

Aphid species populations and their seasonal fluctuation in *Prunus* orchards affected by Sharka disease within the quarantine areas of Argentina

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Poblaciones de especies de pulgones y sus dinámicas estacionales en fincas de *Prunus* afectadas por la enfermedad del Sharka dentro de las áreas cuarentenarias de Argentina

RESUMEN. La enfermedad del Sharka es causada por el virus de la viruela del ciruelo y transmitida mediante áfidos. Fue detectada por primera vez en Argentina en el año 2004 y es de importancia cuarentenaria en frutales de carozo. Para determinar qué especies de áfidos pueden actuar como potenciales vectores de esta enfermedad en nuestro país, se identificaron los ejemplares de pulgones en dos fincas de *Prunus* afectados por Sharka localizadas en la región de Cuyo y se describió su fluctuación estacional. Durante las temporadas 2011-2012 y 2012-2013, mediante el uso de trampas amarillas de agua (TAA), se colectaron semanalmente pulgones desde octubre (primavera) hasta abril (otoño), que fueron identificados mediante claves taxonómicas de áfidos alados. En total, 27 especies de áfidos fueron identificadas. La especie más dominante fue *Myzus persicae* (Sulzer). La población de esta especie mostró una fluctuación estacional con dos picos, uno en noviembre y otro en marzo.

PALABRAS CLAVE. Áfidos. Enfermedad cuarentenaria. Frutales de carozo. Viruela del ciruelo. Trampeo de pulgones. Vector.

ABSTRACT. Sharka is a disease of stone fruits, produced by the Plum Pox Virus and transmitted by aphids. It was detected for the first time in Argentina in 2004. In order to assess the aphid species that could act as a potential vector of this disease, we described the aphids community and seasonal fluctuation within two *Prunus* orchards affected by Sharka disease, located within two quarantine areas in Cuyo Region. Aphids were trapped weekly from October (spring) to April (autumn) during 2011-2012 and 2012-2013, by using yellow water traps (TAA), and identified using winged aphid's taxonomic keys. In total, 27 aphid species were identified. The most dominant species collected was *Myzus persicae* (Sulzer). Orchard-trapped population of this dominant aphid showed seasonal fluctuation with two peaks, November (spring) and March (autumn).

KEYWORDS. Aphids. Aphid-trapping. Plum Pox Virus. Quarantine diseases. Stone fruit orchards.

INTRODUCTION

Plum Pox Virus (PPV) is the causal agent of Sharka disease, an economically important disease of stone fruits. Among the symptoms it shows are leaves deformations,

variable chlorosis, spots, stains and chlorotic rings. In addition, the fruits may show spots, lines and/or chlorotic rings that become necrotic, which ends up affecting the pulp, thus losing flavor and reducing consequently its commercial quality and value. The disease also reduce

fruit production as fruit may fall prematurely. This damage can be up to 100% in highly infected orchards and susceptible cultivars (Nemeth, 1986). Sharka disease had been detected in many areas of the world including Europe, America, Asia and Africa (Nemeth, 1986; Mazyad et al., 1992; Herrera, 1994; Bhardwaj et al., 1995; Llacer & Cambra, 1998; Levy et al., 2000a; Thompson et al., 2001; Navratil et al., 2006; Fujiwara et al., 2011).

PPV was first detected in Argentina in 2004 on plum (*Prunus salicina* Lindl) cv. 'Red Beaut' and apricot (*Prunus armeniaca* L.) cv. 'Bulida' in San Juan province (Dal Zotto et al., 2006), and in 2006 on prune (*Prunus domestica* L.) cv. 'D'Agen' in the south of Mendoza province (Rossini et al., 2009). In 2005, it was considered a quarantine pest under official control by the National Service of Health and Food Quality of Argentina (SENASA, 2005) and quarantine areas were declared. Since then, the movement of plant material from quarantine areas is prohibited (Dal Zotto et al., 2006; Rossini et al., 2012) and nurseries are required to test stone fruit propagation material annually to prevent the spread of the virus. After many years of surveys and destruction of infected material, the incidence of the virus in nurseries is very low, as well as in commercial crops. Currently, there are two quarantine areas in Argentina, one located in Pocitos County, San Juan province, and the other in San Rafael County, south of Mendoza province. The reported virus strain is of the PPV-D type, and none of the detections have been found in peach [*Prunus persica* (L.) Bastch].

