Variation in Resistance Mechanisms to the Green Peach Aphid Among Different Prunus persica Commercial Cultivars

J. A. VERDUGO,1,2 T. MÉNDEZ,1 S. A. ORTIZ–MARTÍNEZ,2 R. CUMSILLE,1 AND C. C. RAMÍREZ1,3

J. Econ. Entomol. 105(5): 1844–1855 (2012); DOI: http://dx.doi.org/10.1603/EC12100

ABSTRACT Peaches and nectarines are frequently attacked by the green peach aphid Myzus persicae (Sulzer), with significant negative impacts on fruit production. The genetic variability of resistance to this aphid among commercial cultivars of Prunus persica (L.) Batsch and Prunus persica variety nectarina was evaluated in this study. In total, 16 cultivars of P. persica were selected to evaluate the occurrence and population growth rate of M. persicae in commercial orchards, as well as in no-choice and probing behavior laboratory assays. The results showed variability between cultivars in resistance and susceptibility to M. persicae, with three cultivars exhibiting different signatures of resistance. The peach cultivar ‘Elegant Lady’ exhibited a low occurrence of aphids in the orchard, a low rate of growth, moderate leaf-rejection in a no-choice test and a higher number and longer period of salivation into sieve elements, suggesting resistance at the phloematic level. The nectarine cultivar ‘August Red’ also exhibited low aphid occurrence in the orchard, a low rate of growth, and resistance at the phloem levels. Finally, the nectarine ‘July Red-NS92’ exhibited a low occurrence of aphids in the orchard, a higher number of rejections in no-choice assays and no ingestion of phloem during the probing behavior experiments, suggesting phloematic resistance. The rest of the cultivars studied exhibited clear susceptibility. Hence, different resistance mechanisms are apparent among the studied cultivars. The information gathered in this study regarding the resistance to M. persicae may assist breeding programs aimed at increasing aphid resistance to peaches and nectarines.

KEY WORDS resistance, performance, probing, no-choice, aphid

Peaches (Prunus persica (L.) Batsch) and nectarines (Prunus persica variety nectarina) are two of the most important fruits in the world. There is a continuous development of new cultivars with the characteristics required by producers and consumers (Sherman et al. 1996, Infante et al. 2008). However, as with all tree fruits, peaches and nectarines are affected by a number of pathogens and insect pests whose inadequate control may affect fruit yields up to 2 yr. One of the most important pests of peaches and nectarines worldwide is the green peach aphid Myzus persicae (Sulzer) (Hemiptera: Aphididae), which is a generalist aphid that uses P. persica trees as a primary host and species from >40 plant families as secondary hosts (Blackman et al. 2000). The green peach aphid produces leaf curling and stunting and devitalization in stems and reduces fruit quality by altering the fruit growth pattern (Pascal et al. 2002, Penvern et al. 2010). In addition, this aphid species is responsible for transmitting the Plum pox potyvirus to P. persica (Isac et al. 1998). A wide range of insecticide resistance mechanisms have also been reported in this aphid species (Field et al. 1988, Moores et al. 1994, Blackman et al. 1995, Foster et al. 1998, Martinez–Torres et al. 1999, Bass et al. 2011). Thus, breeding programs would gain from information regarding resistance variation to M. persicae among different commercial peach and nectarine cultivars.

Peach and nectarine resistance mechanisms to aphids, as well as to other pests and diseases, have been intensively studied (Kervella et al. 1998; Monet et al. 1998; Pascal and Monteux–Caillet 1998; Sauge et al. 1998a,b, 2011; Lambert and Pascal 2011). Studies addressing peach and nectarine resistance to M. persicae have used performance experiments based on the intrinsic rate of natural increase rm (Le Roux et al. 2007), monitoring of probing behavior (EPG) (Sauge et al. 1998a, Pompon et al. 2010) and no-choice testing (Sauge et al. 1998b, Margaritopoulos et al. 2005). EPG studies have produced reliable information concerning the location of the resistance mechanisms. For example, these types of experiments located the resistance mechanism of the ‘Malo Konare’ peach cultivar in the vascular system and found it to provide antibiosis resistance, whereas antixenosis was suggested for the ‘Weeping Flower Peach’ cultivar (Monet and Massonié 1994, Monet et al. 1998, Sauge et al. 1998a,b). Similarly, by monitoring probing behavior of M. persicae on the ‘Rubira’ cultivar, antixenosis was found to be reinforced by induced resistance (Sauge
et al. 1998b, 2002). Genes conferring monogenic resistance to this aphid have been described for the Weeping Flower Peach and Rubira cultivars (Monet and Massoné 1994, Lambert and Pascal 2011). However, studies on the wild Prunus species, P. davidiana, suggested that this cultivar appears to have a phloem-based resistance mechanism (Sauge et al. 1998a,b). Hence, the resistance mechanism of Prunus to M. persicae is likely different depending on the cultivar or species. Information regarding resistance patterns of currently used commercial cultivars of peaches and nectarines would provide knowledge on aphid management for commercial cultivars in general and to breeding programs that include commercial cultivars.

