ORIGINAL PAPER

Impact of scale insect infestation on the content of photosynthetic pigments and chlorophyll fluorescence in two host plant species

Katarzyna Golan · Katarzyna Rubinowska ·
Katarzyna Kmieć · Izabela Kot · Edyta Górska-Drabik ·
Bożena Łagowska · Władysław Michałek

Abstract The aim of this experiment was to determine plant responses to *Coccus hesperidum* L. infestation on two host plants. Groups of five lemon and five fern plants were colonised by various numbers of mobile *C. hesperidum* instar nymphs. After 6 months, all scale insect individuals were counted on each plant. According to the insect density, the plants were divided into a five-degree series. In all density classes of host plants tested, the infestation of scale insects decreased the chlorophyll and carotenoid content as well as the value of three indicators of photosynthetic activity. The strongest decrease in the analysed pigments was observed for the smallest abundance of insects (first-class density) in lemon leaves and in second-class density in fern leaves. The strongest reactions of the chlorophyll fluorescence indicators were observed in density classes III and IV in the fern leaves and density classes IV or V in the lemon leaves. The reactions depended on the specific properties of plants and abundance of insects feeding on them.

Handling Editor: Heikki Hokkanen.

K. Golan (✉) · K. Kmieć · I. Kot · E. Górska-Drabik ·
B. Łagowska
Department of Entomology, University of Life Sciences in Lublin, Leszczyńskiego 7, 20-069 Lublin, Poland
e-mail: katarzyna.golan@up.lublin.pl

K. Rubinowska
Department of Plant Physiology, University of Life Sciences in Lublin, Akademicka 15, 20-950 Lublin, Poland

W. Michałek
Department of General Ecology, University of Life Sciences in Lublin, Akademicka 15, 20-950 Lublin, Poland

Introduction

Coccus hesperidum L. (Sterrnorhyncha; Coccoidea; Coccidae) is one of the most common pests with a host range that includes many field crops worldwide (citrus crops, fruit trees, ornamentals in fields and greenhouses) (Zimmerman 1948; Ebeling 1959; Ben-Dov and Hodgson 1997; Kondo et al. 2008). It damages plants directly and indirectly by excreting more honeydew on the leaves and fruit of the host plants than any other species (Copland and Ibrahim 1985). Because effective management of *C. hesperidum* is still a real problem worldwide, extensive efforts have been undertaken to characterise their impact on the host plants' biochemistry and physiology (Golan and Najda 2011; Golan 2013; Golan et al. 2013).

The feeding of herbivorous insects induces biochemical and physiological changes in the host plants, affecting the life processes of host plants such as photosynthesis (Gomez et al. 2004). The chlorophyll content in plant tissue is one of the primary parameters involved in the interactions between host plants and insects. Chlorophyll levels change during plant development, and nutritional deficiencies occur in response to a wide variety of stresses including biotic stresses such as insect feeding and pathogen infections (Ni et al. 2001, 2002; Heng-Moss et al. 2003; Goł-awska et al. 2010). These changes can be helpful for investigating plants' resistance mechanisms and allow exploration of using photosynthetic pigments as markers for identifying different kinds of chlorosis-causing insects. The available studies include information showing that

plant responses to insect feeding have different effects on chlorophyll *a* and *b* concentrations (Rafi et al. 1996; Heng-Moss et al. 2003; Goławska et al. 2010; Huang et al. 2014). Photosynthesis can be limited by a series of factors, and a growing number of techniques are now available, allowing a better understand of these processes. One of them is chlorophyll *a* fluorescence—a non-invasive technique used to assess physiological responses to herbivory (Bown et al. 2002; Calatayud and Le Rü 2006; Chaerle and Van Der Straeten 2000). With this technique, it is possible to evaluate the impact of insect feeding on light reactions of plants, specifically on photosystem II (PSII) and I (PSI). Chlorophyll fluorescence techniques have been increasingly used to provide additional information on the efficiency of photochemistry, non-photochemical energy dissipation and overall photosynthetic performance (Nabity et al. 2009). Such paired measurements yield information on the extent to which PSII is using the absorbed energy and the degree to which PSII is being damaged by excess light. The use of chlorophyll fluorescence imaging for rapidly detecting indirect perturbations in leaf metabolism before any effects on growth and development have been detected has increased in recent years (Aldea et al. 2006; Tang et al. 2006; Delaney 2008; Nabity et al. 2009). So far, remarkably little is known about the effects of scale insects on the photosynthetic pigment content and on photosynthesis parameters (Vranjic 1997; Retuerto et al. 2004).

In this article, the effects of different densities of *C. hesperidum* per one leaf on the concentrations of plant pigments (chlorophyll *a*, chlorophyll *b* and carotenoids) and the following chlorophyll fluorescence parameters were examined: the maximum quantum yield of photosystem II of dark-adapted leaf (F_v/F_m), effective quantum yield of light-adapted leaf ($\Delta F/F_m'$), and coefficient of non-photochemical and photochemical quenching (qN, qP) in two species of host plants. No similar studies have been conducted. This research represents an initial effort to characterise the effect of the degree of *C. hesperidum* infestation on plant pigment loss and changes in photosynthetic performance in the host plants.

Materials and methods

Plants

The studied plant material consisted of 2-year-old *Citrus limon* var. Ponderosa L. and *Nephrolepis biserrata* (Swartz) Schott. Plants were obtained from the Horticultural Farm in Sokolow Podlaski (Poland). Experimental plants measuring ca. 50-60 cm were grown in 15-cm-diameter plastic pots filled with a standardised horticultural substrate in the laboratory of the University of Life Sciences in Lublin (Poland). Plants were grown in a climatic chamber and kept at 20 ± 2 °C, 65 ± 5 % relative humidity (RH) and a 14-h photoperiod. They were watered regularly and additionally fertilised with mineral fertilisers appropriate for each plant species.

Insects

The scale insects used in the experiments came from cultures kept at the University of Life Sciences in Lublin, Poland. The insects were reared on *C. limon* var. Ponderosa and *N. biserrata* in climatic chambers (20 ± 2 °C, 65 ± 5 % RH and 14-h photoperiod) for 6 months preceding the experiment. This species is mobile only in first and young second instar nymphs, which is why mobile first instar nymphs (crawlers) of this species were used for plant colonisation. The experiment was started in April 2010.

Plant infestation with insects and density classes

Thirty specimens of *C. limon* var. Ponderosa and *N. biserrata* were used for the physiological state analysis of the plants. The plants were divided into six groups of five plants for each species. The control group for each species was represented by five control plants not colonised by scale insects. The other plants were divided into five groups of five plants, each for artificial colonisation with *C. hesperidum*. These groups were separated from each other and then were colonised by various numbers of first instar nymphs of *C. hesperidum*: 10, 30, 50, 100 and 200 individuals/plant. The individuals of this instar were counted and transferred to clean hosts by a thin wooden spatula.

