Universidad
de Talca

Université de Liège

gembloux agro bio tech

FACULTAD DE CIENCIAS AGRARIAS
UNIVERSIDAD DE TALCA - CHILE

AND

COMMUNAUTE FRANCAISE DE BELGIQUE
ACADEMIE UNIVERSITAIRE WALLONIE-EUROPE
UNIVERSITE DE LIEGE – GEMBLOUX AGRO-BIO TECH - BELGIQUE

"DEFENSIVE RESPONSE OF COMMERCIAL VARIETIES OF *Prunus persica* L. TO THE ATTACK OF *Myzus persicae* (Sulzer) APHID"

JAIME VERDUGO LEAL

Thesis presented for obtaining the degree of Doctor en Ciencias Agrarias (U. de Talca) and Docteur en Sciences Agronomiques et Ingénierie Biologique (U. de Liège)

Thesis Directors: Dr. Claudio Ramírez R. (U. de Talca - Chile)
Dr. Frederic Francis (U. de Liège - Bélgica)

TALCA – CHILE
2012

DIRECTOR OF PhD THESIS

Thesis Director: Dr. Claudio C. Ramirez

Thesis Director: Dr. Frederic Francis

COMMITTEE OF EVALUATION:

Dr. Eduardo Fuentes, Universidad de Talca

PhD. Peter Caligari, Universidad de Talca

Dr. Christian Figueroa, Universidad Austral de Chile

Verdugo, J.A. (2011). Defensive response of commercial varieties of *Prunus persica* L. to the attack of *Myzus persicae* (Sulzer) aphid. (PhD Thesis). Facultad de Ciencias Agrarias – Universidad de Talca – Chile and Gembloux, Université de Liège – Gembloux Agro-Bio Tech, Belgium.

Abstract

Peaches and nectarines are among the most important fruit worldwide. Unfortunately, they are heavily attacked by the aphid *M. persicae* causing direct and indirect damages. In Chile, peaches and nectarines are also important fruit products. However, there is little information regarding the variation in susceptibility/resistance among cultivars frequently planted. In this study the interaction between this aphid and commercial genotypes of *P. persica* was addressed. First, commercial cultivars of peaches and nectarines were screened regarding differential resistance in the field (aphid occurrence and performance), as well as in laboratory experiments (no-choice test and feeding behavior). It was found a wide range of different responses in resistance to *M. persicae*, either in the field and laboratory experiments, with some cultivar exhibiting antibiosis, antixenosis or mixed resistances. In a further study, the cultivars with most contrasting resistant patterns were selected with the aim to assess the effect of irrigation on resistance. Two different levels of irrigation were used, well-watered (100% field capacity) and water-deficient (50% field capacity) plants, and the plant defensive response was evaluated. This experiment showed that resistance was modulated by irrigation as under water-deficiency curling produced by aphids was similar between the resistant and susceptible cultivars. It was also found induced resistance and induced susceptibility regardless the cultivar genotypes (resistant or susceptible). In addition, a tolerant response, measured as plant growth after aphid damage, was observed in both cultivars, capacity that was also dependent of irrigation levels with higher tolerance displayed by well-watered plants. Finally, in order to assess how aphids respond to plants with differences in resistant and irrigation, the proteomic profile of aphids that fed on peach cultivars with different resistance and different irrigation treatment was also performed. The proteomic analysis showed that aphids suffered of higher changes in the regulation of proteins after feeding on the susceptible cultivar than on the resistant one. The proteins up-regulated on aphid fed in the susceptible cultivar were mostly involved in the energy

metabolism, whereas on the resistant cultivar the most up-regulated proteins were those associated to the cytoskeleton. All the above helped to a better understanding of both, the defensive response of *Prunus persica* and to the proteomic response of the aphid to plants with different resistance and level of irrigation.

Verdugo, J.A. (2011). Respuesta defensiva de variedades comerciales de *Prunus persica* L. al ataque del áfido *Myzus persicae* (Sulzer). (Tesis doctoral). Facultad de Ciencias Agrarias – Universidad de Talca – Chile y Gembloux, University of Liege – Gembloux Agro-Bio Tech, Bélgica.

Resumen

Durazneros y nectarines son uno de los frutales más importantes en el mundo, los cuales son atacados por el áfido *M. persicae* causando daños directos e indirectos. En Chile, durazneros y nectarines son importantes productos frutícolas los cuales son fuertemente atacados por este áfido. Sin embargo, existe muy poca información enfocada en la variación de la susceptibilidad/resistencia entre los cultivares plantados. En este estudio fue enfocado en la interacción entre este áfido y los genotipos comerciales de *P. persica*. Primero, cultivares comerciales de durazneros y nectarines se seleccionaron buscando la resistencia diferencial en el campo (ocurrencia y desempeño de áfidos), así como en experimentos de laboratorio (pruebas de no elección y comportamiento alimentario). Se pudo encontrar un amplio rango de diferentes respuestas en resistencia a *M. persicae* en experimentos de campo y laboratorio, con algunos cultivares exhibiendo antibiosis, antixenosis o bien una mezcla de ambas. En un estudio adicional, los cultivares con patrones contrastantes en resistencia, fueron seleccionados con el objetivo de evaluar el efecto del riego sobre la resistencia. Se usaron dos niveles diferentes de riego, plantas bien-regadas (100% capacidad de campo) y plantas con déficit de agua (50% capacidad de campo), y se evaluó la respuesta defensiva de la planta. Este experimento mostró que la resistencia fue modulada por el riego, produciendo que plantas resistentes y susceptibles bajo déficit de agua mostraran similar nivel de enrollamiento provocado por áfidos. También se observó resistencia y susceptibilidad inducida, independiente de los cultivares (resistente y susceptible). Una respuesta tolerante, medida como el crecimiento de la planta

después del daño efectuado por el áfido, se observó en ambos cultivares, capacidad que fue dependiente del nivel de riego, particularmente en plantas bien regadas. Finalmente, se evaluó cómo los áfidos responden a plantas con diferencias en resistencia y riego, para lo cual se consideró el perfil proteómico de los áfidos alimentados sobre cultivares de duraznero y tratamiento con diferentes riegos. Los análisis proteómicos mostraron que los áfidos sufrieron más cambios en la regulación de proteínas después de alimentarse en el cultivar más susceptible. Las proteínas sobre-reguladas en áfidos alimentados en el cultivar susceptible actuaban mayoritariamente en el metabolismo de energía, mientras que sobre el cultivar resistente la mayoría fue sobre-regulado en proteínas asociadas al citosqueleto. Todo lo anterior ayudó a entender mejor las respuestas defensivas de *Prunus persica* y la respuesta proteómica del áfido a las plantas con diferente resistencia y nivel de riego.

I dedicate this work to my mom for her support and love

Acknowledgments

To the achievement of this PhD thesis there are many people contributed during these years. I want to acknowledge principally to:

- Grant of Facultad de Ciencias Agrarias of Universidad de Talca.
- Grant Beca de cotutela en el extranjero-Becas Chile-Conicyt.
- Grant of Communauté française de Belgique et la Région wallone.
- Unité et Systèmes de culture Horticoles of INRA-Avignon, France.
- To Conicyt-Project N° 1100746 (Outbreaks and resurgences of green peach aphid *Myzus persicae* (Sulzer) in peaches and nectarines orchards: where do the aphid come from? Responsible research: Claudio Ramirez).
- My thesis director **Dr. Claudio Ramirez** for his trust, help and support during these four years and teach me to trust in my ideas.
- To **Dr. Frederic Francis** for giving me his support and advices during my work in Gembloux.
- To **Dr. Christian Figueroa** which always showed interest in my research and give me the opportunity to work in INRA-Avignon, supported by CONICYT - Scientific Ring ACT-38.
- To **Dr. Marie-Hélène Sauge** by all her advice and help during all these years and be very nice.
- To **Dr. Eduardo Fuentes** for all the advices in my research and give a space in the office of Sanidad Vegetal Department after the earthquake.
- To my friends **Wilson Barros, Esteban Basoalto** and **Juan José Rubiano** for their friendship and those coffee moments speaking about the world and theirs problems (our thesis problems).
- To **Jean-Phillipe Lacroze** to teach and give me all the tips to work with the peaches plants and green peach aphid and his friendship.
- To my friends from Gembloux: **Nicolas Poncelet**, **William Luwaert**, **Julien Bauwens**, **Kacem Lemtiri, Emilie Bosquée, Sophie Vandermoten**, and **Fanny Barsics** for all those good moments.

- To the Laboratorio Interacciones Insecto-Planta team: **Claudio Ramirez, Blas Lavandero, Joceline Barrios, María Eugenia Rubio, Sebastián Ortiz, Marcos Domínguez, Cinthya Villegas and Gabriel Ballesteros**.
- The bachelor students which I worked during my thesis and who contributed to this research: **Tamara Méndez, Rodrigo Bravo, Diego Valenzuela and Rodrigo Cumsille**.
- To **Juliette Goussopoulos** to share unforgettable moments together and her support during a difficult part of my life.
- To **Marta Albornoz, Freddy Ascencio, Carlos de la Fuente, and Mauricio Soto** for their unconditional friendship and **Patricio Ascencio** and **Carolina de la Fuente** (with their dead part of my heart gone with them).

The present thesis has been written according to
Journal of Economic Entomology **requirements**

GENERAL INTRODUCTION

General introduction

Chile is known for its high agricultural production for both domestic consumption and exportation. Every year the fruit cultivated surface increases and some of most important products are grapes, apples, avocados, plums and peaches. However, to become Chile an agricultural power, improvement of quantity and quality of the agricultural products is required. To deliver a good production, optimal water requirements, nutritional, environmental and plant health are needed. The last point involves producing fruit free of disease and pests fruit. Additionally, agricultural production usually suffers from droughts that affect fruit production including their susceptibility to disease and pests.

This study is centered on peaches and nectarines. In Chile, peach and nectarin production in the central region account for the 94% of the national production. Given the climatic conditions, these areas allow adequate accumulation of chilling hours and degree days necessary for the breaking of dormancy and start of production of peaches (Ferreyra et al. 2002, Gratacós 2004). Also in the central valley of Chile, peach production requires adequate availability of irrigation for the appropriate development of fruit growth (Ferreyra et al. 2002). However, peaches and nectarines have a number of related pathogens and pest, whose recognition is important for health measures. Inadequate control may affect up to two year of fruit production yields (Gratacós 2004). One of the most important pests in orchards of peaches and nectarines in Europe is the aphid *Myzus persicae* (Sulzer) (Homoptera: Aphididae) (Sauge 1998a). In Chile, this aphid also causes serious damage in peaches and nectarines, as weakening and shoot losses (Artigas 1994, Cooper et al. 2001). The presence of this pest is an impediment to organic production, because without the application of chemicals to control this pest, the peach production is heavily damaged.

However, in Chile there are no studies on the variation in susceptibility and resistance to this aphid between commercial varieties of peaches and nectarines.

The prediction on the pattern of rainfall in central Chile, where the plantations of peaches and nectarines are mainly located, announce a significant decrease as a consequence of climate change, affecting among other variables, irrigation to crops (Santibañez and Santibañez 2007). This decline, coupled with a reduction in water regulation capacity of the Andean watersheds, could have profound consequences on water availability in spring and summer. Plants under water stress may become more attractive to insects, generating an increase in the population of herbivores, particularly beetles, moths and aphids (Hawkins and Holyoak 1998). Therefore, it is important to determine how peaches and nectarines will respond to the presence of water stress and aphids. That information would be beneficial for preventive purposes aimed to reduce pesticide use and limit crop damage.

Plant-insect interaction

Insect-plant interactions are the result of coevolutionary processes (Fenny 1975, Jermy 1984). The co-adaptation, co-evolution and co-speciation between herbivores and their host plants have been a central topic of modern biology during the last decades. These interactions have been a relevant research topic in recent years, implicating the study of behavioral mechanisms, physiology, genetics and chemistry where insects are involved (Scriber 2002), which also occurs in a complex scenario including different levels of organization from the cellular to the community level (Kessler 2006).

The basic key aspects of insect-plant relationships includes the following process: 1) host plant recognition, 2) plant response to insects, 3) community ecology of herbivore-

plant interactions (natural enemies, competitors, diseases, mutualistism) and 4) abiotic environmental factors mediating insect-plant interactions, generating a geographic mosaic between insects and plants (Scriber 2002).

Host selection mechanisms

Host selection by phytophagous insects usually involves the finding of the right plant to fed on, survive and develop (Bernays and Chapman 1994). Host specialization by insects have leaded to a fine-tune distinction of the correct plant to feed, task where vision, olfaction, mechanosensation and gustation are specifically involved (Kristoffersen 2003). Vision help insects to arrive to the correct host, discriminating shapes, sizes and colors, process that interact with olfaction, because in a wide gamma of plants colors are similar (Bernays and Chapman 1994, Kristoffersen 2003). On the other hand, the olfaction attraction to host plants is primary induced by specific odors (Bernays and Chapman 1994, Kristoffersen 2003). Laboratory studies have shown that insect are attracted and repelled by plant odors which differ between different plants and plant parts, which are kept during host choosing. In aphids it has been described that they are attracted by visual cues, but odors from host plants are more important at short distance. Mechanosensation and gustation are also element of host recognition, which take place even without penetrating the skin or surface, as these can affect the behavior of the insect, accepting the host plant through a sustained feeding and oviposition (Powell et al. 2006).

Once the insects have reached their host plant, the plant does not rest as nothing has happened. Indeed, plant develops a plethora of reactions which has been the subject of extensive research in the last decade.

Plant responses to the attack by herbivores

Resistance and tolerance

In agricultural terms, resistance or tolerance denotes plants that perform better than susceptible ones when facing the invasion of an insect pest (Teetes 1996). Such feature has additional economic benefits as yields reduce crop losses from insect pests and there are lower inputs of pollutant insecticides. Other benefits are of environmental and ecological type, particularly those arising from the increase in species diversity in the agroecosystem resulting from lower effects on non-target species.

Resistance and tolerance are the two main forms of plant defense against attack from herbivores (Agrawal 1998, Strauss and Agrawal 1999, Tiffin 2002). In terms of resistance, this correspond to chemical and mechanical characteristics of the plant that reduce herbivory (antibiosis) and/or preference (antixenosis) (Leimu and Koricheva 2006), which can be also divided into constitutive and induced resistance. While constitutive resistance is expressed independently of the attack, induced resistance is immediately activated once the plant is attacked or damaged (Zhang et al. 2008). Karban and Myers (1989) divided induced responses to three categories: induced responses, induced resistance and induced defense: (1) Induced responses are plant changes that occur after herbivory, these changes may be incidental, such as differences in water content into the plant (Faeth

1992), leaf toughness (Kudo 1996), nitrogen uptake (Jaramillo and Detling 1988), changes in plant secondary metabolism (Baldwin 1994) and trichome density (Baldwin et al. 1990); (2) Induced resistance is a change in the plant that reduces the preference or performance for future attacks produced by herbivores, which may be caused by a variety of biochemical and physical resistance mechanisms (Agrawal 1998); (3) Induced defense is a term reserved for cases where induction results as beneficial in induced plants compared with those not induced. As a result of induction by herbivory may not necessarily be beneficial to plants because, for example, a decreased capacity of larval growth on induced plants may entail a higher herbivory than on non-induced plants (Slanky and Fenny 1977).

Tolerance is defined as the ability of plants to display a high performance despite the negative effects caused by their consumers (herbivores and pathogens) (Strauss and Agrawal 1999, Hurley and Flaspohler 2007). Tolerance has been modeled many times. The compensatory continuum hypothesis (CCH) predicts high tolerance to herbivory in rich environments and lower tolerance under stressed environments (Maschinski and Whitham 1989). On the other hand, the growth rate model (GRM) predict that plants growing under stressful environment will develop below their maximum growth rate and thus have the capacity for regrowth in presence of damage, while plants under normal condition will grow almost in their maximum growth rate and in presence of damage are less capable to recover (Hilbert et al. 1981). Another model is the limiting resource model (LRM), which predict that tolerance is dependent of different non-exclusive aspects such as the resources that are affecting plants, how resources acquisition affects the herbivory and how the herbivory affect resources (Wise and Abrahamson 2005). Other model point out on the relation between tolerance and resistance. For instance, under conditions of deficit of resources plants should display a strong inverse relationship between resistance and

tolerance (Leimu and Koricheva 2006) (trade-off hypothesis between resistance and tolerance). However, resistance and tolerance can occur simultaneously in a species, giving a mixed defense strategy, whose relative importance depends largely on the availability of resources (Nuñez-Farfán et al. 2007).

The resistance and tolerance are costly for plants, which mean exist a tradeoff between plant development or defense and the plants invest in the defense mechanism, but sometime under limited resources have to left the metabolism involved in the defense in prior of the plant development (Herms and Mattson, 1992) and because of that vary in response to different environmental conditions, such as the availability of soil nutrients, herbivory damage, or intra and interspecific competition (Prittinen et al. 2003, Fornoni et al. 2004). Many studies describe how environmental conditions affect the costs of resistance (Bergelson and Purrington 1996, Koricheva 2002). Some theories predict higher costs of resistance in high-stress conditions such as competition or nutrient limitation (Rhoades 1979, Gulmon and Mooney 1986, Zangerl and Bazzaz 1992). Other theories, however, predict low costs of resistance in environments with limited resources (Herms and Mattson 1992). Bergelson and Purrington (1996) conducted a research on the costs of resistance in herbaceous plants, detecting that there are no consistent patterns in environmental effects on costs of resistance. In woody plants the costs of resistance are also influenced by nutritional conditions of the soil (Prittinen et al. 2003). On the other hand, studies addressing how the environment affects costs of tolerance have revealed the presence of costs under stressful conditions and low nutrient availability (Hochwender et al. 2000), in dry and wet environments (Fornoni et al. 2004) and under favorable conditions (Siemens and Zwiazek 2003). In general, the ability to deploy tolerance should be smaller under conditions of limited availability of resources (Nuñez-Farfán et al. 2007).

Most studies of resistance and tolerance have concentrated on annual or short-lived perennial plants. The reasons are due to the need estimate the economic costs of pests on crops and forage plants, which are mostly herbaceous (Haukioja and Koricheva 2000). Good examples in wood plants are in aspen (*Populus tremuloides*) which have revealed that under good quantity of nutrients resistance compete directly with the growth, but not with tolerance (Stevens et al. 2007). Other studies in the genus *Populus* have found that under drought stress resistance to aphids is reduced but tolerance enhanced (Ramírez and Verdugo 2009).

Aphid-plant interactions under different availability of resources

Aphids are adapted to changes in their environment due to its flexible life cycles, including adapting to a wide range of biotic and abiotic stresses through their physiological, behavioral and biochemical responses. However, the population growth rate in aphids depends on the quality of the food supply and stress affect the aphid only if this stress affects the plant, such as drought, plant nutrient content and temperature (Bale et al. 2007). Huberty and Denno (2004) studied the response of various insects species to water stress in plants and showed that insects feeding on phloem tissue are negatively affected by water stress, possibly reducing the firmness and water content interferes the ability of the herbivore to use nitrogen.

Study species:

Prunus persica

Peaches and nectarines, belonging to the Rosaceae family, are very popular fruit grown in the temperate zones worldwide. The scientific name of peach is *Prunus persica* (L.) Batsch and nectarine is *P. persica* var. nectarina, which is a mutant of the peach with smooth skin, and it is almost as old as this, whose origin is unknown. Molecular markers studies have shown that peaches and nectarines belong to different genetic groups (Rojas et al. 2008). Thanks to the continuous work of genetic improvement of peach and nectarine, they have changed considerably from the wild state. Among peaches and nectarines constantly are emerging new cultivars with better characteristics, especially centered in fruit quality. Unlike other fruit species (varieties of which last for longer time periods), the commercial life of peaches or nectarines cultivars usually do not exceed 15 to 20 years, because after that become outdated and new cultivars are produced (Gratacós 2004).

In the next chapters, the study will be centered in the aphid *M. persicae* which cause wilt shoots as some aphids act as vectors for certain viruses, such as the aphid *M. persicae* (Sulzer) transmitted Plum Plox Virus (PPV) (Pascal et al. 2002). Presently the water is a scarce resource in central Chile. More information is needed to use water efficiently during the growing season that affect production and quality of fruit harvested (Gratacós 2004). Plants require a quantity of water on the soil to take advantage of food (mineral salts) for nutrition, as well as irrigation frequency is according to the type of soil (Montaño 2002).

***Myzus persicae* (Sulzer)**

The green peach aphid, *M. persicae* (Sulzer) (Hemiptera: Aphididae) is a generalist aphid using the peach trees, *P. persica* L. (Rosaceae) as its primary host. As secondary host uses

more than 400 plants of different families (Blackman and Eastop 2000). It is considered a serious problem due to the wide spectrum of plant species that can attack and their ability to transmit virus to cause a direct effect on the plant (Foster et al. 2008). In Chile the reproduction is obligate parthenogenetic. Chilean weather conditions appear to determine a probably sexual reproduction, but this have not been reported. On peach orchards, *M. persicae* can cause direct damage by removal of assimilates in the leaves as curly, deformation in the shoots, fruits or flowers fall, weakening the tree, as well as indirect damage caused by the Plum Pox Virus transmission is the principal agent of Sharka disease (Pascal et al. 2002, Sauge et al. 2002, Manachini et al. 2007). A wide range of insecticide resistance mechanisms have also been reported in this aphid species (Moores et al. 1994, Blackman et al. 1995, Martinez-Torres et al. 1999, Foster et al. 2008, Bass et al. 2011).

P. persica- M. persicae interaction

M. persicae has been studied in relation to their secondary hosts but little is known about the resilience of its primary host, peaches and nectarine trees. Mainly in France, since the 70's, this type of interaction has been addressed with field and greenhouses studies using natural aphid populations (Sauge 1998). The Institut National de la Réchèrche Agronomique in France (INRA), in Bordeaux and Avignon, in collaboration with the private sector and producers began by creating new varieties that had a better development and production of those varieties introduced mostly from California, USA (Pascal and Monteaux-Caillet 1998). Also,they began a series of investigations on peach and nectarine resistance to various pests and diseases, one of them is resistance to *M. persicae* (Kervella

et al. 1998, Pascal and Monteaux-Caillet 1998). In addition, it was observed that both the biological and chemical control have had little effectiveness in controlling the pest, therefore host plant resistance in the genus was examine (Massonie et al. 1982) presenting cross-resistance to most insecticides on aphid populations of *M. persicae* against neonicotinoid insecticides (Foster et al. 2008). Resistant varieties to *M. persicae* would reduce production costs and pollution from insecticides toxic waste generated as well as possibly reducing the spread of Sharka disease (Monet and Massonie 1994, Monet et al. 1998).

The genetic determination of resistance to *M. persicae* in *P. persica* seems to be single, dominant and monogenic, as observed in a study of several generations, (Monet and Massonie 1994, Lambert and Pascal 2011). There are cultivars of *P. persicae* as Malo Konare, Weeping Flower Peach and Rubira, which show some resistance to *M. persicae* and also, *P. davidiana*, *P. cerasifera*, *P. kanensis* wild species that are closely related to *P. persica* (Pascal and Monteaux-Caillet 1998). Malo Konare, is a cultivar from Bulgaria which has antibiosis based resistance, which is apparently located in the vascular system and sieve elements, leading to reduced sap ingestion (Sauge et al. 1998a, Sauge et al. 1998b). Weeping Flower Peach is an ornamental peach, native from America, which shows resistance to *M. persicae*, resistance that seems to be controlled by a single dominant gene known as *Rm1*. This resistance is manifested as antixenosis and yellow dots necrotic reactions after four days of colonization (Monet and Massonie 1994, Monet et al. 1998, Sauge et al. 1998b). *P. persica* var. Rubira® is a variety from France, which is used as a rootstock, and presents antixenotic resistance mechanisms, similar to those in Weeping Flower Peach (Sauge et al. 1998a, Sauge et al. 1998b). The infestation of young plants of

this variety causes induced reactions characterized by the appearance of systemic resistance, which are displayed by necrotic damage that would be associated primarily constitutive degradation of substances in response to mechanical injuries and aphid salivary enzymes (Kfoury and Massonie 1995). The gene associated to this resistant was nominated as *Rm2* (Lambert and Pascal 2011) which shows a strong and induced antixenosis-type resistance (Poëssel et al. 2011). Rubira® is characterized by a peach tree with red leaves and it was thought that this feature could be responsible for the resistance. However, a study of crosses and progeny between this variety and the susceptible variety with green leaves "Pamirskij 5" allowed determining that there is no relationship between leaf color and resistance (Pascal et al. 2002). Also the researchers in Bordeaux and Avignon, made the first study to analyze metabolomic interactions between a plant and an insect that feeds on the phloem and could see that Rubira® plants attacked by *M. persicae* decreased carbohydrate levels, amino acids showed no clear answer as glutamine content decreases while phenylalanine increased. Rubira® presented main secondary metabolites such as esters of caffeic acid and chlorogenic acid, which did not change in the presence of aphid attack and dicafeoilquinic 3.5 acid which increases in the presence of aphids (Poëssel et al. 2011).

Other peach species studied in terms of their relationship with *M. persicae* is *P. davidiana*. This species from Lanzhou, an arid region of northwest China is closely related to *P. persica*, exhibit a high resistance to this aphid, which is polygenic type (Lambert et al. 2008). This resistance is based on antibiosis with restricted growth of colonies of aphids. This species is being investigated to use for future crosses with other species of peaches to generate hybrids with resistance to this aphid (Sauge et al. 1998a,

Foulongne et al. 2003, Sauge et al. 2004, Sauge et al. 2006). None of these varieties are grown in Chile. There are not studies about the variation in susceptibility or resistance to *M. persicae* in the varieties of commercial *P. persica* grown in Chile, or how the water availability they affect the status of resistance and susceptibility to aphids.

Aims and thesis chapters

The main aim of this thesis was to study the *P. persica-M. persicae* interaction in the light of plant defenses and insect responses. In the Chapter I several peaches and nectarines cultivated in Chile in commercial orchards were studied in order to screen their susceptibility and resistance to the aphid *M. persicae*. The focus was also to find which type of resistance and at what plant level was located. The study involved field and in laboratory experiments. In Chapter II two selected genotypes of nectarines, which exhibited contrasting resistance in Chapter I, were studied with the aim to understand how the level of irrigation on those cultivar could change the responses of the resistance to the attack of the *M. persicae*, also if this genotypes were able to perform tolerance as other mechanism of defense.

In Chapter III, the peach-nectarine interaction from an "aphid's view" approach was adopted as it was centered in the proteomic response of the aphid *M. persicae* after feeding on two plant genotypes with different levels of resistance to this aphid and also considering the level of water irrigation. Aphid performance was also compared with the proteomic profile exhibited by aphids, with particular interest on which metabolic pathways were

more or less altered on aphids. Finally, main conclusions and perspectives on this study and a resume of the scientific products during the PhD thesis are outlined.

References cited

Agrawal, A. A. 1998. Induced responses to herbivory and increased plant performance. Science 279: 1201-1202.

Alvarez, A. E., M. Dicke, E. Garzo, W. F. Tjallingii, V. Vleeshouwers, and B. Vosman. 2006. Location of resistance factors in the leaves of potato and wild tuber-bearing Solanum species to the aphid *Myzus persicae*. Entomol, Exp. Appl. 157: 145-157.

Artigas, J. N. 1994. Entomología Económica, vol. 1, Universidad de Concepción, Concepción, Chile.

Baldwin, I. T. 1994. Chemical changes rapidly induced by folivory, pp. 1-23. In E. Barnays (ed.), Insect plant interactions. Chemical Rubber Company, Boca Raton, Florida, USA.

Baldwin, I. T., C. L. Sims, and S. E. Kean. 1990. The reproductive consequences associated with inducible alkaloidal responses in wild tobacco. Ecology 71: 252-262.

Bale, J. S., K. L. Ponder, and J. Pritchard. 2007. Coping with stress, pp 287-303. In H.F. Van emden & R. Harrington (eds): Aphids as crop pest. CABI Publishing, London.

Bass, C., A. M. Puinean, M. Andrews, P. Cutler, M. Daniels, J. Elias, V. L. Paul, A. J. Crossthwaite, I. Denholm, L. M. Field, S. P. Foster, R. Lind, M. S. Williamson, and R. Slater. 2011. Mutation of a nicotinic acetylcholine receptor beta subunit is

associated with resistance to neonicotinoid insecticides in the aphid *Myzus persicae*. BMC Neurosci. 12:51.

Bergelson, J., and C. B. Purrington. 1996. Surveying patterns in the cost of resistance in plants. Am. Nat. 48: 536-558.

Bernays, E., and R. Chapman. 1994. Host-plant selection by phytophagus insects, Chapman & Hall, NY, US.

Bjorkman, C. 2000. Interactive effects of host resistance and drought stress on the performance of a gall-making aphid living on Norway spruce. Oecologia 123: 223-231.

Blackman, R. L., and V. F. Eastop. 2000. Aphids on the world's crops: an identification guide, Second edition, Chichester, UK.

Blackman, R. L., J. M. Spence, L. M. Field, and A. L. Devonshire. 1995. Chromosomal location of the amplified esterase genes conferring resistance to insecticides in *Myzus persicae* (Homoptera, Aphididae). Heredity 75: 297-302.

Caffarini, P., A. Pelicano, P. Carrizo, and J. H. Lemcoff. 2006. Impacto del estrés hídrico y la procedencia de *Eucaliptus globulus* Labill. sobre el comportamiento de herbivoría de *Acromymex lundi* Guérin. Idesia Chile 24: 7-11.

Cevik, V., and G. J. King. 2002. High-resolution genetic analysis of the Sd-1 aphid resistance locus in *Malus* spp. Theo. Appl. Gen. 105: 346-354.

Cooper, T., L. Sazo, and K. Sagredo. 2001. Integrated production in Chile: Peaches, nectarines and plums, two years of research and development, pp. 45-50. In W. P. R. S. (WPRS/SROP) (ed.), Bulletin oilb/srop, International Organization for Biological and Integrated Control of Noxious Animals and Plants (OIBC/OILB), Dijon, France.

Dagg, J. 2002. Strategies of sexual reproduction in aphids. PhD Thesis. Georg-August-Universität zu Göttingen, Göttingen.

Delfino, M., and L. Buffa. 2000. Algunas interacciones planta-áfido-hormiga en Córdoba (Argentina). Zool. Baet. 11: 3-15.

Delmotte, F., N. Leterme, J. Bonhomme, C. Rispe, and J. C. Simon. 2001. Multiple routes to asexuality in an aphid species. Proc. Roy. Soc. Biol. Sci. 268: 2291-2299.

Dixon, A. F. G. 1998. Aphid ecology, Chapman and Hall, London, UK.

Dugravot, S., L. Brunissen, E. Letocart, W. F. Tjallingii, C. Vincent, P. Giordanengo, and A. Cherqui. 2007. Local and systemic responses induced by aphids in *Solanum tuberosum* plants. Entomol, Exp. Appl.123: 271-277.

Faeth, S. H. 1992. Do defoliation and subsequent phytochemical responses reduce future herbivory on oak trees. J. Chem. Ecol. 18: 915-925.

Fenny. 1975. Biochemical coevolution between plant and their insect herbivores, pp. 3-15. In G. Raven (ed.), Coevolution of Animals and Plants. University of Texas Press, Texas, USA.

