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Interactions between host plants and *Coccus hesperidum* L. (Hemiptera; Sternorrhyncha; Coccidae)

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SPIS TREŚCI

1. INTRODUCTION

Almost all species of plants are consumed by herbivores, among which insects are especially conspicuous in most terrestrial communities. Insect–plant interactions are temporally, spatially, and ecologically dynamic, resulting in complicated waveforms of associations that are extremely challenging to analyze [Mitter et al. 1991]. There is evidence that host phylogeny, biogeography, chemistry, and within population and within-individual variation influence host selection, specificity, and speciation in phytophagous insects [Bernays and Chapman 1994, Becerra 1997, Janz and Nylin 1998, Berenbaum 2001].

Over 500 000 insects are known to be phytophagous species feeding on growing green plants all over the world. Hemiptera is one of the most abundant and most commonly encounted order of insects. All the insects from Hemiptera order and Sternorrhyncha suborder are plant feeders. In this suborder, nymphs and adults of all species have the same food habits. In the group about 75% of species feed only on a limited range of plant species (monophagous, oligophagous). Polyphagous species feeding on plants from more than one family comprise about 25% of the phytophagous species [Bernays and Chapman 1994]. Phytophagous insects breach the integrity of plant tissues to recover nutrients from all parts of their host plants depending on the species. Herbivorous phloem feeding insects, such as scale insects, aphids or whiteflies cause modest to barely perceptible damage. They provide additional challenges to plants as they deplete photosynthates, vector viruses, introduce chemical protein effectors altering plant defense signaling, infestation symptoms and plant development [Kaloshian and Walling 2005]. When these attributes are combined with broad host ranges, breeding strategies that promote invasiveness, highly evolved feeding strategies, the ability to adapt to a wide range of plant habitats, and the emergence of insecticide-resistant strains, it is not surprising that phloem-feeding insects cause heavy losses in agriculture and horticulture [Goggin 2007].

The host plant selection behaviour of insects provides a variety of related questions at a number of levels [Futuyma 1983, Courtney et al. 1989, Bernays and Chapman 1994, Mayhew 1997]. They are especially complicated when a single plant provides food and shelter for the whole developmental period in many Sternorrhyncha suborder for example wingless aphids and most of the scale insects species [Ben-Dov and Hodgson 1997]. Host selection factors include a lot of factors, among them the host qualities, chemical composition of

the outer and inner tissues including floem sap, and amount of food. The successive phases of the host plant selection process of insects have been called host finding, host acceptance and host suitability [Klingauf 1987]. Acceptance or rejection of a plant is based on a sequence of selection steps in response to a variety of more or less distinct stimuli. The major steps in the host selection process are: attraction (walking, possibly walk to the under surface), testing of the plant surface and outer plant tissues (detection of a suitable probing site, probing) penetraction (withdrawal of the stylets or walking; tapping the floem, or other feeding sites) and testing the phloem (withdrawal of the stylets, leave or walking; ingestion of food) [Klingauf 1987]. Although scale insects belong to the Sternorrhyncha, their life and feeding strategies are distinct from other members of this suborder [Kaloshian and Walling 2005]. They look very different from other hemipterans because the adult females, which are most commonly encountered, often resemble outgrowths on the host plant only with the enlarged dorsum visible, or the body is hidden under wax and/or old exuviae or inside a gall [Kondo et al. 2008]. Small body size and low mobility of older developmental stages and females related to their neotenic structure (absence of wings in females and reduction of the legs) cause that the relations of this group of insects with the plants are often compared to plant-parasite relation [Koteja 1996b, Kaloshian and Walling 2005, Kondo et al. 2008]. Many characteristics of coccoids have been interpreted as adaptations to sedentary life as plant parasites [Miller and Kosztarab 1979].

Although a great number of Sternorrhyncha studies have been carried out on basic plant–insects interactions during the last ten years, the majority of the information concerns aphids, while interactions between plants and scale insects are poorly described [Bogo and Mantle 2000, Calatayud and Le Rü 2006, Fernandes et al. 2011]. Most of attention in the literature available so far is devoted to the problem related to the morphological variation of the scale insects influenced by host plant and site of feeding on the plants [Ebeling 1938, Fonseca 1953, Habib 1957, Danzing 1970, Williams and Kosztarab 1972, Malumphy 1991, Łagowska 1996, 1999, Annecke 1966, Foldi 1978, Stepaniuk and Łagowska 2006]. Papers describing an influence of host plant on the abundance of scale insects colonizing it [Tingle and Copland 1988, Calatayud et al. 1994b, Gantner et al. 2004, Calatayud and Rü 2006, Golan and Górska-Drabik 2005, 2006] and on demographic parameters of these insects, mainly females fertility and nymphs mortality [Dingler 1923, Tereznikowa 1981, Copland and Ibrahim 1985, Smith et al. 1997, Calisir et al. 2005, Golan 2008a, Polat et al. 2010, Kaushik et al. 2012], are equally numerous.

Coccus hesperidum L. commonly known as soft brown scale, was selected for the study. This species belongs to the group of scale insects alien to our fauna, so called "greenhouse scale insects". *C. hesperidum* (soft brown scale) belongs to Coccidae family and is the member of the Sternorrhyncha suborder of Hemiptera. Adult females are elongate oval, ovate to almost rotund, flat to

slightly convex, pale yellowish-green to yellowish-brown, often flecked with brown spots [Gill et al. 1977]. The soft brown scale is according to different authors ovoviviparous [Tereznikowa 1981] or viviparous species [Cancela da Fonseca 1954–1956, Copland and Ibrahim 1985, Ben-Dov et al. 2013]. In tropical zones or when indoors the species reproduces the around-the-year. The number of generations depends on the climatic zone: the species gives 6 generations in Israel [Bodenheimer 1951, Avidov and Harpaz 1969] 3–5 in USA [Ebeling 1959], 3–4 in Azerbaijan [Tereznikowa 1981]. Over 7 generations of *C. hesperidum,* usually overlapping, are observed in greenhouse and indoor rooms, and therefore all its development stages may be observed for the whole vegetation season, often in large amounts [Gill 1988]. The soft brown scale excretes on the leaves and fruit of its host plant more honeydew than any other species [Copland and Ibrahim 1985]. Honeydew coats the plant surface with a thin, impermeable film, to which adhere saprophytic fungi, dust and other pollutants, and this affects the decrease life processes such as photosynthesis and plant assimilation. Moreover, honeydew attracts ants and other insects thriving on it and their presence reduces the plant aesthetics [Bogo and Mantle 2000]. The species attacks the leaves and twigs. The individuals of this species settle on the upper and lower leaf surface mainly along the vein [Borchsenius 1957, Łagowska 1999].

C. hesperidum is one of the most widespread and polyphagous scale insects that attacks a wide variety of field, ornamental, and greenhouse plants worldwide [Gill et al. 1977, Zimmerman 1948, Ben-Dov and Hodgson 1997, Kondo et al. 2008, Ben-Dov et al. 2013]. It is an important pest of various fruit trees, ornamental outdoors and grown in greenhouses. It occurs on many important plants such as cotton, palm, strawberry tree. It is regarded a serious citrus pest in several countries over different regions [Ebeling, 1959, Ben-Dov and Hodgson 1997]. The plant species selected for the study belong to different taxonomic groups. *Ficus benjamina* L. and *Citrus limon* var. Ponderosa L. are classified in Spermatophyta group (seed plants) while *Nephrolepis biserrata* (Swartz) Schott. in Monilophyta (spore-bearing plants). They differed in morphological and anatomical features as well as biochemical composition [Lücker et al. 2002, Ho et al. 2010, Kanaujia et al. 2011, Lee and Shin 2010]. *C. limon* var. Ponderosa belongs to family Rutaceae and probably originates from Asia. The large leaves, thick foliage, large showy fruit, low growing habit and good tolerance of pruning and cutting that make lemon the popular ornamental tree in California and Florida [Lücker et al. 2002]. Citrus fruits have a high content of phenolics, dietary fibre, ascorbic acid and trace elements [Marlett 1992, Marlett and Vollendorf 1994]. *F. benjamina* (family Moraceae) it is native to south and southeast Asia and Australia. This species is widely cultivated in in Hawai. In warmer regions the tree is grown as a specimen, street tree or as a hedge, pot or in the ground. *F. benjamina*, commonly known as the weeping fig, Benjamin's fig, or ficus tree and often sold in stores as just ficus, is a species of flowering plant [Kanaujia et al. 2011]. Several phenolic and flavonoid compounds, in addition to

polysaccharides, anthocyanins, phytosterols, and fatty acids have been characterized in ficus fruits and branches [Oguzhan et al. 2011]. *N. biserrata*, commonly called giant sword or Boston fern, is grown for its impressive foliage. The species is native to Florida and belongs to family *Lomariopsidaceae* (incl. *Nephrolepidaceae*). It can grow from 6 to 8 feet tall in the right conditions, which include high humidity, rich, moist soil, and bright, filtered light [Ho et al. 2010]. *N. biserrata* leaves have a high content of tannins, saponnins, cardiac glycosides, moderate flavonoids, terpenes, phlobatannins and an thraquinones [Ekong et al. 2013].

Despite these differences, lemon, ficus and fern are classified as the most commonly and abundantly species colonized by *C. hesperidum* [Ben-Dov et al. 2013]. Therefore, the studies have been performed to analyze metabolites, which according to many authors, play an important role in various biochemical interactions, especially in plant response to different stress factors, as insects feeding [Wink and Römer 1986, Hahlbrock and Scheel 1989, Bernays and Chapman 1994, Harborne 1997, Leszczyński 2001, Schoonhoven et al. 2005].

In Poland, *C. hesperidum* is one of the most burdensome greenhouse insect species on ornamental plants, which significantly decreases the plant condition and decorative value by mechanical and physical damaging [Dziedzicka 1988, 1990, Łagowska 1995]. Owing to the lack of natural enemies in their new habitat, high reproduction capacity and specific morphology (protective plates, wax powder, body), the effective management is a real problem [Dziedzicka 1988, 1990, Łagowska 1995, Ben-Dov and Hodgson 1997].

Soft brown scale was accepted as a good model example of the scale insects of Coccidae family for the examination of complex relationships between coccids and their hosts due to the common occurrence of this species, usually in large amounts and abundance of honeydew. An additional reason for this choice was the fact, that *C. hesperidum* is often and abundantly observed in Poland on the decorative houseplants indoors and the results of the study may be used in an elaboration of their control programmes which would be safe for humans.

2. PURPOSE OF THE RESEARCH

The understanding of complex relationships between the insects and their hosts is one of the main aims of current ecology and plant protection. This study presents the research on biochemical and behavioural interactions between scale insects and their host plants using the example of *Coccus hesperidum* L. (Hemiptera, Sternorrhyncha, Coccidae). Numerous publications presenting an influence of host plant species specificity on abundance of scale insects colonizing the plants may be found in the literature [Tingle and Copland 1988, Calatayud et al. 1994b, Gantner et al. 2004, Calatayud and Rü 2006, Golan and Górska-Drabik 2005, 2006]. However, the number of papers describing the effect of plants on the life parameters of scale insects is limited even for the common and burdensome Hemiptera species as soft brown scale [Calisir et al. 2005, Polat et al. 2010].

Bilateral aspect of insect–plant interactions has been taken into account, e.g. an influence of host plant on body size, life cycle, mortality of larval stages, scale insects honeydew excretion and colonies size, as well as an effect of these insects feeding on physiological and biochemical state of the host plant. The study explaining the level of host plant acceptance as well as feeding behaviour and frequency of honeydew excretion of this hemipteran species has also been undertaken.

The following research aims were accepted in order to examine the complex interactions between *C. hesperidum* and its hosts:

1. Determination of the effect of host plants on morphometric, demographic parameters and age structure of *C. hesperidum* colonies developing on various host species.
2. Determination of the plant acceptance and colonization by *C. hesperidum*.
3. Description of the process of *C. hesperidum* feeding on various host species monitored in plant tissues using EPG.
4. Examination and description of honeydew excretion dynamics in *C. hesperidum* on various host plant species.
5. Determination of the response of *C. hesperidum* to host plant biochemical properties.
6. Assessment of the effect *C. hesperidum* feeding on biochemical changes of colonized plants.
7. Determination of host plant susceptibility to *C. hesperidum* feeding.

This complex research has been planned to original contribute to the knowledge on the relationships between scale insects and plants. *C. hesperidum* is concurrently a burdensome pest of indoor decorative plants and the results of the research may have a range of practical applications. Based on the honeydew excretion rate and daily excretion of this insect as well as physical properties of the honeydew, it is possible to monitor its presence on the plants, to determine its developmental stage and colonies size as well as to determine an optimum date of its control. The results of the analysis of primary and secondary metabolites content in the sap of healthy plants and those colonized by *C. hesperidum* are the basis to determine biochemical plant resistance to the feeding of this species. The results enable the search for new ways of this pest control that may be safe for humans.

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3. LITERATURE REVIEW

3.1. Host plant–scale insect interactions

The preference of herbivores to plant hosts can significantly influence the phenotype and life history of individuals [Rossiter 1996, Mousseau and Fox 1998, Agrawal 2002]. Populations of the same species developing in different environments can differ in several demographic parameters, which can lead to differences in population growth rates [Caswell 1983]. Specifically, for phytophagous insects, host plant quality can affect fecundity, survivorship and development rates. Coccids occur in nearly all parts of the host. Most of scale insects live on the above–ground parts of plants and many of them are specific to certain parts of plants [Kosztarab and Kozar 1988]. Individual species infest leaves, fruits, branches, main stems, trunks and roots [Tawfeek 2012]. Due to low mobility, the scale insects are very strictly related to the host plant. Therefore, an influence of the structure and biochemical properties of the host plant they colonize on these insects' bionomics and development is very strong [Malumphy 1991, Ben-Dov and Hodgson 1997, Łagowska 1999, Kondo et al. 2008]. Published data on the scale insect morphological variation indicated that the host plant species appeared to be the principal factor. In family Coccidae, the color, shape and size of the body vary depending on the host species. In turn, Hodgson [1967] suggested that some morphological variation in *C. hesperidum* might be correlated with position on the host plant. Łagowska [1999] in her research concerning morphological variability of soft brown scale females also demonstrated that the examined morphological features of soft brown scale were subject to changes depending on the host species and scale insects position on host plant. Other factors which can influence morphological variation of Coccidae are season [Stafford et al. 1948, Miller and Kosztarab 1979], temperature and humidity [Miller 1966, Chatterjee et al. 2000]. The morphological variability of scale insects is affected by the chemical nature or physiology of the host plant. Danzing [1970] hypothesized that nutritional differences in the parts of the host plant may induce dimorphism of scale insects. Host plant species, its biochemical properties and physiological state are important factors affecting also demographic parameters of the insects. However, the number of papers describing the effect of plants on the duration of the pre-reproductive period and reproductive period, fecundity and mortality of nymphs, is limited even for the common scale

insect species [Calisir et al. 2005, Polat et al. 2010]. As was demonstrated, growth and rate of development of scale insects was also influenced by host plant species [Calatayud et al. 1994b, Łagowska 1996, Smith et al. 1997, Golan 2008b]. The species and conditions of the host plant affect the length of generation development and the average female fecundity [Annecke 1959, Metcalf 1962, Borchsenius 1957, Copland and Ibrahim 1985, Ben-Dov and Hodgson 1997, Golan 2008a]. The changes in females fecundity were observed in scale insects on various plant species [Dingler 1923; Tereznikowa 1981, Copland and Ibrahim 1985, Ben-Dov and Hodgson 1997]. The fecundity of *C. hesperidum* females feeding on various host plant species varies and is within the range from 70 to 1000 nymphs [Dingler 1923; Tereznikowa 1981, Copland and Ibrahim 1985, Kaushik et al. 2012]. Scale insect nymphs mortality was strongly affected by specific properties of host plant [Copland and Ibrahim 1985, Ben-Dov 1997, Calatayud et al. 1994a, Kaushik et al. 2012]. Host plant species affects biochemical composition of the honeydew [Golan and Najda 2011] and daily honeydew excretion of *C. hesperidum* [Golan 2008 a].