After 6 months, all the observed individuals of *C. hesperidum* were counted on each plant and the number of observed scale insects per one leaf noted. In the case of *N. biserrata*, the number of compound leaves on plants was taken into consideration. Density classes of experimental plants were established based on this recalculation.

For *C. limon* var. Ponderosa and *N. biserrata*, the density was defined according to a five-degree series: class 0, control (non-infested plants); class I, up to 10 individuals per leaf; class II, from 11 to 30 individuals per leaf; class III, from 31 to 50 individuals per leaf; class IV, from 51 to 100 individuals per leaf; class V, more than 100 individuals per leaf.

The control plants were similarly grown in another climate chamber under the same conditions. The same experimental conditions were used for all the plants used during this study (20 ± 2 °C, 65 ± 5 % RH and 14-h photoperiod). For the physiological analyses, the leaves from different parts of plants with the number of scale insects compatible with established density classes were used.

The pigment contents

Five leaves harvested from the middle part of five randomly chosen plants from each combination were taken for chemical analysis. The material was cut into small pieces and mixed well to obtain the representative assay. The content of pigment: chlorophyll *a*, chlorophyll *b* and carotenoids in plant tissues was assayed according to the method of Lichtenthaler and Wellburn ([1983]) after taking 0.5 g of the leaf fresh weight and extraction in 80 % acetone. The measurement of absorbance was performed with three wavelengths (λ): 470 nm (carotenoids), 646 nm (chlorophyll *b*) and 663 nm (chlorophyll *a*) using a Cecil CE 9500 spectrophotometer. The concentration of particular pigments was calculated according to the following equations:

$$C_{\text{chl.}a} = 12.21 \cdots A_{663} - 2.81 \cdots A_{646}$$

$$C_{\text{chl.}b} = 20.13 \cdots A_{646} - 5.03 \cdots A_{663}$$

$$C_{\text{car.}} = (1{,}000 \cdots A_{470} - 3.27 \cdots C_{\text{chl.}a} - 104 \cdots C_{\text{chl.}b})/227,$$

where A_λ is the absorbance value for wavelength λ.

Next, the concentrations of pigments were converted into their content in the leaf fresh weight.

Photosynthetic activity of plants

In order to determine the effect of *C. hesperidum* feeding on the photosynthetic activity of the studied plant species (*C. limon*, *N. biserrata*) depending on the insect population infesting them, an analysis of chlorophyll fluorescence was conducted. The measurements were performed on each of two plant species in each of the previously enumerated density classes of scale insects colonising the plants. The measurements in five replicates on five different leaves located on five different plants were performed on the control leaves, which were not infested by scale insects, and on the infested leaves at the same location on the plant and with a similar orientation towards the light. Before the measurement, the leaves were shaded for about 20 min by means of manufactured clips. The studies were carried out in a growth chamber with a PAM-2000 fluorometer (Walz GmbH, Germany) using the saturation pulse method (Schreiber et al. [1992]). After a time of dark adaptation, when all PSII reaction centres were open (F_0), the initial fluorescence was measured, and the maximum fluorescence (F_m) was measured at the end of a saturating light pulse (intensity $\sim$2,800 μmol m^{-2} s^{-1}). Light sufficient to drive photosynthesis (actinic light, PPFD = 1,000 μmol m^{-2} s^{-1}) was then applied, and after 10 min the ground fluorescence (F_s) was measured, as evidenced by the unchanging fluorescence levels. Maximum fluorescence after steady-state

conditions (F_m') was determined by applying pulses of the saturated white light every 60 s when the actinic light was on.

Chlorophyll fluorescence measurements were calculated according to the following equations:

Maximum quantum yield of photosystem II

$$F_\text{v}/F_\text{m} = (F_\text{m} - F_0)/F_\text{m}$$

where F_v (variable fluorescence) equals the fluorescence increase induced by the saturation pulse.

The effective quantum yield

$$\Delta F/F_\text{m}' = F_\text{m}' - (F_\text{s}/F_\text{m}')$$

Coefficients of photochemical fluorescence quenching

$$qP = \left[(F_\text{m}' - F_\text{s})/(F_\text{m}' - F_0') \right]$$

The coefficient of non-photochemical quenching

$$qN = \left[(F_\text{m} - F_\text{m}')/(F_\text{m}' - F_0') \right]$$

The calculation of qP and qN requires the parameter of F_0', which was experimentally obtained after a dark red light pulse had been applied to previously light-adapted leaves.

Statistical analyses

The study was designed to measure the effects of different densities of *C. hesperidum* per one leaf on the concentrations of plant pigments and selected chlorophyll fluorescence. The analyses were done in five replicates for each plant series and physiological parameter.

The distribution of obtained data was verified with a chi-square test. One-way ANOVA with Tukey's simultaneous test used for data with a normal distribution was applied at $P = 0.01$ to compare the changes (in control plants and colonised by insects) in the analysed physiological parameters in each host. All statistical analyses used Statistica for Windows, v. 9.1 (StatSoft [2009]).

Results

Changes in the physiological state of host plants

The pigment contents

Statistical analyses showed significant differences in the pigment contents in lemon and fern leaves infested with an increasing number of scale insects (Figs. 1, 2, 3). The results of the ANOVA test were as follows: for chlorophyll *a* content: $F_{5.19} = 12.379$; $P = 0.000215$ in the experiment with *C. limon*; $F_{5.19} = 40.615$; $P = 3.383\text{E}^{-8}$ in the experiment with *N. biserrata*; for chlorophyll *b* content: $F_{5.19} = 11.707$;
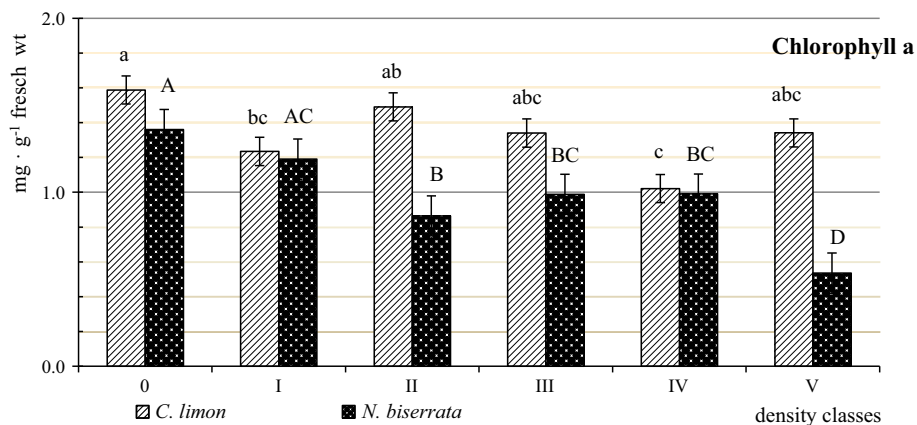
Fig. 1 Chlorophyll *a* content in the leaves of *Citrus limon* var. Ponderosa and *Nephrolepis biserrata* as a result of increasing infestation with *Coccus hesperidum*. Values indicated by *different letters* (*small letters* for *C. limon* and *capital letters* for *N. biserrata*) for each plant species show statistically significant differences at