In Chile, M. persicae produces serious damage to P. persica (peaches and nectarines) plantations (Rosales et al. 1998, Reyes et al. 2003). The implementation of Integrated Fruit Production (IFP), which was adopted to reduce the quantity of chemical inputs used to protect crops from the green peach aphid (Grecchi et al. 2008), is a promising alternative to the conventional pest management system because, although damage and disease is higher in the conventional system, the aphid remains under the tolerable threshold for economic damage (Cooper et al. 2001). IFP is suitable in cases in which resistance variation of commercial cultivars is known.

This study aimed to determine the variation in resistance mechanisms to attack by the M. persicae on several commercial cultivars of P. persica, including peaches and nectarines commonly planted in central Chile. Because measuring herbivore response in terms of performance, preference or probing behavior is an integrative and functionally relevant estimate of resistance (Leimu and Koricheva 2006), herein we report results on 1) the occurrence of M. persicae in commercial orchards, 2) performance variation (population growth rate) of M. persicae on these cultivars during two seasons in the orchards, and 3) laboratory assays assessing the rejection of M. persicae for these cultivars, which included no-choice experiments and probing behavior recordings.

**Materials and Methods**

**Aphid Occurrence on P. persica Orchards.** Between August 2007 and March 2008, 15 orchards of various peach and nectarine cultivars were monitored to assess the presence of the M. persicae (Table 1). These cultivars are widely cultivated in central Chile, and their fruit is exported mostly as fresh fruit to several markets. The orchards were located in the Quinta de Tilcoco district, the Cachapoal province, and the O'Higgins Region in Chile. For each cultivar, 100 randomly selected trees (planted with 3.5 m between rows and 2 m between trees within rows) were sampled every 15 d after a diagonal transect line. One branch per tree was visually assessed for the presence of aphids. The occurrence of aphids was estimated as the mean proportion of aphid-infested trees. In total, 16 sampling dates were undertaken. The data were obtained from orchards under Good Agricultural Practices (GAP) certification. These orchards were under conventional pest management, and aphid occurrence was a result of natural aphid arrival in the orchards.

**Experimental Plants.** All of the experiments were performed with the commercial cultivars of P. persica used for the aphid occurrence study (see above). The age of trees used varied from 6 to 11 yr old, and all trees were grown on ‘Nemaguard’ rootstocks. Different peach and nectarine cultivars were used for different types of assays, as listed in Table 1. The selection of these cultivars reflected their current use by most Chilean growers (Gratacos 2004) and their availability in the commercial orchards during the study period.

**Performance Variation.** During the spring and summer of 2009–2010 and 2010–2011, 10 orchards of various P. persica cultivars were selected for performance assays (Table 1). Within each orchard, five contiguous

---

**Table 1.** Cultivars of P. persica used to study occurrence of aphids in orchards and performance, probing behaviour, and no-choice of M. persicae

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Occurrence in orchards</th>
<th>Performance</th>
<th>Probing behaviour (EPG)</th>
<th>No-choice</th>
</tr>
</thead>
<tbody>
<tr>
<td>'Flavor Crest'</td>
<td>Peach</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>'Cal Red'</td>
<td>Peach</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>'September Sun'</td>
<td>Peach</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>'Elegant Lady'</td>
<td>Peach</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>'White Lady'</td>
<td>Peach</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>'DU23'</td>
<td>Peach</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>'Flame Crest'</td>
<td>Peach</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>'Sweet September'</td>
<td>Peach</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>'Ryan Sun'</td>
<td>Peach</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>'Summer Free-NIS'</td>
<td>Nectarine</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>'August Pearl'</td>
<td>Nectarine</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>'July Red-NS92'</td>
<td>Nectarine</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>'Artic Snow'</td>
<td>Nectarine</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>'Summer Bright'</td>
<td>Nectarine</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>'August Red'</td>
<td>Nectarine</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>'Fire Bright'</td>
<td>Nectarine</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
</tbody>
</table>