Ferreyra, R., and G. Sellés. 1997. Manejo del riego en condiciones de restricción hídrica, pp. 38. In INIA. Platina [ed.], Serie La Platina N° 67, Santiago, Chile.

Ferreyra, R., G. Selles, and G. Lemus. 2002. Efecto del estrés hídrico durante la fase II de crecimiento del fruto del duraznero cv. Kakamas en el rendimiento y estado hídrico de las plantas. Efecto del estrés hídrico durante la fase II de crecimiento del fruto del duraznero cv. Kakamas en el rendimiento y estado hídrico de las plantas. Agri. Téc. 62: 565-573.

Field, L. M., A. L. Devonshire, and B. G. Forde. 1988. Molecular evidence that insecticide resistance in peach potato aphids (*Myzus persicae* Sulz) results from amplification of an esterase gene. Biochem. J. 251: 309-312.

Figueroa, C. C., J. C. Simon, C. A. Dedryver, and H. M. Niemeyer. 2004. Genetic variability of the recently introduced aphid *Sitobion avenae* in Chile, pp. 219-225. In J.C.Simon, C.A. Dedryver, C. Rispe and M. Hulle (ed.), Aphids in a New Millennium, Institut National de la Recherche Agronomique, Rennes, France.

Filajdic, N., T. B. Sutton, J. F. Walgenbach, and C. R. Unrath. 1995. The influence of apple aphid *Spirea* aphid complex on intensity of *Alternaria* Blotch of apple and fruit-quality characteristics and yield. Plant Dis. 79: 691-694.

Fornoni, J., P. L. Valverde, and J. Nunez-Farfan. 2004. Population variation in the cost and benefit of tolerance and resistance against herbivory in *Datura stramonium*. Evolution 58: 1696-1704.

Foster, S. P., I. Denholm, Z. K. Harling, G. D. Moores, and A. L. Devonshire. 1998. Intensification of insecticide resistance in UK field populations of the peach-potato aphid, *Myzus persicae* (Hemiptera: Aphididae) in 1996. Bull. Entomol. Res. 88: 127-130.

Foster, S. P., D. Cox, L. Oliphant, S. Mitchinson, and I. Denholm. 2008. Correlated responses to neonicotinoid insecticides in clones of the peach-potato aphid, *Myzus persicae* (Hemiptera: Aphididae). Pest Manag. Sci. 64: 1111-1114.

Foulongne, M., T. Pascal, P. Arus, and J. Kervella. 2003. The potential of *Prunus davidiana* for introgression into peach *Prunus persica* (L.) Batsch assessed by comparative mapping. Theor. Appl. Genet. 107: 227-238.

Gibert, C., F. Lescourret, M. Genard, G. Vercambre, and A. P. Pastor. 2005. Modelling the effect of fruit growth on surface conductance to water vapour diffusion. Ann. Bot. 95: 673-683.

Gonzales, W. L., C. C. Ramirez, N. Olea, and H. M. Niemeyer. 2002. Host plant changes produced by the aphid *Sipha flava*: consequences for aphid feeding behaviour and growth. Entomol. Exp. Appl. 103: 107-113.

Gotelli, N. 2001. A Primer of Ecology, 3rd ed. Sinauer Associates, Sunderland, Massachusetts.

Gratacós, E. 2004. El cultivo del duraznero *Prunus persica* (L.) Batsch. Pontificia Universidad Católica de Valparaíso, Valparaiso, Chile.

Grechi, I., M. H. Sauge, B. Sauphanor, N. Hilgert, R. Senoussi, and F. Lescourret. 2008. How does winter pruning affect peach tree - *Myzus persicae* interactions?. Entomol. Exp. Appl. 128: 369-379.

Gulmon, S. L., and H. A. Mooney. 1986. Costs of defense and their effects of plant productivity, pp. 681-698. In T.J. Givnish (ed.), On the economy of plant form and function. Cambridge University Press, Cambridge.

Haukioja, E., and J. Koricheva. 2000. Tolerance to herbivory in woody vs. herbaceous plants. Evol. Ecol. 14: 551-562.

Hawkins, B. A., and M. Holyoak. 1998. Transcontinental crashes of insect populations? Am. Nat. 152: 480-484.

Herms, D. A., and W. J. Mattson. 1992. The dilemma of the plants - to growth or defend? Quart. Rev. Biol. 67: 478-478.

Hilbert, D. W., D. M. Swift, J. K. Detling, and M. I. Dyer. 1981. Relative growth rates and grazing optimization hypothesis. Oecologia 51: 14-18.

Hochwender, C. G., R. S. Fritz, and C. M. Orians. 2000. Using hybrid systems to explore the evolution of tolerance to damage. Evol. Ecol. 14: 509-521.

Huberty, A. F., and R. F. Denno. 2004. Plant water stress and its consequences for herbivorous insects: A new synthesis. Ecology 85: 1383-1398.

Hurley, P. F., and D. Flaspohler. 2007. Defense strategies of forest herbs on great lakes island and mainland sites: does ungulate-browse history play role?, pp. 2, School of Forest Resources and Environmental Science. Michigan Technological University, Michigan, USA.

Infante, R., P. Martinez-Gomez, and S. Predieri. 2008. Quality oriented fruit breeding: Peach *Prunus persica* (L.) Batsch. J. Food Agric. Environ. 6: 342-356.

Isac, M., S. Preda, and M. Marcu. 1998. Aphid species - Vectors of plum pox virus. Acta Virol. 42: 233-234.

Jaramillo, V. J., and J. K. Detling. 1988. Grazing history, defoliation, and comtetition - Effects on shortgrass production and nitrogen accumulation. Ecology 69: 1599-1608.

Jermy, T. 1984. Evolution of insect host plant relationships. Am. Nat. 124: 609-630.

Karban, R., and J. H. Myers. 1989. Induced plant-response to herbivory. Ann. Rev. Ecol. Syst. 20: 331-348.

Karban, R., and I. Baldwin. 1997. Induced responses to herbivory, University Chicago Press, Chicago.

Kervella, J., T. Pascal, F. Pfeiffer, and E. Dirlewanger. 1998. Breeding for multiresistance in peach tree, pp. 177-184. In R. Monet (ed.), Fourth International Peach Symposium, Vols 1-2.

Kessler, A. 2006. Plant-Insect interactions in the era of consolidation in biological sciencies. Nicotiana attenuata as an ecological expression system, pp. 19-37. In M. Dicke, W. Takken (ed.), Chemical Ecology: from gene to ecosystem. Springer, FRONTIS, Wageningen, Netherland.

Kfoury, L., and G. Massonie. 1995. Characteristics of the resistance of the peach cultivar Rubira to *Myzus persicae* Sulzer. Agronomie 15: 277-284.

Klingler, J., G. Powell, G. A. Thompson, and R. Isaacs. 1998. Phloem specific aphid resistance in *Cucumis melo* line AR 5: effects on feeding behaviour and performance of Aphis gossypii. Entomol. Exp. Appl. 86: 79-88.

Koricheva, J. 2002. Meta-analysis of sources of variation in fitness costs of plant antiherbivore defenses. Ecology 83: 176-190.

Kristoffersen, L. 2003. The chemical ecology of Homoptera - from host plants to conspecific interactions, pp. 38, Deparment of Ecology. Lund University, Lund.

Kudo, G. 1996. Herbivory pattern and induced responses to simulated herbivory in *Quercus mongolica* var *grosseserrata*. Ecol. Res. 11: 283-289.

Lambert, P., and T. Pascal. 2011. Mapping Rm2 gene conferring resistance to the green peach aphid (*Myzus persicae* Sulzer) in the peach cultivar "Rubira®". Tree Genet. Genomes 7: 1057-1068.

Lambert, P., M. H. Sauge, J. L. Poëssel, and T. Pascal. 2008. Pyramiding mono and polygenic resistances is one strategy to provide lasting control of the resistance to the green peach aphid and Powdery Mildew in peach. In, 4th International Rosaceae Genomics Conference, 2008, Pucón-Chile.

Le Roux, V., E. D. M. Campan, F. Dubois, C. Vincent, and P. Giordanengo. 2007. Screening for resistance against *Myzus persicae* and *Macrosiphum euphorbiae* among wild *Solanum*. Ann. Appl. Biol. 151: 83-88.

Leimu, R., and J. Koricheva. 2006. A meta-analysis of tradeoffs between plant tolerance and resistance to herbivores: combining the evidence from ecological and agricultural studies. Oikos 112: 1-9.

Manachini, B., P. Casati, L. Cinanni, and P. Bianco. 2007. Role of *Myzus persicae* (Hemiptera : Aphididae) and its secondary hosts in plum pox virus propagation. J. Econ. Entomol. 100: 1047-1052.

Margaritopoulos, J. T., C. Tsourapas, M. Tzortzi, O. M. Kanavaki, and J. A. Tsitsipis. 2005. Host selection by winged colonisers within the *Myzus persicae* group: a contribution towards understanding host specialisation. Ecol. Entom. 30: 406-418.

Martinez-Torres, D., S. P. Foster, L. M. Field, A. L. Devonshire, and M. S. Williamson. 1999. A sodium channel point mutation is associated with resistance to DDT and pyrethroid insecticides in the peach-potato aphid, *Myzus persicae* (Sulzer) (Hemiptera : Aphididae). Insect Mol. Biol. 8: 339-346.

Maschinski, J., and T. G. Whitham. 1989. The continuum of plant-responses to herbivory - The influence of plant association, nutrient availability and timing. Am. Nat. 134: 1-19.

Massonie, G., P. Maison, R. Monet, and C. Grasselly. 1982. Resistance to green peach aphid *Myzus persicae* Sulzer (Homoptera, Aphididae) in *Prunus persica* (L) Batsch and others *Prunus*. Agronomie 2: 63-70.

Maureira, M., and A. Lavín. 2005. Enfermedades y plagas más comunes en la frutilla de fruto blanco en la VII Región, pp. 2. In INIA-Raihuén [ed.], Informativo 14. INIA.

Monet, R., and G. Massonié. 1994. Déterminisme génétique de la résistance au puceron vert (*Myzus persicae*) chez le pêcher. Résultats complémentaires. Agronomie: 177-182.

Monet, R., and G. Massonie. 1994. Genetic determinism of resistance to green peach aphid (*Myzus persicae*) in the peach. Further results. Agronomie 14: 177-182.

Monet, R., A. Guye, and G. Massonie. 1998. Breeding for resistance to green aphid *Myzus persicae* Sulzer in the peach, pp. 171-175. In R. Monet (ed.), Fourth International Peach Symposium, Vols 1-2. International Society Horticultural Science, Leuven 1.

Montaño, R. 2002. Cultivos de Durazneros. Hedecom (ed.), Tarija, Bolivia.

Moore, P. D. 1995. How plants deal with what´s eating them. Sci. Watch 6: 7-8.

Moores, G. D., G. J. Devine, and A. L. Devonshire. 1994. Insecticide-insensitive acetylcholinesterase can enhance esterase-based resistance in *Myzus persicae* and *Myzus nicotianae*. Pestic. Biochem. Physiol. 49: 114-120.

Nuñez-Farfán, J., J. Fornoni, and P. Luis Valverde. 2007. The evolution of resistance and tolerance to herbivores. Ann. Rev. Ecol. Evol. Syst. 38: 541-566.

Pascal, T., and R. Monteux-Caillet. 1998. Peach breeding in France, pp. 117-123, Fourth International Peach Symposium, Vols 1-2. International Society Horticultural Science, Louvain.

Pascal, T., and R. Monteaux-Caillet. 1998. Peach breeding in France, pp. 117-123. In A. Horticulturae (ed.), IV International Peach Symposium.

Pascal, T., F. Pfeiffer, J. Kervella, J. P. Lacroze, and M. H. Sauge. 2002. Inheritance of green peach aphid resistance in the peach cultivar 'Rubira'. Plant Breeding 121: 459-461.

Penvern, S., S. Bellon, J. Fauriel, and B. Sauphanor. 2010. Peach orchard protection strategies and aphid communities: Towards an integrated agroecosystem approach. Crop Prot. 29: 1148-1156.

Pettersson, J., W. F. Tjallingii, and J. Hardie. 2007. Host-plant selection and feeding, pp. 331-352. In H.F. Van emden & R. Harrington (eds): Aphids as crop pest. CABI Publishing, London, 2007, 717 pp

.**Poëssel, J.L., M. H. Sauge, M. Staudt, C. Dufour, C. Deborde, Y. Rahbé, B. Jackson, C. Renaud, M. Maucourt, M.N. Corre, H. El-Aouni, J.P. Lacroze, and A. Moing. 2011.** Metabolite profiling and feeding bioassays suggest a major role for a dicaffeoylquinic acid in induced resistance of peach to *Myzus persicae* aphid. In PR-Proteins and induced resistance against pathogens and insects. Neuchâtel, Switzerland.

Pompon, J., D. Quiring, P. Giordanengo, and Y. Pelletier. 2010. Role of host-plant selection in resistance of wild *Solanum* species to *Macrosiphum euphorbiae* and *Myzus persicae*. Entomol. Exp. Appl. 137: 73-85.

Powell, G. 2005. Intracellular salivation is the aphid activity associated with inoculation of non-persistently transmitted viruses. J. Gen. Virol. 86: 469-472.

Powell, G., C. R. Tosh, and J. Hardie. 2006. Host plant selection by aphids: Behavioral, evolutionary, and applied perspectives. Annu. Rev. Entomol. 51: 309-330,

Prado, E., and W. F. Tjallingii. 1997. Effects of previous plant infestation on sieve element acceptance by two aphids. Entomol. Exp. Appl. 82: 189-200.

Price, P. W. 1991. The plant vigor hypothesis and herbivore attack. Oikos 62: 244-251.

Prittinen, K., J. Pusenius, K. Koivunoro, and H. Roininen. 2003. Genotypic variation in growth and resistance to insect herbivory in silver birch (*Betula pendula*) seedlings. Oecologia 137: 572-577.

Prokopy, R. J., J. L. Mason, M. Christie, and S. E. Wright. 1995. Arthropod pest and natural enemy abundance under second-level versus first-level integrated pest management practices in apple orchards: a 4-year study. Agricul. Ecosys. Environ. 57: 35-47.

Quisenberry, S. S., and X. Ni. 2007. Feeding injury, pp. 331-352. . In H.F. Van emden & R. Harrington (eds): Aphids as crop pest. CABI Publishing, London.

Ramírez, C. C., and J. A. Verdugo. 2009. Water availability affects tolerance and resistance to aphids but not the trade-off between the two. Ecol. Res. 24: 881-888.

Ramírez, C. C., and H. M. Niemeyer. 1999. Salivation into sieve elements in relation to plant chemistry: the case of the aphid *Sitobion fragariae* and the wheat, *Triticum aestivum*. Entomol. Exp. Appl. 91: 111-114.

Reyes, F., N. Fiore, M. A. Reyes, P. Sepulveda, V. Paredes, and H. Prieto. 2003. Biological behavior and partial molecular characterization of six Chilean isolates of Plum pox virus. Plant Dis. 87: 15-20.

Rhoades, D. F. 1979. Evolution of plant chemical defense against herbivores. In J. D. H. Rosenthal G.A. (ed.), Herbivores: their interaction with secondary plant metabolites, vol. 4-54. Academic press, Inc.

Rojas, G., M. A. Mendez, C. Munoz, G. Lemus, and P. Hinrichsen. 2008. Identification of a minimal microsatellite marker panel for the fingerprinting of peach and nectarine cultivars. Elec. J. Biotech. 11: 1-12

Rosales, M., P. Hinrichsen, and G. Herrera. 1998. Molecular characterization of plum pox virus isolated from apricots, plums and peaches in Chile, pp. 401-405, 17th International Symposium on Virus and Virus-Like Diseases of Temperate Fruit Crops: Fruit Tree Diseases.

Santibañez, F., and P. Santibañez. 2007. Cambio climático y degradación de tierras en Latinoamérica y Chile. Rev. Amb. Des. 23:54 - 63.

Sarria, E., M. Cid, E. Garzo, and A. Fereres. 2009. Excel Workbook for automatic parameter calculation of EPG data. Comput. Electron. Agric. 67: 35-42.

Sauge, M. H. 1998. Analysis of the mechanisms of resistance to the green peach aphid in several *Prunus* genotypes, pp. 731-739. In R. Monet (ed.), Fourth International Peach Symposium, Vols 1-2. International Society Horticultural Science, Leuven 1.

Sauge, M., J. Kervella, and T. Pascal. 1998a. Settling behaviour and reproductive potential of the green peach aphid *Myzus persicae* on peach varieties and a related wild *Prunus*. Entomol. Exp. Appl. 89: 233-242.

Sauge, M., J. Kervella, and Y. Rahbe. 1998b. Probing behaviour of the green peach aphid *Myzus persicae* on resistant *Prunus* genotypes. Entomol. Exp. Appl. 89: 223-232.

Sauge, M. H., J. P. Lacroze, J. L. Poessel, T. Pascal, and J. Kervella. 2002. Induced resistance by *Myzus persicae* in the peach cultivar 'Rubira'. Entomol. Exp. Appl. 102: 29-37.

Sauge, M. H., T. Pascal, J. P. Lacroze, F. Pfeiffer, and J. Kervella. 2004. Mapping of a genetic factor of partial resistance to *Myzus persicae* in the wild peach *Prunus davidiana*, that impedes phloem sap ingestion by the aphid, pp. 499-505. In J.C.Simon, C.A. Dedryver, C. Rispe and M. Hulle (ed.), Aphids in a New Millennium, Institut National de la Recherche Agronomique, Rennes, France.

Sauge, M. H., F. Mus, J. P. Lacroze, T. Pascal, J. Kervella, and J. L. Poessel. 2006. Genotypic variation in induced resistance and induced susceptibility in the peach *Myzus persicae* aphid system. Oikos 113: 305-313.

Sauge, M. H., J. L. Poessel, T. Guillemaud, and L. Lapchin. 2011. Resistance induction and herbivore virulence in the interaction between *Myzus persicae* (Sulzer) and a major aphid resistance gene (Rm2) from peach. Arthropod-Plant Interact. 5: 369-377.

Scriber, J. M. 2002. Evolution of insect-plant relationships: chemical constraints, coadaptation, and concordance of insect/plant traits. Entomol. Exp. Appl. 104: 217-235.

Sherman, W., P. Lyrene, and R. Sharpe. 1996. Low-chill peach and nectarine breeding at the University of Florida. Proc. Fla. State Hort. Soc. 109: 222-223.

Siemens, J. A., and J. J. Zwiazek. 2003. Effects of water deficit stress and recovery on the root water relations of trembling aspen (*Populus tremuloides*) seedlings. Plant Sci. 165: 113-120.

Slanky, F., and Fenny. 1977. Stabilization of the rate of nitrogen accumulation of larvae of the cabbage butterfly on wild and cultivated food plants. Ecol. Monogr. 47: 209-228.

SPSS, I. 2001. SPSS base version 11.5 for windows user's guide computer program, version By SPSS, I., Chicago.

StatSoft, I. 2004. STATISTICA (data analysis software system), version 7. www.statsoft.com.

Stevens, M. T., D. M. Waller, and R. L. Lindroth. 2007. Resistance and tolerance in *Populus tremuloides*: genetic variation, costs, and environmental dependency. Evol. Ecol. 21: 829-847.

Stone, C., and P. E. Bacon. 1994. Relationship among moisture stress, insect herbivory, foliar cineole content and the growth of river red gum *Eucalyptus camaldulensis*. J. Appl. Ecol. 31: 604-612.

Strauss, S. Y., and A. A. Agrawal. 1999. The ecology and evolution of plant tolerance to herbivory. Trends Ecol. Evol. 14: 179-185.

Teetes, G. 1996. Plant resistance to insects: a fundamental component of IPM. In I. W. T. Radcliff [ed.]. Universidad de Minnesota.

Tifffin, P. 2002. Competition and time of damage affect the pattern of selection acting on plant defense against herbivores. Ecology 83: 1981-1990.

Tjallingii, W. 1995. Aphid-plant interactions: what goes on in the depth of the tissues? Proc. Exp. App. Entomol. 6: 163-169.

Tjallingii, W. F., and T. H. Esch. 1993. Fine-structure of aphid stylet routes in plant-tissues in correlation with EPG signals. Physiol. Entomol. 18: 317-328.

Tjallingii, W. F., E. Garzo, and A. Fereres. 2010. New structure in cell puncture activities by aphid stylets: a dual-mode EPG study. Entomol. Exp. Appl. 135: 193-207.

Van Helden, M., and W. F. Tjallingii. 2000. Experimental design and analysis in EPG experiments with emphasis on plant resistance research, pp. 144-171. In G. P. Walker, Backus, E.A. (ed.), Principles and applications of electronic monitoring and other techniques in the study of homopteran feeding behavior. Entomological Society of America, Lanham.

White, T. C. R. 1984. The abundance of invertebrate herbivores in relation to the availability of nitrogen in stressed food plants. Oecologia 63: 90-105.

Wise, M. J., and W. G. Abrahamson. 2005. Beyond the compensatory continuum: environmental resource levels and plant tolerance of herbivory. Oikos 109: 417-428.

Zangerl, A. R., and F. A. Bazzaz. 1992. Theory and pattern in plant defense allocation. pp 363–392. In: R. Fritz and E.L. Simms (eds.), Plant resistance to herbivores and pathogens. University of Chicago Press, Chicago, Illinois, USA.

Zhang, P.-J., J.-P. Shu, C.-X. Fu, Y. Zhou, Y. Hu, M. P. Zalucki, and S.-S. Liu. 2008. Trade-offs between constitutive and induced resistance in wild crucifers shown by a natural, but not an artificial, elicitor. Oecologia 157: 83-92.

Chapter I

VARIATION IN RESISTANCE MECHANISMS TO GREEN PEACH APHID AMONG DIFFERENT *Prunus persica* COMMERCIAL CULTIVARS

In the Chapter I, several peaches and nectarines cultivated in Chile in commercial orchards were studied in order to screen their susceptibility and resistance to the green peach aphid *M. persicae*. This aphid causes damage in the Chilean orchards and the most effective mechanism to avoid the attack is the application of insecticides which increases the cost of the production and allow to the insect the development of resistance to the insecticides. However, currently in Chile there is not information about this topic. Most of the studies addressing the resistance of *P. persica* to *M. persicae*, come from studies performed in INRA-France, which during the last decades has been developing a genetic breeding program including improvement of peach resistance to pest and diseases. It is important to note, these studies mentioned before related with resistance to the green peach aphid, *M. persicae*, has been used only with ornamental or wild species related with *P. persica* such as *P. davidiana*, but not in commercial cultivars. These cultivars may not also have a defense mechanism because with the artificial selection of the fruit, the characteristics as flavor and color have been considered more than the natural defense. Also is important if the resistance is associated to the specie *P. persica* or *P. persica* var nectarina. Hence, this chapter contributes to fill this gap by performing field (natural occurrence and aphid performance on different peach and nectarines orchards) and laboratory studies (non-choice, EPG) contribute to fill this gap. In addition, with the EPG technique was possible to determine the tissue level in the plant where is presented the resistance.

Running Head:
Verdugo et al.: Resistance of *P. persica* to the green peach aphid

Journal and Section:
Journal of Economic Entomology
Plant Resistance

Corresponding Author and address:
Claudio C. Ramírez
Instituto de Biología Vegetal y Biotecnología, Universidad de Talca, Chile. Código postal 3465548 .
Phone: 56-71-200-289
Fax: 56-71-200-276
e-mail: clramirez@utalca.cl

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

J.A. VERDUGO[1, 2], T. MÉNDEZ [1], S. ORTIZ-MARTÍNEZ [1], R. CUMSILLE [1] AND C.C. RAMÍREZ [1]

[1] Instituto de Biología y Biotecnología Vegetal, Universidad de Talca, 2 Norte 685, Talca, Chile.
[2] Gembloux Agro-Bio Tech - Université de Liège, Unité d'Entomologie fonctionnelle et évolutive, Passage des Déportés 2, B-5030 Gembloux, Belgium.

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* var. *nectarina* was evaluated. A total of sixteen 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 (EPG) 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 prephloem and 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 EPG experiments, suggesting prephloematic 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 in peaches and nectarines.

KEY WORDS: Resistance, performance, probing, no-choice, aphid

Introduction

Peaches (*Prunus persica* (L.) Batsch) and nectarines (*Prunus persica* var. *nectarina*) are two of the most important fruits worldwide. 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 two years. 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 more than 40 plant families as secondary hosts (Blackman et al. 2000). The green peach aphid produces leaf curling, stunting, 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. 1998b, Sauge et al. 1998a, Lambert and Pascal 2011, Sauge et al. 2011). Studies addressing peach and nectarine resistance to *M. persicae* have utilized performance experiments based on the intrinsic rate of natural increase r_m (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 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. 1998b, Sauge et al. 1998a). 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, Sauge et al. 2002). Genes conferring monogenic resistance to this aphid have been described for the Weeping Flower Peach and Rubira® cultivars (Monet and Massonié 1994, Lambert and Pascal 2011). On the other hand, studies on the wild *Prunus* species, *P. davidiana*, suggested that this cultivar appears to have a phloem-based resistance mechanism (Sauge et al. 1998b, Sauge et al. 1998a). 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 (Grechi et al. 2008), is a promising alternative to the conventional pest management system because, although damage and disease is higher than 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 well characterized.

This study is aimed to determine the variation in resistance mechanisms to attack of *M. persicae* aphid on several commercial cultivars of *P. persica*, including peaches and nectarines commonly planted in central Chile. The resistance studied the aphid response as variable, therefore 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* aphid (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 days following 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-infected trees. A total of sixteen

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 the experiments were performed with the commercial cultivars *P. persica* used for the aphid occurrence study (see above). The age of trees used varied from 6 to 11 years 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 (Gratacós 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, ten orchards of various *P. persica* cultivars were selected for performance assays (Table 1). Within each orchard, five contiguous 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 (ca. 30 cm) with at least five extended leaves were selected and marked. The insects used for performance assessment were *M. persicae* adult aphids originating from a multi-clonal stock colony composed by a set of individuals randomly collected the same day of the assays from the ten different *P. persica* cultivars in the field. This approach ensured the availability of a wide genetic variation in the aphid populations. On each of the selected tree branches, ten 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 seven days 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) (Gotelli 2001) as $(\ln N_2 - \ln N_1)/(t_2-t_1)$, where N_1 was the initial number of aphids, N_2 was the final number of aphids, and $(t_2 - t_1)$ was the number of days of the experiment (seven days).

No-choice Test. Young shoots with at least ten leaves were collected from fourteen *P. persica* cultivars (Table 1), placed into pots with 300 ml of water and maintained under controlled conditions (20ºC ± 2 and 16:8, light:dark) 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 multi-clonal stock colony maintained on sweet-pepper *Capsicum annumm* for at least three parthenogenetic generations before the no-choice tests. The number of individual aphids on the leaves, aphids walking outside the leaves and dead aphids were registered at 1, 2, 3, 6, 12 and 24 hours after the start of the experiments. A total of fifteen replicates per cultivar were conducted.

Aphid Probing Behavior. The electronical penetration graph (EPG-DC) technique was used to assess the probing behavior of *M. persicae* on the *P. persica* cultivars. In this technique, the insect and the plant are both part of an electrical circuit. EPG amplifies

voltage fluctuations resulting from the insect-plant interaction, producing waveforms that are stored and analyzed. Different types of signals are emitted depending on the location and activities performed by the aphid stylet inside the plant tissue (Tjallingii and Esch 1993, Alvarez et al. 2006). Acceptance (e.g., sustained phloem ingestion) or rejection (e.g., no penetration of stylet) may reflect differences in the levels of resistance among cultivars (Sauge et al. 2006). Using the EPG technique, it is also possible to recognize different waveforms, such F, which reflects stylet difficulties during penetration at the epidermis/mesophyll level; C, which indicates intercellular penetration by the stylet; E1, which is associated with salivary secretion into the sieve elements; E2, which indicates sap ingestion; and pd, which corresponds to potential drops due to intracellular stylet tip punctures when the stylet passes through membrane cells. These drops can be divided into subphases I, II-1, II-2, II-3 and III (Powell 2005, Tjallingii 2006, Tjallingii et al. 2010). These waveforms are usually recorded as the number of events or their duration, which allows the computation of many non-sequential or sequential behavioral parameters (Van Helden and Tjallingii 2000). These parameters are used to estimate the relative importance of prephloematic, phloematic or all tissue factors affecting aphid probing behavior and accordingly reflecting different levels of plant resistance.

EPGs were performed on branches free of aphids attack before the experience in different *P. persica* cultivars (Table 1), which were collected from the orchards mentioned above and transported to the laboratory. To keep the leaves fresh during the EPGs, the stems of the experimental branches (ca. 15 cm) were submerged in a Pasteur pipette modified to contain water. The aphids used were obtained from a multi-clonal stock-colony of *M. persicae* reared on sweet pepper and maintained under controlled conditions at 20º C ± 2 and 16:8 (light:dark). 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 µm diameter) was attached to the dorsum with a silver conductive adhesive (colloidal silver, Ted Pella, Inc.). 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 four hours. 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 hours. 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, all tissues factors), which can be further analyzed.

Statistical Analysis. Aphid occurrence data were analyzed with Kruskal-Wallis 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 non-parametric 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 the population growth rate of aphids, a multiple regression analysis using these parameters as predictor variables and the population growth rate 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* Orchards. The *P. persica* cultivars that were monitored, including peaches and nectarines, exhibited relatively similar proportions of aphid occurrence, with approximately 1% to 3% of 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 x 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 the Elegant Lady, August Red and Summer Free-N18 cultivars exhibited similar low PGRs (Fig. 3).

No-choice Test. After 24 hours, there were significant differences among cultivars in the number of aphids on 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 out of the leaves, although Artic Snow, Elegant Lady, 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 out 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 non-probe period before the first phloem phase (E) and mean duration of pd showing significant differences among cultivars (Table 2). The duration of the non-probe 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 exhibited the lowest values in August Red and Arctic Snow and the highest values in White Lady and Elegant Lady (Table 2). Following the analysis of the parameters with a non-parametric univariate test, the total duration of 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 stylet difficulties 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 (Table 2). Both parameters also correlated significantly with each other ($r = 0.99$; $P < 0.05$). On the other hand, the mean duration of subphases II-2 of pd (H = 33.73; $P = 0.0002$) and mean duration of subphases II-3 of pd ($H = 26.87$; $P = 0.0027$) were also significantly different among cultivars (Table 2), with the mean duration of subphase II-2 of pd showing the lowest values in August Red, Summer Bright and July Red- NS92 and the highest values in Elegant Lady. The mean duration of subphase II-3 of pd showed the lowest value in Arctic Snow and the highest values in Elegant Lady and White Lady. In addition, the mean duration of subphase II-2 correlated with the mean duration of subphase II-3 ($r = 0.61$; $P < 0.05$). The results for the duration of subphases II-1, II-2 and II-3 of pds in all cultivars are shown in Fig. 5.