$P \leq 0.01$ (Tukey's *HSD* test). Density classes: class 0-control, non-infested plants; class I, up to 10 individuals per leaf; class II, from 11 to 30 individuals per leaf; class III, from 31 to 50 individuals per leaf; class IV, from 51 to 100 individuals per leaf; class V, more than 100 individuals per leaf
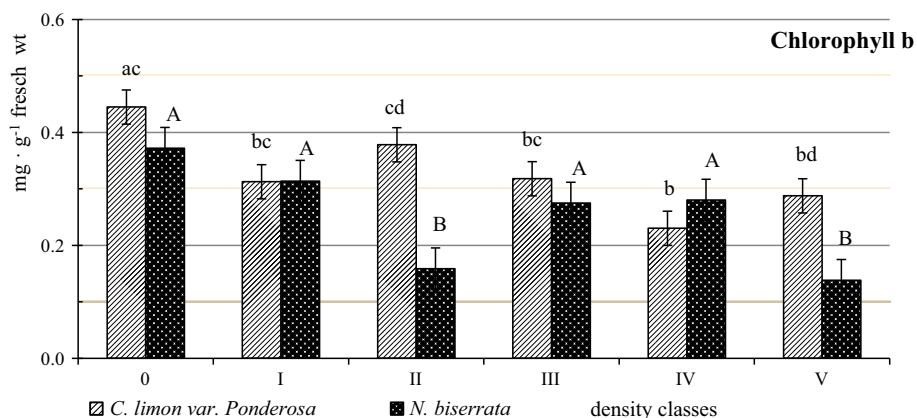

Fig. 2 Chlorophyll *b* content in the leaves of *Citrus limon* var. Ponderosa and *Nephrolepis biserrata* as a result of increasing infestation of *Coccus hesperidum*. Values indicateed by *different letters* (*small letters* for *C. limon* and *capital letters* for *N. biserrata*) for each plant species show statistically significant differences at

$P \leq 0.01$ (Tukey's *HSD* test). Density classes: class 0-control, non-infested plants; class I, up to 10 individuals per leaf; class II, from 11 to 30 individuals per leaf; class III, from 31 to 50 individuals per leaf; class IV, from 51 to 100 individuals per leaf; class V, more than 100 individuals per leaf

$P = 0.000281$ in the experiment with *C. limon*; $F_{5.19} = 31.352$; $P = 1.974E^{-7}$ in the experiment with *N. biserrata*; for carotenoid content: $F_{5.19} = 7.168$; $P = 0.002541$ in the experiment with *C. limon*; $F_{5.19} = 30.175$; $P = 2.553E^{-7}$ in the experiment with *N. biserrata*.

It was found that the small number of *C. hesperidum* individuals on lemon leaves caused over a 22.2 % (I class) and 35.6 % (IV class) statistically significant decrease in chlorophyll *a* content compared to the control (Fig. 1). In the leaves of *C. limon* infested with 11–50 and mass abundance of *C. hesperidum* individuals (classes II, III and V), the level of chlorophyll *a* was still lower compared to the control. The increase in the individual number of scale insects on the leaves of *N. biserrata* in class II to V caused a marked decrease of about 1.9-fold in class II and 1.7-fold

in class III and IV, and the highest decrease of over 3-fold in class V in chlorophyll *a* content (Fig. 1).

In the leaves of *C. limon* infested with *C. hesperidum*, the tendency to a decrease in the chlorophyll *b* level compared to control plants was noted (Fig. 2). The lowest content of the analysed parameter was found in leaves infested with 51–100 *C. hesperidum* individuals on average (class IV). The scale insects caused the strongest decrease (twofold) in chlorophyll *b* content in fern leaves infested with 11–30 (class II) and over 100 individuals on average (class V). Statistical analysis confirmed significant differences in the contents of the analysed parameter in fern leaves in those two classes, but the lowest level of chlorophyll *b* was noted in the class V leaves.
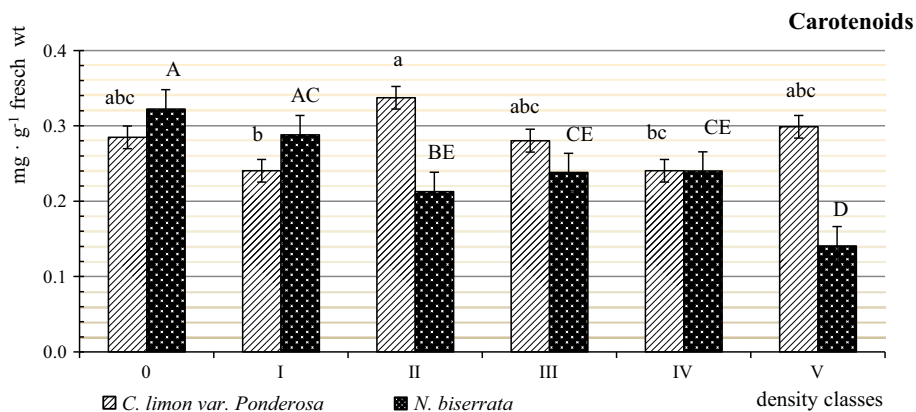
Fig. 3 Carotenoids content in the leaves of *Citrus limon* var. Ponderosa and *Nephrolepis biserrata* as a result of increasing infestation of *Coccus hesperidum*. Values indicated by *different letters* (*small letters* for *C. limon* and *capital letters* for *N. biserrata*) for each plant species show statistically significant differences at $P \leq 0.01$ (Tukey's *HSD* test). Density classes: class 0-control, non-infested plants; class I, up to 10 individuals per leaf; class II, from 11 to 30 individuals per leaf; class III, from 31 to 50 individuals per leaf; class IV, from 51 to 100 individuals per leaf; class V, more than 100 individuals per leaf
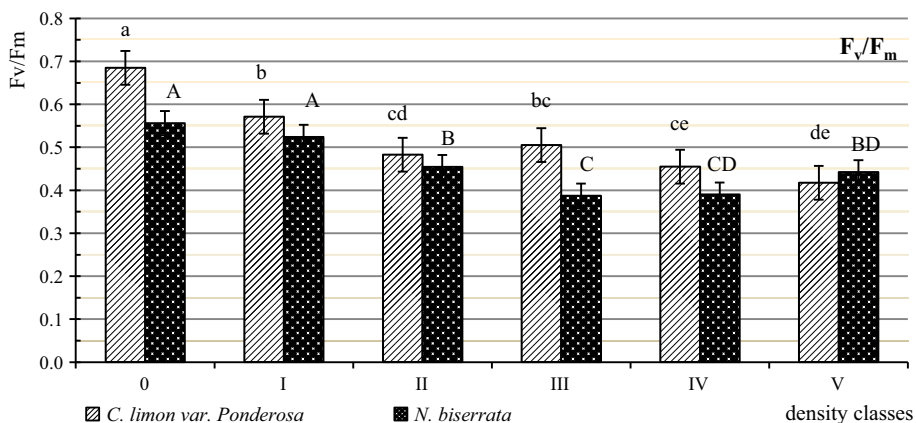