3.2. Host plant selection by insects

3.2.1. The role of physical plant features

The main reason of the acceptance or rejection of plants by phytophagous insects may be physical or chemical properties of host plant. The first, very important in host plant acceptance step, before insects feeding, is the test probing behaviour [Peters 2002, Schoonhoven et al. 2005, Calatayud and Rü 2006]. Physical features of plant organs and tissues can profoundly influence host plant selection behaviour. Morphological characters of plants can influence acceptability, either directly by providing suitable visual cues or by affecting the ability of insects to walk onto certain plant parts. The primary interface in the contact phase of the insect–plant interaction is the plant surface. The most important and the most common properties causing avoidance behaviour are related to the presence of trichomes and wax structures on plant surface, leaf thickness and toughness, sclerotization [Dąbrowski 1988, Smith 1989]. The trichomes limit the access of insects with piercing mouthparts to plant tissues and interfere with small insects attachment to the plant [Reed 1974, Smith 1989, Peters 2002, Schoonhoven et al. 2005]. Glandular trichomes affect the feeding behaviour of the green aphid *Myzus persicae* (Sulzer) by delaying the amount of time to begin feeding [Lapointe and Tingey 1986]. The leaves with heavy wax secretion serve as a resistance barrier against herbivore insects such as aphids, beatles, and other insect gropus [Lupton 1967, Stork 1980]. In contrast, the waxy secretion may stimulate feeding of the cabbage aphid *Brevicorne brassicae* L. and cabbage whitefly *Aleurodes brassicae* Walk. more than glossy-leaved ones [Smith 1989]. The thickness of outer epidermal walls and cuticule layers may in some cases

stand for important mechanical barrier against insects [Juniper and Cox 1973, Niraz et al. 1982, Smith 1989]. Leaves and stems thickness by increasing layer of epidermal cells deter or limit entrance of damaging insects of some cultivars of alfalfa, crucifers, rice, sorgum and wheat [Fiori and Dolan 1981, Tanton 1962, Blum 1968, Wallace et al. 1974]. Niraz et al. [1982] have shown, that the epidermis of the flag leaf of wheat cultivars inhabited by aphids in lesser extent, had fewer stomata, thicker cell walls, larger amounts of cellulose, hemicellulose and pectin, and they were strongly ligninized as compared to aphid-susceptible wheat cultivars. The literature on the influence of leaf morphology on coccid feeding is spare [Renard 1999, Calatayud et al. 2001, Bakr et al. 2009, Kaushik et al. 2012]. Bakr and co-authors [2009] studing the mango scale insects resistant variety demonstrated that the morphological and anatomical plant properties as the thicker epidermis and schlerenchyma, deeper vascular bundle, condense pericyclic fibers and lignin, compared to fewer numbers of resin ducts (food sources), act as physically difficult and energetically cost for the scale insect penetration. However, Renard [1999] restricted the plant characteristic to play the most important role in host plant recognition. Studing the host plant recognition by *Phenacoccus manihoti* Matile-Ferrero (Coccoidea; Pseudococcidae), the author has not observed any relationship between the insect preference for a plant variety and the status of a plant as a non-accepting host in relation to the trichome density or the waxy thin layer on the lower leaf epidermis [Renard 1999].

3.2.2. The importance of plant chemistry

Plant chemical composition is an important determinant of host plant and insect interactions and the decisive factor in plant host selection by insects. There are about 200 000 metabolites of concentration depending on a plant species [Ferne 2007]. Extensive variation in the nutrient and allelochemical composition of foliage within individual plants has been documented, and this variation has been proposed to explain some waveforms of host suitability for phloem-feeding insects [Wink et al. 1982, Whitham 1983, Wink and Römer 1986]. Every plant species and even plant parts vary considerably in their nutritional value for insects [Zimmerman and Ziegler 1975, Bernays and Chapman 1994]. Phloem–feeding insects develop a sustained interaction with sieve elements (SEs). They release saliva that inhibits plant stress responses and prevents closure of pierced SEs by callose or polymerized proteins [Miles 1999]. This allows the insects to ingest large amounts of phloem sap to obtain enough nutrients for their survival. The differences in concentration of host plant quality components (mainly primary metabolites) and the presence or absence of secondary metabolites directly affects the herbivore growth and development, affects fecundity and insect reproductive strategies. Plants' chemical properties have been observed to change upon the feeding of insects [Smith 1989, Bernays and Chapman 1994, Awmack and Leather 2002, Schoonhoven et al. 2005].

The group of metabolites playing an important role in the interaction between phytophagous insects and their host plants, determining a taste-acceptability of a plant to insects are primary metabolites such as sugars, amino-acids and proteins [Bach 1991, Girousse and Bournoville 1994, Schoonhoven et al. 2005, Sempruch 2010]. The phytophagous insects use these metabolites especially as important nutrients needed to synthesize body tissue for their growth and development and to serve as energy sources [Dąbrowski 1988, Smith 1989, Bach 1991, Bernays and Chapman 1994, Girousse and Bournoville 1994, Ananthakrishnan 1990, Ananthakrishnan et al. 1992, Sempruch 2010]. Extensive studies of hemipteran insects, mainly on aphids physiology, have revealed the central role of sugars and amino acids concentration and composition, and sugars:amino acid ratio in aphids performance [Auclair 1963, Dadd 1985, Douglas 1998, Cichocka and Leszczyński 2000, Czerniewicz et al. 2011]. Among them sugars are a major nutrient, mainly carbon source, feeding stimulants and respiratory fuel for many insect species including aphids, whitefly and scale insects [Bernays and Chapman 1994, Harborne 1997, Oleszek et al. 2001, Sempruch 2010, Golan and Najda 2011]. The low concentration of sugars in the plant is one of the most important determinants of the mechanism of plant resistance to pests (lack of acceptance) [Harborne 1997, Oleszek et al. 2001]. Cichocka and Leszczyński [2000] have shown that *Aphis fabae* Scop. (black bean aphids) accepted more broad been cultivar Barton cv. characterized by much higher contents of sugars, nitrogen and amino acids than other studied two cultivars the Neptun cv. and Hangdown Biały cv. Insufficient amount of core components in the plant or their disproportions have negative impact particularly on the demographic parameters of insects causing a decline in fertility, an increased mortality rate and a prolonged life cycle [Boczek 1992, Leszczyński 2001, Kordan et al. 2008]. Phloem-feeding insects assimilate only a portion of the ingested sugar, after hydrolysis by the gut sucrase to its constituent monosaccharides [Ashford et al. 2000]. Disaccharide sucrose and monosaccharides glucose and fructose are the most powerful feeding stimulants. Sucrose is a main component and dominant of plant sap and its concentration varies between 0.5 and 30% w/v [Auclair 1963, Canny 1973, Dixon 1975, Srivastava 1987]. This sugar is an important source of metabolic energy for insects [Fisher 2000]. As was shown by Simpson et al. [1995] the amount of sucrose ingested from phloem by aphids does not vary in a simple fashion with dietary concentration. Aphids require a certain minimal concentration of dietary sucrose for sustained feeding but, above this minimal level, aphids compensate for variation in dietary concentration by feeding faster from diets with lower sucrose concentrations. As was shown by Douglas and others [2006] the lower and upper limits to the dietary sucrose concentrations utilised by *Acyrtosiphon pisum* (Harris) (pea aphids) were modified by a behavioural response, specifically reduced feeding, for the lower limit and osmoregulatory failure for the upper limit.

For many insects, especially for floem feeders these are amino acids that play a more important role in food selection than protein [Hollister and Mullin 1998, Sandström and Moran 1999, Mevi-Sultz and Erhardt 2003, Sempruch 2009, 2010]. Free amino acids make up only about 5% of the nutrient nitrogen in plants and the concentration of total amino acids in phloem sap is generally in the range of 60–200 nmol mL^{-1}, with the nonessential aminoacids [Bernays and Klein 2002, Wilkinson and Douglas 2003]. Their concentration vary depending on plant age, plant part together with abiotic factors. Fluctuations of amino acid content may be reflected by differences in the acceptance of plant by phloem-feeding herbivores [Karley et al. 2002]. Dąbrowski [1988] reported the complete diet of insects to involve nearly 20 different amino acids, mostly egzogenic ones. It has been suggested that aphids utilise amino acids as their predominant energy source [Llewellyn 1972, Dixon 1973, Llewellyn and Qureshi 1979, Van Hook et al. 1980]. Egzogenic amino acids as the substances that cannot be synthesized in the body of the insect thus have to be uptaken with the food, are particularly noteworthy [Sempruch and Ciepiela 1998, Wilkinson and Douglas 2003]. Study of aphid feeding and growth with respect to amino acids content in the floem shows that levels of aspargine and glutamine are usually positively correlated with insect performance [Bernays and Chapman 1994]. In the light of literature [Harrewijn 1970, Wearing 1972, Banerjee and Raychundhuri 1987, Janson et al. 1987, Zhou and Carter 1992, Ciepiela and Sempruch 1993] nitrogen content in tissues of host plants appears to significantly affect the population size, growth, development and reproduction in many aphid species (e.g. *Myzus persicae* (Sulzer), *Aphis gossypii* Glover). Cichocka and co-authors [2002] have shown that *Aphis fabae* accepted much better broad bean (*Vicia fabae* L.) cultivars that showed higher content of free protein amino acids (essential and nonessential). The aphids were characterized by a higher fecundity and longevity when compared to individuals feeding on the cultivars with lower amino acids content. The primary metabolites which are exploited by herbivores also function as precursors of secondary substances, the major elements of plants resistance [Anathakrishnan et al. 1992].

The major and most common nutrient for phytophagous insects are proteins. As the main source of amino acids they determine the growth and development of insect phytophags [Sempruch and Ciepiela 2002, Babic et al. 2008]. Coccidae as phloem-sucking insects are directly exposed not only to nutrients but to all components of the transported fluid. In addition to small molecules like sugars and amino acids phloem sap usually contain more proteins that can be accumulated up to high concentrations [Kehr 2006]. This components of plant tissue is most commonly the limiting nutrient for optimal growth of insects. Proteins also function as precursors of secondary substances, which are major elements of resistance in plants [Whittaker and Feeny 1971, Haslam 1985]. A high proportion of the phloem sap proteins so far identified is predicted to be involved in stress and defence reactions, although their exact physiological functions remain to be

established. The number of scientific works showing either an impact of phloem sap proteins on insects or of insect feeding on phloem sap protein composition or activities remains insufficient. However, due to the direct contact of phloem feeding insects to sieve elements contents, an influence of phloem sap proteins on insects is easily conceivable [Kehr 2006]. The protein levels differ among plant families, age of plants and soil nutrient status. Usually sugars and protein levels are inversely correlated in plants leaves [Bernays and Chapman 1994, Sempruch and Ciepiela 1999, Leszczyński 2001, Wool et al. 2006]. According to Leszczyński [2001] it affects the development, fertility and mortality of aphids. The literature currently available lacks the evidence on the effect of these metabolites on demographic parameters in mealybugs. Nitrogen accumulated in plant tissues may also stimulate the development of insects populations, and the level of plant-herbivore interactions can influence the processes occurring in different populations, as well as whole ecosystems. Nitrogen is present in plant tissues in different forms such as total nitrogen, protein nitrogen, soluble, amine or amide nitrogens [Sempruch 2010]. Some of nitrogen compounds (low-molecular signaling biomolecules, proteins, enzymes and nucleic acids) regulate the process of plant defence reactions. Their activitity potentially generates reactive oxygen species (ROS) or reactive nitrogen species (RNS) responsible for the induction of programmed cell death (PCD) at the attack site, trigers the expression of resistance genes which encode pathogenesis-related proteins, activates biosynthesis of secondary metabolites harmful for herbivores, or changes the structure of plant tissue in a way that impedes the feeding of insects [Karley et al. 2002, Sempruch 2010]. By feeding mainly on phloem sap, characterized by a high content of sugars and low concentration of nitrogen compounds, insects from suborder Sternorhyncha e.g. aphids and scale insects, belong to organisms especially susceptible to the content of nitrogen in plant tissues [Douglas 2006, Radwan 2003]. An increased content of nitrogen in plant tissues, particularly amino-acid proteins, proteins generally and some vitamins is favourable for herbivores [Kusano et al. 2007, Rajuand et al. 2009, Heil 2009, Moloi and Van der Westhuisen 2009].

Low molecular weight molecules belonging to secondary metabolites predominate among semiochemicals found in plant tissues. Their bioactivity is determined by a diverse chemical structure and different concentrations in plant tissues [Harborne 1997]. They are characterized by an extremely diverse chemical structures formed at the biosynthesis of basic secondary metabolites during shikimic acid or active acetate pathways [Leszczyński 2001, Matok 2010]. It is well documented that secondary metabolites play the main role in plant resistance to pests: host ranges of phytophagous insects, determine the suitability of the plant species for colonization and exploitation by the herbivores and thus govern host preferences and acceptability [Bernays and Chapman 1994, Schoonhoven et al. 2005, Ananthakrishnan et al. 1992]. These substances in a given plant species may act both as repellents for polyphagous insect species and as attractants for

as specialists as monophagous, and may thus be largely responsible for host range restriction [Harborne 1997, Urbańska et al. 2002]. The majority of plant secondary compounds are toxic to phytophagous insects and impair their growth and development, however some of them are considered as feeding attractants [Harborne 1997, Urbańska et al. 2002]. Their effect on the insect starts already with the pre-selection of the host plant and continues during active feeding. For phloem-feeding insects, the role of the secondary compounds depends highly on their localization within the plant. The compounds located only in the peripheral tissues (e.g. mesophyll) may have a deterrent effect only during stylet penetration – antixenotic resistance, but those located in the phloem, may influence settling or nutrition dependence expressed in their behavioural or metabolic effectiveness – antixenotic or antibiotic resistance [Givovich et al. 1992, Harborne 1997, Leszczyński 2001]. The ability of plants to produce and accumulate secondary metabolites in response to insect feeding was discovered by biochemists and ecologists in 1970ties, and since then, it attracted attention of entomologists, plant physiologists, and molecular biologists [Karban and Baldwin, 1997, Agrawal et al. 1999]. The most numerous studies on the effect of secondary compounds in plant resistance were based on aphids [Smith 1966, Todd et al. 1971, Schoonhoven and Derksen-Koppers 1976, Dreyer and Jones 1981, Mc Foy and Dąbrowski 1984, Dreyer et al. 1985, Leszczynski et al. 1985, Wink and Witte 1991]. Much less information is available on their effects on scale insects [Newbery et al. 1983, Wargo 1988, Calatayud et al. 1994a, Fernandes et al. 2011]. Newbery et al. [1983] showed that the susceptibility of different trees to *Icerya seychellarutn* Nestw. was inversely correlated with foliar contents in alkaloids and condensed tannins. In turn, Wargo [1988] could not assign any effect to levels of total phenolics in the resistance of *Fagus grandifolia* Ehrh. against the mealybug *Cryptococcus fagisuga* Lindinger. Study of influence of secondary compounds in the phloem sap in cassava on the mealybug *Phenacoccus manihoti* Matile-Ferrero were conducted by Catalayud and co-workers [1994b]. The effects of nutrients and secondary compounds of *Coffea arabica* L. on the behaviour and development of *Coccus viridis* (Green) were studied by Fernandes et al. [2011].

Phenolic compounds synthesized via the shikimate pathway are among the most active allelochemicals found within plants [Leszczyński 2001]. Owing to the diverse structure they had been classified as phenols, phenolic acids, flavonoids, phenylpropanoid acids, coumarins, lignans, and tannins. Phenolic compounds may play important roles in plant physiological processes such as protection against environmental stresses (e.g. herbivory infection); signal molecules in plant-pathogen interactions; structural constituents of cell walls (i.e. lignin or suberin) [Hahlbrock and Scheel 1989, Harborne 1997]. Their condensation was often observed to be higher in plant species resistant to herbivores [Leszczyński 2001]. Phenolic compounds, especially *o*-dihydroxy phenols and tannins, are active inhibitors of the enzymes typical for herbivores. These compounds may

impair the activity of the enzymes particularly important at feeding by reducing the absorption of the food uptaken, causing gastrointestinal irritation and reducing the permeability of nutrients. It refers especially to the enzymes involved in the hydrolysis of cell walls as well as proteases [Leszczyński 2001]. The role of phenolics in plant antiherbivore defense has been a particularly intense by exploited area of study during the past several decades [Appel 1993]. Numerous studies demonstrated phenolics toxicity to herbivores when incorporated into artificial diets [Elliger et al. 1981] or involving the correlation of phenolic content in plants with herbivory or herbivore performance [Dudt and Shure 1994]. However, phenolics (e.g. caffeic acid and protocatechuic acid) are known to stimulate feeding and/or growth of certain insect species [Bernays and Woodhead 1982]. Although many studied, the great difficulties in localizing precisely these substances in plant tissues have often prevented formal demonstration of their defensive function against aphids [Molyneux et al. 1990]. According to data provided by Leszczyński et al. [1985] and Chrzanowski [2007] phenolic acids can affect feeding and development of *Ropalosiphum padi* (L.) and *Sitobion avenae* F. as well as ferulic, caffeic and chlorogenic acids reduced the feeding of *R. padi* on winter wheat. Chlorogenic acid has been suggested as a chemical agent in defence against herbivores due to its pro-oxidative effect. This acid is oxidised to chlorogenoquinone, which binds to amino acids or proteins and thereby reduces the digestion of nutrients [Felton et al. 1989]. Santiago et al. [2005] showed that the resistance of maize (*Zea mays* L.) to the *Sesamia nonagrioides* (Lefèbvre) was associated with high levels of *p*-coumaric and ferulic acids. Chrzanowski [2007] observed 35–45% reduction in numbers of grain aphid population as the influence of caffeic, ferulic and *p*-coumaric acids. Caffeic acid reduced daily fecundity and the intrinsic rate of natural increase, whereas *p*-coumaric acid prolonged the time to maturity in aphid females. However, in studies conducted by Bi et al. [1997] phenolics such as chlorogenic acid did't play a direct role in resistance against lepidopteran insects in tobacco. Whereas, Stevenson et al. [1993] documented that chlorogenic acid inhibit growth and development of the *Spodoptera litura* (F.). Benninger et al. [2004] have shown that chlorogenic acid had more of a negative effect on the growth and development of *Lymantria dispar* (Linnaeus) (gypsy moth) larvae than it did on *Trichoplusia ni* (Hübner) (cabbage looper). Other studies conducted by Ellis [1999] have shown that levels of chlorogenic acid correlate with resistance to *Psila rosae* (F.) (carrot fly). According to Gueldner et al. [1992] this phenolic are also a factor in the resistance of corn to *Spodoptera frugiperda* (J.E. Smith) and *Helicoverpa zea* (Boddie). In studies using EPG tests, Urbańska et al. [2002] showed an increase in the number of probes and a reduction of probe duration by the grain aphid fed on diets containing phenolic acids, especially for gallic and caffeic acids.