All parameters related to phloem factors were analyzed using a non–parametric univariate test, resulting in values for the number of E1 ($H = 24.63$; $P = 0.006$), duration of first E ($H = 21.31$; $P = 0.0191$) and total duration of E1 ($H = 22.90$; $P = 0.01$), with significant differences among cultivars (Table 2). The number of E1 showed the lowest value in August Red and the highest values in Elegant Lady and White lady. The duration of the first E exhibited the longest time in Cal Red, while July Red-NS92 was the shortest (Table 2). The total duration of E1 showed the lowest values 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 non-parametric univariate test, the total duration of the non-phloematic 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 non-phloematic 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 Artic 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 first probe to the first pd and time from the beginning of 1st 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 1st 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 ≥ 1). The PC1 and PC2 components of scores and loading (Table 3; Fig. 6A and 6B) 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). On the other hand, 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 population growth rate 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 salivation into the sieve elements (waveform E1). Thus, Elegant Lady displays signatures of resistance across most of the variables studied. 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 seven days 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 hours, 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 status were defined according to the plant factor involved (Table 2). Because EPG parameters can be useful

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 non-independent parameters, which are associated with different resistant factors, a global multivariate view may help to reduce this complexity. The results from the PCA analysis (Fig. 6a and Fig. 6b) 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 phloematic 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 and Fig. 5), 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 due to phloematic factors. It is particularly interesting to note that in this cultivar, salivation after sieve element puncture (E1) correlated negatively with population growth rate, 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). On the other hand, July Red-NS92, in addition to the lack of phloem ingestion and longer time to commit salivation into the sieve element, exhibited strong prephloematic resistance, as shown by no-choice assays. However, the performance experiment showed a positive population rate of growth 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. 2002).

It is worth noting that the lack of congruence between performance and EPG experiments may be due to 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/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. 1998b, Sauge et al. 1998a, Sauge 1998). In addition, cultivars 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.

It is interesting to note that in this study, peaches and nectarines did not exhibit a major difference in their resistance to aphids. 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. persica*e attacks and aid future breeding programs to improve these cultivars' resistance to pests and diseases.

Acknowledgements

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 PhD Grant from the Facultad de Ciencias Agrarias of the Universidad de Talca awarded to J.V.

References Cited

Alvarez, A. E., W. F. Tjallingii, E. Garzo, V. Vleeshouwers, M. Dicke, and B. Vosman. 2006. Location of resistance factors in the leaves of potato and wild tuber-bearing Solanum species to the aphid *Myzus persicae*. Entomol. Exp. Appl. 121: 145-157.

Bass, C., A. M. Puinean, M. Andrews, P. Cutler, M. Daniels, J. Elias, V. L. Paul, A. J. Crossthwaite, I. Denholm, L. M. Field, S. P. Foster, R. Lind, M. S. Williamson, and R. Slater. 2011. Mutation of a nicotinic acetylcholine receptor beta subunit is associated with resistance to neonicotinoid insecticides in the aphid *Myzus persicae*. BMC Neurosci. 12.

Blackman, R. L., J. M. Spence, L. M. Field, and A. L. Devonshire. 1995. Chromosomal location of the amplified esterase genes conferring resistance to insecticides in *Myzus persicae* (Homoptera, Aphididae). Heredity 75: 297-302.

Cooper, T., L. Sazo, and K. Sagredo. 2001. Integrated production in chile: Peaches, nectarines and plums, two years of research and development, pp. 45-50. In W. P. R. S. (WPRS/SROP) (ed.), Bulletin oilb/srop, International Organization for Biological and Integrated Control of Noxious Animals and Plants (OIBC/OILB), Dijon, France.

Field, L. M., A. L. Devonshire, and B. G. Forde. 1988. Molecular evidence that insecticide resistance in peach potato aphids (*Myzus persicae* Sulz) results from amplification of an esterase gene. Biochem. J. 251: 309-312.

Foster, S. P., I. Denholm, Z. K. Harling, G. D. Moores, and A. L. Devonshire. 1998. Intensification of insecticide resistance in UK field populations of the peach-potato

aphid, *Myzus persicae* (Hemiptera: Aphididae) in 1996. Bull. Entomol. Res. 88: 127-130.

Gonzales, W. L., C. C. Ramírez, N. Olea, and H. M. Niemeyer. 2002. Host plant changes produced by the aphid *Sipha flava*: consequences for aphid feeding behaviour and growth. Entomol. Exp. Appl. 103: 107-113.

Gotelli, N. 2001. A Primer of Ecology, 3rd ed. Sinauer Associates, Sunderland, Massachusetts.

Gratacós, E. 2004. El cultivo del duraznero *Prunus persica* (L.) Batsch, Facultad de Agronomía. Pontificia Universidad Católica de Valparaíso.

Grechi, I., M. H. Sauge, B. Sauphanor, N. Hilgert, R. Senoussi, and F. Lescourret. 2008. How does winter pruning affect peach tree - *Myzus persicae* interactions? Entomol. Exp. Appl. 128: 369-379.

Infante, R., P. Martinez-Gomez, and S. Predieri. 2008. Quality oriented fruit breeding: Peach *Prunus persica* (L.) Batsch. J. Food Agric. Environ. 6: 342-356.

Isac, M., S. Preda, and M. Marcu. 1998. Aphid species - Vectors of plum pox virus. Acta Virol. 42: 233-234.

Karban, R., and I. Baldwin. 1997. Induced Responses to Herbivory, University Chicago Press, Chicago.

Kervella, J., T. Pascal, F. Pfieffer, and E. Dirlewanger. 1998. Breeding for multiresistance in peach tree. Acta Hort. 465: 177-184.

Klingler, J., G. Powell, G. A. Thompson, and R. Isaacs. 1998. Phloem specific aphid resistance in *Cucumis melo* line AR 5: effects on feeding behaviour and performance of *Aphis gossypii*. Entomol. Exp. Appl. 86: 79-88.

Lambert, P., and T. Pascal. 2011. Mapping *Rm2* gene conferring resistance to the green peach aphid (*Myzus persicae* Sulzer) in the peach cultivar "Rubira®". Tree Genet. Genomes 7: 1057-1068.

Le Roux, V., E. D. M. Campan, F. Dubois, C. Vincent, and P. Giordanengo. 2007. Screening for resistance against *Myzus persicae* and *Macrosiphum euphorbiae* among wild Solanum. Ann. Appl. Biol. 151: 83-88.

Leimu, R., and J. Koricheva. 2006. A meta-analysis of tradeoffs between plant tolerance and resistance to herbivores: combining the evidence from ecological and agricultural studies. Oikos 112: 1-9.

Margaritopoulos, J. T., C. Tsourapas, M. Tzortzi, O. M. Kanavaki, and J. A. Tsitsipis. 2005. Host selection by winged colonisers within the *Myzus persicae* group: a contribution towards understanding host specialisation. Ecol. Entom. 30: 406-418.

Martinez-Torres, D., S. P. Foster, L. M. Field, A. L. Devonshire, and M. S. Williamson. 1999. A sodium channel point mutation is associated with resistance to DDT and pyrethroid insecticides in the peach-potato aphid, *Myzus persicae* (Sulzer) (Hemiptera : Aphididae). Insect Mol. Biol. 8: 339-346.

Monet, R., and G. Massonié. 1994. Déterminisme génétique de la résistance au puceron vert (*Myzus persicae*) chez le pêcher. Résultats complémentaires. Agronomie: 177-182.

Monet, R., A. Guye, and G. Massonie. 1998. Breeding for resistance to green aphid *Myzus persicae* sulzer in the peach, pp. 171-175. In R. Monet (ed.), Fourth International Peach Symposium, Vols 1-2. International Society Horticultural Science, Leuven 1.

Moores, G. D., G. J. Devine, and A. L. Devonshire. 1994. Insecticide-insensitive acetylcholinesterase can enhance esterase-based resistance in *Myzus persicae* and *Myzus nicotianae*. Pestic. Biochem. Physiol. 49: 114-120.

Pascal, T., and R. Monteux-Caillet. 1998. Peach breeding in France, pp. 117-123, Fourth International Peach Symposium, Vols 1-2. International Society Horticultural Science, Louvain.

Pascal, T., F. Pfeiffer, J. Kervella, J. P. Lacroze, and M. H. Sauge. 2002. Inheritance of green peach aphid resistance in the peach cultivar 'Rubira'. Plant Breed. 121: 459-461.

Penvern, S., S. Bellon, J. Fauriel, and B. Sauphanor. 2010. Peach orchard protection strategies and aphid communities: Towards an integrated agroecosystem approach. Crop Prot. 29: 1148-1156.

Pompon, J., D. Quiring, P. Giordanengo, and Y. Pelletier. 2010. Role of host-plant selection in resistance of wild Solanum species to *Macrosiphum euphorbiae* and *Myzus persicae*. Entomol. Exp. Appl. 137: 73-85.

Powell, G. 2005. Intracellular salivation is the aphid activity associated with inoculation of non-persistently transmitted viruses. J. Gen. Virol. 86: 469-472.

Powell, G., C. R. Tosh, and J. Hardie. 2006. Host plant selection by aphids: Behavioral, evolutionary, and applied perspectives. Ann. Rev. Entomol. 51: 309-330.

Prado, E., and W. F. Tjallingii. 1997. Effects of previous plant infestation on sieve element acceptance by two aphids. Entomol. Exp. Appl. 82: 189-200.

Ramírez, C. C., and H. M. Niemeyer. 1999. Salivation into sieve elements in relation to plant chemistry: the case of the aphid *Sitobion fragariae* and the wheat, *Triticum aestivum*. Entomol. Exp. Appl. 91: 111-114.

Reyes, F., N. Fiore, M. A. Reyes, P. Sepulveda, V. Paredes, and H. Prieto. 2003. Biological behavior and partial molecular characterization of six Chilean isolates of Plum pox virus. Plant Dis. 87: 15-20.

Rosales, M., P. Hinrichsen, and G. Herrera. 1998. Molecular characterization of plum pox virus isolated from apricots, plums and peaches in Chile, pp. 401-405, 17th International Symposium on Virus and Virus-Like Diseases of Temperate Fruit Crops: Fruit Tree Diseases.

Sarria, E., M. Cid, E. Garzo, and A. Fereres. 2009. Excel Workbook for automatic parameter calculation of EPG data. Comput. Electron. Agric. 67: 35-42.

Sauge, M., J. Kervella, and Y. Rahbe. 1998a. Probing behaviour of the green peach aphid *Myzus persicae* on resistant Prunus genotypes. Entomol. Exp. Appl. 89: 223-232.

Sauge, M., J. Kervella, and T. Pascal. 1998b. Settling behaviour and reproductive potential of the green peach aphid *Myzus persicae* on peach varieties and a related wild Prunus. Entomol. Exp. Appl. 89: 233-242.

Sauge, M. H. 1998. Analysis of the mechanisms of resistance to the green peach aphid in several *Prunus* genotypes, pp. 731-739. In R. Monet (ed.), Fourth International Peach Symposium, Vols 1-2. International Society Horticultural Science, Leuven 1.

Sauge, M. H., J. L. Poessel, T. Guillemaud, and L. Lapchin. 2011. Resistance induction and herbivore virulence in the interaction between *Myzus persicae* (Sulzer) and a major aphid resistance gene (Rm2) from peach. Arthropod-Plant Interact. 5: 369-377.

Sauge, M. H., J. P. Lacroze, J. L. Poessel, T. Pascal, and J. Kervella. 2002. Induced resistance by *Myzus persicae* in the peach cultivar 'Rubira'. Entomol. Exp. Appl. 102: 29-37.

Sauge, M. H., F. Mus, J. P. Lacroze, T. Pascal, J. Kervella, and J. L. Poessel. 2006. Genotypic variation in induced resistance and induced susceptibility in the peach - *Myzus persicae* aphid system. Oikos 113: 305-313.

Sherman, W., P. Lyrene, and R. Sharpe. 1996. Low-chill peach and nectarine breeding at the University of Florida. Proc. Fla. State Hort. Soc. 109: 222-223.

SPSS, I. 2001. SPSS base version 11.5 for windows user's guide computer program, version By SPSS, I., Chicago.

StatSoft, I. 2004. STATISTICA (data analysis software system), version 7. www.statsoft.com.

Tjallingii, W. 1995. Aphid-plant interactions: what goes on in the depth of the tissues? Proc. Exp. App. Entomol. 6: 163-169.

Tjallingii, W. F. 2006. Salivary secretions by aphids interacting with proteins of phloem wound responses. J. Exp. Bot. 57: 739-745.

Tjallingii, W. F., and T. H. Esch. 1993. Fine-structure of aphid stylet routes in plant-tissues in correlation with EPG signals. Physiol. Entomol. 18: 317-328.

Tjallingii, W. F., E. Garzo, and A. Fereres. 2010. New structure in cell puncture activities by aphid stylets: a dual-mode EPG study. Entomol. Exp. Appl. 135: 193-207.

Van Helden, M., and W. F. Tjallingii. 2000. Experimental design and analysis in EPG experiments with emphasis on plant resistance research, pp. 144-171. In G. P. Walker, Backus, E.A. (ed.), Principles and applications of electronic monitoring and other techniques in the study of homopteran feeding behavior. Entomological Society of America, Lanham.

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

Commercial name	**Cultivar**	**Occurrence in orchards** [1]	**Performance**	**Probing behaviour (EPG)**	**No-choice**
Flavor Crest	Peach	X	X	X	X
Cal Red	Peach	X	X	X	X
September Sun	Peach	X	X	X	X
Elegant Lady	Peach	X	X	X	X
White Lady	Peach	X	X	X	X
DU23	Peach	X	X	X	X
Flame Crest [2]	Peach	X			X
Sweet September [2]	Peach	X			X
Ryan Sun	Peach	X			
Summer Free-N18	Nectarine	X	X	X	X
August Pearl	Nectarine		X		X
July Red-NS92	Nectarine	X	X	X	X
Artic Snow	Nectarine	X		X	X
Summer Bright	Nectarine	X		X	X
August Red	Nectarine	X	X	X	X
Fire Bright	Nectarine	X			

[1]: Studies performed during season 2007-2008
[2]: These cultivars were used only in no-choice because insecticides applications in the field could not be avoided.

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

EPG parameter	August Red (N = 10)	Summer Free-N18 (N = 7)	Artic Snow (N = 9)	Flavor Crest (N = 9)	Summer Bright (N = 6)	September Sun (N = 7)
Prephloem factors						
1. Duration of non-probe period before the 1st E (s)	2712.8 ± 1949.8 a	2499.2 ± 2367.0 ab	1687.4 ± 1531.0 ab	2590.0 ± 2711.1 ab	1457.6 ± 1703.0 ab	2687.4 ± 1332.0 ab
2. Mean duration of pd (s)	3.9 ± 0.5 a	4.0 ± 0.4 ab	4.0 ± 0.7 a	4.0 ± 0.8 ab	4.1 ± 0.5 ab	4.4 ± 0.8 ab
3. Total duration of F (s)	2781.3 ± 2603.4 c	3583.3 ± 4858.5 bc	381.2 ± 434.1 abc	386.7 ± 747.5 abc	916.7 ± 2240.0 abc	261.8 ± 444.9 abc
4. Mean duration of F (s)	1413 ± 2291.6 b	966.2 ± 1195.5 ab	67.6 ± 64.2 ab	307.4 ± 763.3 ab	915.5 ± 2240.6 ab	117.1 ± 204.6 ab
5. Mean duration of subphases II-2 of pd (s)	1.4 ± 0.2 a	1.2 ± 0.2 ab	1.4 ± 0.3 ab	1.4 ± 0.4 ab	1.6 ± 0.2 a	1.5 ± 0.3 ab
6. Mean duration of subphases II-3 of pd (s)	1.2 ± 0.3 ab	1.2 ± 0.2 ab	1.4 ± 0.3 a	1.2 ± 0.3 ab	1.6 ± 0.2 ab	1.4 ± 0.2 ab
Phloem factors						
7. Number of E1	0.3 ± 0.5 a	2.3 ± 2.9 ab	1 ± 0.5 ab	3.3 ± 2.6 ab	3.5 ± 4.3 ab	2.4 ± 2.1 ab
8. Duration of first E (s)	422.9 ± 1306.1 ab	105.7 ± 104.7 ab	71.5 ± 58.3 ab	67.2 ± 67.7 ab	180.4 ± 187.8 ab	141.6 ± 195.6 ab
9. Total duration of E1 (s)	17.8 ± 32.0 b	684.0 ± 1026.4 ab	74.3 ± 59.9 ab	325.4 ± 385.1 ab	431.6 ± 504.2 ab	485.3 ± 518.1 ab
All tissue factors						
10. Total duration of pd (s)	354.8 ± 160.9 ab	403.2 ± 210.5 abc	282.2 ± 152.9 a	455.0 ± 277.0 abc	287.1 ± 151.8 ab	281.8 ± 122.3 a
11. Time from the beginning of the 1st probe to first pd (min)	496.3 ± 939.8 ab	988.7 ± 2019.6 ab	593.5 ± 660.0 ab	1599.4 ± 2447.1 b	450.2 ± 780.3 ab	666.2 ± 891.7 ab
12. Total duration of no phloematic phase (min)	64.9 ± 106.2 b	201.3 ± 109.8 ab	212.1 ± 79.5	180.3 ± 102.4 ab	228 ± 19.7 a	163.2 ± 111.7 ab
13. Time from start of EPG to 1st E2 (min)[a]	233.2 ± 21.4 ab	192.7 ± 80.8 ab	239.9 ± 0.0 ab	187.9 ± 79.6 ab	230.1 ± 24.3 b	239.1 ± 0.1 ab
14. Time from 1st probe to 1st E2 (min)[a]	230.0 ± 31.6 ab	189.3 ± 86.5 ab	239.9 ± 0.0 ab	182.5 ± 87.2 ab	230.0 ± 24.4 b	239.1 ± 0.1 ab
15. Time from the beginning of the probe reaching the 1st E2 to that E2 (min)[a]	222.5 ± 55.2 ab	174.3 ± 112.1 ab	239.9 ± 0.0 ab	165.1 ± 112.1 ab	205.1 ± 85.4 b	239.1 ± 0.1 ab
16. Average duration of pd during 3rd hour	2.3 ± 2.0 a	3.9 ± 0.5 ab	2.9 ± 1.8 a	3.5 ± 1.5 ab	3.2 ± 1.6 ab	4.2 ± 0.9 ab

Table 2. Continued.

	July Red-NS92 (N = 7)	DU23 (N = 9)	Cal Red (N = 19)	White Lady (N = 8)	Elegant Lady (N = 8)
Prephloem factors					
1. Duration of non-probe period before the 1st E (s)	3900.1 ± 4435.0 ab	2266.3 ± 1758.7 ab	1337.7 ± 1371.2 ab	3596.9 ± 4448.6 ab	892.2 ± 1468.8 b
2. Mean duration of pd (s)	4.5 ± 0.7 ab	4.6 ± 0.8 ab	4.8 ± 0.8 ab	5.1 ± 0.5 b	5.1 ± 0.6 b
3. Total duration of F (s)	0.0 ± 0.0 a	318.6 ± 912.5 ab	800.3 ± 1567.4 abc	1.9 ± 5.2 a	1.3 ± 3.7 a
4. Mean duration of F (s)	0.0 ± 0.0 b	165.8 ± 454.8 ab	167.2 ± 321.1 ab	1.9 ± 5.2b	0.7 ± 1.8b
5. Mean duration of subphases II-2 of pd (s)	1.2 ± 0.2 a	1.3 ± 0.2 ab	1.2 ± 0.2 ab	1.3 ± 0.3 ab	1.9 ± 0.9 b
6. Mean duration of subphases II-3 of pd (s)	1.5 ± 0.4ab	1.2 ± 0.2 ab	1.5 ± 0.3 ab	1.4 ± 0.3 b	1.6 ± 0.3 b
Phloem factors					
7. Number of E1	4.6 ± 10.8 ab	2.6 ± 2.4 ab	2.4 ± 1.7 ab	4.8 ± 3.8 b	7.6 ± 8.0 b
8. Duration of first E (s)	2.4 ± 5.2 a	132.8 ± 143.5 ab	199.6 ± 398.1 b	71.8 ± 132.9 ab	45.5 ± 34.8 ab
9. Total duration of E1(s)	128.7 ± 273.2 ab	449.9 ± 529.1 ab	364.7 ± 538.3 ab	564.8 ± 738.4 ab	1465.1 ± 2256.7 a
All tissue factors					
10. Total duration of pd (s)	348.3 ± 237.2 ab	697.1 ± 209.5 c	606.7 ± 220.4 bc	504.7 ± 244.7 abc	393.5 ± 235.1 abc
11. Time from the beginning of the 1st probe to first pd (min)	1527.0 ± 3620.1 ab	453.7 ± 856.3 ab	77.2 ± 68.1 a	448.0 ± 571.9 ab	90.8 ± 571.9 ab
12. Total duration of no phloematic phase (min)	66.3 ± ab	174.8 ± 101 ab	194.5 ± 87.2 a	198.6 ± 81.9 ab	181.9 ± 82.8 ab
13. Time from start of EPG to 1st E2 (min)[a]	239.8 ± 0.2 ab	204.5 ± 76.4 ab	210.3 ± 70.3 ab	195.7 ± 74.0 ab	101.4 ± 92.1 a
14. Time from 1st probe to 1st E2 (min)[a]	239.8 ± 0.2 ab	204.5 ± 76.4 ab	240.0 ± 72.3 ab	175.3 ± 88.1 ab	100.7 ± 92.7 a
15. Time from the beginning of the probe reaching the 1st E2 to that E2 (min)[a]	239.8 ± 0.2 ab	190.9 ± 97.2 ab	194.3 ± 91.1 ab	130.7 ± 117.0 ab	77.9 ± 100.9 a
16. Average duration of pd during 3rd hour	2.3 ± 2.2 a	4.5 ± 0.8 ab	3.7 ± 1.7 ab	5.2 ± 0.6 b	4.8 ± 0.5 b

[a] Parameter including total duration of the recording when the E2 waveform was not observed during the recording.

Table 3. Factor loadings of the principal components (PC) with varimax normalized based on correlations. Bold number shows the principal contribution parameters.

EPG parameters	PC1	PC2	PC3	PC4
Total duration of F	0.009711	**-0.872807**	-0.227850	0.174460
Duration of non-probe period before the 1st E	-0.577323	-0.072027	-0.323378	0.494832
Mean duration of pd	0.462663	0.504917	**0.702332**	-0.064875
Mean duration of subphases II-2 of pd	0.586910	0.343965	0.484661	-0.361936
Mean duration of subphases II-3 of pd	0.457490	0.511040	0.641210	0.159219
Mean duration of F	-0.089415	**-0.920257**	-0.248917	0.139960
Number of E1	**0.765640**	0.534075	0.106521	0.105667
Duration of first E	-0.312332	**-0.849457**	0.278503	-0.000183
Total duration of E1	**0.911786**	0.127910	0.132964	-0.217121
Total duration of pd	0.104961	0.036549	0.683951	-0.037987
Time from the beginning of the 1st probe to first pd	-0.174160	0.263109	-0.625230	0.610817
Total duration of no phloematic phase	0.161332	0.248573	-0.057717	**-0.886570**
Time from start of EPG to 1st E2	**-0.962316**	-0.063160	-0.136158	0.118176
Time from 1st probe to 1st E2	**-0.959549**	-0.080112	-0.166855	0.095755
Time from the beginning of the probe reaching the 1st E2 to that E2 (min	**-0.927830**	-0.073887	-0.211031	0.126852
Average duration of pd during 3rd hour	0.549277	0.292054	0.429770	-0.348065
Proportion	0.356316	0.219060	0.162625	0.114667

Figure Legends

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% confidence interval.

Fig. 2. Population rate of growth (PGR, mean ± SE) of the aphid *M. persicae* on ten *P. persica* cultivars as measured in field assays during two consecutive growth seasons.

Fig. 3. Population rate of growth (PGR, mean ± SE) of the aphid *M. persicae* on ten *P. persica* cultivars as measured in field assays including the mean during two consecutive growth seasons.

Fig. 4. Number of aphids (mean ± SE) of *M. persicae* outside the leaves of 14 cultivars of *P. persica* after 24 hours of no choice laboratory experiments.

Fig. 5. Duration of subphases within phase II (mean ± 95% confidence interval) of stylet intracellular punctures performed by *M. persicae* recorded on *P. persica* cultivars. Cultivars are ordered in decreasing order relative to total duration of pds.

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.

Figure 1

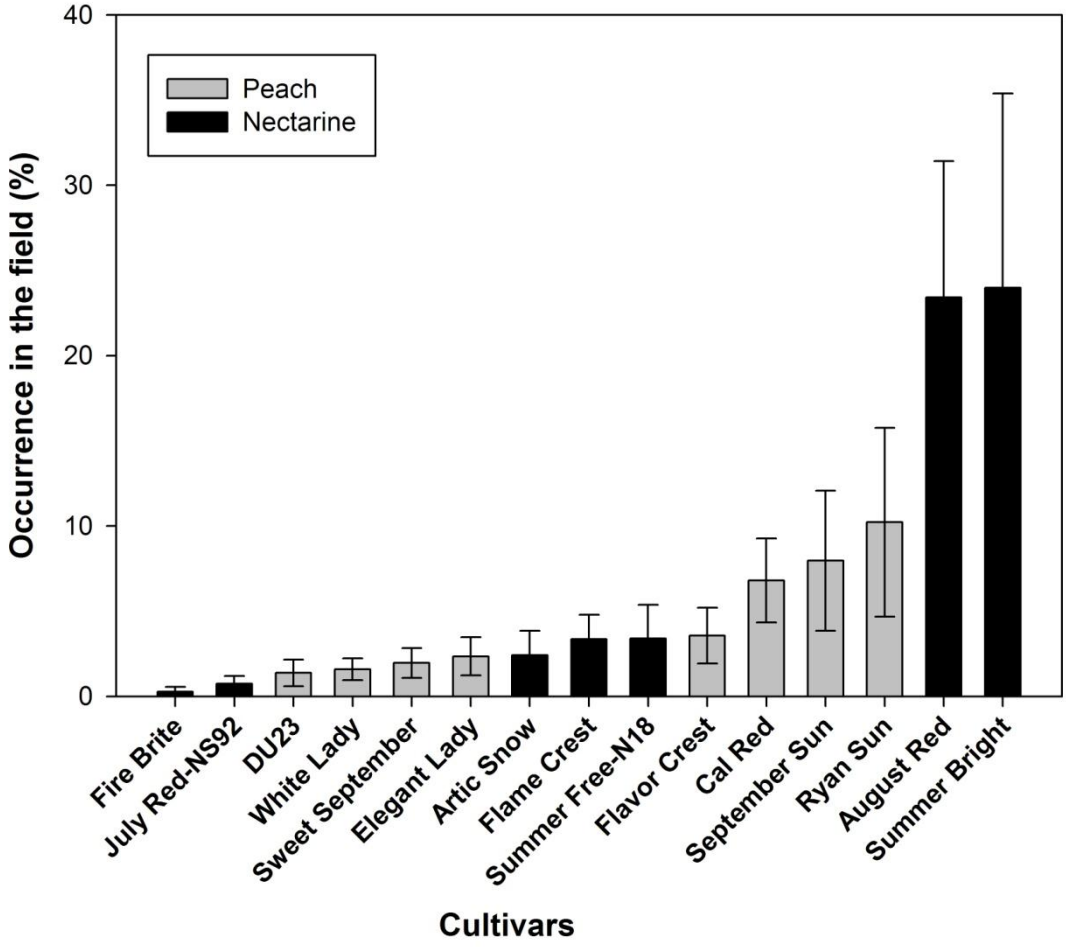

Peach
Nectarine
Occurrence in the field (%)
0
10
20
30
40
Fire Brite
July Red-NS92
DU23
White Lady
Sweet September
Elegant Lady
Artic Snow
Flame Crest
Summer Free-N18
Flavor Crest
Cal Red
September Sun
Ryan Sun
August Red
Summer Bright
Cultivars

Figure 2

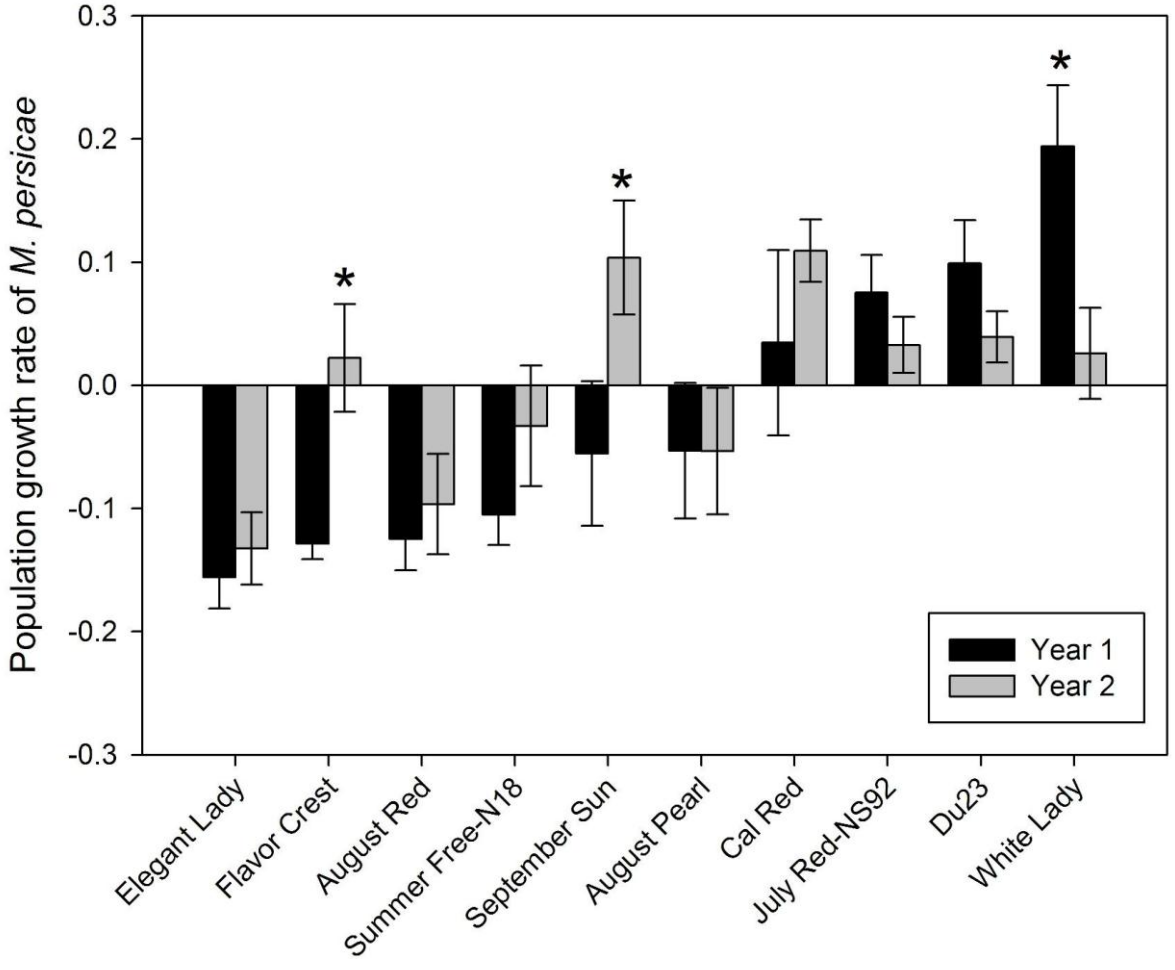

Population growth rate of M. persicae
0.3
0.2
0.1
0.0
-0.1
-0.2
-0.3
*
*
*
Year 1
Year 2
Elegant Lady
Flavor Crest
August Red
Summer Free-N18
September Sun
August Pearl
Cal Red
July Red-NS92
Du23
White Lady

