Effects associated with graft-transmissible agents found in the peach variety 'Ta Tao 5'

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Abstract

The peach variety 'Ta Tao 5' is host to at least three graft-transmissible agents. Extraction and characterization of these agents indicates that they are variants of *Peach latent mosaic viroid* (PLMVd), *Apple chlorotic leaf spot virus* (ACLSV) and a previously uncharacterized Foveavirus referred to as Asian prunus virus 1 (APV1). Each of these agents are utilized separately, in defined combinations and in concert in a field study designed to identify their graft-transmissibility and consequent contribution to phenological changes in the peach varieties 'Springprince' and 'Juneprince.' Field data record variations in bloom date, vegetative growth, and fruiting in both varieties tested. Further, such phenological variation associated with 'Ta Tao 5' differs significantly from artificial combinations of inoculants. The use of 'Ta Tao 5' as an inoculant source to manipulate growth and development of peach trees is unique when compared with other sources.

Keywords: *Prunus persica*, stone fruit, bloom delay, CGRMV, RT-PCR, *trichovirus*

Introduction

The presence of two or more infectious agents has been associated with unique phenological developments in peach (Gibson and Reighard, 2002; Stubbs and Smith, 1971; Scott et al., 2001). *Peach Latent Mosaic Viroid* (PLMVd) is often associated with a delay in bloom, reduced vegetative vigor, and higher fruiting efficiency (Nemeth, 1986; Desvignes et al., 1996; Gibson et al., 2001). In peach, *Apple Chlorotic Leaf Spot Virus* (ACLSV) produces dark green sunken spots or wavy lines on peach leaves, hence the name of the disease; peach dark-green sunken mottle (Nemeth, 1986). Additionally, ACLSV often causes incompatibilities in some peach cultivars (Llacer and Cambra, 1975). *Cherry Green Ring Mottle Virus* (CGRMV) is latent in peach (Parker et al., 1976). Some infectious agents act synergistically within the host to produce novel symptoms. *Prune Dwarf Virus* (PDV) and *Prunus Necrotic Ringspot Virus* (PNRSV) inoculated singly and in combination produce different symptoms in peach (Stubbs and Smith, 1971; Scott et al., 2001).

The peach variety 'Ta Tao 5' is host to at least three graft-transmissible agents. These include isolates of ACLSV; GenBank accession number EU223295 (Marini et al., 2008) and Asian Prunus Virus 1; GenBank accession number FJ824737 (Marini et al., 2009). The PLMVd isolate aligns closely with other variants, but does not contain the 11 nt insertion typically associated with peach calico (Marini, 2007).

Materials and methods

Virus-indexed trees of the peach cultivar 'Springprince' grafted onto Guardian® rootstock were planted January 2005 in a high-density, Y-trained orchard system with 1.8 m spacing in the row and 5.5 m between rows. Trees were inoculated on March 11, 2005 with chip buds to initiate treatments. One control and eleven treatments consisted of two isolates of ACLSV, an unknown high-chill peach (acronym PK), PLMVd, ACLSV & PLMVd, PK & ACLSV, PLMVd & ACLSV, PLMVd & PK, ACLSV & PLMVd & CGRMV, the peach cultivar 'Ta Tao 5,' and PK & ACLSV & PLMVd. Each of the treatments and the control consisted of 4-tree plots randomly assigned within each block and replicated 3 times.

Virus-indexed trees of the peach cultivar 'Juneprince' grafted onto Guardian® rootstock were planted January 2006 in a high-density, Y-trained orchard system with 1.8 m spacing in the row and 5.5 m between rows. Trees were inoculated on September 29, 2006 with chip buds to initiate treatments. One control and seven treatments consisted of 'Ta Tao 5,' ACLSV, PK, PLMVd, Heat-treated 'Ta Tao 5,' ACLSV & PLMVd, Heat-Treated 'Ta Tao 5' & ACLSV. Each of the treatments and the control consisted of 5-tree plots randomly assigned within each block and replicated 3 times.