The literature do not offer many works investigating the effect of phenolic acids on scale insects demographic parameters. Fernandes and co-workers [2011] in their research on scale insects *C. viridis* (Hemiptera; Sternorrhyncha;

Coccidae) documented that caffeine and chlorogenic acid stimulated the locomotory activity of the green scale, thus reducing their feeding. The authors showed increased levels of coffee phenolics and alkaloids in response to feeding of *C. viridis* on *C. arabica*. The concentration of caffeine, the main coffee alkaloid in infested plants was twice as high as in the control plants. Also a significant increase in the concentrations of the main coffee phenolics (caffeic and chlorogenic acid) occurred in plants infested by scale insects.

Tannins are the most abundant plant secondary metabolites, commonly ranging from 5% to 10% dry weight of tree leaves. They can defend leaves against insect herbivores by feeding deterrence and their toxicity. Tannin structure has an important effect on biochemical activity. They are chemically very diverse, and it is important to differentiate at least between two groups: the hydrolysable and the condensed tannins, although they often occur together naturally [Bate-Smith and Metcalfe 1957, Swain 1979]. The ability of insects to tolerate ingested tannins comes from a variety of biochemical and physical defenses in their guts, including surfactants, high pH, antioxidants, and insects anatomy. Most work on the roles of tannins and their impact on insects concern searching for negative associations between tannins and insect performance and reproduction [Mutikainen et al. 2000]. Tannins accumulated in plant tissues may increase the mortality of insects after feeding [Barbenhen and Martin 1994]. Forkner and co-workers [2004] in their studies have showed a significant negative correlations of oak condensed tannins with leaf-chewing herbivore densities. Tannins may induce either acceptance or rejection of the food, generally they may have a great impact on the insects feeding behaviour. As Rhoades [1977] has shown very high concentrations of the tannins in plants of *Larrea* spp were deterrent and that lower concentrations stimulated feeding three grasshopper species (*Astroma quadrilobatum* Mello-Leitão*, Cibolacris parviceps* (Walker)*, Schistocerca Americana* (Drury)) and monophagous on this plant *Semiothisa colorata* Grote. Bennett [1965] showed that tannic acid was deterrent to the alfalfa weevil *Hypera postica* (Gyllenhal). Schoonhoven and Derksen-Koppers [1973, 1976] showed non-preference for artificial diets with tannic acid for several species of Heteroptera, including *Dysdercus koenigii* Fabr. and *Myzus persicae* (Sulzer).

Flavonoids are polyphenolic compounds located in cell vacuoles in green plants. A recent review on flavonoids in insects–plant interaction and plant resistance has been published by Treutter [2006]. They play a variety of biological activities in plants. They are responsible for color, aroma of flowers and fruit, for attracting the pollinators, consequently fruit dispersion and for protecting the plants from different biotic and abiotic stresses, they also act as unique UV-filter, function as signal molecules, allelopathic compounds, phytoalexins, detoxifying agents, antimicrobial defensive compounds [Harborne 1994, Harborne and Williams 2000]. They also showed antibiotic and/or antifeedant effect by reducing the growth and extending developmental cycle and decreasing survivorship of many

herbivorous insects [Stamp 1990, Stamp and Horwath 1992]. Flavonoids can inhibit larval growth of the *Ostrinia nubilalis* (Hübner) (european corn borer) [Abou-Zaid et al. 1993], *Lymantria dispar* (Linnaeus) (gypsy moth) [Beningr and Abou-Zaid 1997] and *Malacosoma disstria* Hübner (forest tent caterpillar) [Abou-Zaid et al. 2000]. Flavonoids interfered with insects molting, reproduction, feeding behaviour [Diaz-Napal et al. 2010]. Kiełkiewicz-Szaniawska and co-workers [2011] observed strongest influence of tannins and flavonols to *Phytoptus tetratrichus* Nalepa (Acari; Eriophyoidea) feeding and negatively affecting their performance. According to the authors *Tilia cordata* Mill. characterized by relatively high amount of anthocyanins and tannins in the leaves is a less suitable host for *P. tetratrichus* than *Tilia tomentosa* Moench.

3.3. Feeding behaviour

The soft scales have a sap-sucking mode of feeding. The structure of the mouthparts as all insects in Coccidae family in general, is related to their specialised feeding behaviour involving acquisition of sap from plant tissues. The basic structure of the soft scales mouthparts consists internally of the tentorium, stylets, pairs of mandibular and maxillary levers, the hypopharynx and also the external labium. The stylets of Coccoidea are long, usually longer than the body. There are four stylets which are modified mandibles and maxillae and have become interlocked to form a tight bundle and constituting a single chain [Koteja 1974, Foldi 1997].

In the Coccidae, the stylets are folded into a loop inside the crumena in the labium [Foldi 1997, Calatayud and Rü 2006]. The mouthparts of soft scale are adopted to sucking sap from plant tissue although it it not clear documented whether these insects feed only on phloem sap or may also use xylem, parenchyma or other tissue [Foldi 1997]. Some valuable information on the mechanisms of feeding behaviour and interactions among insects and their host plants have been provided by EPG techniques (electrical penetration graph system). The development of EPG technique was a major breakthrough in the study of interactions between hemipteran insects and their host plants. The electrical penetration graph system has been developed by Mclean and Kinsey [1964] and then modified by Tjallingii [1978]. This method is based on an electric circuit that includes the insect and the plant. It registers the real-time study of probing (stylet penetration) and ingestion activities inside plant tissues through analyse of electrical stereotypical voltage fluctuations (waveforms) produced by sap-sucking insects on plants [McLean and Kinsey 1967, Tjallingii 1978, Cid and Fereres 2010]. Waveforms are generated after a closed circuit is formed between the insect and plant by insertion of the stylets into the plant tissue [Tjallingii 1978, 1988]. This technique has been very useful to study host plant interactions, among them the identification of stylet activities in plant tissues, insects induced

host plant resistance [Montllor and Tjallingii 1989, Garzo et al. 2002, Alvarez et al. 2006], pathogen transmission and acquisition [Wayadande and Nault 1993, Prado and Tjallingii 1994, Martin et al. 1997], testing of genetically modified plants [Liu et al. 2005] and in plant protection [Nisbet et al. 1993, Harrewijn and Kayser 1997].

EPG method has been for the first time and then widely used in experiments on aphids feeding behaviour. For this reason there are a number of well – characterized EPG waveforms describing plant penetration process for these insects [Tjallingii 1978, 1988]. This method has also been adopted for other sap-sucking insects including planthoppers, whiteflies and thrips [Tjallingii 1978, 1988, Janssen et al. 1989, Backus 1994, Trębicki et al. 2012]. However, until now, there has been little interest in using EPG to study scale insects probing behaviour. Only four works reporting the feeding behaviour of scale insects have been published. All of these concern family Pseudococcidae – *Phenacoccus manihoti*, *Phenacoccus herreni*, *Planococcus citri* (Risso) and *Phenacoccus solenopsis* Tinsley [Calatayud et al. 1994b, Calatayud et al. 2001, Cid and Fereres 2010, Huang et al. 2012]. In scale insects from family coccidae EPG studied haven't been published. Catalayud and co-workers [1994, 2006] and Cid and Fereres [2010] have shown a lot of similarities in EPG parameters between mealybugs and aphids. They documented that mealybugs have a typical phloem-feeding behaviour with exclusively extracellular route to phloem with periodic intracellular punctures. Huang and co-workers [2012] in their studies on stylet penetration behaviour of *P. solenopsis* on cotton identified and characterized typical waveforms of A, B, C, and pd-potential drops (together pathway), E1 and E2 (phloem), F (derailed stylet mechanics) and G (xylem). For the first time waveform F (derailed stylet mechanics) they observed and five novel EPG aspects distinguished. According to those papers the main differences between the EPG's of aphids and mealybugs consisted of higher time of cell punctures and the longer time to the first phloem-ingestion period in mealybugs. Catalayud and co-workers [1994, 2006] documented a lower motility of mealybugs stylets within the phloem searching process as compared to aphids. Cid and Fereres [2010] in their papers showed the similarity of *Planococcus citri* waveforms to those produced by cassava mealybugs (*Phenacoccus* spp.). The main observed differences during *P. citri* probing were the predominance of xylem ingestion activities over the rest of the recorded waveforms and the long time needed to reach the phloem. Calatayud and co-workers [2006], on the other hand, in their studies have pointed out an influence of plant species, a position on the leaf and plant chemistry on the behaviour of two species of cassava mealybugs *Phenacoccus manihoti* and *Phenacoccus herreni*. By comparing the behaviour of mealybugs feeding on different host plants, the authors showed differences in EPG parameters related to the host status. A host plant status had been defined as the accessibility of phloem sap to mealybugs. An early rejection of a plant due to difficulties in finding the phloem may result from antixenosis. According to

the authors the insects position on leaf strongly influences their feeding behaviour what in term is reflected by EPG parameters. Mealybugs and insects from Coccoidea subfamily, are generally found feeding near a major leaf vein what facilitates to determine their phloem feeding behaviour. As Calatayud and coworkers [2001] have shown the most important for host plant acceptance are pre-phloem interactions, mainly the intercellular pathway of the stylets.

3.4. Honeydew excretion

Honeydew is a liquid excreta produced by Sternorrhyncha, a Hemiptera suborder which contains the aphids, scale insects, and psyllids. Insects grouped in these taxons are known to produce a great amounts of honeydew, as a waste product of their feeding. They insert their stylets into phloem cells and feed on the phloem sap, which is rich in photosynthetically derived-carbohydrates but contains only low concentrations of proteins [Koteja 1996]. The insects ingest more carbohydrate than they assimilate or utilize, and excrete excess sugar solution and other waste products of their metabolism through an anal apparatus [Koteja 1996, Ben-Dov and Hodgson 1997].

In the foreign literature, there is quite a lot of attention paid to the problem associated with the chemical composition of honeydew [Gray 1952, Salama and Rizk 1979, Bogo et al. 1998, 2001, Bogo and Mantle 2000], how it is used by beneficial insects to produce honeydew honeys [Crozier 1981, Santas 1985a, b] and attempts to explain relationship between honeydew producers and honeydew users [Bach 1991, Beggs 2001, James et al. 1999, Wang and Tang 1994]. There is little knowledge of the scale insects honeydew production process and few studies that use modern techniques [Bogo and Mantle 2000].

The process of honeydew production is well–documented but most publications focused on aphids [Ashford et al. 2000, Fischer and Shingleton 2001, Wool et al. 2006] and little is known about this process in scale insects. Nishida and Kuramoto [1963], while studing the proces of honeydew production in *Dysmicoccus neobrevipes* Beardsley, the mealybug of Pseudococcidae family, observed the decrease in honeydew production to be correlated with a succeeding developmental stages of individuals. In Poland, only Koteja [1981] and Golan [2008a, b] have analyzed the process of honeydew production by these hemipterans under laboratory conditions. The studies of Koteja [1981] involved the honeydew daily excretion of 8 species among which only *Saissetia hemisphaerica* Targioni is typical for greenhouse plant production. The author pointed out individual differences in the excretion rate and diurnal rhythm of honeydew excretion observed between the studied species in relation to different host plant and ambient temperature. Golan [2008a, b, 2009] proved the honeydew production and physical properties of honeydew droplet to vary during development of *C. hesperidum* individuals depending on the host plant species. According to the

authors the excretion rate of honeydew is the highest in the afternoon and at night to decrease in the morning and at noon [Koteja 1981, Golan 2008b]. Daily intervals in honeydew production observed in each newly emerged developmental stage shall be attributed to molting periods [Golan 2008b].

3.5. Influence of insects on host plant physiology

Herbivorous arthropods induce biochemical and physiological changes in host plants [Gomez et al. 2004]. Modifications in plant protein profiles and alterations in plant oxidative enzyme levels have been reported to be among the first plant responses to insect herbivores feeding [Green and Ryan 1972, Hildebrand et al. 1986, Felton et al. 1994 a, b, Miller et al. 1994, Rafi et al. 1996, Stout et al. 1999, Chaman et al. 2001, Ni et al. 2001]. Plants respond to various stress factors by activating a wide variety of protective mechanisms designed to prevent insects reproduction and dispersion [Leszczyński 2001, Gomez et al. 2004]. It has also been suggested that phloem feeding insects induce responses similar to the pathogen infection and activate the salicylic acid – dependent and jasmonic acid/ethylene-dependent signaling pathways [Walling 2000]. A common phenomenon in many plant responses to insects attacks is an oxidative stress, resulting from the generation of reactive oxygen species (ROS), such as superoxide anion radical, hydrogen peroxide, andhydroxyl radicals [Foyer and Noctor 2005]. The balance between the generation and elimination of ROS is one of the factors determining the performance of insects on the plants [Krishnan and Sehnal 2006, Kulbacka et al. 2009]. The defense mechanisms include the production of ROS, alterations in the cell wall constitution, accumulation of secondary metabolites, activation or synthesis of defense peptides and proteins [Benner 1993, Bennett and Wallsgrove 1994, Heath 2000, De Gara et al. 2003, Agrios 2005, Castro and Fontes 2005]. High ROS concentration damages the absorption of ingested nutrients and can cause oxidative damage to the midgut cells. ROS are the decisive factor that causes lipid peroxidation and enzyme inactivation [Bi and Felton 1995]. Lipid peroxidation is mainly reflected by the damage of cell membranes within chloroplasts and mitochondria what leads to changes in their physical structure [Leszczyński 2001, Mithöfer et al. 2004]. These changes can be determined by using several indices such as electrolyte leakage and malondialdehyde content. Studies concerning lipid peroxidation resulting from oxidative stress, malondialdehyde (MDA) content has been usually used as a biological marker of oxidative stress [Minotti and Aust 1987, Malenčić et al. 2004, Rael et al. 2004, Del Rio et al. 2005]. Aslanturk and coauthors [2011] observed an increase in the content of MDA parameter under the effect of stress caused by gall-forming psyllid on Eucalipt trees. Golan and coauthors [2013] observed differences in plants' reaction to the biotic stress depending on the degree of the plant infestation by *C. hesperidum*. The feeding of

scale insects caused a significant increase in the content of malondialdehyde in fern leaves massively colonized by insects. Biotic stress stimulate the production of ROS and lipid peroxidation of the cell macromolecules. According to Baker and Orlandi [1996] and Aslanturk et al. [2011] the increase in lipid peroxidation may be due to the incapability of antioxidants to capture all the active oxygen species produced by this biotic stress. Mittler et al. [1999] and Liu et al. [2010] suggested that changes in activities and levels of antioxidant enzymes are dependent on ROS and the relationship between ROS and antioxidant enzymes are important in plant response to insect herbivory and other stress factors. Specific antioxidant enzymes such as peroxidase (POX), superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX) participate in ROS metabolism during the insects attack [Bartosz 2009, Leszczyński 2001, Naglaa and Heba 2011]. Superoxide dismutase responsible for catalyzing the dismutation of oxide radical anion, oxide radical and superoxide hydrogen is first to react to the increase of ROS. An excessive amount of superoxide hydrogen is disactivated by the intensified synthesis of ascorbate peroxidase (APX), and also indirectly by guaiacol peroxidase (GP). Peroxidases comprise a large family of related proteins and exist as isoenzymes in individual plant species. Each isoenzyme has variable amino acid sequences and shows diverse expression profiles, suggesting their involvement in various physiological processes. Peroxidases catalyze oxidoreduction between H_2O_2 and various reductants, such as many different fenolic compounds. Indeed, studies have provided evidence that POXs participate in the wall-building processes such as suberization and lignifications oxidation of phenols, auxin catabolism, wound healing and defense against insects infection [Ingham et al. 1998, Hiraga et al. 2001, Maffei et al. 2007]. Several researchers [Miller et al. 1994, Rafi et al. 1996, Jerez 1998, Heng-Moss et al. 2004] have documented changes in enzyme profiles of resistant cultivars in response to insect feeding. As they reported the insects feeding changed the level of oxidative enzymes both in resistant and susceptible plants [Green and Ryan 1972, Hildebrand et al. 1986, Felton et al. 1994a, b, Miller et al. 1994, Rafi et al. 1996, Jerez 1998, Stout et al. 1999, Ni et al. 2001, Chaman et al. 2001, Hiraga et al. 2001, Heng-Moss et al. 2004]. Difference in the expression of peroxidases suggests that plants could have a specific response to insect feeding. Chapman et al. [2001] evaluated the peroxidase activity and found out it was higher in aphid–infested plants comparing to uninfested plants. After removal of aphids from infested plants its the level of peroxidases decreased to the one similar for control plants. Similar results were reported by Argandoña and co-authors [2001]. They documented increased total soluble peroxidase activity in infested barley by spring grain aphid *Schizaphis graminum* (Rondani). Chapman and co-authors [2001] suggested that peroxidase activity increased as time of infestation increased and older plants were more sensitive to aphids feeding than younger plants. Ni et al. [2001] documented the different enzymatic responses in different cereals species to feeding by the *Diuraphis noxia* (Mordvilko) (russian wheat

aphid). Authors observed increased levels of peroxidase activity in wheat leaves of aphids–resistant plants after russian wheat aphid feeding, whereas wheat leaves collected from *Diuraphis noxia* – susceptible plants did not exhibit a similar increase. Also Felton et al. [1994a, b] in their research found increased levels of peroxidase activity in response to *Cerotoma trifurcata* (Forster) (bean leaf beetles), and *Spissistilus festinus* (Say) (three-corned alfalfa leafhopper) in resistant soybean. Stout et al. [1999] observed different biotic stress factors affected the tomato plants, all of these stressors resulted in increasing peroxidases and polyphenol oxidase levels. Cited above results suggest that the synthesis or increased expression of plant enzyme profiles may serve to enhance the plant's resistance to insects. These changes may also be useful as markers for pest resistance [Ni et al. 2001].