These cultivars were used only in no-choice because insecticides applications in the field could not be avoided.
individual trees, planted in the same row, were selected for experimentation and excluded from the application of pesticides. To avoid the effect of pesticide drift resulting from regular sprays on the assays, three additional adjacent rows contiguous to the selected trees were also excluded from pesticide application. In each of the five trees of each cultivar, three branches ($\approx$30 cm) with at least five extended leaves were selected and marked. The insects used for performance assessment were adults of *M. persicae* originating from a multiclonal stock colony composed of a set of individuals randomly collected the same day of the assays from the 10 different *P. persica* cultivars. This approach ensured the availability of a wide genetic variation in the aphid populations. On each of the selected tree branches, 10 wingless adults from the stock colony were placed on the adaxial side of a leaf, while the rest of the branch was protected with mesh bags from natural enemies and from the released aphids. All branches were removed after 7 d and transferred to the laboratory for aphid counting. The number of aphids on three branches was averaged to obtain one value per tree ($n = 5$). Performance of *M. persicae* in each cultivar was calculated using population growth rate (PGR), which allows an estimation of the developmental rate and nymphal mortality (Gottelli 2001), which is calculated as $(\ln N_2 - \ln N_1) / (t_2 - t_1)$, where $N_1$ is the initial number of aphids, $N_2$ is the final number of aphids, and $(t_2 - t_1)$ is the number of days of the experiment (7 d).

**No-Choice Test.** Young shoots with at least 10 leaves were collected from 14 *P. persica* cultivars (Table 1), placed into pots with 300 ml of water and maintained under controlled conditions ($20^\circ C \pm 2$ and a photoperiod of 16:8 [L:D] h) in a growth chamber. One leaf from each cultivar was extracted from the shoot, and the petioles were covered with humid cotton to avoid dehydration. Each leaf was placed in a petri dish (10 cm diameter), and 10 wingless adult aphids of *M. persicae* were gently placed on the adaxial side of the leaves. The *M. persicae* individuals that were used were obtained from a multiclonal stock colony maintained under controlled conditions at $20^\circ C \pm 2$ and a photoperiod of 16:8 (L:D) h. For the EPGs, each aphid was initially immobilized with the help of a vacuum pump, and an electrode (4 cm of gold wire and 18 $\mu$m diameter) was attached to the dorsum with a silver conductive adhesive (colloidal silver, Ted Pella, Inc., Redding, CA). Another electrode (2 mm copper wire) was introduced into the Pasteur pipette containing *P. persica* branches. Before each recording, the aphids were starved for 15 min and then connected to the amplifier Giga 4. Both electrodes were then connected to a DC circuit and recorded continuously for 4 h. The voltage fluctuations were recorded using the program PROBE 3.4 (F. Tjallingii, Laboratory of Entomology, Wageningen University, Netherlands). Six to 18 replications were performed for each cultivar over 4 h. For the data analysis, the Excel Workbook for the automatic calculation of EPG parameters was used (Sarria et al. 2009). This workbook produced sets of parameters according to the factors involved (e.g., epidermal factor, prephloem factors, xylem factors, phloem factors, and all tissues factors), which can be further analyzed.

**Statistical Analysis.** Aphid occurrence data were analyzed with Kruskal–Wallis analysis of variance (ANOVA) on ranks. Performance data from summer 2009 and spring 2010 were analyzed with one-way ANOVA for repeated measures of ranked data, with years as repeated measures. The no-choice test was compared with a generalized linear model with a Poisson distribution using STATISTICA 10.0 (StatSoft 2004). For EPG, multivariate analyses of variance (MANOVA) to determine whether a significant difference existed among cultivars were performed for
each set of parameters, followed by Tukey test for multiple comparisons. EPG parameters with non-normal distribution were normalized using ln (x + 1). Parameters that could not be normalized were analyzed with Kruskal–Wallis nonparametric ANOVA, followed by multiple comparisons. Given that Kruskal–Wallis tests, unlike MANOVA, do not include possible correlations between parameters, correlations were independently assessed. To assess whether any of these EPG parameters correlated with PGR of aphids, a multiple regression analysis using these parameters as predictor variables and the PGR of aphids as the dependent variable was performed. In addition, EPG parameters differing among cultivars (Table 2) were subjected to principal component analysis (PCA) to separate susceptible from resistant cultivars and to find which of these parameter were more associated with resistance. These analyses were performed using SPSS 11.5 for Windows (SPSS 2001).

Results

Aphid Occurrence in P. persica Orachods. The P. persica cultivars that were monitored, including peaches and nectarines, exhibited relatively similar proportions of aphid occurrence, with ≈1 to 3% of each parameter included total duration of the recording when the E2 waveform was not observed during the recording.