Fig. 4 The level of maximum quantum efficiency of the PSII photosystem (F_v/F_m) in the leaves of *Citrus limon* var. Ponderosa and *Nephrolepis biserrata* as a result of increasing infestation of *Coccus hesperidum*. Values indicateded by *different letters* (*small letters* for *C. limon* and *capital letters* for *N. biserrata*) for each plant species show statistically significant differences at $P \leq 0.01$ (Tukey's *HSD* test). Density classes: class 0-control, non-infested plants; class I, up to 10 individuals per leaf; class II, from 11 to 30 individuals per leaf; class III, from 31 to 50 individuals per leaf; class IV, from 51 to 100 individuals per leaf; class V, more than 100 individuals per leaf

The scale insect infestation caused changes in the carotenoid content in lemon and fern leaves compared to controls (Fig. 3). In lemon leaves, the carotenoid content increased in classes II and V, but decreased compared to the control in classes I, III and IV (Fig. 3). In the leaves of *N. biserrata* infested with *C. hesperidum*, a tendency to a decrease in the carotenoid level compared to the control plants was noted.

The lowest level of the analysed parameter was noted in class V. Statistical analyses showed significant differences in the carotenoid content as a result of scale insect infestation (classes II–V) on the fern leaves compared to controls.

Photosynthetic activity

Statistical analyses showed significant differences in the value of all analysed photosynthetic activity indicators in lemon leaves, but only in the value of $\Delta F/F'_m$ in fern leaves infested with an increasing numer of scale insects (Figs. 4, 5, 6, 7). The results of the ANOVA test in the experiment with *C. limon* were as follows: $F_{5.19} = 10.751$; $P = 0.000042$ for F_v/F_m; $F_{5.19} = 10.547$; $P = 0.000459$ for $\Delta F/F'_m$; $F_{5.19} = 18.700$; $P = 0.000027$ for qP; $F_{5.19} = 6.697$; $P = 0.003367$ for qN. In the experiment with *N. biserrata*, the results of the ANOVA test were respectively: $F_{5.19} = 3.320$; $P = 0.0322$ for F_v/F_m; $F_{5.19} = 6.913$;
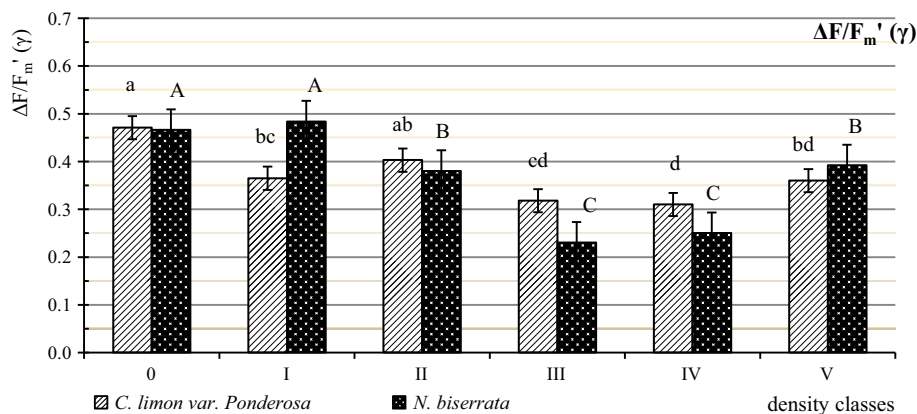
Fig. 5 The level of effective quantum gain of PSII in the leaves of *Citrus limon* var. Ponderosa and *Nephrolepis biserrata* as a result of increasing infestation of *Coccus hesperidum*. Values indicated by *different letters* (*small letters* for *C. limon* and *capital letters* for *N. biserrata*) for each plant species indicate statistically significant differences at $P \leq 0.01$ (Tukey's *HSD* test). Density classes: class 0-control, non-infested plants; class I, up to 10 individuals per leaf; class II, from 11 to 30 individuals per leaf; class III, from 31 to 50 individuals per leaf; class IV, from 51 to 100 individuals per leaf; class V, more than 100 individuals per leaf
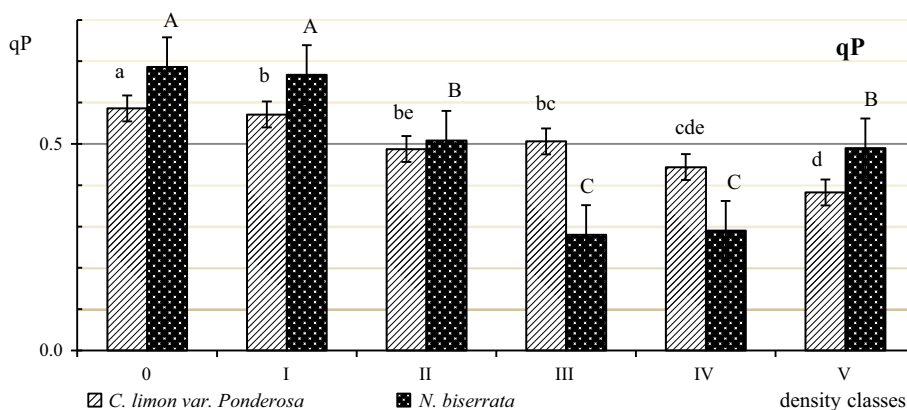

Fig. 6 The value of the coefficient of photochemical quenching (qP) in the leaves of *Citrus limon* var. Ponderosa and *Nephrolepis biserrata* as a result of increasing infestation of *Coccus hesperidum*. Values indicated by *different letters* (*small letters* for *C. limon* and *capital letters* for *N. biserrata*) for each plant species show statistically significant differences at $P \leq 0.01$ (Tukey's *HSD* test). Density classes: class 0-control, non-infested plants; class I, up to 10 individuals per leaf; class II, from 11 to 30 individuals per leaf; class III, from 31 to 50 individuals per leaf; class IV, from 51 to 100 individuals per leaf; class V, more than 100 individuals per leaf

$P = 0.00156$ for $\Delta F/F_m'$; $F_{5.19} = 2.758$; $P = 0.0583$ for qP; $F_{5.19} = 2.831$; $P = 0.0538$ for qN.