4. MATERIAL AND METHODS

4.1. Plant and insect material

The experiments were carried out at the laboratory of the Department of Entomology, University of Life Sciences in Lublin. The air humidity and the temperature measured by a hygrothermograph during the experiment were equal to 65–70%, 20–22°C, respectively. The studied material consisted of two-year old plants of *Citrus limon* var. Ponderosa, *Ficus benjamina* L. and *Nephrolepis biserrata* (Swartz) Schott., measuring ca. 50–60 cm and grown in pots of 15 cm in diameter filled with a standardized horticultural substrate. For artificial colonization of plants, *C. hesperidum* polyphagous pest of greenhouse ornamental plants from superfamily Coccoidea was selected (Phot. 1).

Phot. 1. Adult females and instar nymphs of *Coccus hesperidum* on *Nephrolepis biserrata* leaf
Fot. 1. Dorosłe samice i larwy *Coccus hesperidum* na liściu *Nephrolepis biserrata*

Thirty specimens of *C. limon* var. Ponderosa, *F. benjamina* and *N. biserrata* were aimed at physiological state analysis of the plants. The control group for each species was represented by 5 control plants, not colonized by scale insects. The groups of 5 plants were separated from other plants, which were then colonized by 10, 30, 50, 100 and 200 mobile nymphs of *C. hesperidum*. After six

months, all observed individuals of soft brown scale were counted on each plant, and expressed in the number of observed scale insects per 1 leaf respectively. In the case of *N. biserrata,* the number of compound leaves on plants was taken into consideration. The density classes of insects infestation were established on this basis.

For *C. limon* var. Ponderosa and *N. biserrata* the density was defined according to a five-degree scale: 0 class: control (non-infested plants), I class: up to 10 individuals per leaf; II class: from 11 to 30 individuals per leaf; III class: from 31 to 50 individuals per leaf; IV class: from 51 to 100 individuals per leaf; V class: over than 100 individuals per leaf. Due to the low infestation of *F. benjamina* by scale insects, the density was defined according to a three-degree scale: 0 class: control plants (non-infested by scale insects), I class: up to 10 individuals per leaf, II class: from 11 to 30 individuals per leaf, and III class: from 31 to 50 individuals per leaf.

4.2. Methods of morphometric and demographic analysis

Subsequent developmental stages of *C. hesperidum*: first-instar nymphs (L_1), second-instar nymphs (L_2♀) and females (♀) were isolated on plants colonized by scale insects (*C. limon* var. Ponderosa, *F. benjamina, N. biserrata*). The size of the scale insects body was determined by measurement of its width and length expressed in millimeters (mm), calculating its mean and border values for each stage depending on the species of the host. The study was carried out in 25 replications. The measurement was performed at the laboratory of the Department of Entomology, University of Life Sciences in Lublin using the digital camera DS-Fil/U3 with NIS–D programme for digital analysis of the picture connected up to the Nikon stereoscopic microscope SMZ 800.

Demographic parameters of *C. hesperidum* feeding on plant species studied were determined under laboratory conditions. Microscopic slides according to the method by Williams and Kosztarab [1972] and modified by Łagowska [1996] made in the period of plants colonization by scale insects were used in establishing the length of pre-reproductive and reproductive period. In total, over 400 microscopic sliders were prepared. The experiment was performed in 15 replicates for each plant species. The examination of demographic parameters (females fecundity and instar mortality) involved the rearing of females in Petri dishes. Ten dishes which were considered as replicates, were used in order to determine females fertility and nymphal mortality for each of the examined plant species. The leaves of examined plant species were placed in the dishes on a disc of filtration paper, and humidity was maintained by cotton wool tampon moistened with water which was placed on leaf petiole. Due to the stationary feeding behaviour of females and possibilities damages of proboscis during leaf changes, the dishes at the moment of leaves drying were removed. The fecundity of fe-

males was counted each day. Each first nymphs occurred were moved on the new dishes (1 nymph/1 dish). On three species of host plants, mean female fecundity was calculated basing on the observations. Live and dead instar nymphs were counted during the study. The experiment was carried out in 25 replicates for each plant species.

Five plants of each species were used for the examination of age structure of scale insects colonies. Ten leaves of each plant were observed in 10–14 days intervals under stereoscopic microscope. Live developmental stages of *C. hesperidum* were collected from the leaves during observations. Identification of particular stages was conducted based on microscopic slides. An average percentage share of particular developmental stages in *C. hesperidum* colony on each examined plant species was established based on an observation and microscopic slides.

4.3. Methods to assess acceptance and colonization of plants by *Coccus hesperidum*

Plants acceptance by scale insects (Free-choice test)

The experiment was conducted in the laboratory of the Department of Entomology, University of Life Sciences in Lublin. Non-infested plants of the examined species (*C. limon* var. Ponderosa, *F. benjamina* and *N. biserrata*) were arranged in a circle on the tables in air conditioned chamber, so that their leaves did not touch each other (Phot. 2).

Phot. 2. Three species of plants (*Citrus limon* var. Ponderosa, *Ficus benjamina, Nephrolepis biserrata*) around the arena with mobile first instar nymphs of *Coccus hesperidum* – 'free choice' test

Fot. 2. Trzy gatunki roślin (*Citrus limon* var. Ponderosa, *Ficus benjamina, Nephrolepis biserrata*) ustawione wokół areny z ruchomymi larwami pierwszego stadium *Coccus hesperidum* – test wyboru

The leaves of studied plant species abundantly colonized by *C. hesperidum* were placed in the central point, in the middle of circle, on cardboard platform. The number of instar nymph moving to the plants of the examined species was determined after 5 days. The level of examined species acceptance was calculated as percentage share of nymphs noted on each plant species with respect to the number of all *C. hesperidum* nymphs which passed to all plants. The experiment was established in 6 replicates.

Plants colonization by scale insects

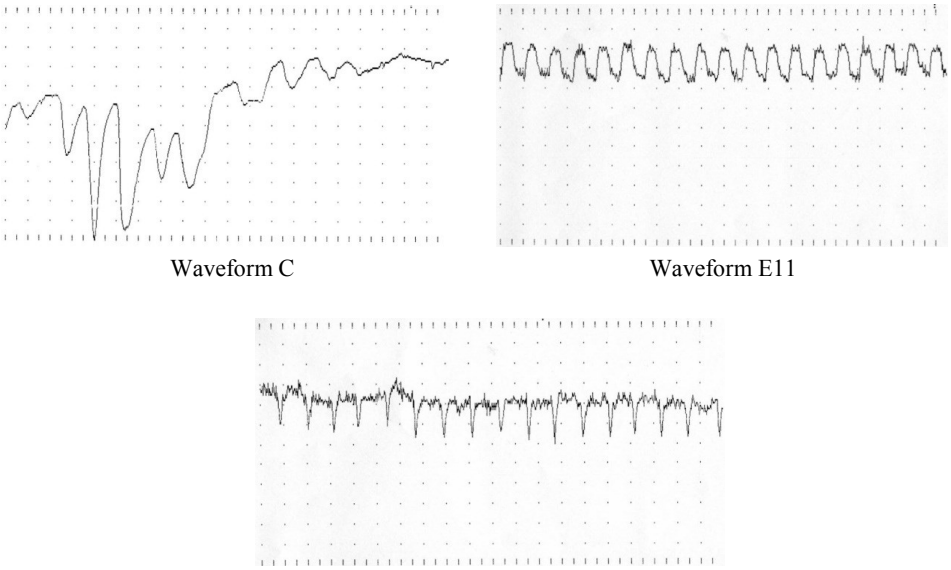
Mobile stages of *C. hesperidum* in the form of first–instar nymphs (crawlers) were placed from the rearing laboratory with a thin wooden spatula (20 individuals L_1/plant) into the plants of the examined species. The abundance of the developing scale insects colony was counting all developmental stages of the scale insects at five terms: 3, 6, 8, 10 and 12 months after plants colonization.

4.4. Methods of assessment of *Coccus hesperidum* feeding behaviour using Electrical Penetration Graphs technique

The experiment was conducted in collaboration with the Department of Biochemistry and Molecular Biology of Siedlce University of Natural Sciences and Humanities.

The recording of feeding behaviour of *C. hesperidum* on plants was monitored using the technique of EPG (electrical penetration graphs) [Tjallingii 1978, 1988, Calatayud et al. 1994b, Leszczyński and Tjallingii 1994, Huang et al. 2012]. The studies used adult individuals of scale insects and the system DC EPG of Giga 4 type. A microelectrode (a gold wire with the diameter of 20 μm and the length of about 2–3 cm) was attached to the dorsal area of the insect body by means of a drop of silver paint (Demetron L2027, Darmstadt, Germany). The observations were continued for 8 hours in 20 replications for each scale insects/plant combination. The records of *C. hesperidum* feeding behaviour were studied using the computer STYLET 2.2 programme, and for analysis the obtained results, a computer EPG ASYST programme was used. The analysis included the number and the mean duration of particular EPG waveforms corresponding to different phases of plant tissue penetration by the stylets of sap-feeding insects, scale insects from family Pseudococcidae in particular [Cid and Fereres 2010, Huang et al. 2012], with minor modifications.

The following, the most characteristic EPG waveforms during *C. hesperidum* feeding behaviour were distinguished (Fig. 1):



EPG waveforms: C – penetration of peripheral tissues, E11 – sieve element salivation, E2 – ingestion of phloem sap
Modele EPG: C – nakłuwanie tkanek peryferyjnych, E11 – nakłuwanie elementów floemu, E2 – pobieranie soku floemowego

Fig. 1. Waveforms recorded for *Coccus hesperidum* feeding

Rys. 1. Graficzny obraz żerowania *Coccus hesperidum*

– Waveform C is the pathway, the activity connected with probing the peripheral tissues (epidermis and mesophyll). The waveform C was characterized by a pattern of cyclic frequency variations and was similar to aphid waveforms A, B, and C. During this study waveforms A, B, and C were not well separated and they were pooled as C for the purpose of analysis.

– Potential drops (pd) were observed during waveform C. During pd waveforms no feeding acitivity of *C. hesperidum* was noted. Pd waveforms observed for *P. solenopsis* [Huang et al. 2012] were connected with the voltage dropped. Pd is probably related to the periods when the stylet is supposed to penetrate the cell membrane and when the stylets are withdrawn. These observations need additional investigation.

– Waveform E11 reflected phloem salivation, observed for *P. solenopsis* [Huang et al. 2012] showing a great similarity to the E1 waveform of aphids. In E11, the clearly positive peaks were superimposed on the waves.

– Waveform E2 reflected passive phloem ingestion with concurrent salivation. The E2 was thus similar to solenopsis mealybug and the aphids E2 waveforms. The waveform E2 was characterized by negative spikes, superimposed on a baseline with regular waves.

4.5. Methods of honeydew excretion analysis

The rate and daily honeydew excretion of *Coccus hesperidum*

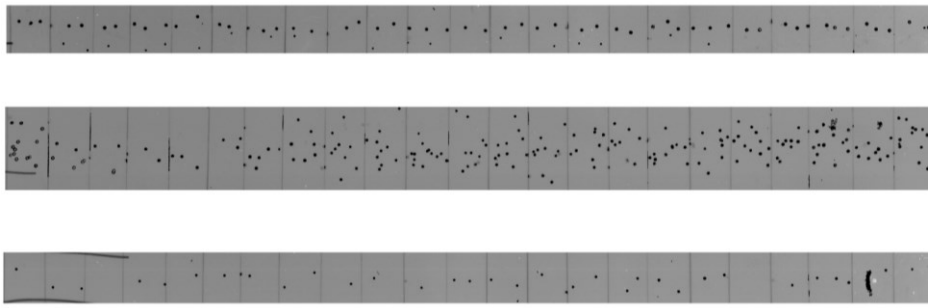
The analysis of *C. hesperidum* honeydew daily excretion was performed following the method described by Koteja [1981] and modified by Golan [2008a]. Three modified types of a hygrothermograph for daily measures were used in the study. Instead of paper, normally serving for recording temperature and humidity readings, an X-ray film was fixed on the cylinder, showing a good absorbance capacity towards honeydew. The film was changed and analyzed after a full rotation (ca. 24 hours).

Phot. 3. Thermohygrograph used for the *Coccus hesperidum* honeydew excretion analysis
Fot. 3. Termohigrograf do analizy procesu spadziowania *Coccus hesperidum*

Above the cylinder, rotating with a constant velocity (one rotation per 24 hours) a transparent plastic plate was attached. Fragments of plants with coccids were then fixed to the interior side of the plate (Phot. 3). The plants used in the study were *C. limon* var. Ponderosa, *F. benjamina*, *N. biserrata*.

Three to five individuals of scale insects were observed at the same time on one hygrothermograph. The experiment was carried out for twenty four hours a day for the developmental time of one *C. hesperidum* generation in three replicates during ca. 60 day period, under laboratory conditions. Honeydew excretion was studied from the moment when the first honeydew droplets were noticed on the film till the moment when the specimen terminated the production of honeydew. Six full paths recorded on the film and several shorter ones were chosen for the analysis. Paths of the honeydew (Phot. 4) excreted by specific individuals were marked on the film with a marker pen, then the number of droplets per

each path was counted. The mean number of honeydew droplets excreted by insects per hour was determined according to honeydew excretion rate and a mean number of honeydew droplets per day as the daily excretion.



Phot. 4. Paths of the honeydew droplets excreted by *Coccus hesperidum* on the X-ray film
Fot. 4. Ścieżki kropli spadzi wydalanych przez *Coccus hesperidum* utrwalone na kliszy rentgenowskiej

The experiment was conducted at the laboratory of the Department of Entomology, University of Life Sciences in Lublin.

The analysis of the physical properties of honeydew

Subsequent developmental stages of *C. hesperidum*: L_1, L_2♀ and ♀ were isolated on the observed plant species: *C. limon* var. Ponderosa, *F. benjamina*, *N. biserrata*. The marked fragments of stems or leaves were placed for the period of about 2 hours directly above the Petri dishes, where honeydew drops excreted by scale insects fell. The size of a honeydew droplet was determined with its diameter expressed in millimeters (mm) and area, expressed in mm^2. The study was carried out in 25 replications for each insect stage on the examined plant species. The measurement was performed using the digital camera DS-Fil/U3 with a NIS–D programme for digital analysis of the picture connected up to the Nikon stereoscopic microscope SMZ 800.

The experiment was conducted in the laboratory of the Department of Entomology, University of Life Sciences in Lublin.

4.6. Methods of examination of anatomical structure and biochemical properties of the plants

Analysis of the anatomical structure of the plants

While analyzing the effect of the anatomical structure of the studied organs of infested plants on the population of scale insects and place of food uptake by them, 100 cross-sections of the leaves and leaf petioles of three observed plant

species were submitted to a microscopic observation under of a light microscope Motic B1. Measurements (50 replicates each) and anatomical observations on selected microtome cross-sections in 50% glycerin and semi-permanent, glycerin--gelatin were performed. The following tissue structures were analyzed: thickness of the leaf blade at the main nerve and about 100 μm axially behind the main nerve, thickness of the epidermis layer at the main nerve of the bottom and upper sides of the leaf, thickness of the external wall of the cell wall of epidermis of the upper and bottom sides of the leaf blade. Microscopic slides from the bottom and upper epidermis of fresh, healthy leaves were made. The number of stomata in 1 mm^2 of the epidermis, length and width of stomata were observed and measured (50 replicates each) Thickness of the leaf blade (50 replications) on control and infested leaves was measured.

