion of lesions greater than 3mm). The phenotyped individuals were genotyped using the 9K peach SNP array (Verde et al., 2012). SNP-based haploblock/haplotypes were determined at previously reported brown rot associated QTL regions (Martinez-Garcia et al., 2013; Pacheco et al., 2014). Statistical differences among different genotypes and haplotypes/alleles were detected by performing ANOVA and Dunnett's T3 test in SPSS v. 23 (IBM®) at the significance of $p<0.05$.

Results and Discussion

In this study, 164 genotypes were evaluated for fruit responses to brown rot inoculation with wounding and non-wounding treatment. The results of the phenotypic evaluations were significantly different between the two treatments, suggesting that wounding increases DSI in the analyzed peach material (Figures 1 and 2). In addition, DSI was positive and significantly correlated between wounded and non-wounded treatments ($r = 0.369$, $p = 0.000$). Lowest DSI, <15, with wounding was observed in the advanced selection 'NC97-45', and 'Contender' and 'June Gold' (Fig. 1). 'NC97-45' has

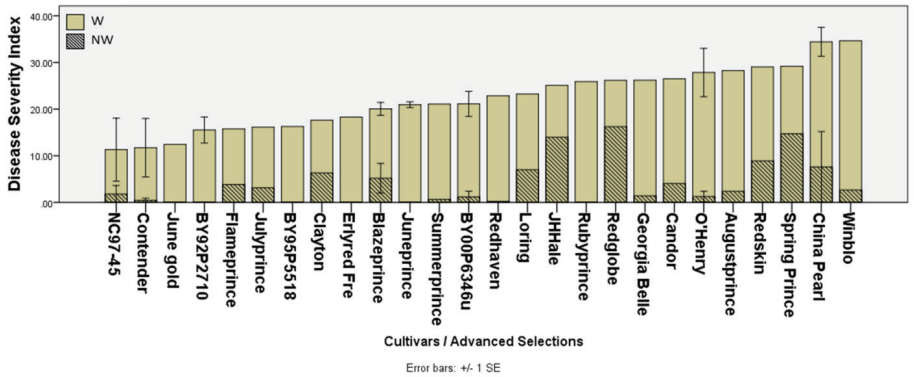

Figure 1. Brown rot disease severity index (DSI) observed in wounded (W) and non-wounded (NW) fruit of peach cultivars and advanced selections. DSI = average lesion diameter incidence (# of lesions greater than 3mm/total # lesions). Bars with standard error indicate average DSI of two years.

'Contender' as a parent which supports the findings of Pacheco et al. (2014) that 'Contender' is a source of resistance/tolerance to brown rot in peach. Advanced selection 'BY00P6346u' from the Byron peach breeding program, is a descendant of 'Bolinha' and is used for introgression of 'Bolinha' resistance/tolerance to brown rot in peach breeding programs (Gradziel et al., 1997). 'BY00P6346u' showed higher DSI with wounding than 'Contender', supporting previous observations that 'Bolinha' resistance/tolerance is mostly skin related, and once fruit ripens flesh becomes susceptible to brown rot (Gradziel and Wang, 1993). The pedigree analysis shows no connections between 'June Gold' ('Flamingo' × 'Springtime') and 'Contender' or 'Bolinha'. In addition, 'Juneprince' ('FV325-58' × 'June Gold'), descendant of 'June Gold', showed similar DSI as 'BY00P6346u' when wounded and better response when not wounded , suggesting that 'June Gold' could also be used as a source of brown rot tolerance in peach.