The relative level of the maximum quantum efficiency of the photosystem PSII (F_v/F_m) in the leaves of both host plants in response to *C. hesperidum* feeding was characterised by a decrease compared to the control plants (Fig. 4). The lowest level of the analysed parameter was noted in lemon leaves infested with scale insects in classes IV and V. Statistical analysis confirmed significant differences in the value of this parameter in lemon leaves in all density classes compared to controls. The feeding of scale insects on *N. biserrata* leaves caused a decrease in the maximum quantum efficiency of the photosystem II level to a minimum value of about 7.14 % (class I) and a maximum of 30.36 % in classes III and IV compared to the control plants (Fig. 4).

The infestation of scale insects on lemon caused a decrease in the effective quantum gain of photosystem II of the light-adapted leaf ($\Delta F/F_m'$) level (Fig. 5). Statistical analyses showed significant differences in the value of $\Delta F/F_m'$ in lemon leaves in density classes I, III, IV and V compared to the control plants. The strongest, over 1.5-fold decrease in the analysed parameter in *C. limon* was noted in the leaves infested with 31–100 *C. hesperidum* individuals on average (class III and IV) compared to the control plants. In the leaves of fern infested with *C. hesperidum* individuals in density classes II–V, the $\Delta F/F_m'$ level decreased compared to the control plants (Fig. 5). The lowest value of the analysed parameter (twofold decrease) was noted in class III compared to the control.

In the leaves of *C. limon* infested with *C. hesperidum* individuals, the level of the coefficient of photochemical
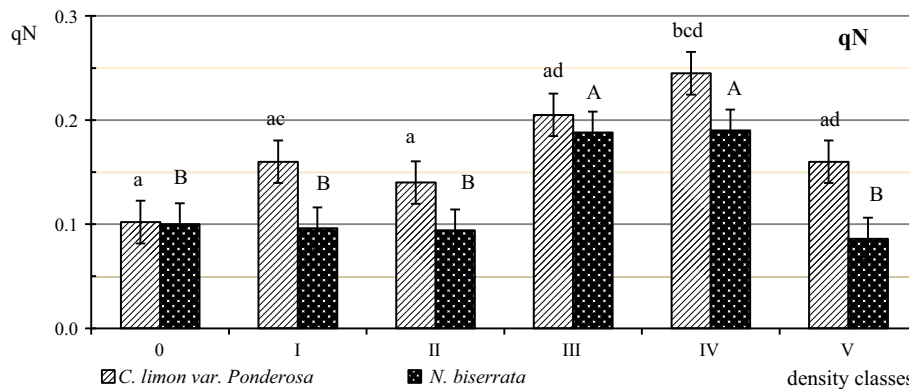
Fig. 7 The value of the coefficient of non-photochemical quenching (qN) of fluorescence in the leaves of *Citrus limon* var. Ponderosa and *Nephrolepis biserrata* as a result of increasing infestation of *Coccus hesperidum*. Values indicated by *different letters* (*small letters* for *C. limon* and *capital letters* for *N. biserrata*) for each plant species show statistically significant differences at $P \leq 0.01$ (Tukey's *HSD* test). Density classes: class 0-control, non-infested plants; class I, up to 10 individuals per leaf; class II, from 11 to 30 individuals per leaf; class III, from 31 to 50 individuals per leaf; class IV, from 51 to 100 individuals per leaf; class V, more than 100 individuals per leaf

quenching (qP) showed a decreasing tendency compared to the control plants (Fig. 6). The lowest level of this parameter was noted in lemon leaves infested with a mass abundance of scale insects (class V). Statistical analysis confirmed significant differences in the value of this parameter in lemon leaves in all density classes compared to controls. The infestation of scale insects on *N. biserrata* leaves caused a decrease in the qP level by almost 60 % in classes III and IV compared to the control plants (Fig. 6).

The level of the coefficient of non-photochemical (qN) quenching of fluorescence in *C. limon* leaves in all the five analysed density classes increased compared to the level in the control leaves (Fig. 7). The maximum level of qN in the lemon leaves was observed in class IV, where the strongest, 2.4-fold higher qN level compared to the control was observed. Statistical analysis confirmed these significant differences. The maximum increase of the qN in fern leaves was noted in classes III and IV, where the strongest was the approximately twofold higher qN level compared to the controls (Fig. 7).

Discussion

The results clearly demonstrate that *C. hesperidum* infestation can have a negative impact on the chlorophyll and carotenoid content as well as the value of three of the four analysed photosynthetic activity indicators (maximum quantum efficiency of photosystem PSII, effective quantum gain of photosystem II of light-adapted leaves and the coefficient of photochemical quenching) in the leaves of two plant species from very distant taxonomic groups: Spermatophyta (seed plants)—*C. limon*; Cryptogamae (spore-bearing plants)—*N. biserrata*. These changes,

measured on individual host plants, varied significantly depending on the scale of the insect density. This information is consistent with earlier studies. Huang et al. (2014) reported that relative chlorophyll loss was related to the amount of feeding damage caused by *Bagrada hilaris* Burmeister. They confirmed that the feeding damage measured on individual host plants varied significantly depending on the adult density and stage of plant growth. In this experiment, a decline in the chlorophyll concentration in the infested leaf tissue of *C. hesperidum*-susceptible lemon and fern was found. The symptoms of scale insect infestation on *N. biserrata* leaves were less visible compared to those observed for lemon (Golan 2013). In this study of chlorosis, local necrosis and leaf falling were observed in class II density on lemon leaves, and these symptoms were intensified with an increase in the scale of insect population abundance on the leaves. A decrease in chlorophyll and carotenoid contents in lemon leaves was observed at lower insect density (class I). Losses of chlorophyll content due to insect herbivory have been quantified in other hemipterans using similar chlorophyll analyses. Other researchers (Buntin et al. 1996; Morales et al. 2002; Goławska et al. 2010) have explored the leaves of stressed plants apparently with less synthesised chlorophyll content, and these changes may be part of the adaptive responses. Chlorophyll *a* and *b* concentrations can be significantly higher in non-infested leaves with both short and long infestations of plants compared to the infested ones (Goławska et al. 2010). As observed by Anderson et al. (1995), these changes often have been regarded as a relatively late mechanism of photosynthetic adaptation. Dai et al. (2009) reported the plants of *Hypericum sampsoni* Hance apparently synthesised less chlorophyll pigment in leaves under *Thrips tabaci*