Treatment verification: Total RNA was extracted from newly emerged peach shoots from all 'Springprince' trees in spring 2006, 2008, and 2009, and from all 'Juneprince' trees in spring 2009; using a modified procedure of Hughes and Galau (1988) (Sara Spiegel, The Volcani Center, Israel, personal communication). The amount of RNA in each sample was measured by recording the absorbance at 260 nm and calculating the concentration using an extinction coefficient

of 25 $(mg/ml)^{-1}cm^{-1}$ (Noordam, 1973). The QIAGEN OneStep™ RT-PCR kit (QIAGEN Inc., Valencia, CA) was used to perform detections of ACLSV, APV1, PLMVd and CGRMV, according to the manufacturer's instructions. Treatments were verified by analysis of RT-PCR fragments.

Field data: Leaf abscission (leaf drop) was recorded by observing the date on which 10%, 50% or 90% defoliation occurred in autumn. Trunk cross-sectional area was calculated from stem caliper measured at the trunk base with a Digimatic Caliper (Mitutoyo Corporation, Japan). Spring bloom was recorded by observing the date on which 10%, 50%, and 90% (full bloom) of flowers were open during spring. Harvest data consisted of individual fruit weight (average of 10 fruit per tree), percent soluble solids as measured with a MT-032ATC Refractometer (International Ripening Company, Norfolk, VA), total number of fruit prior to picking and total weight of fruit. Fruiting efficiency was calculated by dividing total fruit weight by trunk cross-sectional area. Puncture pressure (*i.e.*, firmness) of the fruit was determined with a McCormick FT 327 Penetrometer (International Ripening Company, Norfolk, VA) using a 0.8 cm diameter plunger tip. The skin was sliced off each cheek face perpendicular to the suture of 5 fruit per tree and the mean pressure calculated. Pruning was recorded as the time required to prune each tree and the weight of the clippings removed. Photosynthetically active radiation (par) was recorded 1 m above the ground as an average between one measure perpendicular and one measure parallel to the row between 11:00 AM and 1:00 PM on a clear day in $\mu mol \cdot m^{-2} \cdot s^{-1}$ using a line quantum sensor (LI-191SA; LI-COR, Lincoln, Nebr, USA). All data was analyzed by ANOVA with significance determined using *F* ratios as calculated using mean square across blocks or treatments divided by mean square error.

Results

'Springprince': 'Springprince' trees inoculated with 'Ta Tao 5' were the only treatment exhibiting a striking reduction in trunk cross-sectional area increase for the growing seasons 2006 and 2007, but none of the treatments were significantly different during the 2008 growing season (see Table 1). Leaf drop occurred significantly, 2 to 4 days earlier on 'Ta Tao 5' inoculated trees, but were sometimes similar on other treatments. Pruning data through three growing seasons indicate that 'Ta Tao 5' inoculated trees were significantly less vigorous. Surprisingly, a few other treatments were significantly more vigorous through three growing seasons when compared with the controls. A similar pattern is demonstrated by the light penetration data where significantly more light penetrated the canopy of 'Ta Tao 5' inoculated trees in 2007 and 2009, yet all other treatments exhibit less light penetration during the same years.

Bloom date is the most significant variation recorded in the 'Springprince' trees during the period 2006 to 2009 (see Figure 1). The trees inoculated with 'Ta Tao 5' bloom 1 to 4 days later when compared with all the other treatments and control with the exception of PLMVd inoculated treatments in 2007 which bloomed 3 days later when compared with the control.

Fig. 1 Springprince Bloom 2008. Key left to right: Ta Tao 5/ACLSV, Ta Tao 5/PK/ACLSV, Ta Tao 5, Ta Tao 5, Ta Tao 5/PK, PK, ACLSV, PLMVd/ACLSV, ACLSV, PK/ACLSV, PLMVd/ACLSV/CGRMV, and non-inoculated control. (photo by Dr. Simon Scott).

Fruiting characters measured across the 2006 to 2009 seasons exhibit unremarkable variation with few notable exceptions. These are a significant reduction of total harvest in 'Ta Tao 5' and PLMVd inoculated trees when compared with controls or PK inoculated trees in 2009. Additionally, all treatments had significantly firmer fruit in all 4 seasons when compared with the controls except PK inoculated trees in 2006 and 2007 and PLMVd inoculated in 2006.