'Bolinha' was used as a direct (in C1, 6, 7, 8, 9) or indirect (via 'BY00P6346u' in C2, 3,

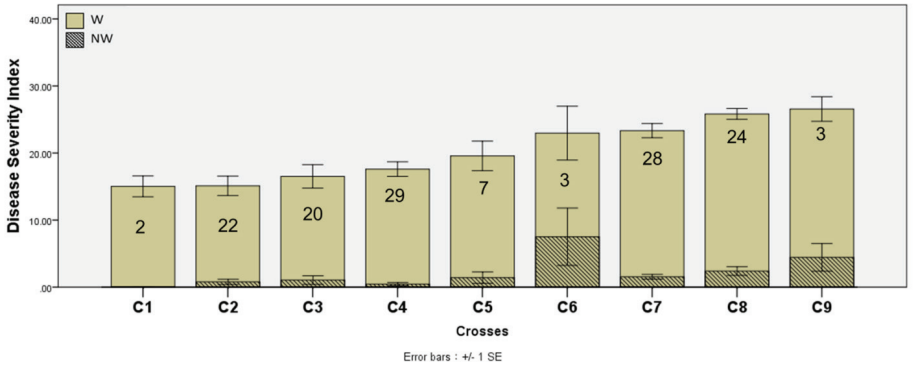

Figure 2. Average brown rot severity for fruits of crosses (C) with 'Bolinha' source of resistance over two seasons (2015-2016). Number of progeny in each cross were shown on the bars. W, wounded; NW, non-wounded.

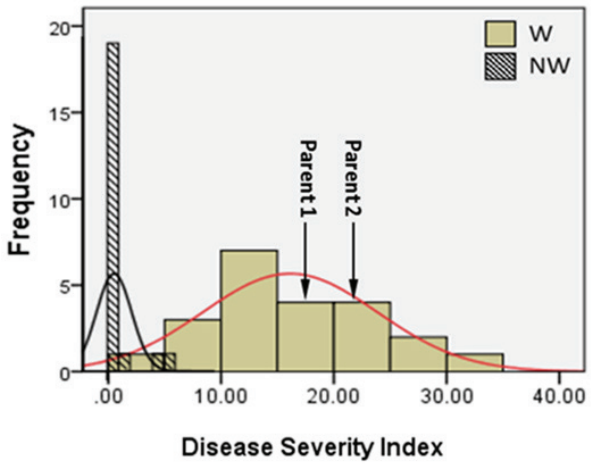

Figure 3. Brown rot disease severity index distribution in cross2 (C2). W, wounded; NW, non-wounded.

4, 5, 7) donor of brown rot resistance/tolerance in the evaluated crosses (C). The number of individuals in crosses varied from 2 to 29 due to already applied selection for fruit quality. Average fruit responses to brown rot infection in the crosses in the wounded treatment were similar to advanced selections and cultivars with narrower range (15 - 28 DSI), and non-wounded treatment elicited no to very low (<5) DSI in crosses (Fig. 2). C1 and C2 had the lowest DSI (>15) under wounded treatment and were among the lowest DSI for non-wounded treatment, while C8 and C9 had the highest DSI under wounded treatment (>25) and were among the highest DSI under non-wounded treatment. Under non-wounded treatment, most of the crosses showed similar DSI (<5), except for the C6 (Figure 2). Individual seedlings within crosses showed segregation for response to brown rot infections with individuals exhibiting lower and higher DSI than the average for the family (data not shown). Transgressive segregations were observed for brown rot DSI in the 'BY00P6346u' derived crosses. In the C2 most progenies showed no symptoms under the non-wounded treatment, while under the wounded treatment they exhibited lower DSIs than both parents (Figure 3). Phenotypic data analysis for C2 under both treatments, revealed advanced selections 5 and 7 with lower DSIs than most of the 'Bolinha' derived breeding material. Similar results were observed in other 'BY00P6346u'-derived crosses evaluated in this study, supporting 'BY00P6346u' as a good choice for incorporation of brown rot resistance/tolerance in peach breeding program (data not shown).