Figure 3

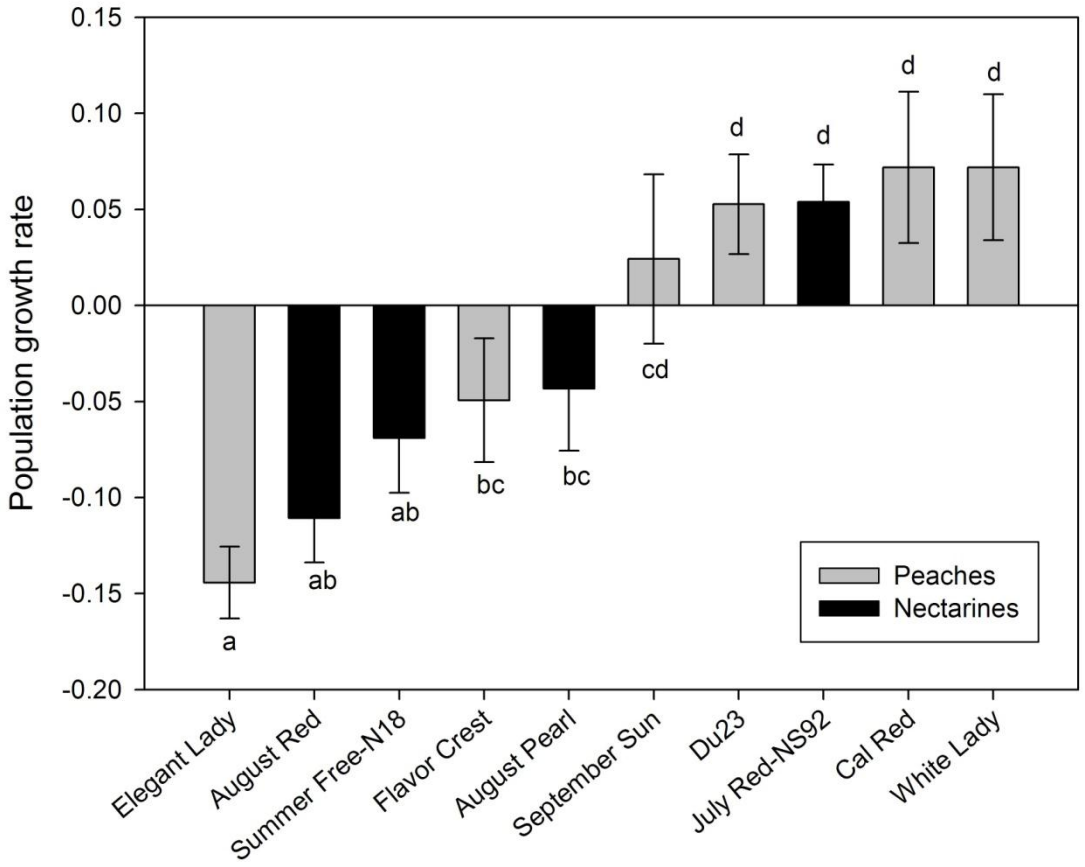

0.15
0.10
0.05
0.00
-0.05
-0.10
-0.15
-0.20
Population growth rate
a
ab
ab
bc
bc
cd
d
d
d
d
Peaches
Nectarines
Elegant Lady
August Red
Summer Free-N18
Flavor Crest
August Pearl
September Sun
Du23
July Red-NS92
Cal Red
White Lady

Figure 4

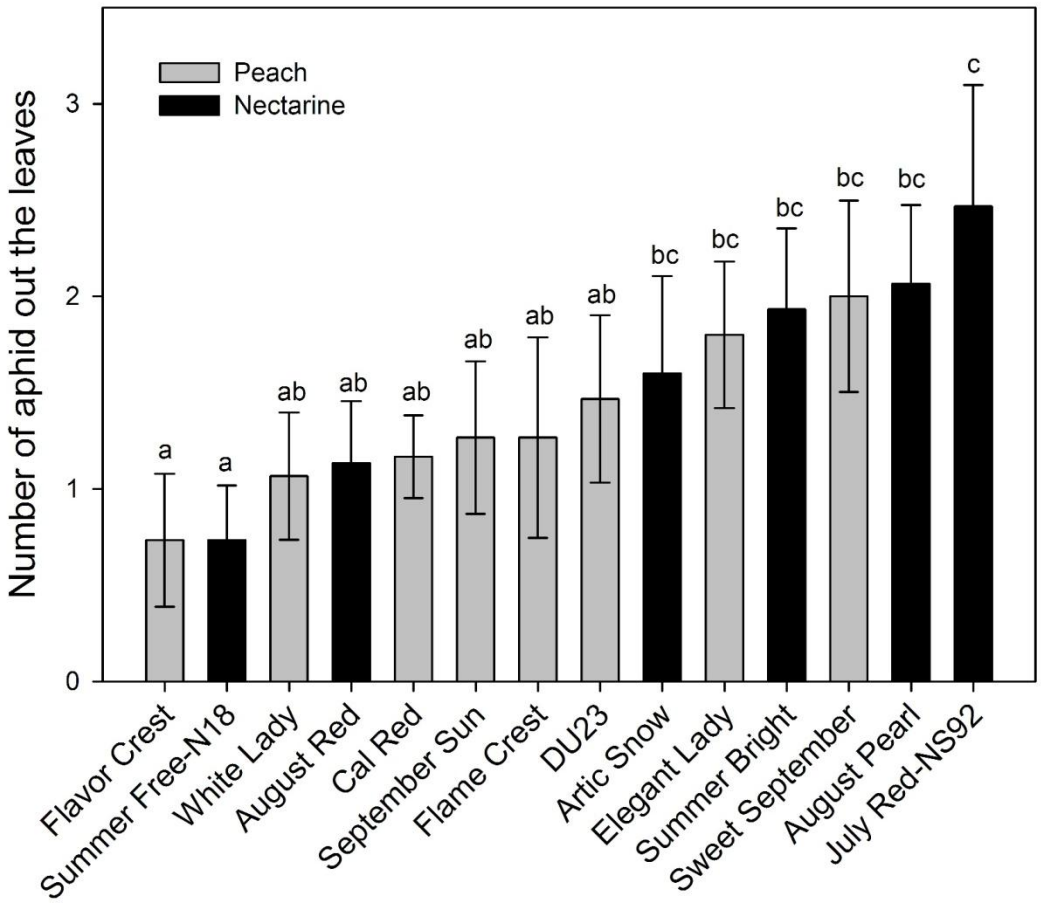

Peach
Nectarine
Number of aphid out the leaves
0
1
2
3
a
a
ab
ab
ab
ab
ab
bc
bc
bc
bc
bc
c
Flavor Crest
Summer Free-N18
White Lady
August Red
Cal Red
September Sun
Flame Crest
DU23
Artic Snow
Elegant Lady
Summer Bright
Sweet September
August Pearl
July Red-NS92

Figure 5

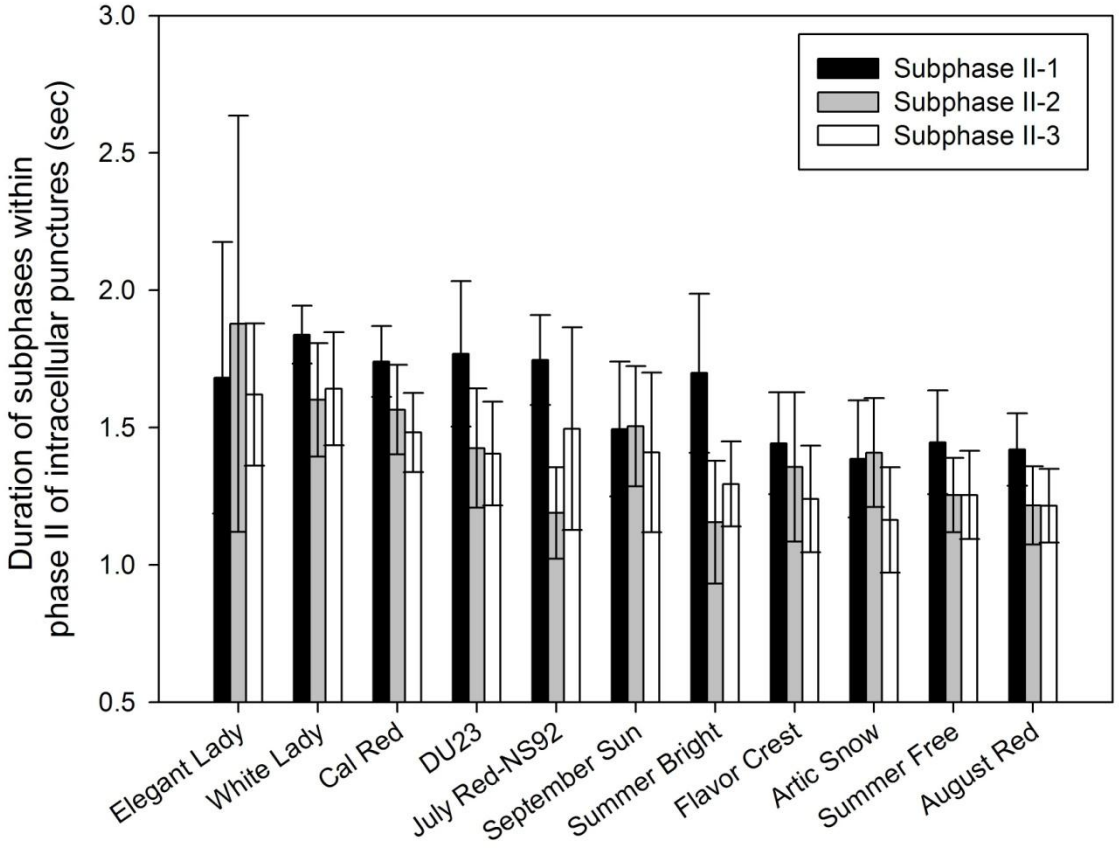

Duration of subphases within phase II of intracellular punctures (sec)
3.0
2.5
2.0
1.5
1.0
0.5
Subphase II-1
Subphase II-2
Subphase II-3
Elegant Lady
White Lady
Cal Red
DU23
July Red-NS92
September Sun
Summer Bright
Flavor Crest
Artic Snow
Summer Free
August Red

Figure 6

(A)

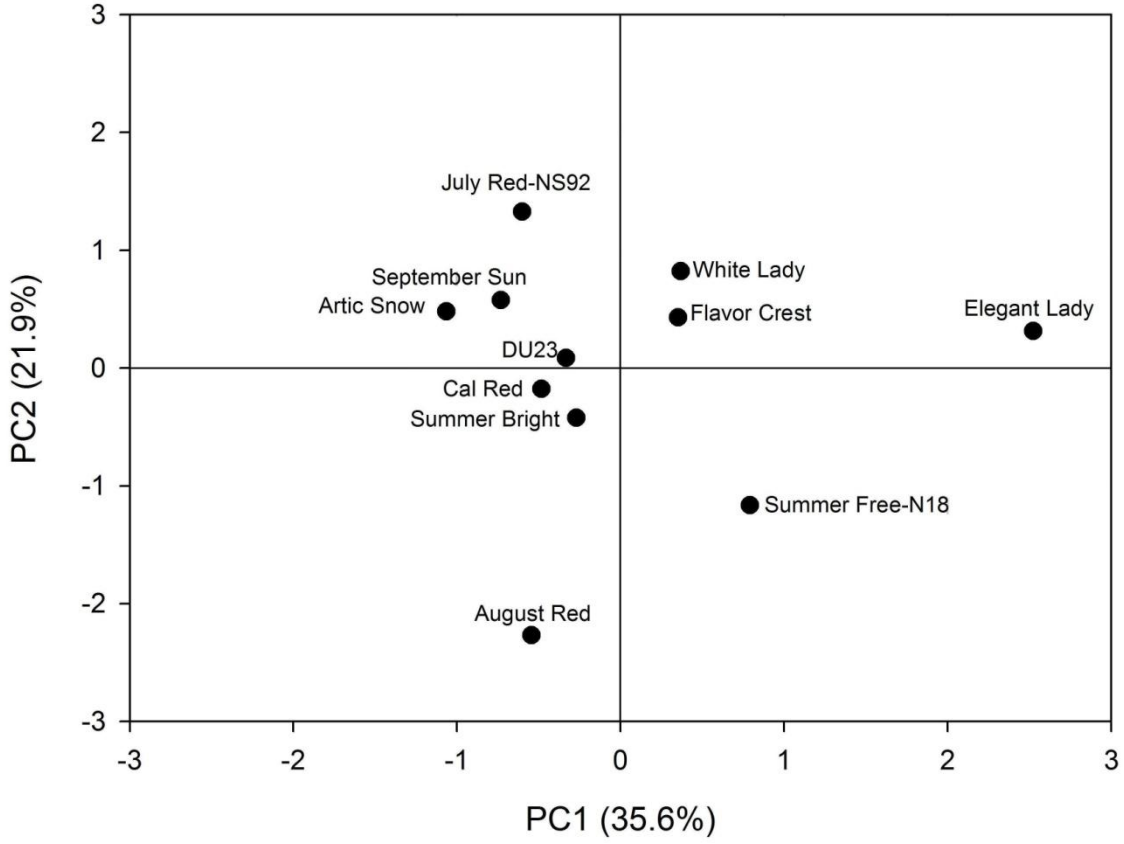
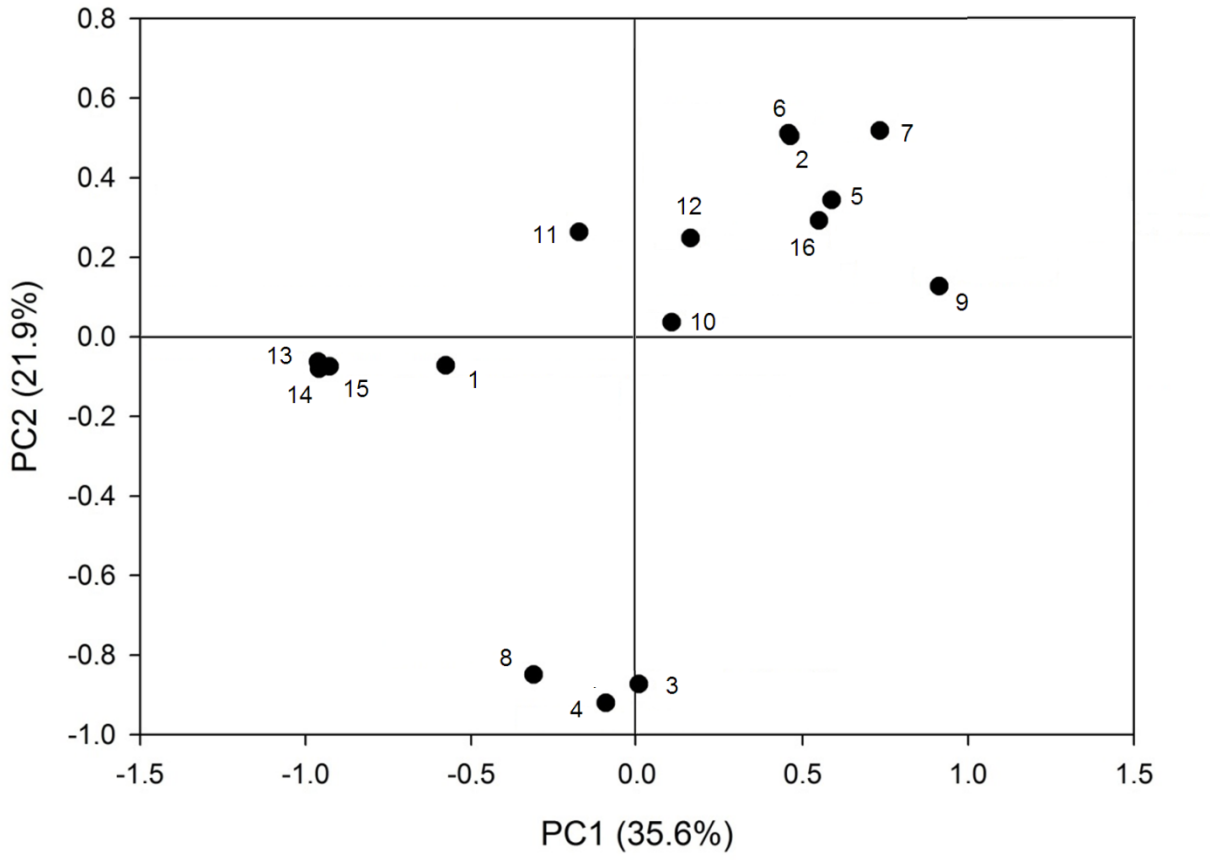

July Red-NS92
September Sun
Artic Snow
DU23
Cal Red
Summer Bright
August Red
White Lady
Flavor Crest
Elegant Lady
Summer Free-N18
PC2 (21.9%)
PC1 (35.6%)
3
2
1
0
-1
-2
-3
-3
-2
-1
0
1
2
3

Figure 6

(B)


0.8
0.6
0.4
0.2
0.0
-0.2
-0.4
-0.6
-0.8
-1.0
-1.5
-1.0
-0.5
0.0
0.5
1.0
1.5
PC2 (21.9%)
PC1 (35.6%)
6
7
2
5
12
11
16
9
10
13
14
15
1
8
4
3

Chapter II

ARE PLANT RESISTANCE AND TOLERANCE ALTERED BY WATER STRESS? NECTARINE-APHID MODEL

In the Chapter II, two commercial genotypes of nectarines exhibiting contrasting resistance in Chapter I, particularly in the non-choice test, were selected to study how irrigation can influence the infestation of *M. persicae*. Because several studies have reported that resources availability can play a special role in the plant defense, we wonder if nectarines can exhibit both tolerance and resistance to *M. persicae*, and how this is influenced by level of irrigation. Summer Free and July Red-NS92 were found the most susceptible and resistant cultivars, respectively (Figure 4, Chapter I), and because of that, they were selected. This last nectarine cultivar was chosen considering its antixenotic type of resistance. Studies in other cultivars have showed the same type of resistance and useful in the resistance studies of *P. persica* to green peach aphid *M. persicae*. Induced resistance of the nectarines to the *M. persicae* attack and the influenced of the level of irrigation were also studied.

Title:

Are plant resistance and tolerance altered by water stress? Nectarine-aphid model

Short title (Running Head): Resistance and tolerance with water stress

Verdugo, J.A.[1,2], Bravo, R.[1], Valenzuela, D.[1] and Ramírez C.C.[1]

1. Laboratorio Interacciones Insecto-Planta, Instituto de Biología Vegetal y Biotecnología, Universidad de Talca, Casilla 747, Talca, Chile. (clramirez@utalca.cl).
2. Gembloux Agro-Bio Tech - Université de Liège, Unité d'Entomologie fonctionnelle et évolutive, Passage des Déportés 2, B-5030 Gembloux, Belgium.

Abstract

Resistance and tolerance are two mechanisms that plant use to display when confronting herbivore attack. Both are known to be affected by environmental fluctuations, and particularly by resource availability. In the present study, two nectarine genotypes with different resistance to the aphid *Myzus persicae* were subjected to the attack of this aphid under variable water supply. The results showed that, independently of the water supply, the resistant genotype exhibited a lower growth in number of leaves in the absence of aphids, which is an indication of the cost of resistance in this cultivar.

Interestingly, aphid attack was compensated and overcompensated in susceptible and resistant cultivars respectively. Population growth rate (PGR) and index of infestation (IF), indicated with water-deficient diminished 2.3 fold in the susceptible cultivar indicating a decrease in the susceptibility and increased 2.4 fold on the resistant cultivar. Independent of the cultivar, under normal irrigation conditions, induced resistant was exhibited and induced susceptibility was caused in the presence of water deficit. Therefore, the resistance and tolerance in this model is affected by the intrinsic traits of the cultivar and also is influenced by the irrigation conditions, an important to consider in future breeding programs.

Key words: resistance, tolerance, overcompensation, water deficit, *Myzus persicae*.

Introduction

Resistance and tolerance are the two main defense strategies developed by plants against the attack of herbivores (Tiffin 2002). In terms of resistance, this corresponds to chemical and mechanical characteristics of the plant affecting herbivore performance (antibiosis) and/or its preference (antixenosis) (Leimu and Koricheva 2006). Resistance can be divided into constitutive and induced resistance. Constitutive resistance is expressed independently of an attack, whereas induced resistance is activated once the plant is attacked or damaged (Cipollini and Heil 2010). However, there are evidences that the display of resistance, either constitutive or induced, is affected by the availability of resources in the environment. Environmental conditions such as nutrient availability are known to affect resistance, although there are conflicting evidences whether this effect is negative or positive (Herms and Mattson 1992, Herms 2002, Nuñez-Farfán et al. 2007). In the case of woody plants, there are some evidences supporting a negative effect on resistance under high-nutrient conditions (Prittinen et al. 2003, Stevens et al. 2007), dependent of the plant genotype (Osier and Lindroth 2006, Silfver et al. 2009) or positive effect on resistance under well-watered conditions (Ramírez and Verdugo 2009).

Tolerance is defined as the ability of plants to develop a high performance despite the negative effects caused by their consumers (herbivores and pathogens), which is usually estimated as the difference in growth or fitness between damaged plants and undamaged plants (Strauss and Agrawal 1999, Hurley and Flaspohler 2007, Stevens et al. 2007). However, tolerance is also dependent on environmental variation. In this sense, the compensatory continuum hypothesis (CCH) proposes that tolerance occurs in a continuum, predicting a high tolerance to herbivory in rich environment and lower tolerance in poor

environment (Maschinski and Whitham 1989). Contrastingly, the growth rate model (GRM) predict plant growing under stressful environment are developing below their maximum growth rate and have the capacity for regrowth in presence of damage, and plant in normal condition are growing almost in their maximum growth rate and in presence of damage are less capable to recover (Hilbert et al. 1981). More recently, the limiting resource model (LRM) states that tolerance will depend of different factors, including the particular resource limiting plant growth and fitness, how the acquisition of resources affect herbivory and how the herbivory can affect the resources (Wise and Abrahamson 2005). Empirically, woody plants are more tolerant than herbaceous plants (Haukioja and Koricheva 2000). Reduction in resources availability such as water variability across environments (Fornoni et al. 2004), reduced CO_2 (Marshall et al. 2008), light scarcity (Baraza et al. 2010) reduce tolerant capacity in woody plants.

Plants are able to be display resistance and tolerance to herbivore simultaneously, although both are costly for the plants (Nuñez-Farfán et al. 2007). The display of resistance and tolerance vary in response to different environmental conditions, such as the availability of soil nutrients, herbivory damage, or intra and interspecific competition (Prittinen et al. 2003, Fornoni et al. 2004). Because both strategies can occur simultaneously in a species as a mixed strategy of defense, their relative importance depends largely on the availability of resources (Nuñez-Farfán et al. 2007, Wise and Abrahamson 2007). Such trade-off, the inverse relationship between resistance and tolerance, has been found mostly in wild plants (Leimu and Koricheva 2006). Most studies on resistance and tolerance have been centered on annuals or short-lived perennial plants, as these are usually crops and forage plants whose economic losses need to be studied (Haukioja and Koricheva 2000). More scarce is the information on tolerance and resistance

in woody plants, and even more the effect of resource availability on resistance and tolerance. Nothing is known about the display of both defensive strategies in *Prunus persica* against herbivores.

In the present study the model conformed by nectarines and green peach aphid (*M. persicae*) was studied in order to assess how aphid attack and water availability affect both resistance and tolerance. In a field experiment, nectarine cultivars differing in the level of resistance to *M. persicae*, were exposed to aphid attack and water deficient conditions and aphid damage and performance (resistance) as well as different plant growth parameters (tolerance) were measured. In order to explore whether or not resistance and tolerance displayed any affected on early plant development in the following season was also assessed.

Materials and Methods

Plants. The experiment was performed using two commercial cultivars of *P. persica* var. nectarina, which have been previously studied as differing in the levels of resistance to the attack of *M. persicae* in terms of antixenotic response: Summer Free-N18 (susceptible) and July Red-NS92 (resistant)(see Chapter I). Young shoots of about 60-90 cm in length and 1-1.5 cm of diameter of these cultivars were pruned in May 2010 from a orchards located in Cachapoal province, Rengo, at Libertador General Bernardo O´Higgins Region, Chile, and maintained at 5º C until grafting (September 2010). Buds were selected and 100 buds from each nectarine cultivar were grafted on the Nemaguard rootstock of two years old and maintained in plastic pots of 20 l containing 2:1 mixture organic soil and sand. All plants

were disposed in an experimental area (30 x 20 m) located on the campus of University of Talca. A total of 144 plants (72 susceptible and 72 resistant) were successfully grafted.

The diameter of the rootstock at 5 cm over the substrate was measured at the day of grafting (September 11, 2010). This value was used as a covariate for the analysis of the other dependent variables. The following dependent variables were measured weekly in main branch resulting from grafting from December 7, 2010 to March 19, 2011. i) diameter at 5 cm from the union of the graft, ii) branch length of the main branch measured from the union of the graft to caulinar apex, iii) number of extended leaves on that branch, all those variables will be consider in the tolerance analysis as indicate (Ramirez and Verdugo, 2009). In addition, in the following season, the iv) number of flower buds and open flowers at blooming and v) number of shoot-buds and open leaves was assessed.

Experimental design. A randomized block factorial design with 2x2x2 arrangement was performed. Blocks were composed by six rows with the eight treatments arranged ramdomly within block with three replicates each. Factors were (i) plant genotype, with susceptible and resistant to attack of *M. persicae* levels, (ii) irrigation, with non-deficient irrigation (100% FC) and deficient irrigation (50% FC) levels, and (iii) aphid damage, with presence and absence of aphid levels.

Irrigation treatment on plants. Experimental plants were subjected to two irrigation treatments which were established based on estimations of the field capacity (FC) of soil. FC was estimated by water saturating the plastic pot containing the plants and soil. After 36 hours the pots with the plants were weighed to obtain the average weight that corresponded to the FC. The permanent wilting point (PWP) was also estimated by leaving one potted

plant without watering until death and subsequently weighted. Thus, FC was estimated to be 18 l and PWP 10 l. With this information, treatments were defined as normal irrigation (100% FC) and deficient irrigation (50% FC). Every four days after potted plant were weighted and water was added to reach either 100% or 50% of FC.

Aphid treatment on plants. Experimental plants exposed to aphid attack were subjected to the natural arriving of *M. persicae* individuals. Control plants that were free of aphids (undamaged plants) were initially treated with the pirethroid insecticide Lambdacihalotrina Karate® and thereafter aphids were removed manually from the plants. The level of curling produced by aphids on leaves was registered all along the season as "degrees of infestation", calculated by the index of relative infestation describe by Grechi et al. (2008).

Aphid performance. Two random plants per treatment were selected to assess the performance of growth in *M. persicae*. Firstly, a group of aphids were randomly collected from the plants treated with aphids to form a stock colony. On one leaf near the apex, one single adult wingless aphid of the stock colony was confined into a clip cage and after seven days the number of individuals was counted. The aphid population growth rate (PGR) was estimated as (ln N2 – ln N1)/(t2-t1), where N1 is the initial number of aphids, N2 the final number of aphids, and (t2 - t1) the number of days of the experiment (Gotelli 2001).

Statistical analysis. Plant traits were all analysed by ANCOVA using GLMM model considering the rootstock diameter as a covariate. Factors were cultivar, irrigation and aphids. A Tukey test was used for multiple comparisons. The GLMM model, fitted by the

Laplace approximation, was: Y (branch length, diameter and number of leaves) = G x I x A + covariate + (1 | Block); where I is the nectarine genotype, R is irrigation and A correspond to aphid. Analyses were executed by statistical software R version 2.12.1.0. Curling and aphid performance were analyzed by two-way anova for ranks followed by LSD Fisher multiple comparison.

Results

The number of extended leaves in a branch was the response variable most affected by the manipulated factors, with a significant effect of genotype, irrigation and aphid (Table 1). Genotype x irrigation, genotype x aphid and irrigation x aphid interactions did also affect the number of leaves (Table 1). The number of leaves was higher in the susceptible genotype, under well-watered conditions and under the presence of aphid (Fig.1). The significant genotype x irrigation interaction revealed that both genotypes exhibited a higher number of leaves under well-watered treatment than water-deficient conditions, although the susceptible genotype reached a higher number that the resistant one (Fig. 1). Contrastingly, under water-deficient both genotypes did not differ in the number of leaves (Fig.1). The significant genotype x aphid interaction revealed that the susceptible genotype attained similar number of leaves regardless the presence of aphids, whereas in the resistant genotype the number of leaves was higher under the presence of aphids (Fig.2). In addition, in the absence of aphids, the resistant genotype exhibited lower number of leaves (Fig. 2). The significant irrigation x aphid interaction showed that under well-watered plants the number of leaves was higher in presence of aphids, while under water-deficient conditions the number of leaves was smaller than in the other treatments and independent of the

presence of aphids (Fig.3). Unlike number of leaves, the branch length and branch diameter were not affected by manipulated factors (Table 1).

Curling exhibited significant cultivar x irrigation interaction ($F_{1, 135} = 4.04$, $P < 0.049$) (Fig. 4), with the susceptible cultivar showing the higher curling under well-watered conditions A lack of differences was found between genotypes under water-deficient conditions. The PGR showed significant irrigation x aphid interaction ($F_{1, 135} = 5.07$, $P < 0.03$), with higher PGR in aphids on plant with well-watered conditions and without previous infestation of aphid (Fig. 5). Differently, PGR was higher in aphids on plants under water-deficient and with previous infestation of aphids (Fig.5).

Number of flower buds showed significant (Table 1 and Fig. 6) in the genotypes with presence of aphids on well watered plants in the past season. In shoot-bud the genotypes exhibited significant aphid x irrigation interaction and aphid x irrigation x cultivar interaction (Fig. 7).

Discussion

These experiments showed that in the nectarines genotypes studied, resistance and tolerance were dependent of the water availability. Mostly of studies on the effect to water irrigation in peaches and nectarines are centered on fruit growth as principal effect for the production (Basiouny 1978, Giannetto and Petillo 1995, Grossman and Dejong 1995a, b, c, Gong et al. 2005), while there are few studies considering water-deficiency (Li and Huguet 1989, Li et al. 1989). Our study is, to our knowledge, the first reporting changes in *Prunus*-aphid interaction mediated by water availability. We found that susceptibility was lowered, although not significantly, under water-deficient conditions in the susceptible genotype as

curling produced by aphids was reduced to similar levels as in the resistant genotype. Under lower water supply, differences otherwise seen under well-watered conditions were cancelled. In addition to this effect on water supply on resistance, there was also an apparent induced susceptibility effect, as the aphid PGR increased on nectarines under water-deficient conditions, but only on plants previously infected (Fig. 5). This induced susceptibility was independent of the genotype (resistant or susceptible). This is different to what was described for other *P. persica* genotypes. For instance, Sauge *et al.* (2006) found that two peach genotypes which were susceptible to aphids increased their susceptibility after aphid damage. The difference between our study and this of Sauge *et al.* (2006) could be associated with the fact that in our study, commercial genotypes of nectarines were used, which are the results of selection for commercial attributes such as size, color, sweetness and other characteristics imposed by the markets. On the other hand, the induction of susceptibility is possibly related with aphid saliva factors injected to the plant during aphid probing which might change chemical contents of sieve element sap and/or the plant physiological status (Prado and Tjallingii 1997). The molecular basis of this mechanism could be also related with induced resistance also exhibited in our study (see below).