(Linderman) damage, while Heng-Moss et al. (2003) stated that the total chlorophyll and carotenoid concentrations differed among Betta wheat isolines in response to aphid feeding. Rafi et al. (1996) reported that susceptible plants have similar chlorophyll concentration levels as their respective non-infested plants after exposure to *Diuraphis noxia* (Mordvilko), whereas resistant plants infested with *D. noxia* had reduced levels of chlorophyll when compared with non-infested plants. The decrease in the chlorophyll level in the host plant leaves affected by *C. hesperidum* compared to the control plants was demonstrated in all density classes in lemon and fern leaves compared to the control level. The chlorophyll content level was dependent on the species of the host plant and scale of insect abundance. However, it was observed that the strongest reduction of all analysed parameters was in fern leaves as compared to lemon. The concentration of photosynthetic pigments can vary with environmental factors (Sachico et al. 1997; Mary et al. 2006), and chlorophyll degradation is a complex phenomenon that often accompanies insect feeding damage of plants (Ni et al. 2001). The impact of insect feeding often varies by plant species.

According to the available information, leaf chlorophyll content is one of the most important factors in determining the rate of photosynthesis and a useful indicator of both potential photosynthetic productivity and general plant vigor (Zarco-Tejada et al. 2002; Mao et al. 2007). *C. hesperidum* infestation was shown to reduce the value of some of the analysed photosynthesis indicators in the leaves of their host plants: maximum quantum efficiency of photosystem PSII (F_v/F_m), effective quantum gain of PSII $[\Delta F/F'_m(\gamma)]$ and the coefficient of photochemical quenching (qP) compared to the control. Only the value of the coefficient of photochemical quenching (qP) in lemon leaves was different, where the lowest level of this parameter was noted in density classes IV and V. The maximum quantum efficiency of PSII of the dark-adapted leaves indicates the efficiency of the photochemical system, specifically, how much captured light energy is being used by the reaction centre and propagated through the photoelectron transport chain. Measuring F_v/F_m provides a rapid method for determining changes in the maximum efficiency of PSII photochemistry (Andrews et al. 1995). The relative decrease in F_v/F_m has also been used in the rapid assessment of plant susceptibility or resistance to aphids (Blanco et al. 1992). This parameter is widely considered to be a sensitive indication of plant photosynthetic performance. Its lower values would be observed with some types of biotic or abiotic stress factors, which reduce the capacity for photochemical quenching of energy within PSII (Kalaji and Guo 2008). An effective quantum yield of the PSII of light-adapted leaves is a good indicator

of the efficiency of light utilisation, i.e. how efficiently absorbed photons are converted into chemical products (Malkin and Niyogi 2000). In this experiment, F_v/F_m and $\Delta F/F'_m$ ratios were significantly affected by *C. hesperidum* damage in both tested plant species. It is typical of the response of many plants to a wide range of environmental stresses and indicates a reduced efficiency of PSII photochemistry (Krause and Weis 1991; Chaerle et al. 2007; Nabity et al. 2009). The present data documented the strongest host plant reaction for the abundance of the scale insect population determined in class III and IV. This abundance of insects affects the maximum reduction in the values of the F_v/F_m and $\Delta F/F'_m$ ratios in both fern and lemon leaves. The substantial decrease in chlorophyll fluorescence parameters indicated that the primary mechanism for photosynthetic rate reduction in damaged leaves is via the interference of the photochemical efficiency at the initial stage of photosynthesis (Dai et al. 2009). Velikova et al. (2010) reported a significant reduction in the chlorophyll fluorescence parameters of herbaceous plants resulting from the feeding and oviposition of two pentatomids, *Murgantia histrionica* (Hahn) and *Murgantia histrionica* (L.). They confirmed that the permanent impairment of photosynthetic photochemistry was restricted to the damaged areas on the leaf. Buntin et al. (1996) showed that feeding injury caused by *Stephanitis pyrioides* (Scott) reduced the chlorophyll content and adversely affected net leaf photosynthesis and transpiration in azalea. The feeding of *Bemisia tabaci* Gennadius reduced the leaf photosynthesis in tomato leaves by decreasing the content and photosynthetic capacity of chlorophyll (Buntin et al. 1993). Different results were presented by Retuerto et al. (2004) who examined the ability of *Ilex aquifolium* L. plants to compensate for scale insect herbivory through increased photosynthesis. They investigated the hypothesis that phloem-sap feeding insects, by acting as additional sinks for photosynthate, might increase the photosynthetic rates of their hosts (Crawley 1999). Contrastingly, Schaffer and Mason (1990) reported that a subtropical tree species (*Guaiacum sanctum* L.) infested by the scale insect *Toumeyella* sp. generally had a lower net CO_2 assimilation than non-infested trees. Tomczyk (2001), on the other hand, observed that poor infection of bean, strawberry, chrysanthemum and cucumber plants by red spiders caused increased intensity of photosynthesis, which contributed to increased metabolism and induction of defensive plant reactions. However, Thomson et al. (2003) showed the increase of photosynthetic intensity as a result of biotic stress caused by *Helix aspersa* Müller feeding on cucumber plants (*Cucumis sativum* L.). As they suggested, under certain conditions and due to the induced defence, plants can tolerate the presence of pests. This phenomenon is

explained by growth stimulation, the increase of photosynthetic intensity, the change in the distribution of assimilative substances or fast necrotisation of tissues. In this way, they are capable of compensating for the losses, balancing the negative effect of phytophagous species, thanks to which the plants remain in good condition and have good yields (Thomson et al. 2003).

The coefficient of photochemical quenching (qN) is an indication of the proportion of PSII reaction centres that are open (Maxwell and Johnson 2000) and is the balance between excitation of PSII centres and removal of electrons from PSII by the electron transport chain (Campbell et al. 1998). The coefficient of non-photochemical quenching was used as a very sensitive indicator of stress-induced limitations (Bilger and Schreiber 1986; Walz 2006). qN accounts for the fact that not only variable fluorescence (induced upon reaction centre closure), but also dark-level fluorescence (all centres open) can be quenched non-photochemically, primarily by heat dissipation stimulated during illumination (Maxwell and Johnson 2000; Walz 2006). Changes in the quenching coefficients, qP and qN, suggest that *C. hesperidum* feeding may influence the photoprotective xanthophyll cycle, altering the thylakoid membrane pH gradient. This cycle plays an important role in protecting PSII under excess light conditions during abiotic and biotic stresses by dissipating excess excitation as heat (Horton et al. 1994; Gilmore et al. 1995; Demming-Adams et al. 1996; Yamamoto and Bassi 1996). Changes in trans-thylakoid pH might compromise the synthesis of zeaxanthin by the xanthophyll deepoxidase enzyme, which could lead to increased formation of triplet-state chlorophyll and singlet-state oxygen, thereby decreasing the efficiency of photosynthesis (Malkin and Niyogi 2000).