Analysis of the reported QTL regions revealed five and two haploblocks on LG1, *QTL1.1* and *QTL1.2*, respectively (Martinez-Garcia et al., 2013), and one haploblock for each of the QTLs reported on LG 2 and 3 (Pacheco et al., 2014) (Table 1). Number of haplotypes/alleles observed in haploblocks ranged from 3 in *QTL1.1* haploblock (H) 2 and 3 (*QTL1.1*_H2 and *QTL1.1*_H3) to 7 in *SK-if-2009* (Table 1). Analysis of phenotypic performance of each detected haplotype/allelic combination (diplotypes/genotypes) revealed significant differences in *QTL1.1, QTL1.2* and *SK-if_2009* under wounded and/or non-wounded treatment. Detailed analysis showed that the individual alleles provided different effects on brown rot resistance/tolerance in the four brown rot associated genomic regions (data not shown).

Seven different diplotypes/genotypes were identified in *QTL1.1*_H3 (Figure 4A). Significantly different (p<0.05) phenotypic performances were detected among different genotypes in this genomic region under the wounded treatment. The non-wounded treatment showed similar fruit responses for brown rot infection among the different genotypes. Trait value analysis of each individual haplotype/allele effect under wounded treatment suggested that the presence of allele 'b' significantly increases DSI (Figure 4B). Identified genotypes in *SK-if_2009* exhibited significantly different responses

Table 1. Haploblocks/haplotypes detected in QTLs associated with brown rot response in peach fruit.

Linkage group	QTL / Haploblock		Haploblock region (Mb)	Flanking SNPs	Number of SNPs	Number of alleles / Haplotypes
LG1	QTL1.1	H1	1.78-1.88	SNP_IGA_5258 SNP_IGA_5726	4	3
		H2	6.95-7.99	SNP_IGA_19818 SNP_IGA_22766	3	6
		H3	8.23-8.31	SNP_IGA_23251 snp_1_7856380	3	4
		H4	9.26-9.71	SNP_IGA_25403 SNP_IGA_26500	5	5
		H5	10.39-10.63	SNP_IGA_28112 SNP_IGA_28465	5	4
	QTL1.2	H1	26.92-27.06	SNP_IGA_88104 SNP_IGA_88772	5	5
		H2	30.86-32.14	SNP_IGA_99110 SNP_IGA_101065	4	5
LG2	SK_if_2009		21.89-22.47	SNP_IGA_274142 SNP_IGA_276426	10	7
LG3	FL_rd_2009		9.28-9.8	SNP_IGA_320761 SNP_IGA_321596	5	5

[1] QTL1.1 and QTL1.2 were detected in peach × almond progeny (Martinez-Garcia et al., 2013).
[2] SK_if_2009 and FL_rd_2009, skin and flesh associated QTLs respectively, were detected in 'Contender' × 'Elegant Lady' progeny (Pacheco et al., 2014).
[3] H - haploblock.

to brown rot infection under both wounded and non-wounded treatments (Figure 4C). The absence of the allele 'c' suggested lower DSI under both treatments (Figure 4D). No significant differences of the phenotypic performance/trait values were observed among the different diplotypes/genotypes in the *FL-rd_2009*, regardless of the treatment. However, analysis of effect of presence or absence of individual haplotypes/alleles showed significant differences under wounded and/or non-wounded treatment and will be further investigated (data not shown). Individual haplotype/allele trait value analysis was hindered by the lack of genotypic data for all phenotyped material. We are currently acquiring additional genotypic and phenotypic ('Contender' derived crosses) data to strengthen our findings.

Even though the source of brown rot resistance in the Clemson peach breeding program is different than in those used to detected the QTLs reported by Martinez-Garcia et al. (2013) and Pacheco et al. (2014) this published information allowed dissection of these genomic regions in haplotypes/alleles relevant for peach. The *QTL1.1* and *QTL1.2* were detected in an interspecific cross using almond background (Martinez-Garcia et al., 2013) and the *SK-if_2009* and *FL-rd_2009* QTLs were detected in an intraspecific F_1 progeny using 'Contender' as source of resistance (Pacheco et al., 2014). Once haplotypes/alleles were determined phenotypic performance or trait values of each allele/genotype were elucidated and statistically significant differences among phenotypic performances of alleles/genotypes were found. In addition, phenotyping for disease by Pacheco et al. (2014) was different in that they analyzed the percentage of infected fruits in non-wounded assay and average rot diameter in wounded assay. The Clemson University peach breeding program used 'Bolinha' derived resistance/tolerance to brown rot as a main source and could offer new insights into genomic regions associated with this trait. Thus, to better understand the genetic mecha-