The experiment was conducted in collaboration with the Department of Botany, University of Life Sciences in Lublin.

Analysis of the primary and secondary metabolites in plant leaves

The biochemical analysis of the content of primary and secondary metabolites of plant extracts was performed after 3 months after the experimental infestation. The material used in this study consisted of the un-infested (control) and infested by *C. hesperidum* leaves of three plant species: *C. limon* var. Ponderosa, *F. benjamina, N. biserrata*. The leaves from all parts of plants were used for analyses. In the case of infested plants the leaves with scale insects were collected.

The majority of the chemical analyses of the plant material were conducted at the Laboratory of the Quality of Vegetables and Herbal Raw Materials of the Department of Vegetable Crops and Medicinal Plants of the University of Life Sciences in Lublin. The content of proteins and total nitrogen was established at the Central Laboratory of Agriecology of the University of Life Sciences in Lublin.

Laboratory analyses of the plant material included primary and secondary metabolites:

Primary metabolites

Following compounds of primary metabolites were analyzed in leaves of three plant species:

(a) the content of reducing sugars and total sugars was determined using the method of Schoorl–Luff [Charłampowicz 1966]. Total sugar content was determined in indirect way after acid hydrolysis to reducing sugars. *Preparation of stock solution*: Weighted portion of plant material – 10 grams of shredded control and colonized leaves was placed in a beaker and filled up with distilled water to 50 cm^3, boiled and heat filtered. After cooling, filled up to 100 cm^3 with distilled water. 25 cm^3 of Luff solution was measured to the conical flask, 10 cm^3 of analyzed solution was added and filled up with distilled water to 50 cm^3. Within 2 minutes the solution was brought to a boil for 10 min and then cooled, after

5 minutes 3 g of KJ was added and addition of 25 cm^3 25% H_2SO_4. When the solution stops to lather, it is titrated with a 0,1 M Na_2SO_4 and 2% starch solution was adding. At the same time blank sample is introduced, in which, instead of studied solution, the same quantity of water is added;

(b) the content of total nitrogen was determined using the method of Kjeldahl with the Kjeltec system 1030, after prior mineralization in a block DS–20 [Wierciński 1999]. *Samples preparation*: 1 g of test materials was weighed on nitrogen free blotting paper. Blotting papers were rolled up and placed in digestion unit tubes adding 10 g of copper catalyst for combustion and 12 cm^3 of concentrated H_2SO_4. Samples were subjected to mineralization for 45–60 min at a temperature of 410^oC, until mixture became clear. To each tube 50–70 cm^3 of water were added. Therefore, samples were diluted by a digest and placed in Kjeltec system. The content of total proteins was established by multiplying the quantity of total nitrogen by coefficient 6.25 [Sikka et al. 1978]. Protein nitrogen was analyzed analogically like total nitrogen, however soluble nitrogen fractions were removed before sample combusting using a boiling acetic acid solution. Soluble nitrogen content was calculated as from the difference between the contents of total and protein nitrogen;

(c) identification of amino acids was performed using the method of High Performance Liquid Chromatography (HPLC) and derivatization method (DABS-Cl). The sum of identified amino acids (total, essential and non-essential ones) was used in the study. 22 derivatized amino acids standards were prepared for the analysis (21 protein amino acids plus glycine). Samples were prepared according to previously developed methodology. The methodology of protein amino acids analysis was prepared based on literature positions [Lin and Chang 1975, Papliński 2002]. *Preparation of standards:* standard amino acids (10 mg) were dissolved in 100 cm^3 of 0.2 M $NaCHO_3$ at pH 9.0. To 100 mm^3 of amino acid solution (10 mg) 300 cm^3 of DABS-Cl acetone solution at a concentration of 10 mm^3/1 cm^3 was added and heated in water bath at a temp of 70°C for 15 min. Then the solution was evaporated to dryness at a temperature of 50°C and dissolved in 10 cm^3 of 70% (v/v) ethanol. *Sample preparation*: 20 mg of shredded and sifted plant material were macerated in 150 cm^3 of methanol (MeOH) for 12 hours stirring from time to time. Extract was filtered and evaporated to dryness under reduced pressure. Dry residue was dissolved in 10 cm^3 of 0.2 M $NaHCO_3$. 2 cm^3 were sampled from the solution and added to 4 cm^3 of acetonitrile in order to remove peptides. Obtained solution was filtered. 1.5 cm^3 of clear solution was heated for 15 min, at a temperature of 70°C of 0.5 cm^3 DABS-Cl acetone solution. The residue was evaporated to dryness at a temperature of 50°C. The dry residue was dissolved in 10 cm^3 of 70% ethanol (EtOH). HPLC analysis was conducted using gradient elution of acetonitrile and methanol (70 plus 30 v/v) with the addition of KH_2PO_4 solution at pH 6.4.

Secondary metabolites

With the aim of establishing the presence of biologically active compounds in the analyzed species of control plants and those infested by scale insects, studies were conducted on the presence of such secondary metabolites as phenolic acids, flavonoids and tannins:

(a) the content of phenolic acids was established using the spectrophotometric method according to Arnov [FP VIII 2009]. The percentage content of phenolic acids was given in conversion into caffeic acid. *Preparation of plant extract*: 1 g of plant material was collected and extracted in 50 cm^3 of analytically pure MeOH for three hours. Extraction procedure was repeated threefold. Supernatant was pooled to a 250 cm^3 volume flask, and then evaporated on a rotary evaporator to a volume of 1 cm^3. Such prepared extract was purified using SPE method and analyzed with the use of high performance liquid chromatography;

(b) isolation and purification of phenolic acids in the studied raw materials were performed using the method based on the literature data [Schmidtlein and Herrmann 1975, Drost-Karbowska et al. 1994, Najda 2004]. The qualitative and quantitative chromatographic analysis was conducted in the system of reversed phases using a liquid chromatographer with a diode detector DAD (L-7450), a steel column Li-Chrospher 100 RP-C18. The mobile phase was a solvent gradient of the solutions of acetronitile + water + 1% acetic acid. Identification of phenolic acids was performed comparing their retention times (tR) with the waveforms and determining their spectra by means of a spectroscope within the range UV (220–400 nm) [Nollet 2000]. The content of particular phenolic acids in the examined raw materials was calculated on the basis of a calibration curve established for each identified phenolic acid;

(c) the content of tannins was determined using the spectrophotometric method according to FP IV [2002]. *Preparation of extract:* to 250 cm^3 volume flask, about 5 g of powdered plant material were weighted, poured with 150 cm^3 of water and kept in boiling water bath for 30 min. Next, solution was cooled, transferred to 250 cm^3 volumetric flasks and filtered through a filter made of blotting paper to dry glassware. Obtained filtrate was used for further studies. General content of polyphenols was determined by spectrophotometric method with Folin reagent at wavelength 760 nm. *Determination of hydrolysable tannins*: to 10 cm^3 of extract, 0.10 g of hide powder was weighted and shaken on a shaker for 1 hour. After this time, the solution was filtered and in obtained filtrate polyphenols content was determined at wavelength 760 nm. To prepare reference solution, 50 mg of pyrogallol was weighted and transferred to a 100 cm^3 volumetric flask, filling to the mark with water. 5 cm^3 of this solution was sampled and dissolved with water to 100 cm^3. 0.5 cm^3 (12.5 μg of pyrogallol) was subjected to the analysis and further procedure as for the determination of polyphenols. The contents of tannins per pyrogallol ($C_6H_6O_3$) (in %) was calculated using the following formula:

$$X\% = 15{,}625\ (A_1 - A_2)m_2 / A_3 m_1$$

A_1 – absorbance of polyphenols in studied sample solution;
A_2 – absorbance of polyphenols not bound to hide powder in studied sample solution;
A_3 – absorbance of reference pyrogallol solution;
m_1 – weighted amount of raw material in gram;
m_2 – weighted amount of pyrogallol in gram.

(d) the content of flavonoids was determined using the spectrophotometric method according to FP VIII [2009] with the percentage of flavonoids given in conversion into quercitin. *Preparation of stock solution:* To weighed 0.5 g of plant material, 20 cm^3 of acetone, 2 cm^3 of hydrochloric acid, 1 cm^3 2% of urotropine solusion were added, and therefore the solution was kept on the boil in a water bath for 30 min. After filtration, 20 cm^3 of acetone was added, re-extracted for 10 min and then filtered. Extracts were filtered and filled up with acetone to 100 cm^3. 20 cm^3 of the solution was transferred to a funnel, 20 cm^3 of distilled water was added and extracted twice with ethyl acetate. Combined organic layers were washed twice with 40 cm^3 of water, filtered into a 50 cm^3 volumetric flask and filled up with ethyl acetate. Organic layers were filtered into a volumetric flask and filled up with ethyl acetate to 50 cm^3. To 10 cm^3 of stock solution, 2 cm^3 of aluminum chloride was added and filled up with a mixture of acetic acid and methanol to 25 cm^3. The absorbance was measured at wavelength 425 nm using reference solution as a reference value.

4.7. Methods of assessing plant physiological state

The analysis of malondialdehyde content

The level of peroxidation of membrane lipids was assessed by determining the content of malondialdehyde (MDA) according to the method by Heath and Packer [1968]. The crushed plant material (0.2 g) was extracted in a 0.1 M potassium phosphate buffer with pH = 7.0, and then centrifuged at 12000 × g for 20 minutes. Next, 2 cm^3 of 0.5% thiobarbituric acid (TBA) was added to 0.5 cm^3 extract of 20% trichloroacetic acid (TCA) and incubated for 30 min in a water bath at the temperature of 95°C. After incubation, the samples were quickly cooled down and centrifuged again at 10000 × g for 10 min. Absorbance was measured at 532 and 600 nm using a Cecil spectrophotometer CE 9500. The concentration of malondialdehyde in a sample was calculated using the molar absorbance coefficient, which for MDA is 155 $nM^{-1} \cdot cm^{-1}$, and it was expressed as nanomoles per 1 g fresh weight.

Determination of the antioxidant enzymes activity

Preparation of extract

The leaves (0.2 g) were homogenized in a mortar in a 0.05 mol dm^{-3} phosphorus buffer with pH = 7.0 at the temperature of 4°C. The homogenate was then centrifuged at the temperature of 4°C at the speed of 10000 × g for 10 min. The supernatant obtained in this way was used for further procedure.

Determination of the activity of ascorbate peroxidase **(APX)**

The activity of ascorbate peroxidase was determined using the method of Nakano and Asada [1981]. The reaction mixture contained 1.8 ml of 0.1 M phosphate buffer with pH = 6.0, 100 µl of enzymatic extract, 20 µl of 5 mM of sodium ascorbate solution, 100 µl 1 mM of hydrogen peroxide. The mixture thus obtained was incubated in a water bath at the temperature of 30°C until it reached that temperature. The measurement of absorbance decline was performed between the first and the fifth minute from the start of the reaction, at the wavelength of 290 nM, using spectrophotometer Cecil CE 9500. The activity of ascorbate peroxidase was established according to the following formula:

$$U \cdot ml^{-1} = (\Delta E/min) \cdot R \cdot V \cdot R' / W_{abs} \cdot V_{en}$$

where: ΔE/min – extinction,
R – reaction coefficient,
V – mixture volume in the cuvette,
R' – dilution ratio,
W_{abs} – mM absorbance coefficient for catalase,
V_{en} – volume of enzymatic extract in the cuvette.

The result was converted to ascorbate peroxidase activity per fresh weight, expressed as U/mg fresh weight.

Determination of the activity of guaiacol peroxidase **(GP)**

The activity of guaiacol peroxidase was measured following to the method given by Małolepsza et al. (1994). The reaction mixture contained 0.5 cm^3 0.05 mol · dm^{-3} of the phosphorus buffer with pH = 5.6, 0.5 cm^3 0.02 mol · dm^{-3} of guaiacol, 0.5 cm^3 0.06 mol · dm^{-3} of H_2O_2 and 0.5 cm^3 of the enzymatic extract. The measurement of extinction was performed for 4 min with one minute intervals using a Cecil spectrophotometer CE 9500, for the wave length of 480 nm. The activity of guaiacol peroxidase was determined using mM of the absorbance coefficient for guaiacol peroxidase, which is 26,6 mM · cm^{-1}. The result was converted to guaiacol peroxidase activity per fresh weight, expressed as U/mg fresh weight.

The analyses of the physiological state of plants were conducted in three repetitions at the laboratory of the Department of Plant Physiology of the University of Life Sciences in Lublin.

4.8. Statistical analysis

The differences between the means for the variables were determined based on an analysis of variance (ANOVA), assuming normality of distribution and homogeneity of variance. Significance of the difference between the means was tested using the method of Tukey's *Honestly Significant Difference* test (*HSD*) and T-test, at a significance level of P = 0.01. The total duration of the *C. hesperidum* feeding behaviour on examined plant species was compared using G test [Sokal and Rohlf 2001].

The value of F – statistics for the examined variables was presented in the tables with the results. The values of the means ($\bar{x}$) on figures and in tables were provided with standard errors values (±SE).

The strength of relationship between two variables was described using Pearson's correlation coefficient (r). The following correlations were calculated during this study:

– metabolite content (primary: total and reducing sugars, total nitrogen, protein nitrogen and soluble nitrogen, total protein, total amino acids, essential amino acids and non-essential amino acids; secondary: phenolic acids, tannins, flavonoids, and identified derivatives of benzoic and trans-cinnamic acids) in relation to plant acceptance, abundance, morphometric parameters (mean scale insects body width and length), demographic parameters (the length of pre-reproductive and reproductive period, fecundity of females and nymphal mortality) as well as *C. hesperidum* feeding behaviour parameters (duration of probing activities of scale insects feeding and mean percentage share of subsequent feeding behaviour activities) and honeydew excretion parameters (excretion rate and daily excretion, dimensions of honeydew droplets);

– feeding behaviour parameters (duration of probing activities of scale insects feeding and mean percentage share of subsequent feeding behaviour activities) in relation to host plant features (leaf blade thickness, epidermis thickness, thickness of external wall of epidermal cells, number of stomata, phloem distance from lower leaf side), morphometric, demographic and honeydew excretion parameters, analyzed primary and secondary metabolite content;

– dimensions of honeydew droplets (diameter and area) in relation to insects age.

Due to the large amount of calculated relationships only statistically significant correlations were described. Statistical analysis was performed using Statistica 9.1 packet (StatSoft, Tulsa).

5. RESULTS

5.1. Host plant effect on morphometric parameters, abundance and life processes of *Coccus hesperidum*

5.1.1. Body size

The influence of host plant species on *C. hesperidum* morphometric parameters (body width and length) of subsequent developmental stages was analyzed. Measurements of body width and length of *C. hesperidum* individuals from three host plant species demonstrated differences in these parameters depending on developmental stage and host plant species of scale insects (Tab. 1).

Among first-instar nymphs the highest mean value of the examined parameters were characteristic for the individuals feeding on ferns. Their body width was about 30% larger than length of 1st-instar nymphs feeding on lemon and ficus, while the length over 20% higher than in the case of nymphs from ficus. Differences between body length of 1st-instar nymphs from lemon and individuals of this developmental stage from other host plants were insignificant (Tab. 1).

Among second-instar nymphs, the highest body size were observed for individuals from *C. limon* var. Ponderosa, whilst the smallest scale insects of this stage feeding on ficus. There were no significant differences in examined parameters between individuals of 2nd-instar nymphs from lemon and fern (Tab. 1).

Among the females of *C. hesperidum,* the largest individuals were observed from *N. biserrata*, and the smallest body size was found for females feeding on ficus. Their body width was over 2-fold, and length about 1.5-fold smaller than in the case of individuals from lemon and fern. Like in the case of 2nd-instar nymphs, no significant differences were observed in examined parameters between individuals of females from lemon and fern (Tab. 1).

5.1.2. Demographic parameters

The study concerning life cycle of *C. hesperidum* demonstrated differences in the values of examined demographical parameters for individuals developing on lemon, ficus and fern (Tab. 2). Significant differences were noted between the time of pre-reproductive period and females average daily fecundity only between scale insects feeding on lemon and ficus. Differences between the length of reproductive period depending on host species were insignificant.