To conclude, this study showed that the infestation of scale insects decreased the chlorophyll $a + b$ and carotenoid content in plant leaves and these changes depended on the specific properties of plants and abundance of insects feeding on them. *C. hesperidum* injury can affect the chlorophyll fluorescence parameters of *C. limon* and *N. biserrata*. Maximum quantum efficiency of the PSII photosystem (F_v/F_m), effective quantum gain of PSII [$\Delta F/F'_m(\gamma)$] and the coefficient of photochemical quenching (qP) of lemon and fern leaves were decreased by increased *C. hesperidum* infestation, while the value of the coefficient of non-photochemical quenching (qN) increased in lemon and fern leaves after feeding of scale insects. Losses of the chlorophyll and carotenoid content and photosynthesis parameters in response to *C. hesperidum* infestation suggest a feeding-induced stress response in their hosts depending on the scale of insect density. The strongest plant responses were observed very rapidly with the smallest abundance of insects (class I) in the leaves of

lemon and somewhat later at a second-class density in the leaves of ferns, where the contents of chlorophyll and carotenoids significantly decreased. The subsequent reaction of ferns may be explained in that *N. biserrata* seems to be a more tolerant host for *C. hesperidum* (Golan 2013).

Because of the economic significance and losses causes by *C. hesperidum*, there is an urgent need to reduce the numbers of this pest. So far, insecticides have been the primary method for *C. hesperidum* management, but due to a lack of data describing density or damage relationships between this pest and commercial host plants, this was not sufficiently effective. Data generated in this study have provided a better understanding of the impacts of the degree of *C. hesperidum* infestation on their hosts and should be useful in developing pest management strategies during crop establishment.

References

Aldea M, Hamilton JG, Resti JP, Zangerl AR, Berenbaum MR, Frank TD, DeLucia EH (2006) Comparison of photosynthetic damage from arthropod herbivory and pathogen infection in understory hardwood saplings. Oecologia 149:221–232

Anderson JM, Chow WS, Park Y-I (1995) The grand design of photosynthesis: acclimation of the photosynthetic apparatus to environmental cues. Photosynth Res 46:129–139

Andrews JR, Fryer MJ, Baker NR (1995) Characterisation of chilling effects on photosynthetic performance of maize crops during early season growth using chlorophyll fluorescence. J Exp Bot 46:1195–1203

Ben-Dov Y, Hodgson CJ (1997) Soft scale insects—their biology, natural enemies and control. Elsevier, Amsterdam

Bilger W, Schreiber U (1986) Energy dependent quenching of dark level chlorophyll fluorescence in intact leaves. Photosynth Res 10:303–308

Blanco LR, Adamson HY, Hales DF (1992) Chlorophyll fluorescence kinetics as a measure of stress in plants infested with aphids: implications for studies of resistance. Aust J Entomol 31:222

Bown AW, Hall DE, MacGregor KB (2002) Insect footsteps on leaves stimulate the accumulation of 4-aminobutyrate and can be visualised through increased chlorophyll fluorescence and superoxide production. Plant Physiol 129:1430–1434

Buntin GD, Gilbertz DA, Oetting RD (1993) Chlorophyll loss and gas exchange in tomato leaves afterfeeding injury by *Bemisia tabaci* (Homoptera: Aleyrodidae). J Econ Entomol 86:517–522

Buntin GD, Braman SK, Gilbertz DA, Phillips DV (1996) Chlorosis, photosynthesis, and transpiration of azalea leaves after azalea lace bug (Heteroptera: Tingidae) feeding injury. J Econ Entomol 89:990–995

Calatayud PA, Le Rü B (2006) Cassava–mealybug interaction. Institut de Recherché Pour le Development, Paris

Campbell D, Hurry V, Clarke AK, Gustafsson P, Öquist G (1998) Chlorophyll fluorescence analysis of cyanobacterial photosynthesis and acclimation. Microbiol Mol Biol Rev 62:667–683

Chaerle L, Van Der Straeten D (2000) Imaging techniques and the early detection of plant stress. Trends Plant Sci 5:495–501

Chaerle L, Leinonen I, Jones HG, Van Der Streaten D (2007) Monitoring and screening plant populations with combined thermal and chlorophyll fluorescence imaging. J Exp Bot 58:773–784

Copland MJ, Ibrahim AG (1985) Biology of glasshouse scale insects and their parasitoids. In: Hussey NW, Scopes N (eds) Biological pest control the glasshouse experience. Cornell University Press, Ithaca, pp 87–90

Crawley MJ (1999) Herbivory. In: Press MC, Scholes JD, Barker MG (eds) Physiological plant ecology. Blackwell Science, Oxford, pp 199–217

Dai Y, Shao M, Hannaway D, Wang L, Liang J, Hu L, Lu H (2009) Effect of Thrips tabaci on anatomical features, photosynthetic characteristics and chlorophyll fluorescence of Hypericum sampsonii leaves. Crop Prot 28:327–332

Delaney KJ (2008) Injured and uninjured leaf photosynthetic responses after mechanical injury on Nerium oleander leaves, and Danaus plexippus herbivory on Asclepias curassovica leaves. Plant Ecol 199:187–200

Demming-Adams B, Gilmore AM, Adams WW III (1996) In vivo functions of carotenoids in higher plants. FASEB 10:203–214

Ebeling W (1959) Subtropical fruit pests. University of California Press, Los Angeles

Gilmore AM, Hazlett TL, Björkman O, Govindjee (1995) Xanthophyll cycle dependent non-photochemical quenching of chlorophyll a fluorescence at low physiological temperatures. In: Mathis P (ed) Photosynthesis: from light to biosphere, vol IV. Kluwer, Dordrecht, pp 825–828

Golan K (2013) Interactions between host plants and Coccus hesperidum L. (Hemiptera; Sternorrhyncha; Coccidae)". Dissertation, University of Life Sciences in Lublin 381

Golan K, Najda A (2011) Differences in the sugar composition of the honeydew of polyphagous brownsoft scale Coccus hesperidum (Hemiptera: Sternorrhyncha: Coccoidea) feeding on various host plants. Eur J Entomol 108:705–709

Golan K, Rubinowska K, Górska-Drabik E (2013) Physiological and biochemical responses of fern Nephrolepis biserrata (Sw.) Schott. to Coccus hesperidum L. infestation. Acta Biol Crac Ser Bot 55:1–6