'Juneprince': The 'Juneprince' trial demonstrated generally unremarkable results across all measured growth and fruiting characters amongst all treatments (see Table 2). Growth rates, winter and summer pruning requirements, and light penetration were exceptionally uniform for all treatments. Most treatments had higher summer clipping weights when compared with the controls in 2007, 2008 and 2009. Leaf drop occurred 2 to 3 days earlier on the 'Ta Tao 5' inoculated trees when compared with all other treatments in 2008. Fruiting efficiency, firmness, fruit count, soluble solids, and individual fruit weight were quite similar in 2008 and 2009. Surprisingly, total harvest was significantly higher for all treatments except PLMVd when compared with controls. Unlike the 'Springprince' trial, bloom date varied less than 2 days in 2008 and not at all in 2007 and 2009.

Tab. 1 Field data for 'Springprince' cultivar.

Agent\trtmt	Units	Control	Ta Tao 5	PK1	ACLSV\PLMVd	α=0.05
08-'09 TCA increase	cm^2	19.1	15.21	17.02	16.57	
07-'08 TCA increase	cm^2	20.13	13.39	17.53	21.41	3.38E-05
06-'07 TCA increase	cm^2	18.12	13.94	20.9	20.77	4.86E-05
08 Leaf drop	JD	310.4	308.1	308.2	308.9	2.36E-09
07 Leaf drop	JD	316.4	312.2	314.5	316.8	2.63E-05
06 Leaf drop	JD	322.7	320.5	325.2	327.1	4.14E-05
09 Winter prune time	m	2.75	2.082	2.716	2.836	4.71E-04
08 Winter prune time	m	1.9	1.387	2.096	1.841	1.26E-08
09 Winter clipping weight	kg	3.2	2.328	3.1	3.204	2.14E-03
08 Winter clipping weight	kg	2.71	2.032	2.664	2.753	2.46E-04
09 Summer prune time	m	1.824	1.525	1.584	1.747	
08 Summer prune time	m	2.326	1.941	2.01	2.624	2.10E-05
07 Summer prune time	m	3.995	3.634	3.92	3.843	
09 Summer clipping weight	kg	3.65	3.017	2.93	3.557	3.95E-02
08 Summer clipping weight	kg	4.575	3.441	3.76	4.832	5.33E-04
07 Summer clipping weight	kg	6.169	5.43	6.275	7.171	2.39E-02
09 Light penetration	par	112.4	111.4	50.94	59.03	4.05E-02
08 Light penetration	par	113.5	135.7	104.3	106.4	
07 Light penetration	par	349.2	361.7	218.2	282	2.77E-02
09 Bloom date - 90%	JD	70.11	71.13	70.6	70.5	4.30E-06
08 Bloom date - 90%	JD	76.06	79.27	76.6	76.64	1.09E-18
07 Bloom date - 90%	JD	76.67	80.67	78.5	79.71	5.71E-11
06 Bloom date - 90%	JD	70.11	70.83	70.6	70.43	5.16E-03
09 Fruiting efficiency	kg/cm^2	0.158	0.113	0.132	0.108	2.00E-03
08 Fruiting efficiency	kg/cm^2	0.101	0.137	0.11	0.089	6.00E-03
09 Puncture pressure	g	7.176	9.835	9.815	9.768	3.00E-04
08 Puncture pressure	g	8.853	10.71	9.815	9.768	1.67E-07
07 Puncture pressure	g	8.285	9.901	8.505	9.463	4.82E-06
06 Puncture pressure	g	8.07	9.855	7.841	8.134	4.30E-05
09 Fruit count	no.	75.27	42.88	65.35	51.81	5.79E-07
08 Fruit count	no.	42.68	45.72	45.93	36.11	
09 Soluble solids	%	8.706	9.278	9.236	9.037	1.60E-02
08 Soluble solids	%	9.856	10.03	10.24	10.43	
07 Soluble solids	%	11.8	12.03	11.74	11.91	
06 Soluble solids	%	9.356	9.25	10.12	9.643	
09 Fruit weight - individual	kg	0.164	0.163	0.155	0.165	
08 Fruit weight - individual	kg	0.137	0.136	0.139	0.151	1.60E-04
09 Total harvest	kg	12.17	6.9	10.02	8.601	7.10E-08
08 Total harvest	kg	5.885	6.239	6.398	5.465	