Table 1. Mean body width and length (mm ± SE) of the developmental stages of *Coccus hesperidum* on studied plant species

Tabela 1. Średnia szerokość i długość ciała (mm ± SE) stadiów rozwojowych *Coccus hesperidum* na badanych gatunkach roślin

Developmental stages Stadium rozwojowe	Host plant Roślina żywicielska	Body width Szerokość ciała (mm)		Body length Długość ciała (mm)	
		$\bar{x}$ ±SE	limiting graniczna	$\bar{x}$ ±SE	limiting graniczna
L_1	*C. limon* var. Ponderosa	0.189 b ± 0.004	0.161–0.232	0.400 ab ± 0.012	0.298–0.513
	F. benjamina	0.171 b ± 0.006	0.116–0.235	0.338 b ± 0.011	0.262–0.467
	N. biserrata	0.238 a ± 0.012	0,175–0,420	0,431 a ± 0.029	0.241–0.779
$F_{2.69}$ P		18.342 $4.092E^{-7}$		6.435 0.00273	
L_2♀	*C. limon* var. Ponderosa	0.739 a ± 0.051	0.402–1.253	1.592 a ± 0.081	0.916–2.277
	F. benjamina	0.334 b ± 0.015	0.187–0.457	0738 b ± 0.019	0.557–0.943
	N. biserrata	0.699 a ± 0.064	0.239–1.433	1308 a ± 0105	0.715–2.591
$F_{2.69}$ P		23.540 $1.607E^{-8}$		47.296 $3.14E^{-11}$	
♀	*C. limon* var. Ponderosa	1.199 a ± 0,060	0.721–2.032	2.224 a ± 0.084	1.314–3.192
	F. benjamina	0.757 b ± 0,070	0.394–1.296	1.552 b ± 0.096	0.986–2.505
	N. biserrata	1.479 a ± 0.080	0.591–2.107	2,448 a ± 0,110	0.925–3.281
$F_{2.69}$ P		22.813 $3.120E^{-8}$		19.304 $2.626E^{-7}$	

Values for a given life stage signed by different letters indicate statistically significant differences at $P \leq 0.01$ (Tukey's *HSD* test)

Wartości dla danych stadiów rozwojowych oznaczone innymi literami różnią się istotnie przy $P \leq 0,01$ (test *HSD* Tukeya)

Elongation of pre-reproductive period, shortening of reproductive period and the lowest daily fecundity of females were characteristic for *C. hesperidum* individuals feeding on *F. benjamina*. In turn, the shortest pre-reproductive period, elongated reproductive period and highest fecundity of females were characteristic for individuals of *C. hesperidum* feeding on lemon compared to individuals from other plant species (Tab. 2).

Table 2. Selected bionomic data of *Coccus hesperidum* L. feeding on three host plant species

Tabela 2. Wybrane parametry demograficzne *Coccus hesperidum* L. żerującego na trzech gatunkach roślin żywicielskich

Host plant Roślina żywicielska	Pre--reproductive period (days) Okres prereprodukcji (dni) $\bar{x}$ ± SE	Reproductive period (days) Okres reprodukcji (dni) $\bar{x}$ ± SE	Daily fecundity of female Płodność dzienna jednej samicy (szt) (sp)		Mortality Śmiertelność (%) $\bar{x}$ ± SE	
			average średnia $\bar{x}$ ± SE	limiting graniczna	L_1	L_2♀
Citrus limon var. Ponderosa	24.50 b ± 1.118	13.00 ± 0.894	11.20 a ± 1.899	1–39	96.29 b ± 0.062	8.13 b ± 0.002
Ficus benjamina	31.50 a ± 0.880	8.00 ± 0.707	3.88 b ± 0.437	1–9	98.02 a ± 0.0004	85.30 a ± 0.012
Nephrolepis biserrata	26.50 ab ± 1.154	11.00 ± 1.702	9.24 ab ± 1.233	2–23	39.55 c ± 0.017	7.64 c ± 0.009
$F_{2.42}$ $F_{2.72}$ P	10.685 0.0023	4.524 0.0343	 8.099 0.0006		 271.180 $1 \cdot 10^{-14}$	 249.440 $1 \cdot 10^{-14}$

Values in columns signed by different letters indicate statistically significant differences at $P \leq 0.01$ (Tukey's *HSD* test)

Wartości w kolumnach oznaczone innymi literami różnią się istotnie przy $P \leq 0{,}01$ (test *HSD* Tukeya)

The analysis demonstrated the highest, over 98% mortality of the first-instar nymphs from *F. benjamina* and over 96% from *C. limon* var. Ponderosa. In turn, very low mortality of first and second-instar nymphs was noted on *N. biserrata*. Statistical analysis confirmed the occurrence of significant differences in values of both nymphal instars mortality between individuals from all host plant species studied (Tab. 2).

The conducted observations demonstrated the occurrence of significant differences in age structure of scale insects colonies on *C. limon* var. Ponderosa ($F_{2.12} = 1202$; $P = 1.543E^{-8}$), *F. benjamina* ($F_{2.12} = 2268.5$; $P = 2.304E^{-9}$) and *N. biserrata* ($F_{2.12} = 511$; $P = 1.988E^{-7}$). High share of the first-instar nymphs and significantly lower of second-instar nymphs and females was noted on all studied plant species (Fig. 2).

Colony of scale insects feeding on *C. limon* var. Ponderosa were characterized by significantly high percentage share of first-instar nymphs (66% of total colony abundance) and nearly 4-fold lower share of second-instar nymphs and females compared to contribution of the first-instar nymphs (Fig. 2). For scale insects colony occurring on *F. benjamina* nearly 80% share of first-instar nymphs was characteristic, while females contribution on this plant was almost 10-fold lower (compared to the first-instar nymphs). Colony of scale insects on *N. biserrata* was composed of about 50% first-instar nymphs, while the share of

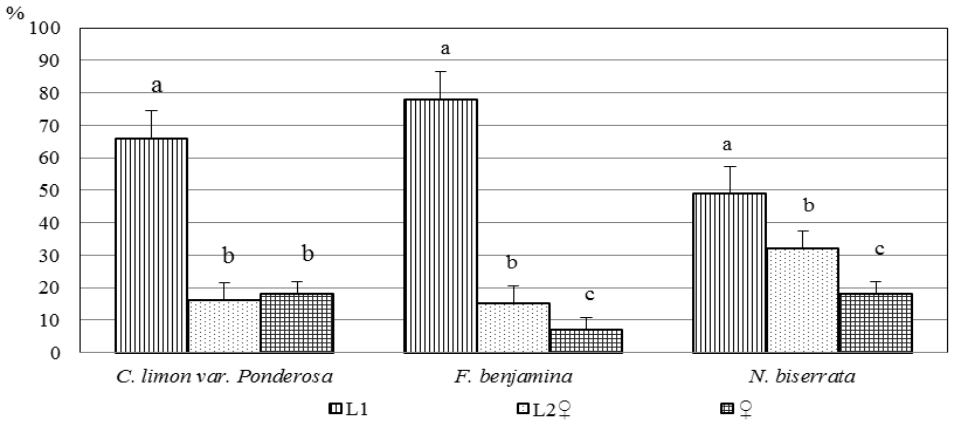


Fig. 2. Age structure of *Coccus hesperidum* colony on three host plant species. Values for a given plant signed by different letters indicate statistically significant differences at $P \leq 0.01$ (Tukey's *HSD* test)

Rys. 2. Struktura wiekowa populacji *Coccus hesperidum* na trzech gatunkach roślin żywicielskich. Dla każdego gatunku rośliny wartości oznaczone innymi literami różnią się istotnie dla $P \leq 0{,}01$ (test *HSD* Tukeya)

second-instar and females on the plant was significantly lower, about 1.3 and 3-fold, respectively, compared to the share of the youngest instar nymphs (Fig. 2).

Age structure of *C. hesperidum* colony varied depending on host plant species. Comparison of percentage contribution of subsequent development stages of *C. hesperidum* from *C. limon* var. Ponderosa, *F. benjamina* and *N. biserrata* demonstrated the occurrence of statistically significant differences in the share of first-instar ($F_{2.12} = 637$; $P = 1.030E^{-7}$) and second-instar nymphs ($F_{2.12} = 273$; $P = 1.284E^{-6}$) as well as females ($F_{2.12} = 34.09677$; $P = 0.000529$).

Colony of *C. hesperidum* on all studied plant species was distinquished by highest percentage share of first-instar nymphs and lower of subsequent developmental stages (Fig. 2). The similar percentage share of second-instar nymphs and females was noted for individuals from *C. limon* var. Ponderosa. Among scale insects feeding on ficus and fern, the percentage of females was the lowest. However, colony of *C. hesperidum* from *N. biserrata* was distinguished by significantly high percentage of second-instar nymphs and females, and significantly low share of first-instar nymphs compared to age structure of brown soft scale occurring on *C. limon* var. Ponderosa and *F. benjamina* (Fig. 2).

5.1.3. The plant acceptance and colonization by scale insects

5.1.3.1. Plants acceptance by scale insects (Free-choice test)

Comparison of mean number of *C. hesperidum* individuals on the plants demonstrated that *C. limon* var. Ponderosa was the most abundantly colonized host species (Fig. 3). Mean number of individuals on lemon was over 3-fold

higher compared to the abundance of insects colony from ficus, and 1.5-fold higher compared to the fern. Statistical analysis confirmed significantly higher colonization of *C. limon* var. Ponderosa only with respect to abundance of scale insects colonizing *F. benjamina* ($F_{1.14} = 8.39$; $P = 0.0003$).

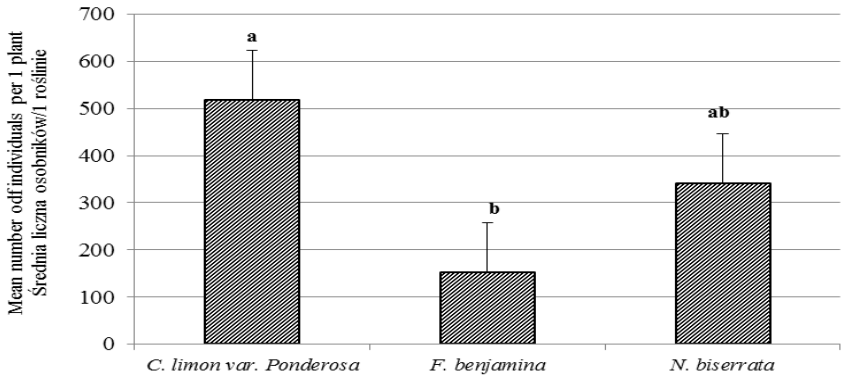


Fig. 3. Mean abundance of *Coccus hesperidum* observed on studied plant species during entire period of observation. Values for a given plant signed by different letters indicate statistically significant differences at $P \leq 0.01$ (Tukey's *HSD* test)

Rys. 3. Średnia liczebność osobników *Coccus hesperidum* na badanych gatunkach roślin w czasie trwania doświadczenia. Średnie oznaczone różnymi literami dla gatunku rośliny różnią się statystycznie dla $P \leq 0{,}01$ (test *HSD* Tukeya)

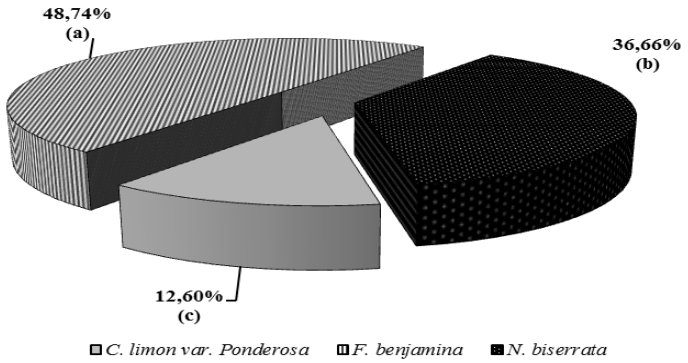


Fig. 4. Level of studied plant species acceptance in a 'free' choice test. Values signed by different letters indicate statistically significant differences at $P \leq 0.01$ (Tukey's *HSD* test)

Rys. 4. Stopień akceptacji badanych gatunków roślin w teście swobodnego wyboru. Średnie oznaczone różnymi literami różnią się statystycznie dla $P \leq 0{,}01$ (test *HSD* Tukeya)

The tested host plants species differed significantly in the attractiveness for *C. hesperidum* ($F_{2.15} = 11077.39$; $P = 1 \cdot 10^{-14}$) (Fig. 4). Nearly 50% of mobile instar nymphs of *C. hesperidum* have chosen *F. benjamina* for a suitable host for feeding. In the free-choice test, *C. limon* var. Ponderosa was accepted almost 4-fold

less as compared to ficus. The feeding on fern and lemon was chosen by 25% and 75%, lower number of mobile instar nymphs than on ficus, respectively.

The observations conducted pointed at the occurrence of differences in degree of *C. limon* var. Ponderosa, *F. benjamina* and *N. biserrata* colonization by *C. hesperidum* depending on plant species ($F_{2.147}$ = 8.398; P = 0.0003) and date of observation ($F_{14.150}$ = 131.482; P = $1 \cdot 10^{-14}$).

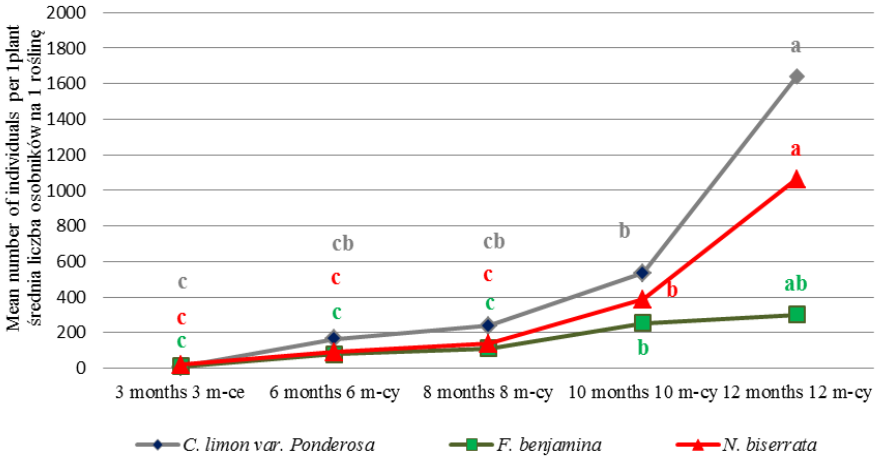


Fig. 5. Population dynamics of *Coccus hesperidum* on studied plant species. Values for host plant signed by different letters at individual dates of observation are statistically different at P ≤ 0,01 (Tukey's *HSD* test)

Rys. 5. Dynamika rozwoju *Coccus hesperidum* na badanych roślinach żywicielskich. Średnie oznaczone różnymi literami dla roślin żywicielskich w określonych datach obserwacji różnią się statystycznie dla P ≤ 0,01 (test *HSD* Tukeya)

Mean abundance of scale insects observed on all plant species at the third month from the beginning of observations was low (Fig. 5). However, since the sixth month from the beginning of the experiment, lemon was the most abundantly colonized plant species. The strongest, over 20-fold increase in *C. hesperidum* abundance was noted on it between the 3rd and 6th month of observation. After 12 months of observation, *C. hesperidum* colony reached the highest abundance on *C. limon* var. Ponderosa. Significantly lower rate of scale insect development was observed on *N. biserrata* and *F. benjamina*. After 12 months of observation, scale insects abundance on fern and on ficus was over 1.5-fold and 5-fold lower, respectively, than on the lemon (Fig. 5).

5.1.3.2. Differences in anatomical structure of host plant leaves and their effect on the level of plant acceptance by *Coccus hesperidum*

Analysis of anatomical structure of the examined plant species leaves demonstrated the occurrence of highly significant differences in features observed such as: leaf blade thickness, thickness of upper and lower leaf blade

epidermis, thickness of external wall of epidermal cells of both sides of leaf blades and number of stomata on lower epidermis and phloem distance from lower leaf side (Tab. 3). No trichomes of mechanical, secretory type or glandular trichomes were observed on the leaves of the examined plant species. Leaves of *C. limon* var. Ponderosa were characterized by nearly 2-fold thicker leaf blade at main nerve, compared to *F. benjamina* and *N. biserrata*. The thickness of leaf blade measured about 100 μm behind the main nerve was the lowest for the leaves of *F. benjamina*. The highest upper epidermis thickness was characteristic for the leaves of *N. biserrata*. The thinnest lower epidermis was observed in the leaves of *C. limon* var. Ponderosa, and it was ca. 20% thinner than in *F. benjamina* and about 30% than in *N. biserrata*. The analysis of leaf tissues cross-sections demonstrated that the upper epidermis of *C. limon* var. Ponderosa and *N. biserrata* was covered outside with about 2-fold thicker wall with respect to analogical cells observed on the lower leaf side. The phloem distance from the lower leaf side was the shortest in lemon as compared to ficus and fern. The longest distance from the lower leaf side was noted in the case of phloem in ficus. Subsequent plant species differed in a number of stomata observed in lower epidermis. Their highest number was noted on the leaves of *C. limon* var. Ponderosa., their number on leaves of *F. beniamina* was 3.5 – fold lower, while on *N. biserrata* it was as much as 15 – fold lower than in *C. limon* var. Ponderosa (Tab. 3).

The analysis of correlation confirmed the occurrence of significant, negative relationship between the degree of plants acceptance and leaf blade thickness at the main nerve ($r = -0.935$; $P = 0.002$) and 100 μm behind the main nerve ($r = -0.854$; $P = 0.003$).

5.1.4. *Coccus hesperidum* feeding behaviour and honeydew excretion on different host plants

5.1.4.1. Feeding behaviour

Observations performed during the feeding of soft brown scale pointed to the occurrence of the main EPG waveforms found in the other groups: C: total pathway, pd: potential drops, E11: sieve element salivation, E2: ingestion of phloem sap. No waveform G: xylem sap ingestion and F: derailed stylet mechanics were observed.