Table 2. Summary results of M. persicae probing behaviour on 11 cultivars of P. persica

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Prephloem factors</td>
<td>45.2 ± 32.5</td>
<td>41.6 ± 39.4ab</td>
<td>28.1 ± 25.5ab</td>
<td>43.2 ± 45.2ab</td>
<td>24.3 ± 28.4ab</td>
<td>44.8 ± 22.1ab</td>
</tr>
<tr>
<td>1. Duration of nonprobe period before the first E (min)</td>
<td>2. Mean duration of pd (s)</td>
<td>4.0 ± 0.4ab</td>
<td>4.0 ± 0.7a</td>
<td>4.0 ± 0.8ab</td>
<td>4.1 ± 0.5ab</td>
<td>4.4 ± 0.8ab</td>
</tr>
<tr>
<td>3. Total duration of F (s)</td>
<td>278.3 ± 260.4c</td>
<td>353.3 ± 485.3c</td>
<td>381.2 ± 434.1abc</td>
<td>386.7 ± 747.5abc</td>
<td>916.7 ± 2240.6dabc</td>
<td>261.8 ± 444.9abc</td>
</tr>
<tr>
<td>4. Mean duration of F’ (s)</td>
<td>1413 ± 2299.6b</td>
<td>966.2 ± 1195.5ab</td>
<td>67.6 ± 64.2ab</td>
<td>307.4 ± 763.3ab</td>
<td>915.5 ± 2240.6dab</td>
<td>117.1 ± 204.6ab</td>
</tr>
<tr>
<td>5. Mean duration of subphases II-2 of pd (s)</td>
<td>14 ± 0.2a</td>
<td>1.2 ± 0.2ab</td>
<td>1.4 ± 0.3ab</td>
<td>1.4 ± 0.4ab</td>
<td>1.6 ± 0.2a</td>
<td>1.5 ± 0.3ab</td>
</tr>
<tr>
<td>6. Mean duration of subphases II-3 of pd (s)</td>
<td>1.2 ± 0.3ab</td>
<td>1.2 ± 0.2ab</td>
<td>1.4 ± 0.3ab</td>
<td>1.2 ± 0.3ab</td>
<td>1.6 ± 0.2ab</td>
<td>1.4 ± 0.2ab</td>
</tr>
<tr>
<td>Phloem factors</td>
<td>7. Number of E1</td>
<td>0.3 ± 0.5a</td>
<td>2.3 ± 2.9a</td>
<td>1 ± 0.5a</td>
<td>3.3 ± 2.6a</td>
<td>3.5 ± 4.3a</td>
</tr>
<tr>
<td>8. Duration of first E (s)</td>
<td>422.9 ± 1306.1abc</td>
<td>105.7 ± 104.7abc</td>
<td>71.5 ± 56.3abc</td>
<td>67.2 ± 67.5ab</td>
<td>180.4 ± 187.6abc</td>
<td>141.6 ± 195.6abc</td>
</tr>
<tr>
<td>9. Total duration of E1 (s)</td>
<td>17.8 ± 32.0b</td>
<td>69.4 ± 1026.4abc</td>
<td>74.3 ± 59.9abc</td>
<td>325.4 ± 385.6abc</td>
<td>431.0 ± 504.2abc</td>
<td>453.5 ± 518.7abc</td>
</tr>
<tr>
<td>All tissue factors</td>
<td>10. Total duration of pd (s)</td>
<td>354.8 ± 160.9ab</td>
<td>403.2 ± 210.5abc</td>
<td>262.2 ± 152.9ab</td>
<td>455.0 ± 277.0abc</td>
<td>287.1 ± 151.8ab</td>
</tr>
<tr>
<td>11. Time from the beginning of the first probe to first pd (min)</td>
<td>496.3 ± 939.8ab</td>
<td>968.7 ± 2019.6abc</td>
<td>593.5 ± 660.0abc</td>
<td>1599.0 ± 2447.1ab</td>
<td>450.2 ± 780.3abc</td>
<td>660.6 ± 891.7abc</td>
</tr>
<tr>
<td>12. Total duration of no phloemasic phase (min)</td>
<td>64.9 ± 106.2b</td>
<td>201.3 ± 109.6abc</td>
<td>212.1 ± 79.5</td>
<td>160.3 ± 102.4abc</td>
<td>228 ± 19.7a</td>
<td>163.2 ± 111.7ab</td>
</tr>
<tr>
<td>13. Time from start of EPG to first E2 (min)</td>
<td>233.2 ± 21.4ab</td>
<td>192.7 ± 80.8abc</td>
<td>239.9 ± 0.0ab</td>
<td>187.9 ± 79.6abc</td>
<td>2301 ± 24.3b</td>
<td>239 ± 0.1ab</td>
</tr>
<tr>
<td>14. Time from first probe to first E2 (min)</td>
<td>230.0 ± 31.6ab</td>
<td>189.3 ± 56.5abc</td>
<td>239.0 ± 0.0ab</td>
<td>182.5 ± 87.2abc</td>
<td>230.0 ± 24.4a</td>
<td>239.1 ± 0.1ab</td>
</tr>
<tr>
<td>15. Time from the beginning of the probe reaching the first E2 to that E2 (min)</td>
<td>222.5 ± 55.2ab</td>
<td>1714.3 ± 112.1abc</td>
<td>239.9 ± 0.0ab</td>
<td>165.1 ± 112.1abc</td>
<td>2051 ± 85.4b</td>
<td>239.1 ± 0.1ab</td>
</tr>
<tr>
<td>16. Average duration of pd during third hour</td>
<td>2.3 ± 2.0a</td>
<td>3.9 ± 0.5ab</td>
<td>2.9 ± 1.8a</td>
<td>3.5 ± 1.5ab</td>
<td>3.2 ± 1.6ab</td>
<td>4.2 ± 0.9ab</td>
</tr>
</tbody>
</table>