A given area becomes infested when the virus is introduced by infested propagation material. After the introduction of infested plants, trees around the infested tree become infested by means of several species of aphids (Hemiptera: Aphididae) that act as vectors of the disease. This leads to a typical pattern of dispersion through aerial vectors that transmit viruses. Aphids can acquire the virus even during rapid testing into epidermal cells of the infested plant tissue. Effective transmission can be carried out by transient aphid species that do not necessarily colonize the host plant (Ortego, 1991; Labonne et al., 1995). Therefore, colonizing and migratory aphids using the trees as food resources can both transmit the disease. Around 20 different species of aphids have been reported to transmit PPV around the world. Transmission efficacy depends on many factors, such as the virus strain, host species and cultivar, plant age, time of the year and aphid species (Levy et al., 2000a).

The species composition of aphid vector community in orchards varies depending on its location. For example, in Eastern Europe, *Hyalopterus pruni* Geoffroy and *Phorodon humuli* Schrank are the prevalent species when to maximum PPV spread was recorded (Isac et al., 1998). In Western Europe, *Aphis spiraecola* Patch and *Myzus persicae* Sulzer (Hemiptera: Aphididae) were reported as the prevalent vector species (Labonne et al., 1995; Capote et al., 2010). In Pennsylvania orchards, North of the USA,

Rhopalosiphum maidis Fitch (Hemiptera: Aphididae), *A. spiraecola*, and *M. persicae* were consistently dominant (Wallis et al., 2005). Although there are some technical reports on aphids on fruit trees in Argentina (Ortego, 2008), there is currently little knowledge on the species composition and population dynamics of aphid species in *Prunus* orchards in Argentina.

Therefore, in order to understand how PPV could spread, it is relevant to determine the aphid species composition from different *Prunus* orchards. With this aim, we described aphid species composition and population seasonal fluctuation of the dominant species in two *Prunus* orchards within the two Argentinean quarantine areas.

MATERIAL AND METHODS

Studied areas

The study was conducted in two orchards within the Sharka disease quarantine areas in Argentina. In San Juan province, the study site was a 0.5 ha peach orchard (*P. persica* cv. 'O'Henry') of 15-year-old plants located in San Martín County (S31°53'57.6", W68°38'0.81"). In Mendoza province, the study site was a 0.5 ha prune orchard (*P. domestica* cv. 'D'Agen') of 18-year-old plants located in Rama Caída Research Station from INTA, in San Rafael County (S34°39'33.3", W68°23'26.7"). The orchards were located 385 km apart. San Martín orchard was surrounded by alfalfa and numerous species of weeds, unlike the Rama Caída orchard, surrounded by land managed by tillage, with little vegetation cover. In addition, in the Rama Caída orchard, two insecticide treatments with imidacloprid and deltamethrin were carried out during the spring months while no insecticide applications were undertaken in San Martín orchard.

Aphid trapping

Sampling was carried out during two growing seasons from October (spring) to April (autumn) in 2011-2012 and 2012-2013. One square Moericke water trap, 0.60 m long, 0.60 m wide and 0.12 m deep, placed 0.70 m above the ground, was located in each orchard. Traps were filled with water and a few drops of detergent. Aphids were collected 2-3 times per week and stored in 70% alcohol for future identification.

Aphid species identification

Aphids were identified using winged aphids taxonomic key (Remaudière & Seco Fernández, 1990) under a binocular stereoscopic microscope Zeiss model Stemi 2000 C. Specimens were mounted for species identification using a modified method of Eastop & Van Emden (1972). Aphids were placed in test tubes of 10% potassium hydroxide, boiled in water bath for 5 minutes and cooled up to 20 °C. Test tubes were filled with 70% alcohol (the aphids precipitated to the bottom of the tube) and washed as many times as necessary to eliminate the

potassium hydroxide. Once the aphids had been washed, the alcohol was removed again and the clarifying liquid was added, boiled in Bain Marie for 5 to 10 minutes, shaking the tube from time to time to distribute homogeneously the insects in the liquid. A drop of Faure liquid was placed in the center of a labeled glass microscope slide, and the specimen was mounted directly into the drop covered with a slide cover, taking care to maintain aphid appendages in a proper orientation for clear viewing.

Data analysis

Community species richness in both locations were estimated using R software (biodiversity Package) (Kindt & Coe, 2005) by employing the abundance-based estimators Chao and ACE indices (Villarreal et al., 2004). As no striking changes were observed in aphid abundances over the two years for the different sampling dates (data not shown), the sum of the abundances of the two seasons was used for each location. Populations of the most dominant species were graphed to examine their seasonal fluctuation. The criterion for choosing the dominant species was determined by considering that the sum of the relative abundances of the dominant species for each locality was 70% of the total relative abundance (Wallis et al. 2005).