We also detected induced resistance as the aphid PGR was reduced when plants were previously infected (Fig. 5). This induction was independent of the plant genotype. It has been described that *M. persicae* can generate stronger defensive responses in resistant peach genotypes (Sauge et al. 2002, Sauge et al. 2006, Sauge et al. 2011). Such changes occur apparently at the level of primary and secondary metabolites (Poëssel et al. 2011). The mechanism underlying this induced resistance might be also due to changes induced by aphid probing. In particular, changes at the site of stylet penetration may serve as chemical signals of defensive response involving elicitors which may activate the action of resistance

gene (Sauge et al. 2002). Indeed, resistance QTLs of *Prunus davidiana* have been found to be co-located with QTLs underlying aphid feeding behavior, with a resistance allele at the major QTL associated with drastic reductions in phloem sap ingestion by aphids, suggesting a phloem-based resistance mechanism (Sauge et al. 2012).

Tolerant response was evident in the number of leaves in both cultivars with also an effect of water supply. Here, the susceptible genotype exhibited full compensation as plants with or without aphids were not different in number of leaves. This genotype was evidently susceptible as curling was larger than the resistant (Fig. 4), however, aphid infestation do not entail reduction of leaves. The presence of aphids may reduce either defoliation or leaf senescence. Indeed, the resistance genotype exhibited overcompensation as under aphid damage increased the number of leaves. In both cases, aphids may be acting as the sink-source system usually found in galling insects (Larson and Whitham 1991), generating an arresting of assimilates to the leaves where they feed and/or prolonging the permanence of those leaves in the plants, including changes in metabolic pathways (Thompson and Goggin 2006). In our work, this capacity of compensation was larger in the resistant genotype, which is different to that found in other trees species (Compson et al. 2011). Interestingly, this tolerance observed in the resistance genotype may involve an increase in plant tissue quality or quantity after damage which may have a positive effect on the insect's performance (Fornoni 2011). In some cases this compensation can be considered as mutualism, because both (the plants and insects) are benefited (Agrawal 2000). Nevertheless, it is necessary to study other characteristics of the leaves such as photosynthetic capacity, which were not studied in this work, together with the quantity and quality of the mature fruit resulting from this treatment, which is particularly relevant for fruit trees.

We also found that tolerance was modulated by water supply. Compensation occurred even under water-deficient plants, but overcompensation was found only in the well-watered plants (Fig. 3). This suggests that compensation to some extend is dependent of current plant resources. On the other hand, this reduced tolerance under water-deficient conditions could be related to the lower photosynthetic rate which aphids and other sap-feeders insect use to provoke on their host plants (Retuerto et al. 2004, Eyles et al. 2011). The opposite response of compensation to water-deficiency has been described in a resistant-to-aphid poplar hybrid. In this case, samplings with aphid attack under drought stress showed a overcompensation in number of leaves (Ramírez and Verdugo 2009). More studies are needed to unravel how tolerance is modulated by water availability. It worth noting that non negative association between resistance and tolerance was found.

The assessment of early stages of plant development during the next growth season following the aphid and irrigation treatments revealed that in plants of the susceptible genotypes, that were subjected to well-watered conditions and aphid damage, the number of flower buds (Fig. 6) and number of shoot buds (Fig. 7) developed earlier. It is likely that this earlier flowering during bloom foliar bud break was triggered by the influence of the attack of the aphid. As mentioned above, the sick-source model may explain the larger number of leaves in aphid attacked plants, process that could have also increased locally the abundance of photoassimilates or the reallocation of resources to the aphid damaged area. Such concentration of resources could have elicited faster access for those flower and foliar buds located in the area of previous damage. An aphid-induced phytohormonal change could have been also occurred. That effect of previous aphid infestation on flowering has not been previously reported. Interestingly, this result suggests that aphids may be even beneficial for plants because they may act as flowering inductor, enhancing plant fitness.

Future studies could look for the specific metabolites and genes are regulated by aphid feeding and leading to a faster plant development.

Acknowledgements

This research was supported by Fondecyt Grant 1100746 to C.R. and PhD Grant of Facultad de Ciencias Agrarias of Universidad de Talca to J.V.

References

Agrawal, A. A. 2000. Overcompensation of plants in response to herbivory and the by-product benefits of mutualism. Trends Plant Sci. 5: 309-313.

Baraza, E., R. Zamora, and J. A. Hodar. 2010. Species-specific responses of tree saplings to herbivory in contrasting light environments: An experimental approach. Ecoscience 17: 156-165.

Basiouny, F. M. 1978. Response of peach seedlings to water stress and saturation conditions. Proceedings of the Florida State Horticultural Society 90: 261-263.

Cipollini, D., and M. Heil. 2010. Costs and benefits of induced resistance to herbivores and pathogens in plants. CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nut. Nat. Resour. 5: 1-25.

Compson, Z. G., K. C. Larson, M. S. Zinkgraf, and T. G. Whitham. 2011. A genetic basis for the manipulation of sink-source relationships by the galling aphid Pemphigus batae. Oecologia 167: 711-721.

Eyles, A., D. Smith, E. A. Pinkard, I. Smith, R. Corkrey, S. Elms, C. Beadle, and C. Mohammed. 2011. Photosynthetic responses of field-grown Pinus radiata trees to artificial and aphid-induced defoliation. Tree Physiol. 31: 592-603.

Fornoni, J. 2011. Ecological and evolutionary implications of plant tolerance to herbivory. Funct. Ecol. 25: 399-407.

Fornoni, J., P. L. Valverde, and J. Nuñez-Farfán. 2004. Population variation in the cost and benefit of tolerance and resistance against herbivory in *Datura stramonium*. Evolution 58: 1696-1704.

Giannetto, G. B., and M. G. Petillo. 1995. Effects of irrigation and two soil management schemes on yield and fruit quality of peaches in Uruguay. pp 431-435. In Proceedings of the Fifth International Microirrigation Congress. Florida, USA.

Gong, D. Z., S. Z. Kang, and J. H. Zhang. 2005. Responses of canopy transpiration and canopy conductance of peach (*Prunus persica*) trees to alternate partial root zone drip irrigation. Hydrol. Processes 19: 2575-2590.

Gotelli, N. 2001. A Primer of Ecology, 3rd ed. Sinauer Associates, Sunderland, Massachusetts.

Grechi, I., M.-H. Sauge, B. Sauphanor, N. Hilgert, R. Senoussi, and F. Lescourret. 2008. How does winter pruning affect peach tree - *Myzus persicae* interactions? Entomol. Exp. Appl. 128: 369-379.

Grossman, Y. L., and T. M. Dejong. 1995a. Maximum fruit growth potential following resource limitation during peach growth. Ann. Bot. 75: 561-567.

Grossman, Y. L., and T. M. Dejong. 1995b. Maximum fruit growth potential and seasonal patterns of resource dynamics during peach growth. Ann. Bot. 75: 553-560.

Grossman, Y. L., and T. M. Dejong. 1995c. Maximum vegetative growth potential and seasonal patterns of resource dynamics during peach growth. A Ann. Bot. 76: 473-482.

Haukioja, E., and J. Koricheva. 2000. Tolerance to herbivory in woody vs. herbaceous plants. Evol. Ecol. 14: 551-562.

Herms, D. A. 2002. Effects of fertilization on insect resistance of woody ornamental plants: Reassessing an entrenched paradigm. Environ. Entomol. 31: 923-933.

Herms, D. A., and W. J. Mattson. 1992. The dilemma of plants- to grow or defend. Q. Rev. Biol. 67: 283-335.

Hilbert, D. W., D. M. Swift, J. K. Detling, and M. I. Dyer. 1981. Relative growth rates and the grazing optimization hypothesis. Oecologia 51: 14-18.

Hurley, P. F., and D. Flaspohler. 2007. Defense strategies of forest herbs on great lakes island and mainland sites: does ungulate-browse history play role?, pp. 2, School of Forest Resources and Environmental Science. Michigan Technological University, Michigan, USA.

Larson, K. C., and T. G. Whitham. 1991. Manipulation of food resources by a gall-forming aphid - the physiology of sink-source interactions. Oecologia 88: 15-21.

Leimu, R., and J. Koricheva. 2006. A meta-analysis of tradeoffs between plant tolerance and resistance to herbivores: combining the evidence from ecological and agricultural studies. Oikos 112: 1-9.

Li, S. H., and J. G. Huguet. 1989. Production, fruit quality and development of peach trees under different irrigation regimes. Fruits (Paris) 44: 225-232.

Li, S. H., J. G. Huguet, P. G. Schoch, and P. Orlando. 1989. Response of peach tree growth and cropping to soil water deficit at various phenological stages of fruit development. J. Hort. Sci. 64: 541-552.

Marshall, C. B., G. Avila-Sakar, and E. G. Reekie. 2008. Effects of nutrient and CO2 availability on tolerance to herbivory in *Brassica rapa*. Plant Ecol. 196: 1-13.

Maschinski, J., and T. G. Whitham. 1989. The continuum of plant-responses to herbivory - The influence of plant association, nutrient availability and timing. Am. Nat. 134: 1-19.

Nuñez-Farfán, J., J. Fornoni, and P. Luis Valverde. 2007. The evolution of resistance and tolerance to herbivores. Ann. Rev. Ecol. Evol. Syst. 38: 541-566

Osier, T. L., and R. L. Lindroth. 2006. Genotype and environment determine allocation to and costs of resistance in quaking aspen. Oecologia 148: 293-303.

Poëssel, J.L., M. H. Sauge, M. Staudt, C. Dufour, C. Deborde, Y. Rahbé, B. Jackson, C. Renaud, M. Maucourt, M.N. Corre, H. El-Aouni, J.P. Lacroze, and A. Moing. 2011. Metabolite profiling and feeding bioassays suggest a major role for a dicaffeoylquinic acid in induced resistance of peach to *Myzus persicae* aphid. In PR-Proteins and induced resistance against pathogens and insects. Neuchâtel, Switzerland.

Prado, E., and W. F. Tjallingii. 1997. Effects of previous plant infestation on sieve element acceptance by two aphids. Entomol. Exp. Appl. 82: 189-200.

Prittinen, K., J. Pusenius, K. Koivunoro, and H. Roininen. 2003. Genotypic variation in growth and resistance to insect herbivory in silver birch (*Betula pendula*) seedlings. Oecologia 137: 572-577.

Ramírez, C. C., and J. A. Verdugo. 2009. Water availability affects tolerance and resistance to aphids but not the trade-off between the two. Ecol. Res. 24: 881-888.

Retuerto, R., B. Fernandez-Lema, R. Rodriguez, and J. R. Obeso. 2004. Increased photosynthetic performance in holly trees infested by scale insects. Funct. Ecol. 18: 664-669.

Sauge, M.H., J.-L. Poessel, T. Guillemaud, and L. Lapchin. 2011. Resistance induction and herbivore virulence in the interaction between *Myzus persicae* (Sulzer) and a major aphid resistance gene (Rm2) from peach. Arthropod-Plant Interact. 5: 369-377.

Sauge, M.-H., F. Mus, J.-P. Lacroze, T. Pascal, J. Kervella, and J.-L. Poessel. 2006. Genotypic variation in induced resistance and induced susceptibility in the peach - *Myzus persicae* aphid system. Oikos 113: 305-313.

Sauge, M. H., P. Lambert, and T. Pascal. 2012. Co-localisation of host plant resistance QTLs affecting the performance and feeding behaviour of the aphid *Myzus persicae* in the peach tree. Heredity 108: 292-301.

Sauge, M. H., J. P. Lacroze, J. L. Poessel, T. Pascal, and J. Kervella. 2002. Induced resistance by *Myzus persicae* in the peach cultivar 'Rubira'. Entomol. Exp. Appl. 102: 29-37.

Silfver, T., H. Roininen, E. Oksanen, and M. Rousi. 2009. Genetic and environmental determinants of silver birch growth and herbivore resistance. For. Ecol. Manage. 257: 2145-2149.

Stevens, M. T., D. M. Waller, and R. L. Lindroth. 2007. Resistance and tolerance in *Populus tremuloides*: genetic variation, costs, and environmental dependency. Evol. Ecol. 21: 829-847.

Strauss, S. Y., and A. A. Agrawal. 1999. The ecology and evolution of plant tolerance to herbivory. Trends Ecol. Evol. 14: 179-185.

Thompson, G. A., and F. L. Goggin. 2006. Transcriptomics and functional genomics of plant defence induction by phloem-feeding insects. J. Exp. Bot. 57: 755-766.

Tiffin, P. 2002. Competition and time of damage affect the pattern of selection acting on plant defense against herbivores. Ecology 83: 1981-1990.

Wise, M. J., and W. G. Abrahamson. 2005. Beyond the compensatory continuum: environmental resource levels and plant tolerance of herbivory. Oikos 109: 417-428.

Wise, M. J., and W. G. Abrahamson. 2007. Effects of resource availability on tolerance of herbivory: A review and assessment of three opposing models. Am. Nat. 169: 443-454.

Table 1. GLMM logistic parameter estimates (Estimates), *Z* values and *P* values for the different measures on the two genotypes of nectarines with different level of irrigation and aphid attack. Rootstock diameter was used as a covariate.

	Number of leaves			Flower bud			Shoot-bud		
	Estimates	*Z*	P	Estimates	Wald stat.	P	Estimates	Wald stat.	P
Rootstock diameter	0.03	7.23	0.001	0.68	41.94	0.000	0.308	0.69	0.406
Genotype	0.13	3.14	0.001	0.04	1.755	0.185	0.276	13.77	0.000
Irrigation	0.43	10.67	0.001	0.03	0.874	0.350	-0.127	2.91	0.088
Aphid	0.12	2.67	0.01	0.02	0.354	0.552	-0.005	0.00	0.950
Genotype x Irrigation	-0.26	-4.51	0.001	0.05	3.356	0.067	0.169	5.16	0.023
Genotype x Aphid	-0.28	-4.45	0.001	0.04	1.485	0.223	0.248	11.07	0.001
Irrigation x Aphid	-0.18	-3.1	0.01	0.09	9.337	0.002	0.246	10.91	0.001
Genotype x Irrigation x Aphid	0.03	0.31	0.8	0.10	10.81	0.001	0.180	5.82	0.016

Figure captions

Fig. 1. Number of leaves (mean ± SE) in the susceptible and the resistant genotype under two different water supplies (well-watered and water- deficient plants). Different letters indicate significant differences followed Tukey test ($p < 0.05$).

Fig. 2. Number of leaves (mean ± SE) in nectarines genotypes (susceptible and resistant) in relation to the presence of aphid attack. Different letters indicate significant differences by Tukey ($p < 0.05$).

Fig. 3. Number of leaves (mean ± SE) on water treatment x aphid interaction. Different letters indicate significant differences by Tukey ($p < 0.05$).

Fig.4. Curling grade (mean ± SE) in nectarines cultivars: N18 (susceptible) and NS92 (resistant) on cultivar x water treatment interaction. Different letters indicate significant differences by Tukey ($p < 0.05$).

Fig. 5. Population growth rate (PGR) (mean ± SE) in water treatment x aphid interaction. Different letters indicate significant differences by LSD Fisher ($p < 0.05$).

Fig. 6. Number of flower buds in cultivars of *P. persica* (R: resistant genotype and S: susceptible genotype) in the next season to the irrigation and aphid treatments. The measures were in August 30 y September 6.

Fig. 7. Number of shoot buds in cultivars of *P. persica* (R: resistant genotype and S: susceptible genotype) in the next season to the irrigation and aphid treatments. The measures were in August 30.

Fig.1

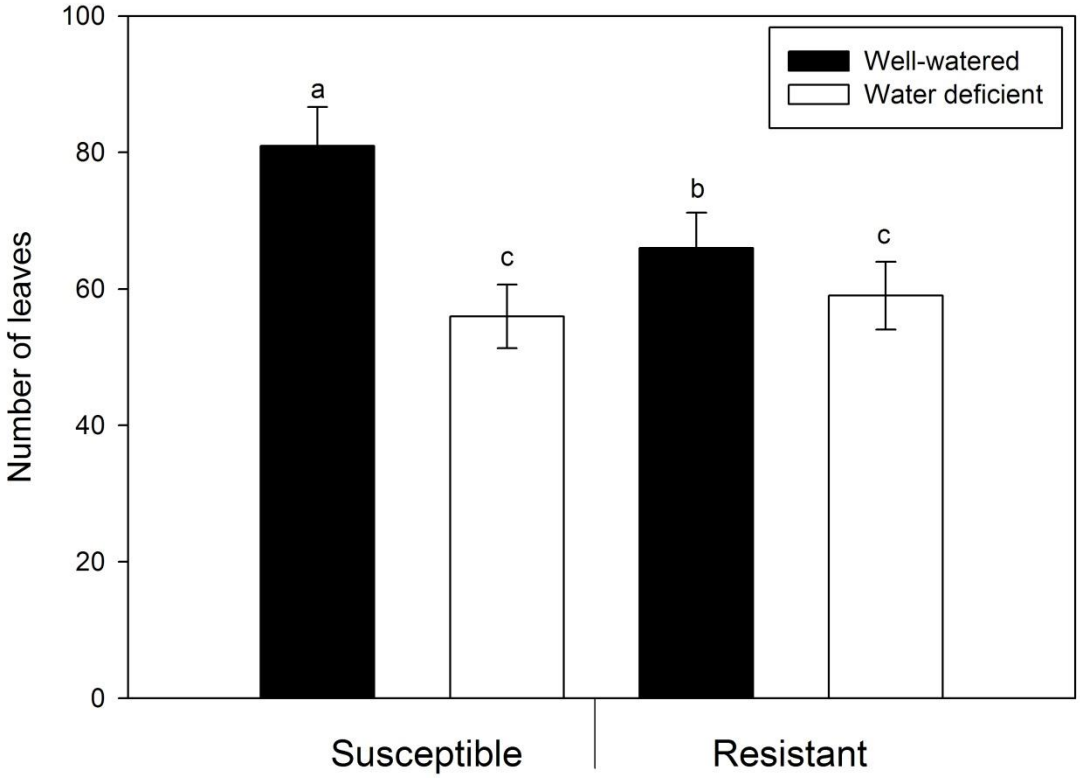

100
80
60
40
20
0
Number of leaves
Well-watered
Water deficient
a
c
b
c
Susceptible
Resistant

Fig.2

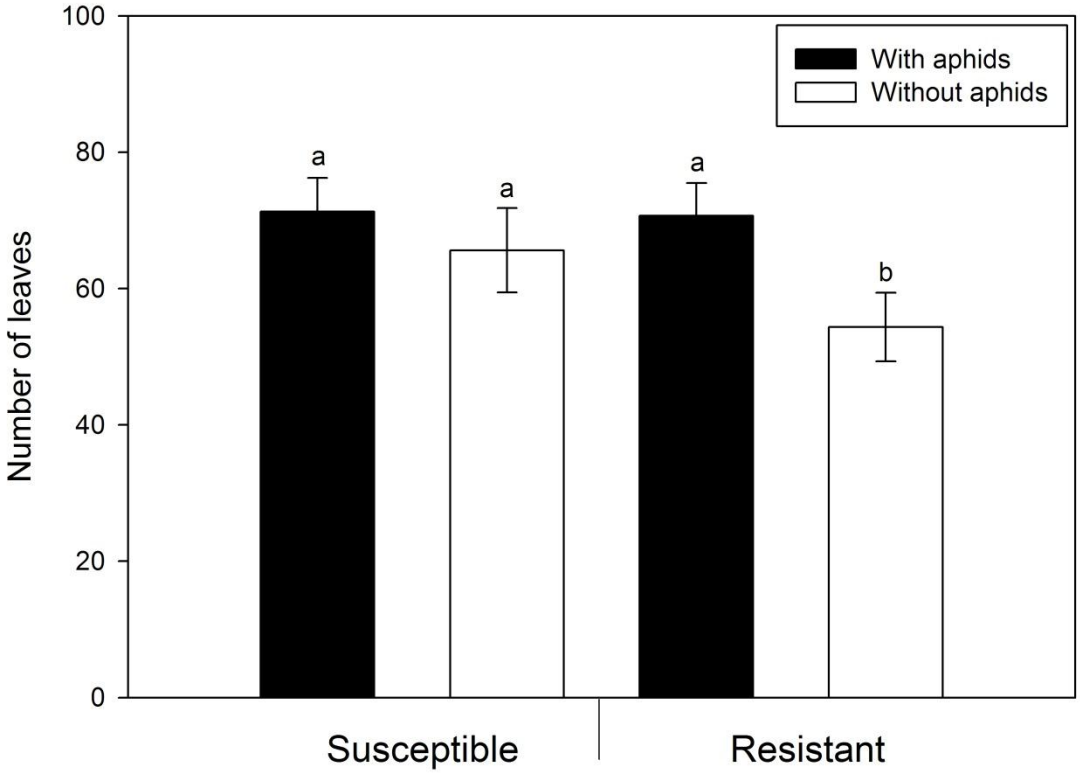

100
80
60
40
20
0
Number of leaves
With aphids
Without aphids
a
a
a
b
Susceptible
Resistant

Fig. 3

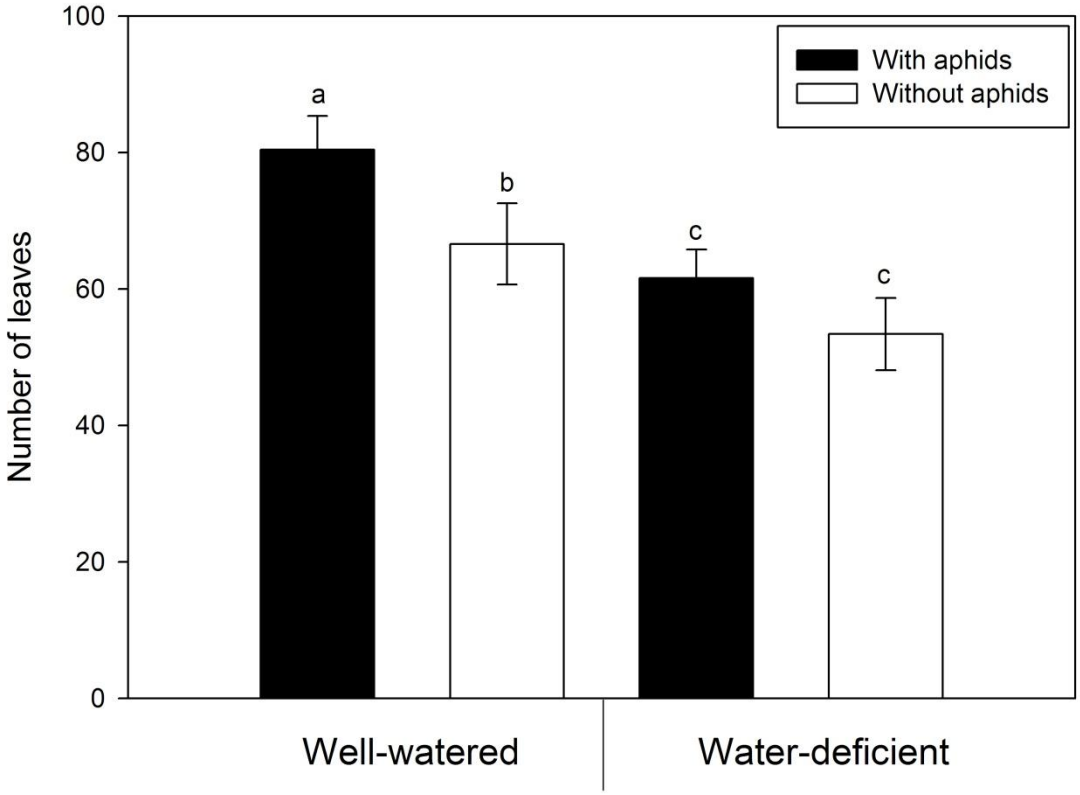

100
80
60
40
20
0
Number of leaves
With aphids
Without aphids
a
b
c
c
Well-watered
Water-deficient

Fig. 4

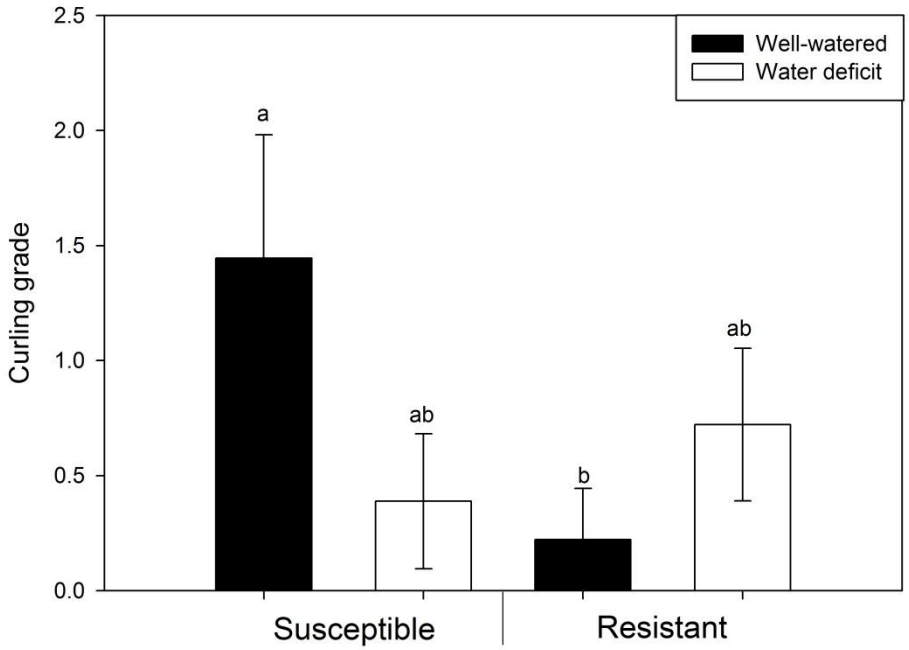

Well-watered
Water deficit
Curling grade
2.5
2.0
1.5
1.0
0.5
0.0
a
ab
b
ab
Susceptible
Resistant

Fig. 5

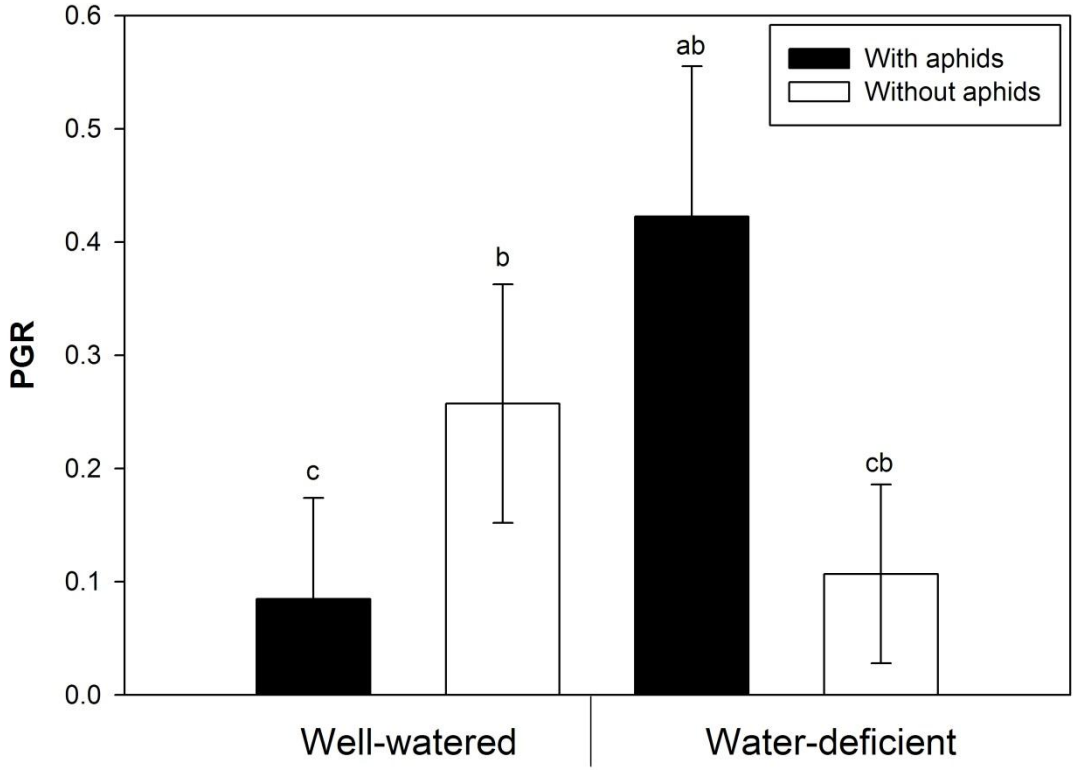

0.6
0.5
0.4
0.3
0.2
0.1
0.0
PGR
With aphids
Without aphids
c
b
ab
cb
Well-watered
Water-deficient