*Parameter including total duration of the recording when the E2 waveform was not observed during the recording.
buds infected by aphids (Fig. 1), although there was significant variation among cultivars ($H = 24.76; P = 0.037$). Only the nectarine cultivars ‘August Red’ and ‘Summer Bright’ exhibited a higher proportion of infestation, with over 20% occurrence. It is important to mention that the presence of the parasitoids *Aphidius colemani* and *Aphidius rhopalosiphi* (Hymenoptera: Braconidae) was detected in low abundances in some cultivars, but their prevalence could not be assessed.

**Performance Variation.** *M. persicae* displayed significant variation in PGR on different *P. persica* cultivars ($F = 5.32; df = 9.39; P < 0.001$), with significant effects for year ($F = 7.49; df = 1.39; P < 0.001$) and the year × cultivar interaction ($F = 10.65; df = 9.39; P < 0.001$). Only ‘Flavor Crest,’ ‘September Sun,’ and ‘White Lady’ exhibited a different PGR between years (Fig. 2). However, independent of the year (Fig. 3), the cultivars White Lady, ‘Cal Red,’ ‘July Red-N92,’ ‘Du23,’ and September Sun exhibited similar high PGRs, while ‘August Red’ and ‘Summer Free-N18’ cultivars exhibited similar low PGRs (Fig. 3).

![Fig. 1. Aphid occurrence on commercial cultivars of *P. persicae* in central Chile (O’Higgins Region) during the spring-summer season of 2008–2009. Bars indicate the 95% CI. Different letters above bars represent significant differences ($P < 0.05$) among cultivar based on multiple comparison test after Kruskal-Wallis.](image1)

![Fig. 2. Population growth rate (PGR, mean ± SE) of the aphid *M. persicae* on 10 *P. persica* cultivars as measured in field assays for two consecutive years. Asterisks indicate significant difference ($P < 0.05$) between years within cultivar based on Tukey multiple comparison test after ANOVA for repeated measures.](image2)
No-Choice Test. After 24 h, there were significant differences among cultivars in the number of aphids off of the leaves (GLM, Poisson: Wald $\chi^2 = 35.4$; df = 14; $P = 0.001$; Fig. 4). ‘July Red-NS92’ had the highest number of aphids off of the leaves, although ‘Artic Snow,’ ‘Summer Bright,’ ‘Sweet September,’ and ‘August Pearl’ had also higher values of rejection. Flavor Crest and Summer Free-N18 had the lowest number of aphids off of their leaves (Fig. 4). All other cultivars exhibited values between these contrasting cultivars.

Aphid Probing Behavior. Among the parameters related to prephloem factors, MANOVA showed significant differences among cultivars (Wilks lambda = 0.2980; $F = 1.906$; df = 60; $P < 0.001$), with the duration of the nonprobe period before the first phloem phase (E) and mean duration of pd showing significant differences among cultivars (Table 2). The duration of the nonprobe period before the first E exhibited the lowest value in ‘Elegant Lady’ and the highest value in August Red, whereas the mean duration of pd exhib-
Duration of the first E (duration of E1) showed the lowest value in August Red and the highest values in Elegant Lady and White Lady (Table 2). Following the analysis of the parameters with a nonparametric univariate test, the total duration of the parameter F (H = 35.52; P = 0.0001) and mean duration of F (H = 34.64; P = 0.0001) showed differences among cultivars. These two parameters are associated with difficulty during penetration at the epidermis/mesophyll level; both were similarly lower in the July Red-NS92, Elegant Lady, and White Lady cultivars, while August Red exhibited the highest values in Elegant Lady and White Lady. The duration of the first E2 showed the lowest value in August Red and the highest values in Elegant Lady. Correlations were found between the following parameters: the number of E1 with duration of first E (r = 0.85; P < 0.05), the number of E1 with total duration of E1 (r = 0.74; P < 0.05), and the duration of first E with total duration of E1 (r = 0.98; P < 0.05). It is worth noting that M. persicae did not exhibit phloem ingestion (E2) in July Red-NS92, Arctic Snow, and September Sun during the recording phase.