RESULTS

In total, 2520 aphids were caught in the San Martín peach orchard (Table I) and 1136 aphids in the Rama Caída prune orchard (Table II). Of these, 2341 individuals were identified at the species level in San Martín and 612 in Rama Caída. The remaining 179 individuals and 524 individuals in San Martín and in Rama Caída, respectively were identified at the genera level. The identification at the species level revealed 25 aphid species in San Martín and 11 in Rama Caída.

Total species richness predicted by Chao and ACE indices for San Martín orchard were 27 and 28, respectively. Species richness indices predicted two to three additional species of aphids compared with the actual number identified in San Martín. The Chao and ACE indices for Rama Caída had a value of 11 in both cases, indicating that no additional aphid species were predicted compared to the number collected in the field.

Three species were considered dominant in San Martín (Table I) and four in Rama Caída (Table II). In contrast, 22 species in San Martín and seven in Rama Caída were considered rare species.

The dominant species in the San Martín peach orchard were *Brevicoryne brassicae* L. *Myzus persicae* and *Uroleucon sonchi* L. (Table I). In the Rama Caída prune orchard, the four dominant species were: *M. persicae*, *Aphis craccivora* Koch, *B. brassicae* and *Hyperomyzus lactucae* L. Those individuals in which the identification

was reached at the genus level in Rama Caída belonged to the genus *Aphis*.

Table I. Total (N) and relative abundance (%) of number of individuals of aphid species/genus trapped in the San Martín peach orchard during two growing seasons. Dominant species/genus are highlighted in bold.

SPECIES LEVEL	N°	%
<i>Brevicoryne brassicae</i> L.	1022	40,56
<i>Myzus persicae</i> Sulzer***	613	24,33
<i>Uroleucon sonchi</i> L.	180	7,14
<i>Hyperomyzus lactucae</i> L.	148	5,87
<i>Lipaphis erysimi</i> Kaltendbach	126	5
<i>Hyalopterus pruni</i> Geoffroy**	110	4,37
<i>Macrosiphum euphorbiae</i> Thomas	23	0,91
<i>Sipha maydis</i> (Passerini)	21	0,83
<i>Metopolophium dirhodum</i> Walker	19	0,75
<i>Brachycaudus helichrysi</i> Kaltendbach***	16	0,63
<i>Chaitophorus leucomelas</i> Koch	13	0,52
<i>Hyadaphis coriandri</i> Das	10	0,4
<i>Acyrtosiphon kondoi</i> Shinji	7	0,28
<i>Aphis nerii</i> Boyer	6	0,24
<i>Aphis craccivora</i> Koch*	5	0,2
<i>Terioaphis riehmi</i> Börner	4	0,16
<i>Terioaphis trifolii</i> Monell	4	0,16
<i>Aphis spiraeicola</i> Patch**	3	0,12
<i>Rhopalosiphum maidis</i> Fitch	3	0,12
<i>Brachycaudus rumexicolens</i> Patch	2	0,08
<i>Eucarazzia elegans</i> Ferrari	2	0,08
<i>Capitophorus elaeagni</i> Del Guercio	1	0,04
<i>Dysaphis aucupariae</i> Buckton	1	0,04
<i>Hyadaphis foeniculi</i> Passerini	1	0,04
<i>Uroleucon ambrosiae</i> Thomas	1	0,04
TOTAL SPECIES	2341	92,9
GENUS LEVEL		
<i>Aphis</i> sp. L	62	2,46
<i>Terioaphis</i> sp. Walker	38	1,51
<i>Uroleucon</i> sp. Mordvilko	37	1,47
<i>Pemphigus</i> sp. Hartig	26	1,03
<i>Acyrtosiphon</i> sp. Mordvilko	14	0,55
<i>Dysaphis</i> sp. Börner	2	0,08
TOTAL GENUS	179	7,1
TOTAL	2520	

* Recognized PPV vectors

** Most important vector

colonizes *Prunus* (*Aphis spiraeicola*: occasionally).

The three dominant species collected in the San Martín peach orchard showed two abundance peaks during the season. The first one occurred in November (spring in the Southern Hemisphere) and the second one in March (autumn). In contrast, the third dominant species, *U. sonchi*, showed the second peak in February (Fig. 1). The dominant species collected in the Rama Caída prune orchard from Mendoza province showed the same abundance peaks as the most frequent species found in San Martín (Fig. 2).

Table II. Total (N) and relative abundance (%) of number of individuals of aphid species/genus trapped in the Rama Caída plum orchard during two growing seasons. Dominant species/genus are highlighted in bold.