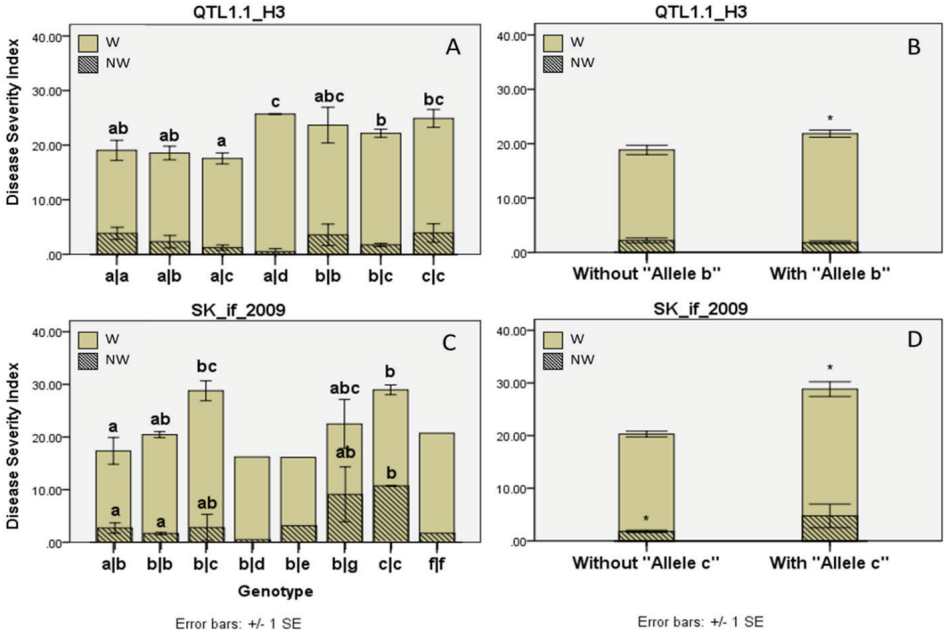

Figure 4. Trait values of brown rot associated genotypes (A) and haplotypes/alleles (B) detected in Clemson University peach breeding germplasm on *QTL1.1*_Haploblock3 (*QTL1.1*_H3) (Martinez-Garcia et al., 2013). Different letters/* indicate significant differences at P<0.05 according to Dunnett's T3 test. W, wounded; NW, non-wounded.

nisms that control brown rot fruit resistance/tolerance, further QTL mapping studies using pedigree based analysis (PBA) approach will be performed. This could uncover additional regions in peach genome associated with brown rot DSI and or provide additional resolution in elucidating trait values of brown rot associated haplotypes/alleles.

Conclusion

In this study, we presented the responses of 164 pedigreed germplasm from the Clemson University peach breeding program to inoculations with *Monilinia fructicola*. Significant differences in brown rot fruit infection responses were observed. Genotypes/diplotypes with different phenotypic performance/trait values were detected for three published brown rot associated QTLs, *QTL1.1*, *QTL1.2* and *SK-if_2009*, and haplotypes/alleles with trait values were detected in all reported brown rot associated genome regions (*QTL1.1*, *QTL1.2*, *Sk-if_2009* and *FL-rd_2009*). The analyzed peach germplasm exhibited sufficient brown rot tolerance/resistance variability for novel detection of genomic regions associated with brown rot tolerance/resistance in peach applying PBA approach. This work represents an important basis for developing predictive DNA information tools for brown rot resistance / tolerance.