The results of EPG tests proved that during feeding on the studied plant species, *C. hesperidum* spent the most time on phloem sap ingestion: E2 waveform (31.67–67.01%) (Fig. 6). The frequency of the other feeding activities in decreasing order were: penetration of peripheral tissues (epidermis and mesophyll): C waveform (pathway) (24.69–53.48%), waveform pd (potential drops): (3.91–12.36%) and sieve element salivation: E11 waveform (2.49–4.60%) (Fig. 4).

Table 3. Selected features of anatomical structure of leaves of the examined plant species

Tabela 3. Wybrane cechy budowy anatomicznej liści badanych gatunków roślin

Host plant Roślina żywicielska	Leaf blade thickness Grubość blaszki liściowej (µm) $\bar{X}$ ±SE		Epidermis thickness Grubość warstwy epidermy (µm) $\bar{X}$ ±SE		Thickness of external wall of epidermal cells Grubość zewnętrznej ściany komórek epidermy (µm) $\bar{X}$ ±SE		Number of stomata per 1 mm^2 of lower epidermis Liczba aparatów szparkowych na 1 mm^2 epidermy dolnej $\bar{X}$ ±SE	Phloem distance from lower leaf side Odległość floemu od dolnej strony liścia (µm) $\bar{X}$ ±SE
	at main nerve w nerwie głównym	ca.100 µm behind main nerve około100 µm za nerwem głównym	upper górna	lower dolna	upper górna	lower dolna		
Citrus limon var. Ponderosa	977.09 a ± 3.028	322.87 a ± 0.049	17.00 b ± 0.004	12.18 c ± 0.154	5.17 a ± 0.004	2.66 b ± 0.025	543.87 a ± 3.878	155.09 c ± 2.03
Ficus benjamina	533.02 b	241.60 c	14.50 c	15.22 b	5.06 a	4.49 a	150.82 b	181.38 a
Nephrolepis biserrata	522.60 c ± 1.366	308.38 b ± 0.023	19.16 a ± 0.344	16.90 a ± 0.217	4.74 b ± 0.069	2.56 b ± 0.061	36.30 c ± 0.141	167.72 b ± 2.357
$F_{(2.27)}$ P	14853.83 $1{\cdot}10^{-14}$	18790.90 $1{\cdot}10^{-14}$	88.40 $1.41E^{-12}$	209.02 $1{\cdot}10^{-14}$	14.13 0.00006	653.36 $1{\cdot}10^{-14}$	13172.51 $1{\cdot}10^{-14}$	34,85 $3.774E^{-8}$

Values in columns signed by different letters indicate statistically significant differences at $P \leq 0.01$ (Tukey's *HSD* test)

Średnie w kolumnach oznaczone innymi literami różnią się istotnie przy $P \leq 0{,}01$ (test *HSD* Tukeya)

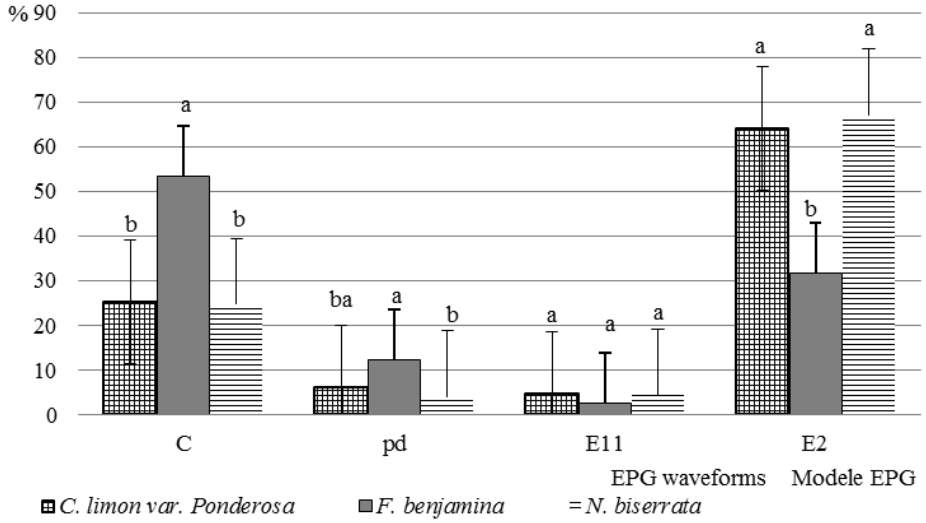


Fig. 6. Mean percentage share of subsequent feeding activities of *Coccus hesperidum* on three host plant species; C – penetration of peripheral tissues, pd – potential drops, E11 – sieve element salivation, E2 – ingestion of phloem sap. Means for waveform signed by different letters are different at $P \leq 0.01$ (Tukey's *HSD* test)

Rys. 6. Porównanie średniego procentowego udziału poszczególnych aktywności związanych z żerowaniem *Coccus hesperidum* na trzech badanych gatunkach roślin żywicielskich; C – nakłuwanie tkanek peryferyjnych, pd – spadki napięcia, E11 – nakłuwanie elementów floemu, E2 – pobieranie soku floemowego. Średnie dla modeli żerowania oznaczone różnymi literami różnią się istotnie przy $P \leq 0{,}01$ (test *HSD* Tukeya)

Statistical analysis confirmed significance of differences in percentage share of C waveform ($F_{2.57} = 7.565$; $P = 0.0012$), pd waveform ($F_{2.57} = 4.787$; $P = 0.0119$), and E2 waveform ($F_{2.57} = 9.507$; $P = 0.0002$). No differences in E11 waveform share ($F_{2.57} = 0.95460$; $P = 0.391$) were noted on the examined plant species (Fig. 6).

No statistically significant differences in the total duration of the *C. hesperidum* feeding behaviour on *C. limon* var. Ponderosa and *N. biserrata* (G test = 0.6; df = 3; P = 0.896) were found. The duration of EPG waveforms observed on the two plant species differed significantly from this noted on *F. benjamina*. *C. hesperidum* females feeding on *C. limon* var. Ponderosa and *N. biserrata* started plant tissues penetration sooner, penetrated peripheral tissues shorter and ingested phloem sap longer as compared to individuals from *F. benjamina* (Tab. 4).

5.1.4.2. Honeydew excretion

Honeydew excretion rate and daily excretion

Changes in honeydew excretion process of subsequent developmental stages of *C. hesperidum* were noted during individual development of scale insects (Tab. 5). Statistical analysis confirmed significant differences in honeydew excretion rate and daily excretion between subsequent developmental stages on studied

Table 4. Mean duration (min ± SE) of probing activities of *Coccus hesperidum* feeding on host plant species during 8 hours of registration

Tabela 4. Czas trwania (min ± SE) poszczególnych aktywności związanych z żerowaniem *Coccus hesperidum* na trzech badanych gatunkach roślin żywicielskich podczas 8 godzin rejestracji

EPG waveforms Modele EPG	Duration (min) of EPG waveforms on three host plants Czas trwania (min) modeli EPG na roślinach żywicielskich $\bar{x}$ ±SE		
	Citrus limon var. Ponderosa	*Ficus benjamina*	*Nephrolepis biserrata*
C	121.08 b ± 19.492	256.700 a ± 32.400	118.524 b ± 32.407
pd	29.323 c ± 5.950	59.330 b ± 13.258	18.770 c ± 8.160
E11	22.070 c ± 5.117	11.952 c ± 5.330	21.061 c ± 6.566
E2	307.526 a ± 24.768	152.020 b ± 31.330	321.644 a ± 34.686
E11 + E2	329.596 a ± 23.447	163.972 b ± 31.449	342.705 a ± 34.143
$F_{3.76}$ $F_{3.65}$ P	67.018 $1 \cdot 10^{-14}$	20.933 $5.51E^{-10}$	 25.335 $5.68E^{-11}$

EPG waveforms: C – penetration of peripheral tissues, pd – potential drops, E11 – sieve element salivation, E2 – ingestion of phloem sap

Modele EPG: C – nakłuwanie tkanek peryferyjnych, pd – spadki napięcia, E11 – nakłuwanie elementów floemu, E2 – pobieranie soku floemowego

Means values signed by different letters in rows indicate statistically significant differences at $P \leq 0.01$ (Tukey's *HSD* test)

Średnie dla modelu żerowania oznaczone innymi literami w rzędach różnią się istotnie dla $P \leq 0{,}01$ (test *HSD* Tukeya)

Table 5. Average values of honeydew excretion rate and daily excretion of particular developmental stages *Coccus hesperidum*

Tabela 5. Średnie wartości intensywności i aktywności spadziowania poszczególnych stadiów rozwojowych *Coccus hesperidum*

Developmental stages Stadium rozwojowe	Honeydew excretion rate (droplets per 1 hour) Intensywność spadziowania (krople/1 h)		Honeydew daily excretion (droplets per 24 hours) Aktywność spadziowania (krople/dobę)	
	$\bar{x}$ ± SE	limiting graniczna	$\bar{x}$ ± SE	limiting graniczna
L_1	4.905 a ± 0.399	0–26	120.498 a ± 10.96	35–401
L_2♀	2.416 b ± 0.189	0–14	55.501 b ± 5.927	11–128
♀	0.526 c ± 0.115	0–3	12.677 c ± 2.819	1–39
$F_{2.213}$ $F_{2.341}$ P	67.077 $1 \cdot 10^{-14}$		 178.507 $1 \cdot 10^{-14}$	

Values in columns signed by different letters indicate statistically significant differences at $P \leq 0.01$ (Tukey's *HSD* test)

Wartości w kolumnach oznaczone innymi literami różnią się istotnie dla $P \leq 0{,}01$ (test *HSD* Tukeya)

host plants. The values of honeydew excretion rate and daily excretion of *C. hesperidum* decreased with insect's age. Mean honeydew excretion rate was by two-fold greater in first–instar nymphs than in second–instar nymphs, and over by 9-fold greater than in females (Tab. 5).

Table 6. Honeydew excretion rate and daily excretion of subsequent developmental stages of *Coccus hesperidum* feeding on three host plant species

Tabela 6. Intensywność i aktywność spadziowania poszczególnych stadiów rozwojowych *Coccus hesperidum* na trzech gatunkach roślin żywicielskich

Developmental stages Stadium rozwojowe	Host plant Roślina żywicielska	Honeydew excretion rate (droplets per 1 hour) Intensywność spadziowania (krople/1 h)		Honeydew daily excretion (droplets per 24 hours) Aktywność spadziowania (krople/doba)	
		$\bar{x}$ ±SE	limiting graniczna	$\bar{x}$ ± SE	limiting graniczna
L_1	*Citrus limon* var. Ponderosa	5.330 a ± 0.179	1–26	127.920 a ± 12.25	52–401
	Ficus benjamina	4.107 b ± 0.145	0–18	98.568 b ± 7.62	45–212
	Nephrolepis biserrata	5.280 a ± 0.136	0–18	126.72 a ± 9.484	35–254
$F_{2.69}$ $F_{2.107}$ P		24.129 $1.134E^{-8}$		3.201 0.04464	
$L_2♀$	*Citrus limon* var. Ponderosa	2.355 d ± 0.057	0–7	56.520 c ± 3.00	14–90
	Ficus benjamina	2.123 d ± 0.126	0–14	50.952 c ± 5.256	11–120
	Nephrolepis biserrata	2.772 c ± 0.075	0–10	66.528 c ± 4.215	13–128
$F_{2.69}$ $F_{2.107}$ P		10.945 0.00007		1.973 0.1438	
♀	*Citrus limon* var. Ponderosa	0.324 g ± 0.015	0–3	7.776 e ± 0.825	1–22
	Ficus benjamina	0.531 f ± 0.181	0–3	12.744 e ± 1.286	1–27
	Nephrolepis biserrata	0.725 e ± 0.0163	0–1	17.400 d ± 1.668	1–39
$F_{2.69}$ $F_{2.107}$ P		144.438 $1 \cdot 10^{-14}$		19.192 $5.862E^{-8}$	

Values for a given life stage signed by different letters indicate statistically significant differences at $P \leq 0.01$ (Tukey's *HSD* test)

Wartości dla danego stadium oznaczone innymi literami różnią się istotnie dla $P \leq 0{,}01$ (test *HSD* Tukeya)

The honeydew excretion rate and daily excretion were by 22% greater in 1st-instar nymphs feeding on lemon and fern than on ficus (Tab. 6). Statistical analysis confirmed significantly lower value of honeydew indicators for the 1st-instar nymphs feeding on ficus compared to the same developmental stage from *C. limon* and *N. biserrata* (Tab. 6). Second-instar nymphs feeding on fern were characterized by significantly high values of honeydew excretion rate compared to the values of this parameter for individuals from lemon and ficus. Honeydew daily excretion of 2nd-instar nymphs were the highest for scale insects feeding on fern, but did not differ significantly compared to honeydew daily excretion of this developmental stage feeding on lemon and ficus. Among females, significantly high excretion rate and daily excretion were characteristic for individuals from *N. biserrata*. Compared to them, individuals from *C. limon* var. Ponderosa excreted almost 40%, and from ficus 30% lower amount of honeydew in analyzed time units. Statistical analysis confirmed significant differences in honeydew excretion rate of females feeding on the examined plant species, and high honeydew daily excretion of females from *N. biserrata* compared to females from lemon and ficus (Tab. 6).

Dimensions of honeydew droplets

The occurrence of differences in mean values of dimensions expressed as its diameter and area of honeydew droplets excreted by subsequent developmental stages of *C. hesperidum* were demonstrated (Tab. 7).

Table 7. Average value of diameter and area of honeydew droplets excreted by particular developmental stages *Coccus hesperidum*

Tabela 7. Średnie wartości średnicy i powierzchni kropli spadzi wydalanej przez poszczególne stadia rozwojowe *Coccus hesperidum*

Developmental stages Stadium rozwojowe	Dimensions of honeydew droplets Właściwości fizyczne kropli spadzi			
	diameter – średnica (mm)		area – powierzchnia (mm^2)	
	$\bar{x}$ ± SE	limiting graniczna	$\bar{x}$ ± SE	limiting graniczna
L_1	0.305 c ± 0.072	0.180–0.439	0.075 c ± 0.003	0.023–0.156
L_2♀	0.527 b ± 0.019	0.280–0.833	0.241 b ± 0.016	0.062–0.545
♀	0.627 a ± 0.015	0.426–0.878	0.320 a ± 0.015	0.152–0.633
$F_{2.222}$ P	128.358 $1 \cdot 10^{-14}$		93.892 $1 \cdot 10^{-14}$	

Values in columns signed by different letters indicate statistically significant differences at $P \leq 0.01$ (Tukey's *HSD* test)

Wartości w kolumnach oznaczone innymi literami różnią się istotnie dla $P \leq 0{,}01$ (test *HSD* Tukeya)

The growth in analyzed parameters values was noted with the age of insects. The droplets of the smallest diameter were excreted by the first-instar nymphs of *C. hesperidum*. Honeydew droplets of the highest analyzed physical properties were excreted by females. Statistical analysis confirmed the occurrence of significantly higher values of diameter and surface of honeydew droplet for older developmental stages of *C. hesperidum* (Tab. 7).

The differences in honeydew droplet size of the same developmental stage depending on host plant species were demonstrated (Tab. 8).

Table 8. Comparison of the mean value of diameter and area of honeydew droplets excreted by subsequent developmental stages of *Coccus hesperidum* on three host plants

Tabela 8. Porównanie średnich wartości średnicy i powierzchni kropli spadzi wydalanej przez poszczególne stadia rozwojowe *Coccus hesperidum*

Developmental stages Stadium rozwojowe	Host plant Roślina żywicielska	Dimensions of honeydew droplets Właściwości fizyczne kropli spadzi			
		diameter – średnica (mm)		area – powierzchnia (mm^2)	
		$\bar{x}$ ±SE	limiting graniczna	$\bar{x}$ ±SE	limiting graniczna
L_1	*Citrus limon* var. Ponderosa	0.237 c ± 0.077	0.180–0.305	0.045 c ± 0.003	0.023–0.073
	Ficus benjamina	0.371 a ± 0.071	0.306–0.439	0.107 a ± 0.044	0.070–0.156
	Nephrolepis biserrata	0.307 b ± 0.019	0.294–0.331	0.074 b ± 0.012	0.057–0.086
$F_{2.72}$ P		118.920 $1 \cdot 10^{-14}$		94.380 $1 \cdot 10^{-14}$	
L_2♀	*Citrus limon* var. Ponderosa	0.710 d ± 0.118	0.609–0.833	0.399 d ± 0.013	0.292–0.545
	Ficus benjamina	0.555 e ± 0.522	0.512–0.596	0.244 e ± 0.005	0.196–0.299
	Nephrolepis biserrata	0.317 fb ± 0.325	0.280–0.340	0.079 fb ± 0.002	0.061–0.091
$F_{2.72}$ P		660.280 $1 \cdot 10^{-14}$		364.875 $1 \cdot 10^{-14}$	
♀	*Citrus limon* var. Ponderosa	0.761 g ± 0.114	0.701–0.878	0.461 g ± 0.014	0.386–0.633
	Ficus benjamina	0.614 h ± 0.152	0.472–0.785	0.300 h ± 0.017	0.177–0.482
	Nephrolepis biserrata	0.506 i ± 0.148	0.426–0.667	0.198 i ± 0.015	0.015–0.349
$F_{2.72}$ P		84.725 $1 \cdot 10^{-14}$		76.127 $1 \cdot 10^{-14}$	

Values in columns for a given life stage indicate statistically significant differences at $P \leq 0.01$ (Tukey's *HSD* test)
Wartości w kolumnach oznaczone innymi literami różnią się istotnie dla $P \leq 0{,}01$ (test *HSD* Tukeya)

For first-instar nymphs, honeydew droplets excreted by individuals from lemon were characterized by the lowest diameter and area while the highest

values were obtained for the droplets excreted by these developmental stages feeding on ficus. Statistical analysis confirmed differences in diameter and area of honeydew droplets excreted by first-instar nymphs feeding on lemon, ficus and fern (Tab. 8).