Goławska S, Krzyżanowski R, Łukasik I (2010) Relationship between infestation and chlorophyll content in Fabaceae species. Acta Biol Crac Ser Bot 52:76–80

Gomez KS, Oosterhuis DM, Rajguru SN, Johnson DR (2004) Molecular biology and physiology. Foliar antioxidant enzyme responses in cotton after aphid herbivory. J Cotton Sci 8:99–104

Heng-Moss TM, Ni X, Macedo T, Markwell JP, Baxendale FP, Quisenberry SS, Tolmay V (2003) Comparison of chlorophyll and carotenoid concentrations among Russian wheat aphid (Homoptera: Aphididae)—infested wheat isolines. J Econ Entomol 96:475–481

Huang TI, Reed DA, Perring TM, Palumbo JC (2014). Feeding damage by Bagrada hilaris (Hemiptera: Pentatomidae) and impact on growth and chlorophyll content of Brassicaceous plant species. Arthropod–Plant Interact. doi:10.1007/s11829-014-9289-0

Horton P, Ruban AV, Walters RG (1994) Regulation of light harvesting in green plants. Plant Physiol 106:415–420

Kalaji MH, Guo P (2008) Chlorophyll fluorescence: a useful tool in barley plant breeding programs. In: Sanchez A, Gutierrez SJ (eds) Photochemistry research progress. Nova Publishers, NY, pp 439–463

Kondo T, Gullan PJ, Williams DJ (2008) The study of scale insects (Hemiptera: Sternorrhyncha: Coccoidea). Rev Corpoica 9:55–61

Krause GH, Weis E (1991) Chlorophyll fluorescence and photosynthesis: the basics. Annu Rev Plant Physiol Plant Mol Biol 42:313–349

Lichtenthaler HK, Wellburn AR (1983) Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. Biochem Soc Trans 11:591–592

Malkin R, Niyogi K (2000) Photosynthesis. In: Buchanan B, Gruissem W, Jones R (eds) Biochemistry and molecular biology of plants. American Society of Plant Physiologists, Rockville

Mao LZ, Lu HF, Wang Q, Cai MM (2007) Comperative photosynthesis characteristics of Calycanthus chinensis and Chimonanthus praecox. Photosynthetica 45:601–605

Mary EP, Maria R, Paymon A (2006) Response of photosynthesis to high light and drought for Arabidopsis thaliana grown under a UV-B enhanced light regime. Photosynth Res 90:79–90

Maxwell K, Johnson GN (2000) Chlorophyll fluorescence—practical guide. J Exp Bot 51:659–668

Morales F, Abadía A, Abadía J, Montserrat G, Gil-Pelegrín E (2002) Trichomes and photosynthetic pigment composition changes: responses of Quercus ilex subsp. Ballota (Desf.) Samp. and Quercus coccifera L. to Mediterranean stress conditions. Trees 16:504–510

Nabity PD, Zavala JA, DeLucia EH (2009) Indirect suppression of photosynthesis on individual leaves by arthropod herbivory. Ann Bot 103:655–663

Ni X, Quisenberry SS, Markwell J, Heng-Moss T, Hgley L, Baxendale F, Sarath G, Klucas R (2001) In vitro enzymatic chlorophyll catabolism in wheat elicited by cereal aphid feeding. Entomol Exp Appl 101:159–166

Ni X, Quisenberry SS, Heng-Moss T, Markwell J, Higley L, Baxendale F, Sarath G, Klucas R (2002) Dynamic change in photosynthetic pigments and chlorophyll degradation elicited by cereal aphid feeding. Entomol Exp Appl 105:43–53

Rafi MM, Zemetra RS, Quisenberry SS (1996) Interaction between Russian wheat aphid (Homoptera: Aphididae) and resistant and susceptible genotypes of wheat. J Econ Entomol 89:239–246

Retuerto R, Fernández-Lema B, Roiloa SR, Obeso JR (2004) Increased photosynthetic performance in Ilex aquifolium trees infested by scale insects. Funct Ecol 18:664–669

Sachico F, Kintake S, Ichiro T (1997) Photosynthetic properties of leaves of Eupatorium makinoi infected by a geminivirus. Photosynth Res 53:253–261

Schaffer B, Mason LJ (1990) Effects of scale insect herbivory and shading on net gas exchange and growth of a subtropical tree species (Guaiacum sanctum L.). Oecologia 84:468–473

Schreiber U, Neubauer C, Schliwa U (1992) PAM fluorometer based on mediumfrequency pulsed Xe-flash measuring light: a highly sensitive new tool in basic and applied photosynthesis research. Photosynth Res 36:65–72

Statsoft Inc (2009) Statistica (Data Analysis Software System), version 9.1. www.statsoft.com

Tang JY, Zielinski RE, Zangerl AR, Crofts AR, Berenbaum MR, DeLucia EH (2006) The differential effects of herbivory by first and fourth instars of Trichoplusia ni (Lepidoptera: Noctuidae) on photosynthesis in Arabidopsis thaliana. J Exp Bot 57:527–536

Thomson VP, Cunningham SA, Ball MC, Nicotra AB (2003) Compensation for herbivory by Cucumis sativus through increased photosynthetic capacity and efficiency. Oecologia 134:167–175

Tomczyk A (2001) Physiological and biochemical responses of plants to spider mite feeding. In: Haliday RB, Walter DE, Proctor HC, Norton RA, Collof MJ (eds) Acarology. CSIRO Publishing, Melbourne, pp 306–313

Velikova V, Salerno G, Frati F, Peri E, Conti E, Colazza S, Loreto F (2010) Influence of feeding and oviposition by phytophagous pentatomids on photosynthesis of herbaceous plants. J Chem Ecol 36:629–641

Vranjic JA (1997) Ecology: effects on host plants. In: Ben-Dov Y, Hodgson CJ (eds) Soft scale insects—their biology, natural enemies and control. Elsevier Science, Amsterdam, pp 323–336

Walz H (2006) IMAGING-PAM chlorophyll fluorometer, 2.152/7.06 edn. Effeltrich, Germany

Yamamoto HY, Bassi R (1996) Carotenoids: localisation and function. In: Ort DR, Yocum CF (eds) Oxygenic photosynthesis: the light reactions: advances in photosynthesis, vol 4. Kluwer, Dordrecht, pp 539–563

Zarco-Tejada PJ, Miller JR, Mohammed GH, Noland TL, Sampson PH (2002) Vegetation stress detection through chlorophyll a + b estimation and fluorescence effects on hyperspectral imagery. J Env Qual 31:1433–1441

Zimmerman EC (1948) Insects of Hawaii, vol 2. University of Hawaii Press, Apterygota to Thysanoptera