For the parameters related to all tissue factors, MANOVA showed significant differences among cultivars (Wilks lambda = 0.214; F = 1.53; df = 90; P < 0.003; Table 2). Among these parameters, total duration of pd showed the highest values in White Lady and Elegant Lady and the lowest value in Arctic Snow. Time from the beginning of the first probe to first pd exhibited the highest value in Flavor Crest and the lowest value in Cal Red (Table 2). Following analysis of the parameters using a nonparametric univariate test, the total duration of the nonphloematic phase (H = 22.83; P = 0.0114), time from start of EPG to first E2 (H = 17.12; P = 0.0716), time from first probe to first E2 (H = 16.94; P = 0.0756), time from the beginning of the probe reaching to first E2 (H = 15.50; P = 0.1148) and average duration of pd during the third hour (H = 35.7; P = 0.0001) exhibited significant differences among cultivars (Table 2). Total duration of the nonphloematic phase showed the highest value in Summer Bright, while the lowest value was found in August Red. Time from start of EPG to the first E2, time from the first probe to the first E2 and time from the beginning of the probe reaching the first E2 exhibited the highest value in Arctic Snow and the lowest value in Elegant Lady. In addition, average duration of pd during the third hour showed the longest value in the cultivars White Lady and the shortest values in August Red and July Red-NS92. A significant correlation was found for time from the beginning of the
Table 3. Factor loadings of the principal components (PC) with varimax normalized based on correlations

<table>
<thead>
<tr>
<th>EPG parameters</th>
<th>PC1</th>
<th>PC2</th>
<th>PC3</th>
<th>PC4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total duration of F</td>
<td>0.590711</td>
<td>-0.572507</td>
<td>-0.227590</td>
<td>0.174460</td>
</tr>
<tr>
<td>Duration of nonprobed period before the first E</td>
<td>-0.577323</td>
<td>-0.073207</td>
<td>-0.323178</td>
<td>0.494832</td>
</tr>
<tr>
<td>Mean duration of pd</td>
<td>0.462663</td>
<td>0.508917</td>
<td>0.702332</td>
<td>0.064575</td>
</tr>
<tr>
<td>Mean duration of subphases II-2 of pd</td>
<td>0.569910</td>
<td>0.349965</td>
<td>0.454661</td>
<td>0.361936</td>
</tr>
<tr>
<td>Mean duration of subphases II-3 of pd</td>
<td>0.457400</td>
<td>0.511040</td>
<td>0.641210</td>
<td>0.139960</td>
</tr>
<tr>
<td>Mean duration of F</td>
<td>-0.089415</td>
<td>-0.928257</td>
<td>-0.249817</td>
<td>0.139960</td>
</tr>
<tr>
<td>Number of E1</td>
<td>0.765640</td>
<td>0.534073</td>
<td>0.105211</td>
<td>0.105967</td>
</tr>
<tr>
<td>Duration of first E</td>
<td>-0.312332</td>
<td>-0.549457</td>
<td>0.275053</td>
<td>0.000183</td>
</tr>
<tr>
<td>Total duration of E1</td>
<td>0.911786</td>
<td>0.127910</td>
<td>0.132964</td>
<td>-0.217121</td>
</tr>
<tr>
<td>Total duration of pd</td>
<td>0.104611</td>
<td>0.038549</td>
<td>0.663951</td>
<td>-0.037987</td>
</tr>
<tr>
<td>Time from the beginning of the first probe to first pd</td>
<td>-0.174095</td>
<td>0.263109</td>
<td>-0.625230</td>
<td>0.610817</td>
</tr>
<tr>
<td>Total duration of no phloematic phase</td>
<td>0.161332</td>
<td>0.249573</td>
<td>0.057177</td>
<td>-0.856570</td>
</tr>
<tr>
<td>Time from start of EPG to first E2</td>
<td>-0.962316</td>
<td>-0.063160</td>
<td>-0.136158</td>
<td>0.118176</td>
</tr>
<tr>
<td>Time from first probe to first E2</td>
<td>-0.959549</td>
<td>-0.080112</td>
<td>-0.168655</td>
<td>0.095755</td>
</tr>
<tr>
<td>Time from the beginning of the probe reaching the first E2 to that E2 (min)</td>
<td>-0.927830</td>
<td>-0.073587</td>
<td>-0.210301</td>
<td>0.126552</td>
</tr>
<tr>
<td>Average duration of pd during third hour</td>
<td>0.540277</td>
<td>0.292054</td>
<td>0.429770</td>
<td>-0.348065</td>
</tr>
<tr>
<td>Proportion</td>
<td>0.592316</td>
<td>0.190900</td>
<td>0.162925</td>
<td>0.114667</td>
</tr>
</tbody>
</table>

Bold no. shows the principal contribution parameters.

first probe to the first pd and time from the beginning of first probe to the first E2 ($r = 0.23; P < 0.05$). Similarly, time from the start of EPG to the first E2 and time from the beginning of first probe to the first E2 correlated significantly ($r = 0.81; P < 0.05$). The mean duration of subphase II-1 was the only EPG parameter that correlated significantly with PGR ($r = 0.32; P < 0.001$).