Fig. 6

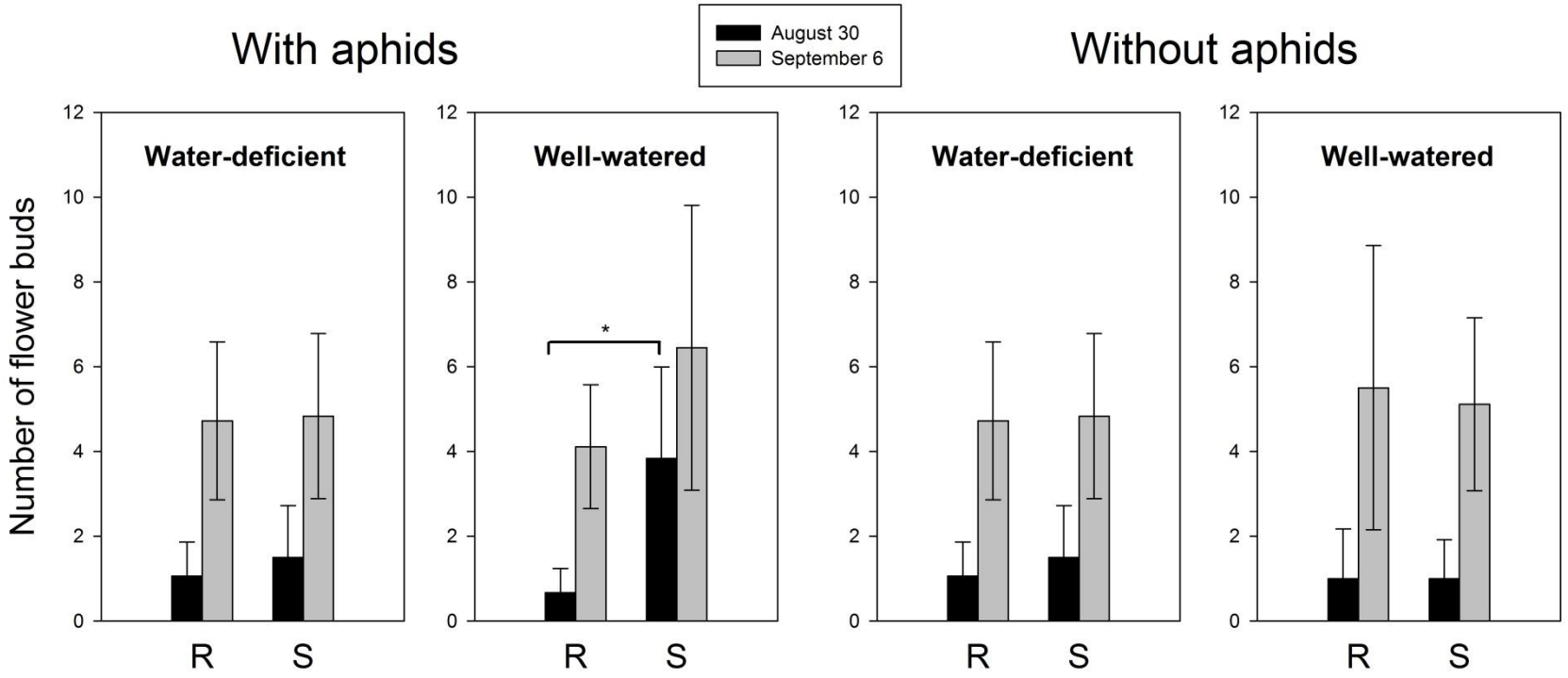
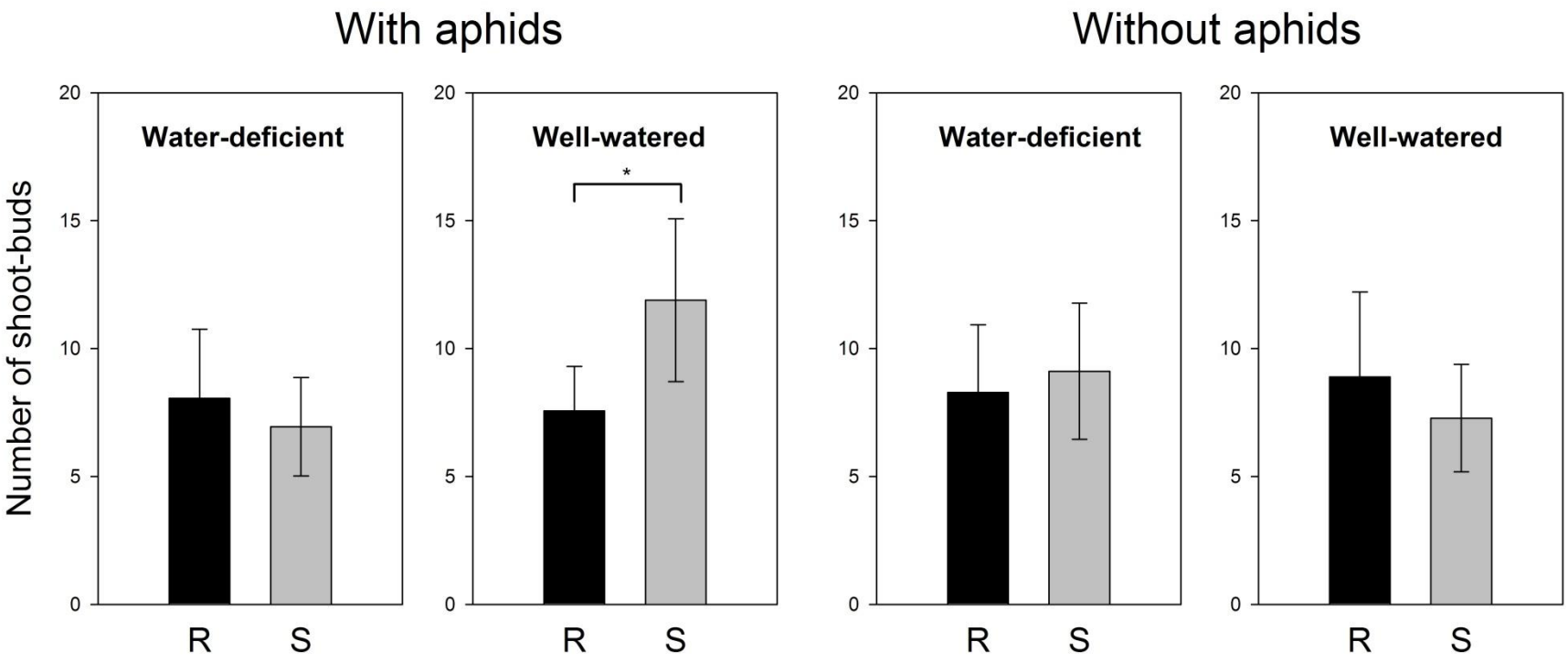

With aphids
August 30
September 6
Without aphids
Water-deficient
Well-watered
Water-deficient
Well-watered
Number of flower buds
0
2
4
6
8
10
12
*
R
S
R
S
R
S
R
S

Fig. 7


With aphids
Without aphids
Water-deficient
Well-watered
Water-deficient
Well-watered
Number of shoot-buds
20
15
10
5
0
*
R
S

Chapter III

REDUCED WATER SUPPLY ON APHID-RESISTANT PLANTS ELICITS LOWER CHANGES IN THE PROTEOMIC PROFILE OF ITS HERBIVORE: PEACH-APHID INTERACTION

This chapter 3 is centered in the response of the green peach aphid *M. persicae* to the peach *P. persica*. Instead of studying the plants, here the studied was in the insect. With this, we could have a complete view in the interaction *P. persica – M. persicae,* including under water deficit. In this chapter we consider how the irrigation in *P. persicae* could module physiological changes in the aphid *M. persicae*, to adapt its metabolism to the conditions of the host plant. To elucidate these changes, a proteomics approach was used. Only one study with aphids has addressed similar problems, but none in aphid-Prunus interaction. We analyzed the proteomics profile of one clone of *M. persicae* fed on two different cultivars of *P. persica* with different resistance level. With this study we will be able to determine if there is differences among the aphid fed on different cultivars and between these aphids fed on cultivars with different irrigation treatments. Differences will be expressed in the protein regulation (up or down regulated proteins) followed determining the protein functionality using comparative proteomics. Finally, the biochemical pathways involved in aphid's response to plant resistance and plant water stress will be outlined.

Reduced water supply to aphid-resistant plants elicits lower changes in the proteomic profile of its herbivore: peach-aphid interaction

Short title (Running Head): Proteomics in *M. persicae* fed on peaches under water deficit

J.A. Verdugo [1,2], Lacroze J.P.[3], Sauge M.H.[3], Ramírez C.C.[1] and Francis F.[2]

1. Laboratorio Interacciones Insecto-Planta, Instituto de Biología Vegetal y Biotecnología, Universidad de Talca, Casilla 747, Talca, Chile. (clramirez@utalca.cl).
2. Gembloux Agro-Bio Tech - Université de Liège, Unité d'Entomologie fonctionnelle et évolutive, Passage des Déportés 2, B-5030 Gembloux, Belgium.
3. INRA, Unité et Systèmes de culture Horticoles, Domaine St. Paul, Site Agroparc, Avignon Cedex 9, 84914 France.

Abstract

The effect of water supply on plant defense to herbivores has been intensively studied. However, the herbivore response to herbivore-resistant plants under water deficit is poorly understood. In this study, the proteomic profile of one clone of the green peach aphid *Myzus persicae* which was exposed to two genotype of *Prunus persica*, one susceptible to this aphid (GF305) and another resistant (Rubira®), which were both also subjected to normal and water deficient irrigation was studied. The results showed that after 48 h, as compared to aphids fed on control plants (well-watered), the aphids fed on the susceptible peach genotype under water-deficit exhibited 19 proteins up-regulated, whereas on the resistant genotype only 8 proteins were up-regulated. Most of the proteins exhibiting changes on the susceptible genotypes were involved in energy metabolism (as Regulator of G-protein signaling 7-like and RNA 3' terminal phosphate cyclase), whereas those mainly associated to cytoskeleton functionality (as actin related protein 1 and F-actin capping protein subunit beta) changed in the resistant genotype. Five proteins exhibited similar regulation changes as a consequence of water-deficit in both peach genotypes, proteins mostly related with cytoskeleton. Only one of these proteins was down-regulated, the mitochondrial-processing peptidase, which is associated with aphid response to toxicity. In a parallel experiment using similar treatments as in the proteomic study, the population rate of growth (PGR) of this clone was only affected by peach genotype, finding, as expected, that PGR was higher in the susceptible peach. Thus, these findings suggest that susceptible plant, under water deficit, more than resistance ones, suffers of physiological changes which in turn elicit (or "transferred to") a significant proteomic changes on aphids.

Key words: resistance, water stress, *Myzus persicae*, *Prunus persica*, proteomic

Introduction

Herbivorous insects are exposed to many abiotic stress such as solar radiation, extreme temperature, water or nutrients deficiencies that directly may reduce their fitness and indirectly throughout the effects of that stressor on their host plants (Bale et al. 2007, Nguyen et al. 2007). There are a number of theoretical and empirical studies showing that, under water stress, plant resistance to herbivores is reduced (White 1984, Price 1991, Herms and Mattson 1992, Zangerl and Bazzaz 1992, Hamilton and Coleman 2001, Wise and Abrahamson 2005, Bale et al. 2007, White 2009, Simpson et al. 2012). However, in woody plants this effect is less evident that in annual plants (Koricheva et al. 1998). Plants subjected to drought stress use to lose their green color, face major temperature on foliar tissues and higher infrared light reflectance, characteristics that would make them more acceptable or attractive to the insects (Mattson and Haack 1987, Moore 1995, Li et al. 2008). This would be possible provided that the arthropods possess sensitive receptors to the water conditions (Mattson and Haack 1987). Stresses such as drought and temperature can affect the biology of herbivore insects and biochemical composition of plants, altering the suitability of the plants. For example, aphid damage on plants produce higher content of some flavonoids as kaempferol in broccoli with drought and water logged, or lower content of gluconisolate in drought plant (Cole 1997, Khan et al. 2010, 2011). In addition, plant defense allocation patterns may be modified by the amount of resources available, altering, among other things, the chemical cues used by the aphid to locate its feeding site in the phloem (Bale et al. 2007) or allocated resources in root growth (Hunt and Nicholls 1986). The variation in water supply or nutrients affects plants defense strategies such as resistance and tolerance (Ramírez and Verdugo 2009).

Among phytophagous insects, aphids feed from phloem sap and they are very sensitive to variation in plant nutrients (Jansson and Ekbom 2002), which is also depending of the aphid-plant system and biotic and abiotic conditions. It have been reported that aphid will develop better on vigorous plant (Price 1991) them stressed plant (White 1984). Water availability is one of the principal resources for plants and aphids, and this may affect the concentration of amino acids and carbohydrates in the phloem sap, affecting positively or negatively the aphid performance (Nguyen et al. 2007). On water-stressed plants, aphid performance has been found to be enhanced (*M. persicae* on *B. oleracea*) or unaltered (*B. brassicae* on *B.oleracea*), suggesting that aphid species differ in their sensitivity to plant water-stress (Khan et al. 2010). However, little is known about how aphid's metabolism reacts when feeding on water-stressed plants which may also differ in their degree of susceptibility or resistance to aphids. Metabolic changes in the potato aphid (*Macrosiphum euphorbiae*) on water-stressed plants potato produced up-regulation of proteins involved on energy metabolism (Nguyen et al. 2007). Nothing is know about this kind of proteomic changes of aphids after feeding on resistance and susceptible which are also subjected to water-stress.

The green peach aphid *Myzus persicae* (Sulzer) (Hemiptera: Aphididae), is a very generalist aphid species whose primary host are peach trees, *P. persica* L. (Rosaceae), and use more than 400 plant species as secondary host (Blackman and Eastop 2000). In springtime, peaches and nectarines are heavily attacked by the green peach aphid *M. persicae* and it can produce direct loss by removal of assimilated, symptoms in the leaves as leaf curling, deformation in the fruit and/or fall of the flowers, besides the indirect damage produced by the transmission of the Plum Pox Virus (PPV) that is the principal agent of Sharka disease (Sauge et al. 1998d, Sauge et al. 1998c). This aphid has also

developed resistance to a wide variety of insecticides (Devonshire et al. 1998). This aphid is the most studied species, only followed by the pea aphid *Acyrthosiphon pisum*, whose genome is currently available. However, we know little about what metabolic changes are induced in this aphid when exposed to both plants with different degrees of resistance that are also subjected to different levels of irrigation. Resistant plants subjected to lower water supply could entail changes in their defense physiology, which in turn could affect the aphid physiology.

In the present work, the proteomic profile of *M. persicae* individuals after feeding on two cultivars of *P. persica* which differ on its resistance to this aphid (GF305, susceptible cultivar; Rubira®; resistant cultivar) and treated with two water irrigations (well-watered and water-deficit irrigation) was analyzed. Hence, the aim of this work was to find whether or not aphid exhibited metabolic differences after feeding on plants with different resistance and irrigation treatments.

Materials and Methods

Plants: Two peach genotypes of two months of age were used in experiment 1 and 2 (see below): cultivar Rubira® (clone S2605) and GF305, which were previously reported as resistant and susceptible to *M. persicae* were used (Sauge et al. 2002, Sauge et al. 2006). All plants were grafted on GF305 rootstock in pots under conditions indicated by Sauge *et al*. (2006a). The plants were reared in a greenhouse at INRA-Avignon. .

Aphids: Female adults of *Myzus persicae* clone Mp05, that were collected on peach orchard in the south of France (Avignon), were reared on peach cultivar GF305 under controlled

conditions (19±1ºC with a photoperiod 16L: 8D). Experiments were performed only with wingless adult aphids, which were age-synchronized by placing 20 wingless adults females on GF305 apex and after 48 hours these adults were recollected and only new produced nymphs (4580 nymphs were used for the experiments) were maintained in GF305 to adulthood.

Plant irrigation treatments: Healthy plants of each cultivar were subjected to well-watered irrigation (100% field capacity (FC) and to water-deficient irrigation (30% FC). FC was estimated by firstly weighting the plastic pot containing the plants and soil, followed by water saturating the pot, which was weighed once again after 24 hours in order to obtain the difference in weight that corresponded to the FC (0.6 l). With this information, treatments were defined as "well-watered" by supplying the 100% of FC and "water-deficit" adding the 30% of FC. In both treatments, water was supplied every two days until the end of the experiments, which lasted nine days. In order to assess the degree of water stress of the experimental plants, at seventh day at the midday, the leaf water potential (Ψ_{pd}) of each plant was measured using a Scholander pressure chamber. One leaf per measurement was performed. The leaf water potential under 100% FC and 30% FC were 1.17 ± 0.05 MPa and 1.78 ± 0.12 MPa for the susceptible and 0.99 ±0.03 MPa and 1.63 ± 0.07 MPa for the resistant cultivar. The Ψ_{pd} was significantly reduced by different irrigations in both cultivars ($p < 0.0001$ and $p < 0.0001$ for susceptible and resistant cultivars, respectively; Tukey test), and were not different between the cultivars.

Two main experiments were performed with plants subjected to the irrigation treatments described above: i) experiment 1, aphid proteomics, and ii) experiment 2, aphid performance.

Experiment 1: Aphid proteomic on water-deficient plants

Plant of the susceptible and resistant genotypes of 40 and 50 cm of height with three shoots were put in a chamber with controlled conditions (22° C, 60% R.H. and 16L:8D photoperiod) with the irrigations treatments as explained above. A total of 40 wingless adult aphids were placed in the first 5-7 open leaves on each shoot in both cultivars. Aphids were allowed to freely move within each plant. After 48 hours, the populations of aphids were weighted and maintained at -20 ºC until proteomic analysis. Previous studies have determined that followed 48 h of aphid attack, the resistant cultivar Rubira® showed differences in the primary and secondary metabolites (Poëssel et al. 2011). Ten replicates per treatment were used.

For the proteomic analysis, aphids collected separately from each treatment and weighted using 100 mg of aphids were crushed in 7 M urea, 2 M thiourea 20 mM Tris pH 8.5 buffer including 1% CHAPS and 1% ASB-14, and centrifuged at 15000 g at 4 ºC during 15 min. Supernatants were collected and proteins precipitated using the 2D Clean Up Kit according instructions of the manufacturer (GE Healthcare). Quantification of the precipitated proteins was performed using the RCDC quantification kit from BioRad. The protein extracts (aliquot of 25 µg) were labeled with one of three Cydye (GE Healthcare) following standard DIGE protocol and dye-swap design with four technical replicates per treatment. Comparison of the treatments was made according to labeling protein samples with either Cye3 or Cye5 and were mixed with a standard protein mixture labeled with Cye2. The mix of labeled proteins was adjusted to a volume of 450 µl that was used to rehydrate 24 cm IPG strips (pH 3-10 NL from GE Healthcare) for 12 h at 20ºC and constant voltage of 50 V. Isoelectric focusing (IEF) was carried out at 200 V for 200 Vh, 500 V for 500 Vh, 1000 V for 1000 Vh and 8000 V for 60000 Vh at 20ºC and a maximum

current setting of 50 µA/strip in an isoelectric focusing unit from BioRad. Following IEF, the IPG strips were equilibrated for 15 min in 375 mM Tris (pH 8.8) containing 6 M urea, 20% v/v glycerol, 2% w/v SDS, and 130 mM DTT and then for a further 15 min in the same buffer except that DTT was replaced with 135 mM iodoacetamide following the protocol for FS 1800 fiber wicks (Gelcompany). The second-dimensional electrophoresis was performed in the HPE Flat Top Tower for horizontal electrophoresis (Serva Electrophoresis). The second dimension was carried out at 100 V, 28 mA, 4W during 30 min after 200 V, 52 mA, 12 W during 30 min; 300 V, 80 mA, 20 W during 10 min; 1500 V, 160 mA, 120 W during 3 h 50 min. Finally gel was put in a fixation solution 15% ethanol, 10% acetic acid and 75% high purity water overnight and after the gels were scanned in the Thyphoon fluorcence imager (GE Amersham) at wavelengths corresponding to each cydye. The analyses of the images were made with SAMESPOT 2D Software version 3.5 (Nonlinear dynamics) according the manufacturer´s instructions. Thus, comparisons between samples from all the treatments and a total of eight 2D-gels were produced.

For protein identifications a non-labeled 500µg sample of aphid protein mixture was added in one of the analytical gel and the protein spots were excised from the gel using an Ettan spotpicker robot (GE Healthcare). Selected gel pieces were collected in 96-well plates designed for the Proteineer dp automated digester (Bruker, Bremen, Germany). Briefly, gels pieces were washed with three alternative soaking in 100% ammonium hydrogenocarbonate 50mM, and a mix of 50% Acetonitrile 50% ammonium hydrogenocarbonate 50mM. Two additional washes were performed with 100% acetonitrile to dehydrate the gel. 3µl of freshly activated trypsin (Roche, porcine, proteomics grade) 10ng /µl in ammonium hydrogenocarbonate was used to rehydrate the gel pieces at 8°C for

30 minutes. Trypsin digestion was performed for 3h at 30°C. Peptides extraction was performed with 10µl of 1% formic acid for 30 minutes at 20°C.

Protein digests (3µl) were adsorbed for 3 minutes on prespotted anchorchips(R) using the Proteineer dp automaton. Spots were washed "on-target" using 10mM dihydrogeno-ammonium phosphate in 0.1% TFA-MilliQ water to remove salts. High throughput spectra acquisition is performed using an Ultraflex II MALDI mass spectrometer (Bruker) in positive reflectron mode, with close calibration enabled, Smartbeam laser focus set to medium, and a laser fluency setting of 65 to 72% of the maximum. Delayed extraction is set to 30 ns. Steps of 100 spectra in the range of 860 to 3800 Da are acquired at a 200 Hz LASER shot frequency with automated evaluation of intensity, resolution and mass range. 600 successful spectra per sample are summed, treated and de-isotoped in line with an automated SNAP algorithm using FLEX ANALYSIS 2.4 software (Bruker), and subsequently submitted in the batch mode of the Biotools 3.0 software suite (Bruker) with an in-house hosted MASCOT search engine (MatrixScience.com) to the database (public NCBI non redundant released from 2011/07/17). A mass tolerance of 80 ppm with close calibration and one missing cleavage site are allowed. Partial oxidation of methionine residues and complete carbamylation of cystein residues are considered. The probability score calculated by the software was used as one criterion for correct identification. Experimental and Mascot results molecular weights and pI were also compared.

Experiment 2: Aphid performance on water-deficient plants

Plant of the susceptible and resistant genotypes between 30 and 40 cm with one shoot were placed in a chamber with the same condition as described for Experiment 1.On these plants,

aphid population growth rate (PGR) was measured, which is estimated as (ln N2 – ln N1)/(t2-t1), were N1 is the initial number of aphids, N2 the final number of aphids, and (t2 - t1) the number of days of the experiment (12 days) (Gotelli 2001). Between 5 to 10 wingless adults were deposited on each plant as initial number of aphids (N1). Ten replicates per treatment were used.

Because at the end of this experiment a larger amount of aphid were available, aphids were collected from each treatment and maintained at -20°C in order to identify the endosymbiont community by PCR technique using specific primers described by Tsuchida *et al.* (2002). A total 100 mg of aphids were weight and homogenized on 400 µl of solution buffer with 0,4 M NaCl 10 mM, Tris-HCl pH 8,0 and 2 mM EDTA pH 8,0, after are add 40 µl of 20% of SDS and 8 µl of proteinase K (20mg/ml). Samples were incubated at 60°C during 1 h, and after 300 µl of NaCl 6M and vortex 30 s, centrifuge 30 min at 10000 g. Keep the supernatant and add the same volume of isopropanol and maintain at -20° C during 1 h and centrifuge 20 min at 4° C at 10000g after throw out the liquid and wash the precipitate with 1 ml of ethanol 70%, throw the ethanol and leave dry the pellet and finally add 100 µl of sterile water. In order to characterize the community of secondary endosymbionts in the clone used (MP05),

Statistical analysis:

For proteomic analysis, normalized spot intensities from gels were compared by Student–*t* statistical test ($p < 0.05$) implemented in SAMESPOT 2D Software version 3.5 (Nonlinear dynamics). PGR was analyzed with two-way ANOVA followed by Tukey for multiple comparisons.

Results

Experiment 1: Aphid proteomic on water-deficient plants

More than 2000 spots were identified were identified according to cydyes labelling in 2D-PAGE (Fig. 1). However, only 50 proteins significantly varied among treatments (Table 1). Three proteins were up-regulated among the genotypes independent of the water treatments: phosphoglycerate mutase in the susceptible cultivar GF305 (dot 134; enzyme involved in the glycolysis related to carbon fixation), guanylate cyclase (dot 54; neurotransmissor and involved insect muscle formation) and uroporphyrinogen decarboxylase (updo, isoform B) (dot 122; porphyrin metabolism) in the resistant genotype Rubira® (Table 1).

Regarding the comparison in regulation between water conditions within genotypes (Fig. 2), in the susceptible genotype a total of 24 protein were identified with differential regulation: 19 were up-regulated exclusively in susceptible genotype under water-deficit (spots 14, 21, 30, 38, 47, 54, 66, 72, 102, 128, 134, 138, 142, 179, 956 and 990 in *M. persicae* and dots 64, 119 and 149 in *Buchnera*; Table 1), Five proteins were share with Rubira® (spots 80, 114 and 140 in *M. persicae* and dots 94 in *Buchnera*; and only one protein down-regulated in susceptible genotype (dot 127; Fig. 2). The magnitude of this regulation also varied with water treatments (Fig. 3a). The protein group exhibiting the higher number of up-regulated proteins was that associated to energy metabolism (10 proteins). Among these proteins, the regulator of G-protein signaling 7-like (dot 21) was the most up-regulated one with 3.2 fold, followed by RNA 3' terminal phosphate cyclase (dot 47) with 2.7 folds and the ATPase AAA domain-containing protein 2-like (dot 38) with 2.4 folds. The remaining seven proteins of this group range between 1.2 and 1.9 folds. The other thirteen proteins up-regulated in the susceptible genotype belonged to several

groups, including proteins associated to exoskeleton, cytoskeleton, amino acid metabolism, carbohydrate metabolism, protein synthesis and others (Fig. 3a). It is worth noting that among exoskeleton proteins, the cuticular protein CPG12 (dots 14) was the most up-regulated one with 3.5 folds. The only down- regulated protein was the mitochondrial-processing peptidase (dot 127) with -1.7 folds.

In the resistant genotype Rubira®, a total of 13 protein were identified with differential regulation: eight were up-regulated exclusively in this genotype under water-deficit (spots 75, 79, 91, 93, 124, 147 and 175 in the *M. persicae* and dots 132 in *Buchnera*; Table 1), the others five proteins were share with the susceptible genotype, mentioned above. The magnitude of this regulation also varied with water treatments (Fig. 3a and 3b).

The protein group exhibiting the higher number of up-regulated proteins was that associated to the cytoskeleton (seven proteins), actin proteins (dots 75, 91, 114, 124, 140,147 and 175) which are associated with cytoskeleton and cytoplasm related with exocytosis were up-regulated between 1.4 to 1.6 folds. The other five protein belong to a different groups, but is important to note the protein phospho-2-dehydro-3-deoxyheptonate aldolase (dot 132) was the protein more up-regulated expressed 1.8 fold in *Buchnera* on aphid feed on water stressed plant. The only protein down-regulated on both genotypes was the mitochondrial-processing peptidase (dot127).

2) *Aphid performance on water stress plants:*

Significant differences in aphid performances (PGR) were found only between the cultivars ($F_{1, 36}$ = 45.306, P < 0.001), with no interaction between peach genotype x irrigation of main effect of irrigation. As expected, on the resistant peach genotype aphids showed the lowest population increment (Fig. 4).

Discussion

The aphid response when were fed on two peach cultivars, one susceptible and other resistant to aphids and subjected to two level of irrigation, was not evident in terms of the population growth rate. The only significant difference was associated with the nectarine genotypes. Differently, aphid proteomic showed large differences between treatments specially on aphids fed on the susceptible genotypes, which were related to regulation of protein related to energy metabolism, exoskeleton and cytoskeleton. Aphid fed on the resistant showed less differences although most differences were associated to proteins up-regulated on the cytoskeleton, and only three proteins were associated to the differences among genotypes. One protein up-regulates in the susceptible genotype was associated to energy metabolism phosphoglycerate mutase (dot 134), which is an enzyme present in the glycolysis related to carbon fixation (Carreras et al., 1982). The resistant genotype also presented one protein associated to this process, guanylate cyclase (dot 54), act in the formation of insect muscle (Robinson et al., 1982), and lastly the uroporphyrinogen decarboxylase (updo, isoform B; dot 122) was classified in other functions which act in the porphyrin metabolism .

Considering the performance of *M. persicae* based on PGR during 12 days, there were differences among the aphid growing in both genotypes (Fig. 4), but there were not differences associated to the water deficit to generate differences in the population growth. It is likely that and additional stress (i.e. nutrient as nitrogen; M.H. Sauge, personal communication), could have been effective. Nevertheless, others genotypes of *P. persica var* nectarine with different level of resistance to *M. persicae* were shown to elicit

difference in aphid PGR up 43% of change (see Chapter II) after 12 days, which is a larger period compared to what was used in the current experiment, which involved only 48 h of response. Nevertheless, that period was enough to trigger relevant changes in aphid proteomic profile. More proteins were deregulated on the susceptible genotype compared to the resistance genotype (Fig 3a and 3b).

Why *M. persicae* responded more in the susceptible genotypes under water-deficit? The answer may be related with changes in plant physiology which are "transferred" to the aphid as under this circumstance the otherwise susceptible plant become physiologically altered and possibly harmful. The resistant plant instead could be less sensible to water changes and then more resilient in their physiology. Aphids have already been shown to response to secondary metabolic modifications in *Brassica oleracea* var. italica under water stress due to quantitative changes in glucosinolates which altered the population of aphid *M. persicae* (Khan et al. 2010). In our case, most up-regulated proteins were found to be involved in energy metabolism; therefore, plant water stress seems to induce changes in the insect physiology. One of the proteins associated to the energy metabolism with a high fold changes was the regulator of G-protein signaling 7-like (dot 21), which was up regulated 3.2 fold. This is an essential protein for behavioural adaptations, allowing reproduction and feeding behaviour in a complex environment (Wilkie 2000). In addition, RNA 3' terminal phosphate cyclase (dot 47) was an enzyme up-regulated with unknown function in aphids, although its properties are similar in bacteria and human (Genschik et al. 1997, Genschik et al. 1998) and probably in this case in regulated by water deficit. Proteins associated to exoskeleton as cuticle proteins, which are rich glycine protein, showed high fold rate change which may be involve in cuticle restoration (Zhang et al. 2008, Wang et al. 2010)., which may be related to avoid dehydration. Up-regulated proteins in metabolism of

cytoskeleton were also implicated in adaptation to different stresses (Francis et al. 2010). Some other deregulated aphid proteins were found to be implicated in the amino acid pathway, such as the anthranilate synthase acting in the biosynthesis of the tryptophan in *Buchnera*, which increases the production of the amino acid (Baumann et al. 1995, Plague et al. 2003), which under stress its function increases. Several proteins acting in the carbohydrate and synthesis of proteins were also found to be changed in the tested conditions herein.

Considering the up-regulated proteins in the resistant genotype, most of the up-regulated protein were related with cytoskeleton (dots 75, 91, 114, 124, 140,147 and 175), which work on the adaptation to many stresses, providing internal structure to the cells and working in the transport, cellular division andintracellular traffic via filaments within cells. Also is was important the phospho-2-dehydro-3-deoxyheptonate aldolase (dot 132) expressed 1.8 fold in *Buchnera* on aphid fed on water stressed plant, which is involved in the metabolism of various amino acid as alanine, aspartate, glutamate, glycine, serine and threonine, therefore the water stress on the plant reduced the quantity of the amino acids in the aphid and his symbiont had to metabolize more these components. It remind unknown why these changes in the endosymbiont related genes were observed in aphids fed on water-deficient resistant plants. Looking at plant metabolic changes would help to elucidate this phenomenon.

The mitochondrial-processing peptidase was the only one found to be down-regulated similarly in both genotypes subject to stress, this protein is contaminant from mitochondria (Bayyareddy et al. 2009), has been found in salivary glands (Konishi et al. 2009) and also was identified be down-regulated in insect exposed to carbamate

insecticides (Sharma et al. 2004), therefore this could be a protein which is downregulated under stressed conditions.

Independently of the plant genotype, three proteins associated to cytoskeleton were up-regulated (dots 94, 114 and 140), which would be reacting to the water stress as adaptation (Francis et al. 2010). It is important to note that two proteins from the endosymbiont *Buchnera* were regulated. These were involved in the amino acid metabolism as anthranilate synthase component I (dot 64), a protein which is part of the biosynthesis of the tryptophan in *Buchnera*, which is also found in limited concentration, but an increase of this enzyme allow more production of the amino acid (Baumann et al. 1995, Plague et al. 2003, Douglas 1998), In addition, phospho-2-dehydro-3-deoxyheptonate aldolase associated in the metabolism of alanine, aspartate, glutamate, glycine, serine and threonine was modulated. The specific role of endosymbiont in the aphid response to plant resistance and water availability need further studies.

In summary, water treatment did not have a strong influence in the aphid population, but at the proteomic level they showed large responses. The aphids fed on the susceptible genotype have higher number and fold rate of protein expressed, which were mainly involved in the energy metabolism, exoskeleton, cytoskeleton and amino acid from the symbiont *Buchnera*. More works on the secondary metabolites in *Prunus persicae* under water stress and the consequence on aphids would help to identify what aphids do to face plants with altered physiology.