Acknowledgments

This work was supported by USDA's National Institute of Food and Agriculture for the Specialty Crop Research Initiative through the competitive project "RosBREED: Combining disease resistance with horticultural quality in new rosaceous cultivars" (2014-51181-22378). The authors thank Musser Farm staff for their technical assistance.

Literature cited

Chen, F., S.E. Everhart, P.K. Bryson, C. Luo, X. Song, X. Liu, and G. Schnabel. 2015. Fungicide-induced transposon movement in *Monilinia fructicola*. Fungal Genet. and Biol. 85: 38-44.

Chen, F., X. Liu, and G. Schnabel. 2013. Field strains of *Monilinia fructicola* resistant to both MBC and DMI fungicides isolated from stone fruit orchards in the eastern United States. Plant Dis. 97: 1063-1068.

Feliciano, A., A.J. Feliciano, and J. Ogawa. 1987. *Monilinia fructicola* resistance in the peach cultivar Bolinha. Phytopathol. 77: 776-780.

Gradziel, T., R. Bostock, and J.P. Adaskaveg. 2002. Resistance to brown rot disease in peach is determined by multiple structural and biochemical components. Acta Hort. 622: 347-35.

Gradziel, T. and D. Wang. 1993. Evaluation of brown rot resistance and its relation to enzymatic browning in clingstone peach germplasm. J. Amer. Soc. Hort. Sci. 118: 675-679.

Gradziel, T., M. Thorpe, R. Bostock, and S. Wilcox. 1997. Breeding for brown rot (*Monilinia fructicola*) resistance in clingstone peach with emphasis on the role of fruit phenolics. Acta Hort.. 465: 161- 170.

Luo, C. and G. Schnabel. 2008. Adaptation to fungicides in *Monilinia fructicola* isolates with different fungicide resistance phenotypes. Phytopathol. 98: 230-238.

Martínez-García, P.J., D.E. Parfitt, R.M. Bostock, J. Fresnedo-Ramírez, A. Vazquez-Lobo, E.A. Ogundiwin, T.M. Gradziel, and C.H. Crisosto. 2013. Application of genomic and quantitative genetic tools to identify candidate resistance genes for brown rot resistance in peach. PloS One 8: e78634.

Pacheco, I., D. Bassi, I. Eduardo, A. Ciacciulli, R. Pirona, L. Rossini, and A. Vecchietti. 2014. QTL mapping for brown rot (*Monilinia fructigena*) resistance in an intraspecific peach (*Prunus persica* L. Batsch) F1 progeny. Tree Genetics and Genomes 10: 1223-1242.

Rungjindamai, N., P. Jeffries, and X. Xu. 2014. Epidemiology and management of brown rot on stone fruit caused by *Monilinia laxa*. Eur. J. Plant Pathol. 140: 1-17.

Sharma, R.L. 2005. Management of brown rot (Monilinia laxa) in peaches in warmer arears. Acta Hort.. 696: 359-362.

Tate, K. and P. Wood. 2000. Potential ascospore production and resulting blossom blight by *Monilinia fructicola* in unsprayed peach trees. New Zealand J. Crop and Hort. Sci. 28: 219-224.

Verde, I., N. Bassil, S. Scalabrin, B. Gilmore, C.T. Lawley, K. Gasic, D. Micheletti, U.R. Rosyara, F. Cattonaro, and E. Vendramin. 2012. Development and evaluation of a 9K SNP array for peach by internationally coordinated SNP detection and validation in breeding germplasm. PLOS ONE 7(6), 10.

Zehr, E.I. 1982. Control of brown rot in peach orchards. Plant Dis. 66: 1101-1105.

Ziosi, V., M. Noferini, G. Fiori, A. Tadiello, L. Trainotti, G. Casadoro, and G. Costa. 2008. A new index based on vis spectroscopy to characterize the progression of ripening in peach fruit. Postharvest Biol. Technol. 49: 319-329.