The PCA analysis with varimax normalized rotation, including 16 EPG parameters and 11 cultivars, showed four principal components with 35.6, 21.9, 16.3, and 11.5% explained variance and a cumulative variance of 85.3% (eigenvalues $\geq 1$). The PC1 and PC2 components of scores and loading (Table 3; Fig. 6A,B) showed that the Elegant Lady cultivar was mostly associated with a longer total duration of E1 and a higher number of E1 (high score on PC1). In addition, August Red was associated with a longer total duration and mean duration of F and duration of first E (high score on PC2). However, July Red-NS92, Arctic Snow, and September Sun were associated with a longer time from the beginning of the first probe, longer probe periods before the first E and a longer time from the start of EPG to first E2 (high scores on PC1).

Discussion

By estimating its occurrence in peach and nectarine orchards, measuring PGR, monitoring leaf rejection (no-choice test), and monitoring probing behavior in laboratory assays, we determined an integrated assessment of variation in resistance to the aphid *M. persicae* among a set of commercial cultivars of *P. persica*. Considering the evidence, the resistance or susceptibility status of a given peach or nectarine cultivar depends on the data type. Nevertheless, taken together, some cultivars exhibited distinct resistance to *M. persicae*.

Aphid occurrence in the orchards yielded important insight into susceptibility rather than resistance. Here, August Red and Summer Bright showed the highest values of aphid occurrence (Fig. 1), although most of the cultivars exhibited a low occurrence. However, aphid occurrence in the orchards may mask the genetic variation in resistance to aphids because the orchards studied were under conventional pest management. To determine the status of resistance of each cultivar, it is useful to evaluate the results from different manipulative experiments at the performance, no-choice or probing behavior level. For instance, the cultivar Summer Bright exhibited the highest occurrence of aphids in the orchards, that is, the highest susceptibility to aphids. However, a no-choice test showed an intermediate resistance (Fig. 4) for Summer Bright, while probing behavior assays revealed moderate susceptibility (Table 2) for this cultivar. Nevertheless, some cultivars exhibited a similar tendency both in aphid occurrence in the orchards and in manipulative experiments. For example, the cultivar Elegant Lady exhibited a low aphid occurrence with 2.3% of infested buds (Fig. 1), while it ranked as the lowest cultivar in PGR of *M. persicae*. The no-choice experiment showed an intermediate resistance for Elegant Lady (Fig. 4), and EPG data were characterized by a higher frequency and longer duration of saliva into the sieve elements (waveform E1). Thus, Elegant Lady displays signatures of resistance across most of the studies performed. However, this was not the case for all cultivars.

An overview of the evidence obtained using the manipulative experiments (performance, no-choice, and EPG) may facilitate the discovery of the most resistant cultivars. Regarding the performance variation experiments (PGR), two tendencies were apparent among cultivars (Fig. 3): White Lady and Cal Red exhibited susceptibility to aphids, while Elegant Lady was the most resistant to aphids. Because these results reflect 7 d of reproduction, such resistance is most likely the result of an antibiotic effect. The results from the no-choice assay showed a completely different trend, with only 20% of aphids rejecting the leaves after 24 h, which is an indicator of high susceptibility.
However, in these experiments, the aphid’s rejection of the July Red-NS92 cultivar was higher, suggesting a strong antixenotic effect.

For the EPG results, varying resistance or susceptibility statuses were defined according to the plant factor involved (Table 2). Because EPG parameters can be useful in identifying the tissues containing putative resistance factors (Tjallingii 1995), a detailed analysis of the EPG results may help elucidate the antibiotic and antixenotic components of the resistance mechanisms. Because EPG data are used to produce many different nonindependent parameters, which are associated with different resistant factors, a global multivariate view may help reduce this complexity. The results from the PCA analysis (Fig. 6A,B) suggest that Elegant Lady is a resistant cultivar that is associated with the variables correlated with frequent and longer salivation into the sieve elements (E1), which is an indication of phloemematic factor acting on resistance. August Red exhibited resistance associated with the presence of the stylet’s penetration difficulties (F) and a longer duration of the first phloem phase, indicating both the presence of prephloematic and phloematic factors. Finally, July Red-NS92 showed resistance associated with a lack of phloem ingestion (E2) and all tissues parameters, indicating the presence of prephloematic factors.