Acknowledgements

This research was supported Grant of Facultad de Ciencias Agrarias of Universidad de Talca to J.V. and Grant Becas Chile Conicyt-Chile to JV, Grant of Communauté française de Belgique et la Région wallone, also to thank to Unité et Systèmes de culture Horticoles of INRA-Avignon, France.

References

Agrawal, A. A. 1998. Induced responses to herbivory and increased plant performance. Science 279: 1201-1202.

Agrawal, A. A. 2000. Overcompensation of plants in response to herbivory and the by-product benefits of mutualism. Trends Plant Sci. 5: 309-313.

Alvarez, A. E., M. Dicke, E. Garzo, W. F. Tjallingii, V. Vleeshouwers, and B. Vosman. 2006a. Location of resistance factors in the leaves of potato and wild tuber-bearing Solanum species to the aphid *Myzus persicae*. Entomol. Exp. Appl. 157: 145-157.

Alvarez, A. E., W. F. Tjallingii, E. Garzo, V. Vleeshouwers, M. Dicke, and B. Vosman. 2006b. Location of resistance factors in the leaves of potato and wild tuber-bearing *Solanum* species to the aphid *Myzus persicae*. Entomol. Exp. Appl. 121: 145-157.

Artigas, J. N. 1994 Entomología Económica, vol. 1, Universidad de Concepción, Concepción, Chile.

Baldwin, I. T. 1994. Chemical changes rapidly induced by folivory, pp. 1-23. In E. Barnays (ed.), Insect plant interactions. Chemical Rubber Company, Boca Raton, Florida, USA.

Baldwin, I. T., C. L. Sims, and S. E. Kean. 1990. The reproductive consequences associated with inducible alkaloidal responses in wild tobacco. Ecology 71: 252-262.

Bale, J. S., K. L. Ponder, and J. Pritchard. 2007. Coping with stress, pp 287-303. In H.F. Van emden & R. Harrington (eds): Aphids as crop pest. CABI Publishing, London.

Baraza, E., R. Zamora, and J. A. Hodar. 2010. Species-specific responses of tree saplings to herbivory in contrasting light environments: An experimental approach. Ecoscience 17: 156-165.

Basiouny, F. M. 1978. Response of peach seedlings to water stress and saturation conditions. Proc. Flor. State Hort. Soc. 90: 261-263.

Bass, C., A. M. Puinean, M. Andrews, P. Cutler, M. Daniels, J. Elias, V. L. Paul, A. J. Crossthwaite, I. Denholm, L. M. Field, S. P. Foster, R. Lind, M. S. Williamson, and R. Slater. 2011. Mutation of a nicotinic acetylcholine receptor beta subunit is associated with resistance to neonicotinoid insecticides in the aphid *Myzus persicae*. BMC Neurosci. 12:51.

Baumann, P., C. Y. Lai, L. Baumann, D. Rouhbakhsh, N. A. Moran, and M. A. Clark. 1995. Mutalistic associations of aphidsand prokaryotes - Biology of the genus *Buchnera*. Appl. Environ. Microbiol. 61: 1-7.

Bayyareddy, K., T. M. Andacht, M. A. Abdullah, and M. J. Adang. 2009. Proteomic identification of *Bacillus thuringiensis* subsp israelensis toxin Cry4Ba binding proteins in midgut membranes from *Aedes aegypti* Linnaeus (Diptera, Culicidae) larvae. Insect Biochem. Mol. Biol. 39: 279-286.

Bergelson, J., and C. B. Purrington. 1996. Surveying patterns in the cost of resistance in plants. Am. Nat. 148: 536-558.

Bernays, E., and R. Chapman. 1994. Host-plant selection by phytophagus insects, Chapman & Hall, NY, US.

Blackman, R. L., and V. F. Eastop. 2000. Aphids on the world's crops: an identification guide, Second edition, Chichester, UK.

Blackman, R. L., J. M. Spence, L. M. Field, and A. L. Devonshire. 1995a. Chromosomal location of the amplified esterase genes conferring resistance to insecticides in *Myzus persicae* (Homoptera, Aphididae). Heredity 75: 297-302.

Cipollini, D., and M. Heil. 2010. Costs and benefits of induced resistance to herbivores and pathogens in plants. CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources 5: 1-25.

Cole, R. A. 1997. The relative importance of glucosinolates and amino acids to the development of two aphid pests *Brevicoryne brassicae* and *Myzus persicae* on wild and cultivated brassica species. Entomol. Exp. Appl. 85: 121-133.

Compson, Z. G., K. C. Larson, M. S. Zinkgraf, and T. G. Whitham. 2011. A genetic basis for the manipulation of sink-source relationships by the galling aphid *Pemphigus batae*. Oecologia 167: 711-721.

Cooper, T., L. Sazo, and K. Sagredo. 2001. Integrated production in Chile: Peaches, nectarines and plums, two years of research and development, pp. 45-50. In W. P. R. S. (WPRS/SROP) (ed.), Bulletin oilb/srop, International Organization for Biological and Integrated Control of Noxious Animals and Plants (OIBC/OILB), Dijon, France.

Devonshire, A. L., L. M. Field, S. P. Foster, G. D. Moores, M. S. Williamson, and R. L. Blackman. 1998. The evolution of insecticide resistance in the peach-potato aphid, *Myzus persicae*. Phil. Trans. Roy. Soc. Biol. Sci. 353: 1677-1684.

Douglas, A. E. 1998. Nutritional interactions in insect-microbial symbioses: Aphids and their symbiotic bacteria *Buchnera*. Ann. Rev. Entomol. 43: 17-37.

Eyles, A., D. Smith, E. A. Pinkard, I. Smith, R. Corkrey, S. Elms, C. Beadle, and C. Mohammed. 2011. Photosynthetic responses of field-grown *Pinus radiata* trees to artificial and aphid-induced defoliation. Tree Physiol. 31: 592-603.

Faeth, S. H. 1992. Do defoliation and subsequent phytochemical responses reduce future herbivory on oak trees. J. Chem. Ecol. 18: 915-925.

Fenny. 1975. Biochemical coevolution between plant and their insect herbivores, pp. 3-15. In G. Raven (ed.), Coevolution of Animals and Plants. University of Texas Press, Texas, USA.

Ferreyra, R., G. Selles, and G. Lemus. 2002. Efecto del estrés hídrico durante la fase II de crecimiento del fruto del duraznero cv. Kakamas en el rendimiento y estado hídrico de las plantas. Efecto del estrés hídrico durante la fase II de crecimiento del fruto del duraznero cv. Kakamas en el rendimiento y estado hídrico de las plantas. . Agri. Téc. 62.: 565-573.

Field, L. M., A. L. Devonshire, and B. G. Forde. 1988. Molecular evidence that insecticide resistance in peach potato aphids (*Myzus persicae* Sulz) results from amplification of an esterase gene. Biochem. J. 251: 309-312.

Fornoni, J. 2011. Ecological and evolutionary implications of plant tolerance to herbivory. Funct. Ecol. 25: 399-407.

Fornoni, J., P. L. Valverde, and J. Nunez-Farfan. 2004. Population variation in the cost and benefit of tolerance and resistance against herbivory in *Datura stramonium*. Evolution 58: 1696-1704.

Foster, S. P., I. Denholm, Z. K. Harling, G. D. Moores, and A. L. Devonshire. 1998. Intensification of insecticide resistance in UK field populations of the peach-potato aphid, *Myzus persicae* (Hemiptera: Aphididae) in 1996. Bull. Entomol. Res. 88: 127-130.

Foster, S. P., D. Cox, L. Oliphant, S. Mitchinson, and I. Denholm. 2008. Correlated responses to neonicotinoid insecticides in clones of the peach-potato aphid, *Myzus persicae* (Hemiptera: Aphididae). Pest Manag. Sci. 64: 1111-1114.

Foulongne, M., T. Pascal, P. Arus, and J. Kervella. 2003. The potential of *Prunus davidiana* for introgression into peach *Prunus persica* (L.) Batsch assessed by comparative mapping. Theor. Appl. Gen. 107: 227-238.

Francis, F., F. Guillonneau, P. Leprince, E. De Pauw, E. Haubruge, L. Jia, and F. L. Goggin. 2010. Tritrophic interactions among *Macrosiphum euphorbiae* aphids, their host plants and endosymbionts: Investigation by a proteomic approach. J. Insect Physiol. 56: 575-585.

Genschik, P., K. Drabikowski, and W. Filipowicz. 1998. Characterization of the *Escherichia coli* RNA 3 '-terminal phosphate cyclase and its sigma(54)-regulated operon. J. Biol. Chem. 273: 25516-25526.

Genschik, P., E. Billy, M. Swianiewicz, and W. Filipowicz. 1997. The human RNA 3'-terminal phosphate cyclase is a member of a new family of proteins conserved in Eucarya, Bacteria and Archaea. Embo Journal 16: 2955-2967.

Giannetto, G. B., and M. G. Petillo. 1995. Effects of irrigation and two soil management schemes on yield and fruit quality of peaches in Uruguay. pp 431-435. In Proceedings of the Fifth International Microirrigation Congress. Florida, USA.

Gong, D. Z., S. Z. Kang, and J. H. Zhang. 2005. Responses of canopy transpiration and canopy conductance of peach (*Prunus persica*) trees to alternate partial root zone drip irrigation. Hydrol. Processes 19: 2575-2590.

Gonzales, W. L., C. C. Ramirez, N. Olea, and H. M. Niemeyer. 2002. Host plant changes produced by the aphid *Sipha flava*: consequences for aphid feeding behaviour and growth. Entomol. Exp. Appl. 103: 107-113.

Gotelli, N. 2001. A Primer of Ecology, 3rd ed. Sinauer Associates, Sunderland, Massachusetts.

Gratacós, E. 2004. El cultivo del duraznero *Prunus persica* (L.) Batsch. Pontificia Universidad Católica de Valparaíso, Valparaiso, Chile.

Grechi, I., M. H. Sauge, B. Sauphanor, N. Hilgert, R. Senoussi, and F. Lescourret. 2008a. How does winter pruning affect peach tree - *Myzus persicae* interactions? Entomol. Exp. Appl. 128: 369-379.

Grechi, I., M.-H. Sauge, B. Sauphanor, N. Hilgert, R. Senoussi, and F. Lescourret. 2008b. How does winter pruning affect peach tree - *Myzus persicae* interactions? Entomol. Exp. Appl 128: 369-379.

Grossman, Y. L., and T. M. Dejong. 1995a. Maximum vegetative growth potential and seasonal patterns of resource dynamics during peach growth. Ann. Bot. 76: 473-482.

Grossman, Y. L., and T. M. Dejong. 1995b. Maximum fruit growth potential and seasonal patterns of resource dynamics during peach growth. Ann. Bot. 75: 553-560.

Grossman, Y. L., and T. M. Dejong. 1995c. Maximum fruit growth potential following resource limitation during peach growth. Ann. Bot. 75: 561-567.

Gulmon, S. L., and H. A. Mooney. 1986. Costs of defense and their effects of plant productivity, pp. 681-698. In G. TJ (ed.), On the economy of plant form and function. Cambridge University Press, Cambridge.

Hamilton, E. W., and J. S. Coleman. 2001. Heat-shock proteins are induced in unstressed leaves of *Nicotiana attenuata* (Solanaceae) when distant leaves are stressed. Am. J. Bot. 88: 950-955.

Haukioja, E., and J. Koricheva. 2000. Tolerance to herbivory in woody vs. herbaceous plants. Evol. Ecol. 14: 551-562.

Hawkins, B. A., and M. Holyoak. 1998. Transcontinental crashes of insect populations? Am. Nat. 152: 480-484.

Herms, D. A. 2002. Effects of fertilization on insect resistance of woody ornamental plants: Reassessing an entrenched paradigm. Environ. Entomol. 31: 923-933.

Herms, D. A., and W. J. Mattson. 1992. The dilemma of the plants- To growth or defend?. Q. Rev. Biol. 67: 478-478.

Hilbert, D. W., D. M. Swift, J. K. Detling, and M. I. Dyer. 1981. Relative growth rates and grazing optimization hypothesis. Oecologia 51: 14-18.

Hochwender, C. G., R. S. Fritz, and C. M. Orians. 2000. Using hybrid systems to explore the evolution of tolerance to damage. Evol. Ecol. 14: 509-521.

Huberty, A. F., and R. F. Denno. 2004. Plant water stress and its consequences for herbivorous insects: A new synthesis. Ecology 85: 1383-1398.

Hunt, R., and A. O. Nicholls. 1986. Stress and the coarse control of growth and root-shoot partitioning in herbaceous plants. Oikos 47: 149-158.

Hurley, P. F., and D. Flaspohler. 2007. Defense strategies of forest herbs on great lakes island and mainland sites: does ungulate-browse history play role?, pp. 2, School of

Forest Resources and Environmental Science. Michigan Technological University, Michigan, USA.

Infante, R., P. Martinez-Gomez, and S. Predieri. 2008. Quality oriented fruit breeding: Peach *Prunus persica* (L.) Batsch. J. Food Agric. Environ. 6: 342-356.

Isac, M., S. Preda, and M. Marcu. 1998. Aphid species - Vectors of plum pox virus. Acta Virol. 42: 233-234.

Jansson, J., and B. Ekbom. 2002. The effect of different plant nutrient regimes on the aphid *Macrosiphum euphorbiae* growing on petunia. Entomol. Exp. Appl. 104: 109-116.

Jaramillo, V. J., and J. K. Detling. 1988. Grazing history, defoliation, and comtetition - Effects on shortgrass production and nitrogen accumulation. Ecology 69: 1599-1608.

Jermy, T. 1984. Evolution of insect host plant relationships. Am. Nat. 124: 609-630.

Karban, R., and J. H. Myers. 1989. Induced plant-response to herbivory. Ann. Rev. Ecol. Syst. 20: 331-348.

Karban, R., and I. Baldwin. 1997. Induced Responses to Herbivory, University Chicago Press, Chicago.

Kervella, J., T. Pascal, F. Pfieffer, and E. Dirlewanger. 1998a. Breeding for multiresistance in peach tree. Acta Hort. 465: 177-184.

Kervella, J., T. Pascal, F. Pfeiffer, and E. Dirlewanger. 1998b. Breeding for multiresistance in peach tree, pp. 177-184. In R. Monet (ed.), Fourth International Peach Symposium, Vols 1-2.

Kessler, A. 2006. Plant-Insect interactions in the era of consolidation in biological sciencies. *Nicotiana attenuata* as an ecological expression system, pp. 19-37. In M.

D. a. W. Takken (ed.), Chemical Ecology: from gene to ecosystem. Springer, FRONTIS, Wageningen, Netherland.

Kfoury, L., and G. Massonie. 1995. Characteristics of the resistance of the peach cultivar Rubira to *Myzus persicae* Sulzer. Agronomie 15: 277-284.

Khan, M. A. M., C. Ulrichs, and I. Mewis. 2010. Influence of water stress on the glucosinolate profile of *Brassica oleracea var. italica* and the performance of *Brevicoryne brassicae* and *Myzus persicae*. Entomol. Exp. Appl. 137: 229-236.

Khan, M. A. M., C. Ulrichs, and I. Mewis. 2011. Water stress alters aphid-induced glucosinolate response in *Brassica oleracea var. italica* differently. Chemoecology 21: 235-242.

Klingler, J., G. Powell, G. A. Thompson, and R. Isaacs. 1998. Phloem specific aphid resistance in *Cucumis melo* line AR 5: effects on feeding behaviour and performance of *Aphis gossypii*. Entomol. Exp. Appl. 86: 79-88.

Konishi, H., H. Noda, Y. Tamura, and M. Hattori. 2009. Proteomic analysis of the salivary glands of the rice brown planthopper, *Nilaparvata lugens* (Stal) (Homoptera: Delphacidae). Appl. Entomol. Zool. 44: 525-534.

Koricheva, J. 2002. Meta-analysis of sources of variation in fitness costs of plant antiherbivore defenses. Ecology 83: 176-190.

Koricheva, J., S. Larsson, and E. Haukioja. 1998. Insect performance on experimentally stressed woody plants: A meta-analysis. Ann. Rev. Entomol. 43: 195-216.

Kristoffersen, L. 2003. The chemical ecology of Homoptera - from host plants to conspecific interactions, pp. 38, Deparment of Ecology. Lund University, Lund.

Kudo, G. 1996. Herbivory pattern and induced responses to simulated herbivory in *Quercus mongolica* var grosseserrata. Ecol. Res. 11: 283-289.

Lambert, P., and T. Pascal. 2011. Mapping *Rm2* gene conferring resistance to the green peach aphid (*Myzus persicae* Sulzer) in the peach cultivar "Rubira®". Tree Genet. Genomes 7: 1057-1068.

Lambert, P., M. H. Sauge, J. L. Poëssel, and T. Pascal. Year. Published. Pyramiding mono and polygenic resistances is one strategy to provide lasting control of the resistance to the green peach aphid and Powdery Mildew in peach. *In*, 4th International Rosaceae Genomics Conference, 2008, Pucón-Chile.

Larson, K. C., and T. G. Whitham. 1991. Manipulation of food resources by a gall-forming aphid - the physiology of sink-source interactions. Oecologia 88: 15-21.

Le Roux, V., E. D. M. Campan, F. Dubois, C. Vincent, and P. Giordanengo. 2007. Screening for resistance against *Myzus persicae* and *Macrosiphum euphorbiae* among wild Solanum. Ann. Appl. Biol. 151: 83-88.

Leimu, R., and J. Koricheva. 2006. A meta-analysis of tradeoffs between plant tolerance and resistance to herbivores: combining the evidence from ecological and agricultural studies. Oikos 112: 1-9.

Li, H., W. A. Payne, G. J. Michels, and C. M. Rush. 2008. Reducing plant abiotic and biotic stress: Drought and attacks of greenbugs, corn leaf aphids and virus disease in dryland sorghum. Environ. Exp. Bot. 63: 305-316.

Li, S. H., and J. G. Huguet. 1989. Production, fruit quality and development of peach trees under different irrigation regimes. Fruits (Paris) 44: 225-232.

Li, S. H., J. G. Huguet, P. G. Schoch, and P. Orlando. 1989. Response of peach tree growth and cropping to soil water deficit at various phenological stages of fruit development. J. Hort. Sci. 64: 541-552.

Manachini, B., P. Casati, L. Cinanni, and P. Bianco. 2007. Role of *Myzus persicae* (Hemiptera : Aphididae) and its secondary hosts in plum pox virus propagation. J. Econ. Entomol. 100: 1047-1052.

Margaritopoulos, J. T., C. Tsourapas, M. Tzortzi, O. M. Kanavaki, and J. A. Tsitsipis. 2005. Host selection by winged colonisers within the *Myzus persicae* group: a contribution towards understanding host specialisation. Ecol. Entom. 30: 406-418.

Marshall, C. B., G. Avila-Sakar, and E. G. Reekie. 2008. Effects of nutrient and CO2 availability on tolerance to herbivory in *Brassica rapa*. Plant Ecol. 196: 1-13.

Martinez-Torres, D., S. P. Foster, L. M. Field, A. L. Devonshire, and M. S. Williamson. 1999. A sodium channel point mutation is associated with resistance to DDT and pyrethroid insecticides in the peach-potato aphid, *Myzus persicae* (Sulzer) (Hemiptera : Aphididae). Insect Mol. Biol. 8: 339-346.

Maschinski, J., and T. G. Whitham. 1989. The continuum of plant-responses to herbivory - The influence of plant association, nutrient availability and timing. Am. Nat. 134: 1-19.

Massonie, G., P. Maison, R. Monet, and C. Grasselly. 1982. Resistance to green peach aphid *Myzus persicae* Sulzer(Homoptera, Aphididae) in *Prunus persica* (L) Batsch and others *Prunus*. Agronomie 2: 63-70.

Mattson, W. J., and R. A. Haack. 1987. The role of drought stress in provoking outbreaks of phytophagous insects. Bioscience 37: 110-118.

Monet, R., and G. Massonié. 1994. Déterminisme génétique de la résistance au puceron vert (*Myzus persicae*) chez le pêcher. Résultats complémentaires. Agronomie: 177-182.

Monet, R., and G. Massonie. 1994. Genetic determinism of resistance to green peach aphid (*Myzus persicae*) in the peach. Further results. Agronomie 14: 177-182.

Monet, R., A. Guye, and G. Massonie. 1998a. Breeding for resistance to green aphid *Myzus persicae* sulzer in the peach, pp. 171-175. In R. Monet (ed.), Fourth International Peach Symposium, Vols 1-2. International Society Horticultural Science, Leuven 1.

Monet, R., A. Guye, and G. Massonie. 1998b. Breeding for resistance to green aphid *Myzus persicae* sulzer in the peach, pp. 171-175. In R. Monet (ed.), Fourth International Peach Symposium, Vols 1-2.

Montaño, R. 2002. Cultivos de Durazneros. In Hedecom (ed.), Tarija, Bolivia.

Moore, P. D. 1995. How plants deal with what´s eating them. Sci. Watch 6: 7-8.

Moores, G. D., G. J. Devine, and A. L. Devonshire. 1994a. Insecticide-insensitive acetylcholinesterase can enhance esterase-based resistance in *Myzus persicae* and *Myzus nicotianae*. Pestic. Biochem. Physiol. 49: 114-120.

Moores, G. D., G. J. Devine, and A. L. Devonshire. 1994b. Insecticide ionsensitive acetylcholinesterase can enhance esterase based resistance in *Myzus persicae* and *Myzus nicotiane*. Pest. Biochem. Physiol. 49: 114-120.

Nguyen, T. T. A., D. Michaud, and C. Cloutier. 2007. Proteomic profiling of aphid *Macrosiphum euphorbiae* responses to host-plant-mediated stress induced by defoliation and water deficit. J. Insect Physiol. 53: 601-611.

Nuñez-Farfán, J., J. Fornoni, and P. Luis Valverde. 2007. The evolution of resistance and tolerance to herbivores. Ann. Rev. Ecol. Evol. Syst. 38: 541-566

Osier, T. L., and R. L. Lindroth. 2006. Genotype and environment determine allocation to and costs of resistance in quaking aspen. Oecologia 148: 293-303.

Pascal, T., and R. Monteaux-Caillet. 1998. Peach breeding in France, pp. 117-123. *In* A. Horticulturae [ed.], IV International Peach Symposium.

Pascal, T., and R. Monteux-Caillet. 1998. Peach breeding in France, pp. 117-123, Fourth International Peach Symposium, Vols 1-2. International Society Horticultural Science, Louvain.

Pascal, T., F. Pfeiffer, J. Kervella, J. P. Lacroze, and M. H. Sauge. 2002. Inheritance of green peach aphid resistance in the peach cultivar 'Rubira'. Plant Breed. 121: 459-461.

Penvern, S., S. Bellon, J. Fauriel, and B. Sauphanor. 2010. Peach orchard protection strategies and aphid communities: Towards an integrated agroecosystem approach. Crop Prot. 29: 1148-1156.

Plague, G. R., C. Dale, and N. A. Moran. 2003. Low and homogeneous copy number of plasmid-borne symbiont genes affecting host nutrition in *Buchnera aphidicola* of the aphid Uroleucon ambrosiae. Mol. Ecol. 12: 1095-1100.

Poëssel, S. M. H., Staudt M., Dufour C., Deborde C., Rahbé Y.,, R. C. Jackson B., Maucourt M., Corre M.N., El-Aouni H., Lacroze J.P.,, and M. A. Year. Published. Metabolite profiling and feeding bioassays suggest a major role for a dicaffeoylquinic acid in induced resistance of peach to *Myzus persicae* aphid. *In*, PR-Proteins and Induced Resistance Against Pathogens and Insects, Neuchâtel, Switzerland.

Pompon, J., D. Quiring, P. Giordanengo, and Y. Pelletier. 2010. Role of host-plant selection in resistance of wild Solanum species to *Macrosiphum euphorbiae* and *Myzus persicae*. Entomol. Exp. Appl. 137: 73-85.

Powell, G. 2005. Intracellular salivation is the aphid activity associated with inoculation of non-persistently transmitted viruses. J. Gen. Virol. 86: 469-472.

Powell, G., C. R. Tosh, and J. Hardie. 2006. Host plant selection by aphids: Behavioral, evolutionary, and applied perspectives. Ann. Rev. Entomol. 51: 309-330.

Prado, E., and W. F. Tjallingii. 1997. Effects of previous plant infestation on sieve element acceptance by two aphids. Entomol. Exp. Appl. 82: 189-200.

Price, P. W. 1991. The plant vigor hypothesis and herbivore attack. Oikos 62: 244-251.

Prittinen, K., J. Pusenius, K. Koivunoro, and H. Roininen. 2003. Genotypic variation in growth and resistance to insect herbivory in silver birch (Betula pendula) seedlings. Oecologia 137: 572-577.

Ramírez, C. C., and J. A. Verdugo. 2009. Water availability affects tolerance and resistance to aphids but not the trade-off between the two. Ecol. Res. 24: 881-888.

Ramírez, C. C., and H. M. Niemeyer. 1999. Salivation into sieve elements in relation to plant chemistry: the case of the aphid *Sitobion fragariae* and the wheat, *Triticum aestivum*. Entomol. Exp. Appl. 91: 111-114.

Retuerto, R., B. Fernandez-Lema, R. Rodriguez, and J. R. Obeso. 2004. Increased photosynthetic performance in holly trees infested by scale insects. Funct. Ecol. 18: 664-669.

Reyes, F., N. Fiore, M. A. Reyes, P. Sepulveda, V. Paredes, and H. Prieto. 2003. Biological behavior and partial molecular characterization of six Chilean isolates of Plum pox virus. Plant Dis. 87: 15-20.

Rhoades, D. F. 1979. Evolution of plant chemical defense against herbivores. In J. D. H. Rosenthal G.A. (ed.), Herbivores: their interaction with secondary plant metabolites, vol. 4-54. Academic press, Inc.

Rojas, G., M. A. Mendez, C. Munoz, G. Lemus, and P. Hinrichsen. 2008. Identification of a minimal microsatellite marker panel for the fingerprinting of peach and nectarine cultivars. Electro. J. Biotech. 11: 1-12.

Rosales, M., P. Hinrichsen, and G. Herrera. 1998. Molecular characterization of plum pox virus isolated from apricots, plums and peaches in Chile, pp. 401-405, 17th International Symposium on Virus and Virus-Like Diseases of Temperate Fruit Crops: Fruit Tree Diseases.

Santibañez, F., and P. Santibañez. 2007. Cambio climático y degradación de tierras en Latinoamérica y Chile. Revista Ambiente y Desarrollo: 54 - 63.

Sarria, E., M. Cid, E. Garzo, and A. Fereres. 2009. Excel Workbook for automatic parameter calculation of EPG data. Comput. Electron. Agric. 67: 35-42.

Sauge, M. H. 1998. Analysis of the mechanisms of resistance to the green peach aphid in several Prunus genotypes, pp. 731-739. In R. Monet (ed.), Fourth International Peach Symposium, Vols 1-2. International Society Horticultural Science, Leuven 1.

Sauge, M., J. Kervella, and Y. Rahbe. 1998a. Probing behaviour of the green peach aphid *Myzus persicae* on resistant Prunus genotypes. Entomol. Exp. Appl. 89: 223-232.

Sauge, M., J. Kervella, and T. Pascal. 1998b. Settling behaviour and reproductive potential of the green peach aphid *Myzus persicae* on peach varieties and a related wild Prunus. Entomol. Exp. Appl. 89: 233-242.

Sauge, M. H., J. P. Lacroze, J. L. Poessel, T. Pascal, and J. Kervella. 2002. Induced resistance by *Myzus persicae* in the peach cultivar 'Rubira'. Entomol. Exp. Appl. 102: 29-37.

Sauge, M. H., T. Pascal, J. P. Lacroze, F. Pfeiffer, and J. Kervella. 2004. Mapping of a genetic factor of partial resistance to *Myzus persicae* in the wild peach *Prunus*

davidiana, that impedes phloem sap ingestion by the aphid, pp. 499-505. In J.C.Simon, C.A. Dedryver, C. Rispe and M. Hulle (ed.), Aphids in a New Millennium, Institut National de la Recherche Agronomique, Rennes, France.

Sauge, M.-H., F. Mus, J.-P. Lacroze, T. Pascal, J. Kervella, and J.-L. Poessel. 2006. Genotypic variation in induced resistance and induced susceptibility in the peach - *Myzus persicae* aphid system. Oikos 113: 305-313.

Sauge, M.-H., J.-L. Poessel, T. Guillemaud, and L. Lapchin. 2011. Resistance induction and herbivore virulence in the interaction between *Myzus persicae* (Sulzer) and a major aphid resistance gene (Rm2) from peach. Arthropod-Plant Interac. 5: 369-377.

Sauge, M. H., P. Lambert, and T. Pascal. 2012. Co-localisation of host plant resistance QTLs affecting the performance and feeding behaviour of the aphid Myzus persicae in the peach tree. Heredity 108: 292-301.

Scriber, J. M. 2002. Evolution of insect-plant relationships: chemical constraints, coadaptation, and concordance of insect/plant traits. Entomol. Exp. Appl. 104: 217-235.

Sharma, R., S. Komatsu, and H. Noda. 2004. Proteomic analysis of brown planthopper: application to the study of carbamate toxicity. Insect Biochem. Mol. Biol. 34: 425-432.

Sherman, W., P. Lyrene, and R. Sharpe. 1996. Low-chill peach and nectarine breeding at the University of Florida. Proc. Fla. State Hort. Soc. 109: 222-223.

Siemens, J. A., and J. J. Zwiazek. 2003. Effects of water deficit stress and recovery on the root water relations of trembling aspen (*Populus tremuloides*) seedlings. Plant Sci. 165: 113-120.

Silfver, T., H. Roininen, E. Oksanen, and M. Rousi. 2009. Genetic and environmental determinants of silver birch growth and herbivore resistance. For. Ecol. Manage. 257: 2145-2149.

Simpson, K. L. S., G. E. Jackson, and J. Grace. 2012. The response of aphids to plant water stress - the case of *Myzus persicae* and *Brassica oleracea var. capitata*. Entomol. Exp. Appl. 142: 191-202.

Slanky, F., and Fenny. 1977. Stabilization of the rate of nitrogen accumulation of larvae of the cabbage butterfly on wild and cultivated food plants. Ecol. Monogr. 47: 209-228.

SPSS, I. 2001. SPSS base version 11.5 for windows user's guide computer program, version By SPSS, I., Chicago.

StatSoft, I. 2004. STATISTICA (data analysis software system), version 7. www.statsoft.com.

Stevens, M. T., D. M. Waller, and R. L. Lindroth. 2007. Resistance and tolerance in Populus tremuloides: genetic variation, costs, and environmental dependency. Evol. Ecol. 21: 829-847.

Strauss, S. Y., and A. A. Agrawal. 1999. The ecology and evolution of plant tolerance to herbivory. Trends Ecol. Evol. 14: 179-185.

Teetes, G. 1996. Plant Resistance to Insects: A Fundamental Component of IPM. *In* I. W. T. Radcliff (ed.). Universidad de Minnesota.

Thompson, G. A., and F. L. Goggin. 2006. Transcriptomics and functional genomics of plant defence induction by phloem-feeding insects. J. Exp. Bot. 57: 755-766.