SPECIES LEVEL	N°	%
<i>Myzus persicae</i> Sulzer**#	237	20,86
<i>Aphis craccivora</i> Koch*	95	8,36
<i>Brevicoryne brassicae</i> L.	63	5,55
<i>Hyperomyzus lactucae</i> L.	63	5,55
<i>Lipaphis erysimi</i> Kaltentbach	56	4,93
<i>Terioaphis trifolii</i> Monell	41	3,61
<i>Macrosiphum euphorbiae</i> Thomas	22	1,94
<i>Aphis nerii</i> Boyer	14	1,23
<i>Aulacorthum solani</i> Kaltentbach	7	0,62
<i>Metopolopium dirhodum</i> Walker	7	0,62
<i>Terioaphis riehmii</i> Börner	7	0,62
TOTAL SPECIES	612	53,87
GENUS LEVEL		
<i>Aphis</i> sp. L	345	30,37
<i>Terioaphis</i> sp. Walker	99	8,71
<i>Pemphigus</i> sp. Hartig	36	3,17
<i>Brachycaudus</i> sp. van der Goot	21	1,85
<i>Acyrtosiphon</i> sp. Mordvilko	15	1,32
<i>Uroleucon</i> sp. Mordvilko	8	0,7
TOTAL GENUS	524	46,13
TOTAL	1136	

* Recognized PPV vectors

** Most important vector

colonizes *Prunus* (*Aphis spiraeicola*: occasionally).

DISCUSSION

In this work, we studied the aphid species composition and seasonal population fluctuation of the dominant species in two *Prunus* orchards within the two Sharka disease quarantine areas from Argentina. We obtained relevant information on aphid species and genera associated with two different hosts in two localities. The number of aphid species found in the San Martín peach orchard was significantly higher than in the Rama Caída prune orchard.

Expected richness indices (Chao and ACE) in the Rama Caída prune orchard were the same as those obtained during our sampling, suggesting that our data is close to the maximum expected (Villarreal et al., 2004). In contrast, species richness indices in the San Martín peach orchard predicted two to three additional aphid species compared to the actual number identified. Furthermore, the species richness value of 25 species obtained in San Martín is similar to that obtained using yellow pan traps in other temperate cropping systems (Di Fonzo et al., 1997; Nault et al., 2004), suggesting that our trapping data were effective to represent the aphid community.

In the locality of San Martín, the dominant species (taking 70% of the total abundance) were *B. brassicae* (40.56%), *Myzus persicae* (24.33%) and *Uroleucon sonchi* (7.14%), of which only *M. persicae* and *U. sonchi* are

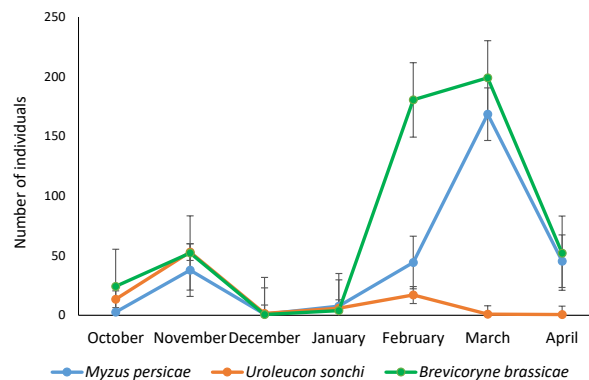


Fig. 1. Abundance fluctuation of the dominant aphid species collected in yellow water trap catches in a peach orchard in San Martín, San Juan, during two consecutive growing seasons (2011-2012 and 2012-2013). Mean ± se.

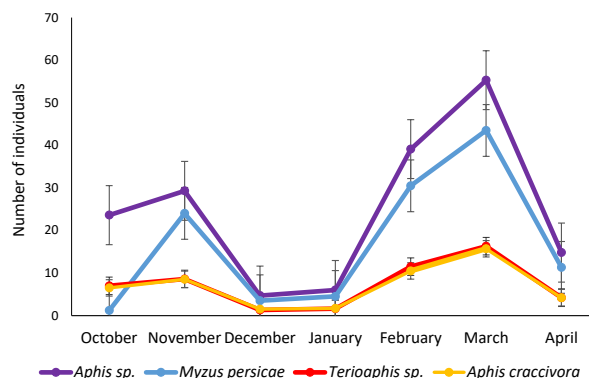


Fig. 2. Abundance fluctuation of the dominant aphid species/genus collected in yellow water trap catches in a prune orchard in Rama Caída, Mendoza, during two consecutive growing seasons (2011-2012 and 2012-2013). Mean ± se.

recorded as important vector species of PPV (Labonne et al., 1995; Levy et al., 2000b., Wallis et al., 2005). Of these two species, only *M. persicae* has been observed colonizing *Prunus* plantations in Mendoza, Argentina (Ortego, 2008). Other species that are recorded as vectors of PPV and were found in this locality, yet with low abundance, were *Hyalopterus pruni*, *Aphis craccivora* and *Aphis spiraeicola*. Of those only *H. pruni* has been reported to colonize *Prunus* in France (Levy et al., 2000b).