EPG allowed a detailed view of the pattern of cell punctures within plant tissues (Tjallingii et al. 2010), which may aid in discerning factors involved in resistance. Using this information, the mean duration of the subphases of pds were analyzed. August Red, which was found to possess prephloematic resistance, showed a short duration of pds (Table 2; Fig. 5),

Fig. 6. Principal components analysis with varimax normalized rotation for the EPG study of M. persicae probing on several P. persica cultivars. Panels shows two principal components (PC1 vs. PC2) for (A) scores of cultivars and (B) loading for parameters. EPG parameters are numbered as described in Table 2.
particularly in subphase II-2. It is interesting to note that *M. persicae* showed the second lowest performance in August Red. A short pds may be associated with the presence of intracellular metabolites that induce the rapid withdrawal of stylets (Powell et al. 2006). The role of subphase II-2 is unknown (Tjallingii et al. 2010). No further conclusion could be drawn from this detailed view for the Elegant Lady and July Red-NS92 cultivars.

Elegant Lady exhibited resistance mostly because of phloemetic factors. It is particularly interesting to note that in this cultivar, salivation after sieve element puncture (E1) correlated negatively with PGR, which has been found to occur more frequently in aphid-resistant plants (Klingler et al. 1998, Ramírez and Niemeyer 1999, Tjallingii 2006). A higher number and duration of E1 were also found in other peach cultivars, such as the wild peach *P. davidiana*, which is highly resistant to aphids (Sauge et al. 1998a). However, July Red-NS92, in addition to the lack of phloem ingestion and longer time to commit salivation into the sieve element, exhibited strong prephloemetic resistance, as shown by no-choice assays. However, the performance experiment showed a positive PGR in this cultivar, suggesting that despite an initial antixenotic effect, aphids are likely able to develop induced susceptibility under such experimental conditions, as shown in other aphid-plant systems (Karban and Baldwin 1997, Prado and Tjallingii 1997, Gonzales et al. 2010). No further conclusion could be drawn because of the large difference in the time windows involved between both assays. However, the studies performed allowed the identification of the putative enzymes involved between both assays. However, the studies performed allowed the identification of the putative enzymes involved between both assays. However, the studies performed allowed the identification of the putative enzymes involved between both assays. However, the studies performed allowed the identification of the putative enzymes involved between both assays. However, the studies performed allowed the identification of the putative enzymes involved between both assays.

It is worth noting that the lack of congruence between performance and EPG experiments may be because of the large difference in the time windows involved between both assays. However, the studies performed allowed the identification of the putative origins of the resistance. Other studies addressing the *M. persicae-P. persica* interaction have also identified cultivars with contrasting susceptibility or resistance statuses based on different experiments. A low resistance for the peach cultivar “GF305,” minor resistance for the ‘Summergrand’ and Malo Konare cultivars, and moderate resistance for the Rubira and Weeping Flower Peach cultivars to *M. persicae* were found (Sauge et al. 1998a,b; Sauge 1998). In addition, accesses of the wild species *P. davidiana* were found to be highly resistant to *M. persicae*. In these cultivars, resistance appears to be based on antibiosis (Malo Konare and *P. davidiana*) and antixenosis (Rubira and Weeping Flower Peach). The genetic basis of this response has previously been identified (Monet and Massonié 1994, Lambert and Pascal 2011). The commercial cultivars studied herein are very likely to possess the same genetic basis; however, additional studies are necessary to determine the presence of such a genetic basis.

Peaches and nectarines did not exhibit a major difference in their resistance to aphids. Nevertheless, this may not be the case when aphids attack directly on fruits. Nectarine fruits are glabrous and are apparently more attacked by aphids than peaches (data not shown). However, a slightly higher resistance was found in nectarines because more cultivars of this variety showed low performance and leaf-rejections in no-choice testing. It should be noted that this study only assessed constitutive resistance, although induced resistance has been shown to occur on different cultivars of *P. persicae* and related species (Sauge et al. 2006). Further studies should attempt to identify induced responses to the attacks of *M. persicae* on these cultivars, which would aid in understanding the resistance of *P. persica* varieties and cultivars to the *M. persicae* attacks and aid future breeding programs to improve these cultivars’ resistance to pests and diseases.

Acknowledgments

The authors are grateful to “Don” Gustavo Fernandez from Agricola JHM and Freddy Ascencio for their valuable help in the field. This research was supported by Fondecyt Grant 1100746 awarded to C.R. and by a Ph.D. Grant from the Facultad de Ciencias Agrarias of the Universidad de Talca awarded to J.V.

References Cited


SPSS, Inc. 2001. SPSS base version 11.5 for windows user’s guide computer program, version by SPSS, I., Chicago, IL.


Received 9 March 2012; accepted 19 July 2012.