Tab. 2 Field data for 'Juneprince' cultivar

Measure	Units	Ta Tao 5	PK	HT TT5	ACLSV	HT TT5 & ACLSV	PLMVd	PLMVd & ACLSV	Control	*α=0.05*
08-'09 TCA increase	cm^2	17.04	23.704	23.87	25.2	21.7	18.092	26.48	22.06	
09 Winter prune time	m	1.824	2.2033	2.194	2.292	1.951	2.0844	2.178	1.97	
08 Winter prune time	m	1.617	2.2167	1.97	1.55	1.31	1.2633	1.45	1.74	3.37E-07
09 Winter clipping we	kg	2.339	3.1333	2.789	3.317	2.489	2.2778	3.011	2.428	3.44E-02
08 Winter clipping we	kg	1.043	1.1967	1.05	0.947	0.72	0.6067	0.85	0.96	1.69E-05
09 Summer prune tim	m	2.293	2.4967	2.847	2.58	2.737	2.02	2.44	2.357	5.47E-08
08 Summer prune tim	m	1.483	1.8767	2.067	2.057	1.967	1.7833	2.11	1.467	6.74E-03
07 Summer prune tim	m	1.667	2.58	2.303	3.203	3.47	2.1756	2.546	3.219	4.17E-05
09 Summer clipping w	kg	3.667	4.5833	4.072	5.25	4.1	3.3944	5.211	3.278	8.98E-05
08 Summer clipping w	kg	2.917	4.5667	3.46	3.877	2.823	2.94	4.133	2.41	6.27E-08
07 Summer clipping w	kg	3.944	5.9222	5.15	6.156	4.661	4.3444	5.956	4.178	3.48E-03
09 Light penetration	par	65.66	57.074	62.29	39.77	71	55.82	25.42	95.09	
08 Light penetration	par	268.4	146.11	145.2	148.8	155.9	222.22	149.2	266.2	
09 Bloom date - 90%	JD	70	69.333	69	69	69.33	69.667	69	69.33	3.49E-08
08 Bloom date - 90%	JD	76	75.444	76	74	76	74.667	74.22	73.89	1.83E-14
07 Bloom date - 90%	JD	72.89	74.556	72.78	72.89	72.56	73.556	73.44	73.33	1.93E-03
08 leaf drop	JD	315.8	317.78	318.1	320.7	317.4	318.11	317.9	319.6	1.74E-03
09 Fruiting efficiency	kg/cm	0.182	0.1185	0.146	0.128	0.165	0.159	0.127	0.146	
08 Fruiting efficiency	kg/cm	0.096	0.082	0.13	0.076	0.127	0.0765	0.088	0.064	1.60E-09
09 Puncture pressure	kg	4.948	4.5533	4.668	4.311	4.339	4.3344	4.644	4.172	3.65E-03
09 Fruit count	no.	52.6	41.603	53.71	44.49	53.92	43.037	46.99	45.32	
08 Fruit count	no.	35.11	37	54.67	33	51.22	27.111	36.44	25.33	1.53E-11
09 Soluble solids	%	11.1	11.36	11.01	11.06	11.35	11.351	11.43	11.8	
09 Fruit weight - indiv	kg	0.175	0.1897	0.173	0.189	0.171	0.1974	0.18	0.181	2.18E-03
08 Fruit weight - indiv	kg	0.097	0.1021	0.097	0.096	0.087	0.1	0.094	0.087	
09 Total Harvest	kg	9.024	7.8608	9.269	8.305	9.228	8.3871	8.457	8.304	
08 Total Harvest	kg	3.549	3.7522	5.279	3.036	4.373	2.7533	3.451	2.249	5.21E-11

Discussion

Graft-transmissible agents utilized in this study are stable and detectable by RT-PCR in 'Springprince' and 'Juneprince' cultivars of peach. Phenological affects are measurable and minimal in both cultivars from 2006 to 2009. Bloom delay is desirable to avoid the risks of late spring frosts which frequently occur in the southeastern United States. Additionally, vegetative growth reduction is desirable to reduce labor requirements. This study suggests there are accelerations in vegetative growth and fruiting associated with some combinations of graft-transmissible agents. Further study is required to determine which agents or combination of agents is responsible for such increases in growth and development.

Most notable from the study is the uniqueness of 'Ta Tao 5' inoculation. Artificial combinations of PLMVd, ACLSV and APV1 failed to reproduce the effects found with 'Ta Tao 5' inoculation. Possible explanations include: PLMVd, ACLSV, and APV1 in 'Ta Tao 5' are sufficiently variable from other isolates to result in unique effects; there are additional, yet to be discovered agents present; or, genetic factors occur between the agents and 'Ta Tao 5.' Further molecular characterization and continued field studies are required.

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