In Rama Caída, the dominant species were *M. persicae* (20.86%) and *Aphis craccivora* (8.36%). This location also included a large number of specimens that were identified up to genus level such as *Aphis* sp. (30.37%) and *Terioaphis* sp. (8.71%) as already noticed by Avila et al. (2014). As in San Martín, *M. persicae* presented a high abundance. In contrast, in Rama Caída, within the dominant species, we found *A. craccivora*, which in San Martín was found in low abundance. In the case of the specimens that were identified at the genus level, such as *Aphis* spp., additional sampling and identification efforts

should be a priority since they may contain potential vectors of PPV.

Comparisons between the two sampling areas is not possible for several reasons. First, they had different *Prunus* species and host species has been shown to affect aphids' community composition and richness (Levy et al., 2000a). Second, the surrounding vegetation was not similar. We suspect that the greater richness and abundance of aphid species in the San Martín peach orchard could be due to the surrounding vegetation. Most of the aphid species found in this orchard feed on weeds (mainly from the families Asteraceae, Brassicaceae, Solanaceae, Fabaceae and Poaceae) and in this location, weeds prevail in the surroundings of the orchard. Third, the use of insecticides on Rama Caída may have affected the abundance of aphid species (Bravo Sayes, 2021), which not surprisingly, showed the lowest abundance. In this sense, further surveys are required to confirm such differences and determine the relevant factors that generate these differences.

Only three species reported here colonize frequently *Prunus* sp.: *M. persicae*, *B. helichrysi* and *H. pruni* (Levy et al., 2000b). However, nonpersistently spread viruses can be acquired and transmitted rapidly by transient aphids moving rapidly through orchards; therefore, transient aphids can be also relevant in the way Sharka disease may spread (Labonne et al., 1995). This is of relevance considering that controlled PPV transmission tests in the laboratory have shown that many aphid species can transmit the virus at very low levels of PPV in the plant, especially if large numbers of aphids are used per plant (Labonne et al., 1995; Gildow et al., 2004; Piglionico et al., 2021). Therefore, the contribution to PPV spread by inefficient vector species occurring in high densities at the right time in the orchard during infrequent immigrations should not be disregarded (Wallis et al., 2005). In previous works, in peach crops, *M. persicae*, *U. sonchi*, *H. pruni* were demonstrated to be able to spread PPV (Levy et al., 2000b; Gildow et al., 2004). In addition, three species that occurred in low prevalence in San Martín A. *spiraecola*, *Brachycaudus helichrysi* Kaltenbach and *Dysaphis aucupariae* Buckton have been recorded as vectors of PPV on *Prunus* cultivars (Isac et al., 1998; Gildow et al., 2004). Our results in relation to species found were like those previously described by several authors in Spain, France and North America (Labonne et al., 1995; Wallis et al., 2005; Capote et al., 2010). Moreover, *M. persicae* was reported as the most efficient vector in experimental transmission of PPV-D isolates in North America (Gildow et al., 2004).

Our findings showed two population's peaks, one in spring (November) and the other in autumn (March) for *M. persicae* that could explain the highest viral incidence observed during spring and early autumn in fruit orchards in Mendoza (Marini et al., 2015). In general, most of the aphid species sampled in this study showed the same

seasonal fluctuation. It remains to be seen whether successful management of these aphids would adequately control the spread of PPV if it becomes endemic. Future local PPV transmission studies are also needed to know PPV vector transmission efficacy among different PPV hosts. This is of high importance given that very little is known about PPV transmission efficacy and the mechanisms of how PPV spreads under field conditions. Surveys reporting which *Aphis* species colonize *Prunus* spp. are also needed as well as the impact of migratory non-colonizing aphids on PPV transmission.

In all, our results provide novel and valuable baseline information related to species richness and time of occurrence of aphids in *Prunus* orchards inside two quarantine areas in Argentina. Although data should be updated and validated extending the survey area, we propose that due to the effective PPV vector capacity and its consistent dominant presence as transient insects in peach and prune orchards during spring, *M. persicae*, may be the most important potential vector of PPV (Sharka disease) in *Prunus* orchards in Argentina.

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