Tiffin, P. 2002. Competition and time of damage affect the pattern of selection acting on plant defense against herbivores. Ecology 83: 1981-1990.

Tjallingii, W. 1995. Aphid-plant interactions: what goes on in the depth of the tissues? Proc. Exp. App. Entomol. 6: 163-169.

Tjallingii, W. F., and T. H. Esch. 1993. Fine-structure of aphid stylet routes in plant-tissues in correlation with EPG signals. Physiol. Entomol. 18: 317-328.

Tjallingii, W. F., E. Garzo, and A. Fereres. 2010. New structure in cell puncture activities by aphid stylets: a dual-mode EPG study. Entomol. Exp. Appl. 135: 193-207.

Tsuchida, T., R. Koga, H. Shibao, T. Matsumoto, and T. Fukatsu. 2002. Diversity and geographic distribution of secondary endosymbiotic bacteria in natural populations of the pea aphid, *Acyrthosiphon pisum*. Mol. Ecol. 11: 2123-2135.

Van Helden, M., and W. F. Tjallingii. 2000. Experimental design and analysis in EPG experiments with emphasis on plant resistance research, pp. 144-171. In G. P. Walker, Backus, E.A. (ed.), Principles and applications of electronic monitoring and other techniques in the study of homopteran feeding behavior. Entomological Society of America, Lanham.

Wang, Y., J. C. Carolan, F. Hao, J. K. Nicholson, T. L. Wilkinson, and A. E. Douglas. 2010. Integrated Metabonomic-Proteomic Analysis of an Insect-Bacterial Symbiotic System. J. Prot. Res. 9: 1257-1267.

White, T. C. R. 1984. The abundance of invertebrate herbivores in relation to the availability of nitrogen in stressed food plants. Oecologia 63: 90-105.

White, T. C. R. 2009. Plant vigour versus plant stress: a false dichotomy. Oikos 118: 807-808.

Wilkie, T. M. 2000. G-protein signaling: Satisfying the basic necessities of life. Current Biol. 10: R853-R856.

Wise, M. J., and W. G. Abrahamson. 2005. Beyond the compensatory continuum: environmental resource levels and plant tolerance of herbivory. Oikos 109: 417-428.

Wise, M. J., and W. G. Abrahamson. 2007. Effects of resource availability on tolerance of herbivory: A review and assessment of three opposing models. Am. Nat. 169: 443-454.

Zangerl, A. R., and F. A. Bazzaz. 1992. Theory and pattern in plant defense allocation. In R.S. Fritz, E.L Simms (ed.). Plant Resistance to Herbivores and Pathogens: Ecology, Evolution and Genetics, University of Chicago Press, Chicago.

Zhang, J., C. Goyer, and Y. Pelletier. 2008a. Environmental stresses induce the expression of putative glycine-rich insect cuticular protein genes in adult *Leptinotarsa decemlineata* (Say). Insect Mol. Biol. 17: 209-216.

Zhang, P.-J., J.-P. Shu, C.-X. Fu, Y. Zhou, Y. Hu, M. P. Zalucki, and S.-S. Liu. 2008b. Trade-offs between constitutive and induced resistance in wild crucifers shown by a natural, but not an artificial, elicitor. Oecologia 157: 83-92.

Figure captions

Fig. 1. 2D-PAGE () of protein of *M. persicae* labelled with Cydye 2 separated on a 12.5% acrylamide gel. Number indicates the proteins showing significant expression, complete are given in Table 1.

Fig. 2. Ven Diagram of proteins *M. persicae* expressed in differentially when is feeding in two different cultivars: GF305 and Rubira® with water stress condition. Symbols represent differential regulation of proteins.

Fig. 3. Comparison of protein expression between aphids response in a) the susceptible genotype (GF305) with normal irrigation (100%FC) versus water stress (30%FC), b) the resistant genotype (Rubira®) with normal irrigation (100%FC) versus water stress (30%FC).

Fig. 4. Population growth rate (PGR) (mean ± SE) of *M. persicae* in two cultivar of *P. persica* with two irrigation treatments. Different letter indicate significantly differences by Tukey.

Table 1. Proteins identified and related metabolic pathways in aphid which differ in quantity among different treatments [a]

Spot number	MW	pI	Mowse score	MS coverage	Protein name	Accession number	Source organism
Cytoskeleton							
14	62983	8.49	69	0	Cuticular protein CPG12	gi|193706873	*Acyrthosiphon pisum*
30	62983	8.49	123	n.d.	Cuticular protein CPG12	gi|193706873	*Acyrthosiphon pisum*
75	42158	5.29	83	30	Actin related protein 1	gi|217330650	*Acyrthosiphon pisum*
78	72602	7.97	133	29	Auticular protein 15 from Low Complexity family	gi|193620175	*Acyrthosiphon pisum*
79	63909	7.06	101	16	Cuticular protein CPG12	gi|19371385	*Acyrthosiphon pisum*
91	42158	5.29	106	44	Actin related protein 1	gi|217330650	*Acyrthosiphon pisum*
94	72602	7.97	93	25	Cuticular protein 15	gi|19362017	*Acyrthosiphon pisum*
114	42194	5.30	135	52	Actin 5	gi|67782283	*Aedes aegypti*
124	38171	5.36	123	48	Actin-87E	gi|156542177	*Tribolium castaneum*
128	41934	5.44	78	27	Muscle-specific actin 3	gi|33642245	*Aedes aegypti*
140	31118	5.35	57	35	F-actin capping protein subunit beta	gi|170049079	*Culex quinquefasciatus*
147	17079	7.62	48	35	Cofilin/actin depolymerizing factor-like protein	gi|187179329	*Acyrthosiphon pisum*
175	42177	5.23	84	44	Actin 6	gi|71383976	*Aedes aegypti*
176	175906	5.48	86	15	Collagen alpha-1(IV) chain-like	gi|328723513	*Acyrthosiphon pisum*
Amino acid metabolism							
4	59461	9.04	50	13	Anthranilate synthase component I	gi|260779942	*Buchnera aphidicola*
80	76376	5.93	55	12	Protein tyrosine phosphatase, non-receptor type 11	gi|193617708	*Acyrthosiphon pisum*
115	43215	6.23	54	31	l-allo-threonine aldolase-like isoform 2	gi|328720750	*Acyrthosiphon pisum*
132	39363	9.72	55	24	Phospho-2-dehydro-3-deoxyheptonate aldolase	gi|116515018	*Buchnera aphidicola*
Energy metabolism							

21	56751	7.98	61	44	Regulator of G-protein signaling 7-like	gi| 328716109	*Acyrthosiphon pisum*
38	149701	8.44	72	14	ATPase AAA domain-containing protein 2-like	gi|350416751	*Bombus impatiens*
47	42656	7.02	88	22	RNA 3' terminal phosphate cyclase	gi|157114207	*Aedes aegypti*
54	155092	6.57	47	3	Guanylate cyclase	gi|193657089	*Acyrthosiphon pisum*
76	30333	8.22	n.d.	n.d.	Regulator of G-protein signaling 17-like	gi|193631927	*Acyrthosiphon pisum*
87	18567	8.73	58	26	Deterin isoform 1	gi|253314420	*Acyrthosiphon pisum*
102	32871	9.29	81	28	ATP synthase-gamma chain, isoform A	gi|24651125	*Drosophila melanogaster*
119	51775	9.20	52	10	Flagellum-specific ATP synthase	gi|25008474	*Buchnera aphidicola*
127	53257	5.66	54	15	Mitochondrial-processing peptidase	gi|193683602	*Acyrthosiphon pisum*
134	28771	8.70	62	27	Phosphoglycerate mutase	gi|157116217	*Aedes aegypti*
138	58329	8.92	54	16	Fatty acyl-CoA reductase	gi|335892852	*Apis mellifera*
149	26561	9.59	55	36	Orotidine 5'-phosphate decarboxylase	gi|21672540	*Buchnera aphidicola*
154	16459	10.08	67	33	Endothelial differentiation-related factor 1	gi|240849174	*Acyrthosiphon pisum*
158	232338	6.80	74	10	Myotubularin-related protein 13-like	gi|328714925	*Acyrthosiphon pisum*
173	39024	6.67	62	20	Replication factor C subunit 2-like	gi|350401447	*Bombus*
177	25245	6.16	63	45	Peroxiredoxin	gi|60300018	*Gryllotalpa orientalis*
179	24400	5.28	64	26	Ferritin-like precursor	gi|24084857	*Acyrthosiphon pisum*
191	14576	8.74	54	15	Acyl-CoA thioesterase	gi|345538649	*Acyrthosiphon pisum*
630	23986	5.61	60	39	Thymidylate kinase-like isoform 3	gi|193631931	*Acyrthosiphon pisum*
956	34800	8.07	66	21	Retinol dehydrogenase 12-like	gi|193582347	*Acyrthosiphon pisum*
1515	109871	4.91	74	17	Blastoderm-specific gene 25D	gi|110771129	*Apis mellifera*
Xenobiotic degradation							
84	63230	6.13	115	36	Carboxyl-Esterase FE4	gi|544256	*Myzus persicae*
Carbohydrate metabolism							
990	52307	7.22	54	18	Knockdown CG3861-PA	gi|193676562	*Acyrthosiphon pisum*

Continued from Table 1

Stress response							
1267	37150	4.68	66	13	Eukaryotic translation initiation factor 2-alpha kinase	gi|328714887	*Acyrthosiphon pisum*
Protein synthesis							
72	64873	9.56	51	33	Protein phosphatase 1D-like	gi|328716050	*Acyrthosiphon pisum*
108	30566	6.54	57	0	E3 ubiquitin-protein ligase SIAH1	gi|340710245	*Bombus terrestres*
Others							
66	73114	8.63	66	16	MAU2 chromatid cohesion factor homolog	gi|328706676	*Acyrthosiphon pisum*
93	50995	6.05	77	26	Elongator component	gi|170036115	*Culex quinquefasciatus*
120	28496	8.40	70	23	OCIA domain-containing protein	gi|193598993	*Acyrthosiphon pisum*
122	28496	6.09	59	25	Updo, isoform B	gi|221330099	*Drosophila melanogaster*
142	28496	5.67	49	23	Nicotinic acetylcholine receptor a2 subunit	gi|218669716	*Tribolium castaneum*
188	28496	5.66	55	18	Mitochondrial processing peptidase beta subunit	gi|193683602	*Acyrthosiphon pisum*

MW, molecular weight; pI, isoelectric point; Score, Mowse score according to Mascot search; MS coverage, percentage of the protein sequence identified; Accession, accession number on NCBI; Organism, related original organism for the protein identification.

[a]: Proteins that significantly varied ($p<0.05$) between different treatments are listed by spot number according to the gel image analysis.

Fig. 1

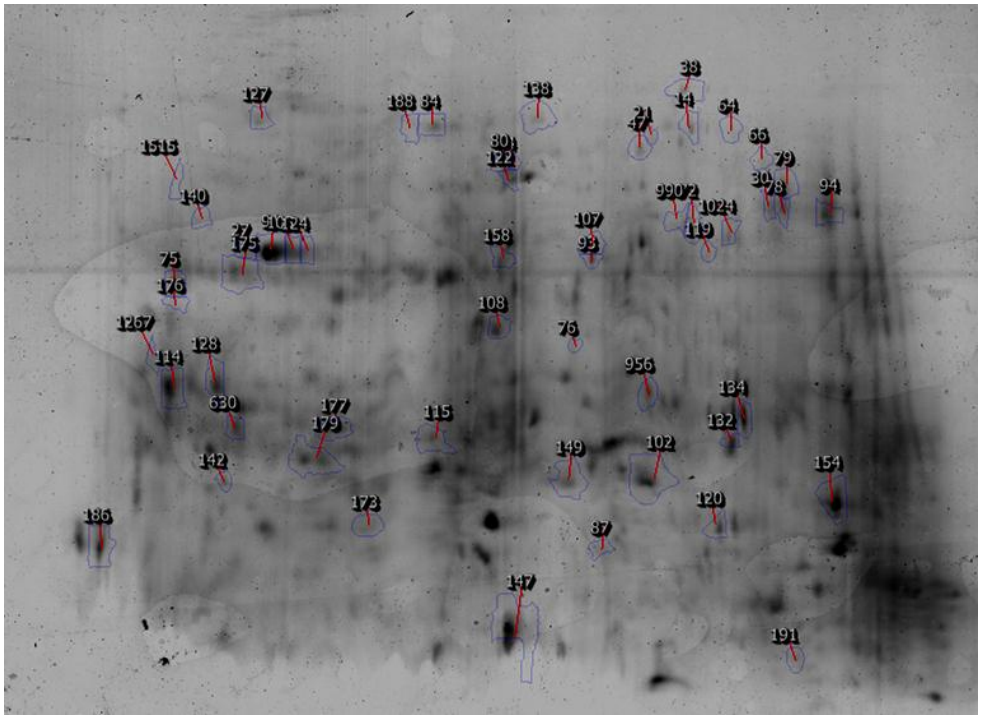

38
127
188
84
138
14
64
21
47
80
122
55
79
30
78
94
99
72
102
41
119
107
93
158
27
175
75
176
108
76
126
7
114
128
956
134
630
177
179
115
132
149
102
142
154
173
120
186
87
147
191

Fig. 2

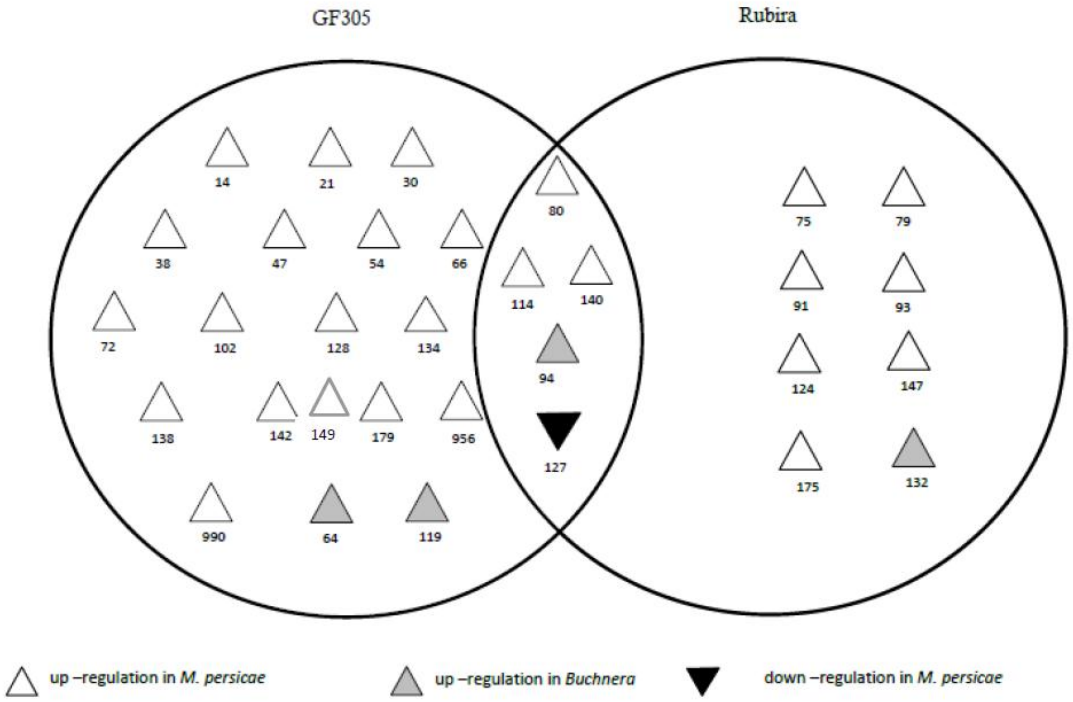

GF305
Rubira
14
21
30
38
47
54
66
72
102
128
134
138
142
149
179
956
990
64
119
80
114
140
94
127
75
79
91
93
124
147
175
132
up –regulation in M. persicae
up –regulation in Buchnera
down –regulation in M. persicae

Fig. 3a

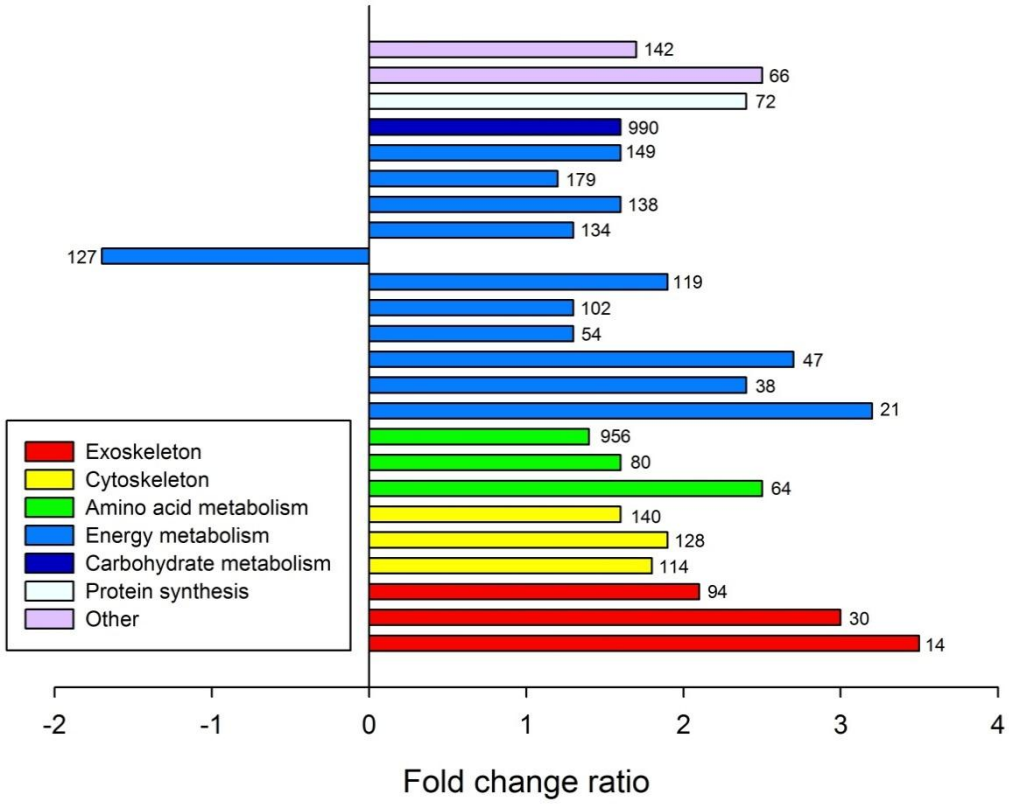

142
66
72
990
149
179
138
134
127
119
102
54
47
38
21
956
80
64
140
128
114
94
30
14
Exoskeleton
Cytoskeleton
Amino acid metabolism
Energy metabolism
Carbohydrate metabolism
Protein synthesis
Other
-2
-1
0
1
2
3
4
Fold change ratio

Fig. 3b

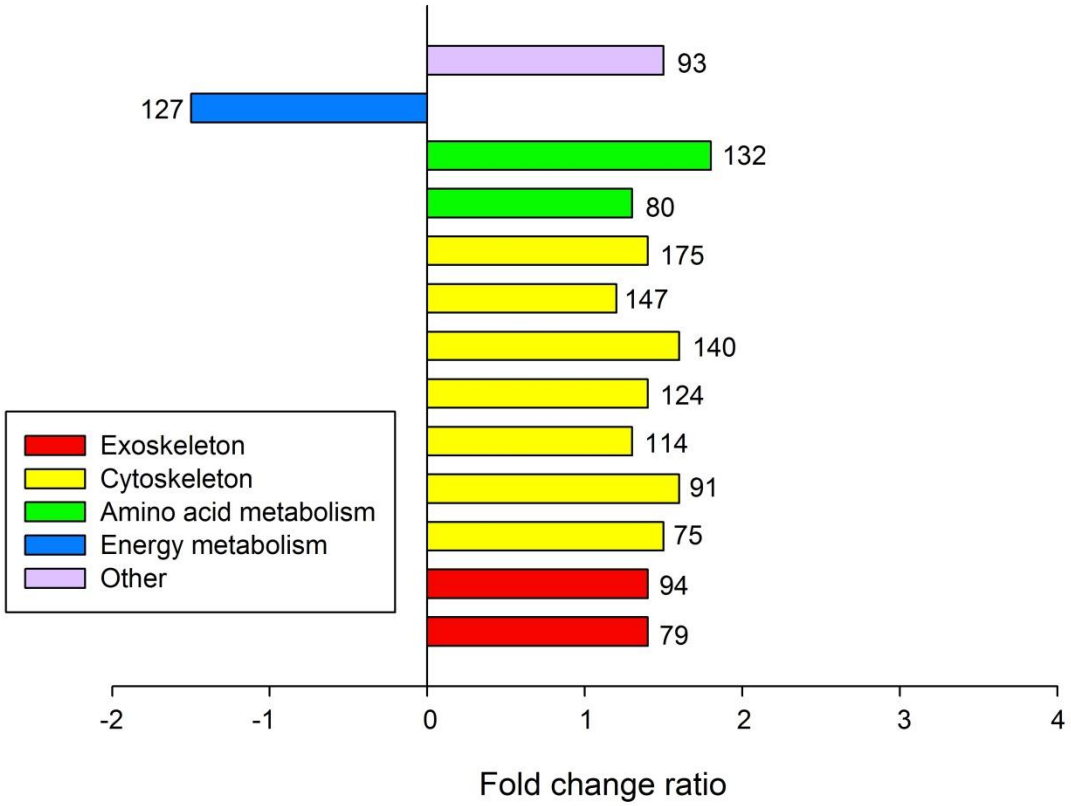
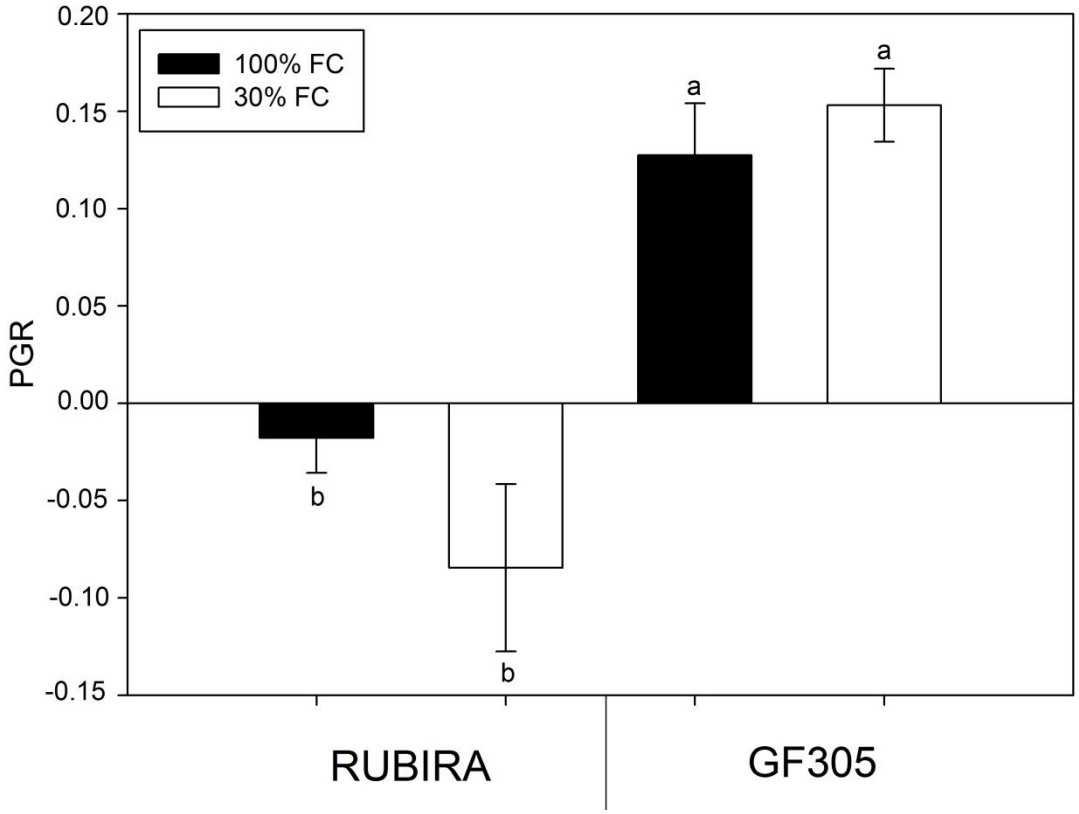

93
127
132
80
175
147
140
124
114
91
75
94
79
Exoskeleton
Cytoskeleton
Amino acid metabolism
Energy metabolism
Other
-2
-1
0
1
2
3
4
Fold change ratio

Figure 4


0.20
0.15
0.10
0.05
0.00
-0.05
-0.10
-0.15
PGR
100% FC
30% FC
a
a
b
b
RUBIRA
GF305

CONCLUSION AND PERSPECTIVES

Conclusions and perspectives

This PhD thesis was aimed to contribute to the understanding of the mechanisms of defenses of *P. persica* and the response of *M. persicae*. To reach this principal objective, field and laboratory research work was conducted. Firstly (Chapter I), the study of commercial cultivars of peaches and nectarines showed genetic variability of resistance. Specifically, the monitoring in the field, population rate of growth, probing behavior and no-choice tests exhibited wide variability across cultivars. Actually, some cultivar exhibited major resistant. This study was centered only on constitutive resistance, but it would be important to address the induced responses to the attack in the same cultivar studied to describe in a better way the global defense response of *P. persica*. From that study, it is worthwhile to highlight the results obtained with two nectarine cultivars which exhibited large differences in resistance to *M. persicae*. These cultivars were selected to a further study (Chapter I), where how irrigation affected resistance/susceptibility against the aphid attack and its influence in the growth of the next season was addressed. In this study the tolerance was also studied. The most resistant genotype, which exhibited antixenotic responses (Chapter I), presented a higher aphid attack under water deficit conditions, whereas in the susceptible cultivar the attack was lower. This study illustrated the importance of the irrigation and also how can modulate the aphid attack. Indeed, well-watered plants exhibited induced susceptibility and under water deficit there was an induced resistance, effect that was independent of the plant genotype, therefore plants well-watered are more affected by a previous aphid attack. This result indicates that resistance and tolerance are affected by the intrinsic genetic of the cultivar and also the influence of irrigation conditions. It is interesting to note that the susceptible genotype showed

compensation under well-watered conditions, which probably entailed higher accumulation of photoassimilates on the branches which in turn could have been the responsible factor accounting for by the increased number of flower buds and shoot buds exhibited in the season after the infestation. In the first two chapters, the study was clearly centered in the response of the plant to the aphid attack and how water availability can modulate the defensives responses and in the Chapter III completely changed to an insect-centered approach. Thus, the aphid response, assessed in terms of changes in the proteomic response, was studied after feeding on two peach cultivars, also one susceptible and another resistant to the aphid, including variation in the level of irrigation. Aphid displayed differences in regulation of a number of proteins followed different treatment. In general, these changes were associated to energy metabolism on the susceptible genotype and cytoskeleton on the resistant genotype. A common response (down-regulation) was observed in both genotypes, which was associated with general stress response. This showed that aphids are affected by stressed conditions of its host, in this case the water deficit of the plant elicit metabolic changes in the aphid due the internal changes in the plant generate an adaptation in the host, although in this case the changes in the aphid were modulated by the genetic of the host. It worth noting this result consider the response of the aphid fed on non-commercial genotypes and it will be interesting to contrast this results with aphids on commercial genotypes because the results can be modulated by the artificial selection of the fruit, Therefore, it will be interesting to continue the proteomic analysis in aphids on other genotypes including those displaying different mechanism of resistance as antibiosis. Moreover, the aphid proteomic response to induced resistance need to be determine, which would be a novel issue in plant-insect interactions.

The results obtained so far, describe the importance of the water supply in the plant defense strategies, their dependence of the plant genotype and the metabolic changes elicits on the aphid. Hopefully this information will contribute in our understanding regarding plant defenses to the attack of aphids and could also provide new insight to peach breeding programs, particularly those centered in the mechanism of resistance to aphids.

LIST OF SCIENTIFIC PRODUCTS

List of scientific products

1. Publications

- **Verdugo, J.A**., Méndez, T., Ortíz-Martínez S.A., Cumsille R. and Ramírez C.C. 2012. Variation in resistance mechanisms to the green peach aphid among different *Prunus persica* commercial cultivars. Submitted to Journal of Economic Entomology.
- **Verdugo, J.A.**, Bravo, R. Valenzuela, D. and Ramírez C.C. Are plant resistance and tolerance altered by water stress? Nectarine-aphid model. In preparation
- **Verdugo J.A.**, Lacroze J.P., Sauge M.H., Ramírez C.C. and Francis F. Reduced water supply on aphid-resistant plants elicits lower changes in the proteomic profile of its herbivore: peach-aphid interaction. In preparation

2. International/national meetings

2011

Verdugo, J.A., Bravo, R., Sauge, M.H, Francis, F. y Ramírez, C.C. Influencia del ataque de *Myzus persicae* (Hemiptera:Aphididae) en cultivares de *Prunus persica* (Rosales:Rosaceas) con diferentes niveles de estrés hídrico. XXXIII Congreso Nacional de Entomología y I Congreso Sudamericano de Entomología. La Serena, Chile. Nov 30- Dec 2.

Verdugo, J.A., Rubio-Meléndez, M.E., Ramírez, C.C. and Francis, F. Resistance of *Prunus persica* (L.) Batsch to the attack of the aphid *Myzus persicae* (Sulzer) in Chile. 63rd International Symposium on Crop Protection. Gent, Belgium. May, 24

Rubio-Meléndez, M.E., **Verdugo, J.A.** and Ramírez, C.C. Defensive response of *Tritricum aestivum* to the attack of *Sitobion avenae*: aphid genotype, cultivar and

irrigation system effects. 63rd International Symposium on Crop Protection. Gent, Belgium. May, 24.

2010

Ramírez C.C., **Verdugo J.A**., Rubio-Meléndez, Barrios-San Martín J., Rubiano-Rodriguez J.A. and Figueroa C. Identificación de morfos sexuales de *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) en huertos de duraznero en la región de O`Higgins y del Maule, Chile. XXXII Congreso Nacional de Entomología, Arica, Chile, December 1-3.

Verdugo J.A., Rubio-Meléndez, Barrios-San Martín J. and Ramírez C.C. Resistencia entre cultivares de *Prunus persica* (L.) Batsch (Rosale: Rosaceae) al ataque del áfido *Myzus persicae* (Sulzer) (Hemiptera: Aphididae). XXXII Congreso Nacional de Entomología, Arica, Chile, December 1-3.

2009

Verdugo J.A. y Ramírez C.C. ¿Existen variedades resistentes al áfido *M. persicae* en durazneros y nectarines en Chile?. 60 Congreso Agronómico de Chile. Talca, Chile, 27-October 30.

2008

Cabrera-Brandt, M., **Verdugo, J.**, Fuentes-Contreras, E., Ramírez, C.C., Sauge, M-H., Lacroze, J-P. & Figueroa, C.C. Respuestas adaptativas del áfido *Myzus persicae* (Sulzer) alimentados sobre plantas con diferentes niveles de defensas: evidencias de co-evolución?. Reunión Anual de la Sociedad de Biología de Chile, Pucón, Chile, November. 26-29.