Among second-instar nymphs, the honeydew droplets of the highest diameter and area were excreted by individuals from lemon. Their diameter was 2,2-fold and 1.3-fold higher, while area 5-fold and 1,6-fold higher, respectively, compared to the dimensions of honeydew droplet excreted by individuals from fern and ficus. Honeydew droplets of the lowest value of diameter and area were excreted by second-instar nymphs feeding on fern (Tab. 8).

Similar relationships were noted in the size of honeydew droplets excreted by females. The highest diameter and area was found in the case of droplets excreted by females feeding on lemon while females from fern excreted honeydew droplets of the lowest diameter and area (Tab. 8).

5.2. The response of *Coccus hesperidum* to host plant biochemical properties

5.2.1. Primary metabolites concentration in the non-infested host plant leaves

The results of chemical analysis demonstrated that the leaves of *C. limon* var. Ponderosa, *F. benjamina* and *N. biserrata* differed significantly in their content of primary metabolites such as total sugars, reducing sugars, protein nitrogen, total amino acids, essential and non-essential amino acids. No significant differences were noted for total nitrogen, soluble nitrogen and total protein (Tab. 9).

F. benjamina leaves contained significantly higher amount of sugars (total and reducing ones) and protein nitrogen compared to their concentration in the leaves of other two plant species. The leaves of *C. limon* var. Ponderosa and *N. biserrata* were characterized by significantly lower concentration of mentioned primary metabolites. About 2-fold higher concentration of total sugars and about 3-fold higher content of reducing sugars was noted in leaves of *F. benjamina* compared to the content of these metabolites in the leaves of other two species. Considerably higher content of protein nitrogen was also found in leaves of ficus compared to its content in the leaves of *C. limon* var. Ponderosa and *N. biserrata*. However, high content of essential and non-essential amino acids in non-infested plants (control) was characteristic for the leaves of *N. biserrata*. The content of essential amino acids in fern leaves was nearly four times higher compared to lemon, and about three fold higher compared to ficus. The leaves of *N. biserrata* were also distinguished by higher concentration of non-essential amino acids compared to these metabolites concentration in leaves of lemon and ficus, of 37% and 48%, respectively.

Table 9. Concentration of primary metabolites (sugars. nitrogen and protein) and content of amino acids in leaves of non-infested plants
Tabela 9. Stężenie metabolitów pierwotnych (cukrów, azotu i białka) oraz zawartość aminokwasów w liściach roślin kontrolnych

Host plant Roślina żywicielska	Sugars – Cukry (%) $\bar{x}$ ±SE		Nitrogen – Azot (%) $\bar{x}$ ±SE			Protein Białko (%) $\bar{x}$ ±SE	Amino acids – Aminokwasy (mg · g) $\bar{x}$ ±SE		
	total ogółem	reducing redukujące	total ogółem	protein białkowy	soluble rozpuszczalny	total ogółem	total ogółem	essential egzogenne	non-essential endogenne
Citrus limon var. Ponderosa	3.98 b ± 0.0371	2.15 b ± 0.300	2.684 a ± 0.031	0.020 b ± 0.001	2.663 a ± 0.002	16.760 a ± 0.026	3.078 b ± 0.069	0.309 c ± 0.008	2.768 b ± 0.061
Ficus benjamina	7.43 a ± 0.0633	6.03 a ± 0.470	2.804 a ± 0.052	0.059 a ± 0.0008	2.747 a ± 0.032	17.513 a ± 0.205	2.99 b ± 0.034	0.43 b ± 0.013	2.561 c ± 0.021
Nephrolepis biserrata	3.63 c ± 0.056	2.05 b ± 0.123	2.69 a ± 0.012	0.015 b ± 0.0009	2.675 a ± 0.011	16.812 a ± 0.078	4.99 a ± 0.0003	1.186 a ± 0.0001	3.805 a ± 0.0002
$F_{2.6}$ P	1553.931 $7.154E^{-9}$	1877.815 $4.058E^{-9}$	4.83 0.0561	580.333 $1.360E^{-7}$	4.930 0.0542	10.890 0.0108	636.054 $1.035E^{-7}$	2635.221 $1.40E^{-9}$	320.722 $7.959E^{-7}$

Values in columns signed by different letters indicate statistically significant differences at $P \leq 0.01$ (Tukey's *HSD* test)
Wartości w kolumnach oznaczone tymi samymi literami nie różnią się istotnie dla $P \leq 0{,}01$ (test *HSD* Tukeya)

Table 10. Statistically significant correlations between the biochemical content of host plant leaves and the body size, the length of the pre-reproductive and reproductive period of *Coccus hesperidum*

Tabela 10. Statystycznie istotne korelacje pomiędzy właściwościami biochemicznymi roślin a rozmiarami ciała, długością okresu prereprodukcji i reprodukcji *Coccus hesperidum*

Analyzed parameters Analizowane parametry		Pearson's correlation coefficients Wartości współczynników korelacji r – Pearsona	
		r	P
Total amino acids Aminokwasy ogółem	body size wielkość ciała	0.8133	0.007
Essential amino Aminokwasy egzogenne		0.828	0.005
Non-Essential amino acids Aminokwasy endogenne		0.788	0.01
Total sugars : Total protein Cukry ogółem : Białko ogółem		-0.688	0.04
Total sugars Cukry ogółem	pre-reproductive period okres prereprodukcji	0.704	0.03
Reducing sugars Cukry redukujące		0.724	0.02
Protein nitrogen Azot białkowy		0.717	0.02
Tannins Garbniki		-0.696	0.03
p-hydroxybenzoic acid Kwas *p*-hydroksybenzoesowy		-0.749	0.02
Total sugars Cukry ogółem	reproductive period okres reprodukcji	-0.698	0.03
Reducing sugars Cukry redukujące		-0.727	0.02
Total protein Białko ogółem		-0.769	0.01
Total nitrogen Azot ogółem		-0.778	0.001
Soluble nitrogen Azot rozpuszczalny		-0.773	0.01
Tannins Garbniki		0.759	0.01
p-hydroxybenzoic acid Kwas *p*-hydroksybenzoesowy		0.726	0.02

Table 11. Statistically significant correlations between the biochemical content of host plant leaves and instar nymphs mortality, scale insects colony abundance, plants acceptance, feeding behaviour of *Coccus hesperidum*

Tabela 11. Statystycznie istotne korelacje pomiędzy właściwościami chemicznymi roślin żywicielskich a śmiertelnością stadiów larwalnych i liczebnością kolonii czerwców, stopniem akceptacji roślin oraz modelami żerowania *Coccus hesperidum*

Analyzed parameters Analizowane parametry		Pearson's correlation coefficients Wartości współczynników korelacji r – Pearsona	
		r	P
Total amino acids Aminokwasy ogółem	instar nymphs mortality śmiertelność stadiów larwalnych	-0.783	0.004
Essential amino acids Aminokwasy egzogenne		-0.783	0.004
Non-essential amino acids Aminokwasy endogenne		-0.783	0.004
Flawonoids Flawonoidy		0.987	$7.686E^{-7}$
Syringic acid Kwas syryngowy		0.789	0.01
Essential amino acids Aminokwasy egzogenne	abundance liczebność	r = 0.685	0.04
Total sugars Cukry ogółem	host plant acceptance akceptacja roślin żywicielskich	0.698	0.03
Reducing sugars Cukry redukujące		0,741	0.02
Total nitrogen Azot ogółem		0.706	0.03
Protein nitrogen Azot białkowy		0,707	0.03
Total protein Białko ogółem		0,708	0.03
Tannins Garbniki		-0,997	$2.05E^{-10}$
p-hydroxybenzoic acid Kwas *p*-hydroksybenzoesowy		-0.781	0.01
Total sugars Cukry ogółem	E11 waveform model E11	-0.999	0.01
Flawonoidy Flawonoidy	pd waveform model pd.	0.997	0.04

Table 12. Statistically significant correlations between biochemical content of host plant leaves and *Coccus hesperidum* honeydew rate excretion

Tabela 12. Statystycznie istotne korelacje pomiędzy właściwościami chemicznymi roślin żywicielskich a tempem wydalania spadzi przez *Coccus hesperidum*

Analyzed parameters Analizowane parametry		Pearson's correlation coefficients Wartości współczynników korelacji r – Pearsona	
		r	P
Sugars Cukry	total ogółem	-0.972	0.00001
	reducing redukujące	-0.984	0.000001
Nitrogen Azot	total ogółem	-0.869	0.002
	protein białkowy	-0.974	0.074
	soluble rozpuszczalny	-0.757	0.01
Protein Białko	total ogółem	-0.863	0.002
Tannins Garbniki		0.787	0.01
Total sugars : Total nitrogen Cukry ogółem : Azot ogółem		-0.975	0.000007
Total sugars : Total protein Cukry ogółem : Białko ogółem		-0,778	0,01

The analysis of correlations confirmed the existence of strong positive relationship between content of total amino acids (total: r = 0.813; P = 0.007), essential amino acids (r = 0.828; P = 0.005) and non-essential amino acids (r = 0.788; P = 0.01) in plant leaves and scale insects body size (width) (Tab. 10). The size of scale insects body was affected by proportions between the content of total sugars and total protein (r = -0.688; P = 0.04)

The increased content of total sugars (r = 704; P = 0.03) and reducing sugars (r = 0.724; P = 0.02) as well as protein nitrogen (r = 0.717; P = 0.02) in the leaves of the plant species affected elongation of pre-reproductive period of insects (Tab. 10).

Negative relationship between the length of reproductive period and content of total sugars (r = -0.698; P = 0.03) and reducing sugars (r = -0.727; P = 0.02) in the leaves was noted. Decreased content of protein and nitrogen in leaves of examined plant species influenced the elongation of reproductive period

(total protein: r = -0.769; P = 0.01, total nitrogen: r = -0.778; P = 0.001; soluble nitrogen: r = -0.773; P = 0.01) (Tab. 10).

Table 13. Statistically significant correlations between the biochemical content of host plant leaves and dimension of honeydew droplets excreted by *Coccus hesperidum*

Tabela 13. Statystycznie istotne korelacje pomiędzy właściwościami chemicznymi roślin żywicielskich a wielkością kropli spadzi wydalanych przez *Coccus hesperidum*

Analyzed parameters Analizowane parametry			Pearson's correlation coefficients Wartości współczynników korelacji r – Pearsona	
			r	P
Sugars Cukry	total ogółem	diameter średnica	0.860	0.003
		area powierzchnia	0.916	0.0005
	reducing redukujące	diameter średnica	0.874	0.002
		area powierzchnia	0.929	0.0002
Nitrogen Azot	total ogółem	diameter średnica	0.843	0.004
		area powierzchnia	0.798	0.009
	protein białkowy	diameter średnica	0.861	0.003
		area powierzchnia	0.920	0.0004
	soluble rozpuszczalny	diameter średnica	0.772	0.01
		area powierzchnia	0.684	0.04
Total protein Białko ogółem		diameter średnica	0.848	0.003
		area powierzchnia	0.797	0.01
Tannins Garbniki		diameter średnica	-0.878	0.002
		area powierzchnia	-0.847	0.003
Total sugars : Total nitrogen Cukry ogółem : Azot ogółem		diameter średnica	0.853	0.003
		area powierzchnia	0.911	0.0006
Total amino acids : Reducing sugars Aminokwasy ogółem : Cukry redukujące		diameter średnica	-0.713	0.03

Main role of various nitrogen forms as a factor affecting insects development was also confirmed in high linearly negative correlation between mortality of instar nymphs and content of total amino acids ($r = -0.783$; $P = 0.004$), essential amino acids ($r = -0.783$; $P = 0.004$) as well as non-essential ones ($r = -0.783$; $P = 0.004$) in the leaves. The higher content of essential amino acids positively affected the increase in scale insects abundance ($r = 0.685$; $P = 0.04$) (Tab. 11).

The analysis of correlations confirmed an existence of strong positive relationship between total sugars ($r = 0.698$; $P = 0.03$), reducing sugars ($r = 0.741$; $P = 0.02$), nitrogen (total: $r = 0.706$; $P = 0.03$, protein: $r = 0.707$; $P = 0.03$) and total protein ($r = 0.708$; $P = 0.03$) concentration in the leaves of host plants and level of acceptance of the plant species by mobile stages of *C. hesperidum* (Tab. 11).

Total sugars content in the leaves was negatively correlated with total time of sieve element salivation (E11 waveform) ($r = -0.999$; $P = 0.01$) (Tab. 11).

The role of sugars and nitrogen in *C. hesperidum* relations with the examined plant species is confirmed by high and statistically significant correlations between their rate in plants leaves (Tab. 12). High ratio between content of total sugars and total nitrogen ($r = -0.975$; $P = 0.000007$) as well as total sugars and total protein ($r = -0.778$; $P = 0.01$) affected the decrease in honeydew excretion rate of scale insects (Tab. 12).

Positive influence of primary metabolites (sugars, nitrogen and protein) on physical properties of honeydew droplets excreted by scale insects was confirmed (Tab. 13). High proportions between the content of total sugars and nitrogen in leaves also affected higher diameter and area of honeydew droplets excreted by scale insects.

5.2.1. Secondary metabolites concentration in the non-infested host plant leaves

The results of chemical analysis of control plants leaves demonstrated that *C. limon* var. Ponderosa, *F. benjamina* and *N. biserrata* differed in concentration of all examined secondary metabolites (Tab. 14). Significantly higher content of tannins was noted in lemon leaves. It was about 4-fold higher compared to *F. benjamina* and 2-fold higher than in *N. biserrata*. Leaves of *F. benjamina* were characterized by significantly higher content of flavonoids and the lowest content of phenolic acids and tannins compared to two other plant species. Leaves of *N. biserrata* demonstrated significantly higher content of phenolic acids and the lowest of flavonoids compared to lemon and ficus leaves. Content of phenolic acids in fern leaves was over 3-fold higher that these metabolites content in *F. benjamina* and about 2-fold compared to *C. limon* var. Ponderosa.

Table 14. Concentration of secondary metabolites in non-infested (control) leaves of host plants
Tabela 14. Stężenie wtórnych metabolitów (%) w liściach trzech gatunków roślin kontrolnych

Host plant Roślina żywicielska	Secondary metabolites – Metabolity wtórne (%) $\bar{x}$ ±SE		
	phenolic acids kwasy fenolowe	tannins garbniki	flavonoids flawonoidy
Citrus limon var. Ponderosa	0.367 b ± 0.002	9.17 a ± 0.221	0.378 b ± 0.0006
Ficus benjamina	0.219 c ± 0.003	2.40 c ± 0.110	0.412 a ± 0.0003
Nephrolepis biserrata	0.729 a ± 0.001	4.56 b ± 0.0002	0.245 c ± 0.00033
$F_{2.6}$ P	10237.96 $2.51E^{-11}$	587.72 $1.310\ E^{-7}$	35150.2 $6.22E^{-13}$

Values in columns signed by different letters indicate statistically significant differences at $P \leq 0.01$ (Tukey's *HSD* test)
Wartości w kolumnach oznaczone innymi literami różnią się istotnie przy $P \leq 0{,}01$ (test *HSD* Tukeya)

Correlation analysis showed that increased content of tannins affected shortening of pre-reproductive period (r = -0.696; P = 0.03) and elongation of reproductive period of soft brown scale (r = 0.759; P = 0.01) (Tab. 10). The analysis of correlation demonstrated an existence of strong, linearly negative relationship between the level of plant acceptance by *C. hesperidum* and tannins content in leaves of the plant species (r = -0.997; $P = 2.05E\text{-}^{10}$) (Tab. 11). In turn, an existence of linearly positive relationship between flavonoids content and mortality of instar nymphs was found (r = 0.987; $P = 7.686E^{-7}$) (Tab. 11). Positive relationship between flavonoids content and the duration of waveform pd was demonstrated (r = 0.997; P = 0.04) (Tab. 11). Scale insects honeydew excretion rate increased with growing tannins content in the leaves (r = 0.787; P = 0.01), however diameter (r = -0.878; P = 0.001) and area (r = -847; P = 0.003) of honeydew droplets excreted by *C. hesperidum* decreased (Tab. 12, 13).

Chromatographic analysis of the leaves of various plant species demonstrated the presence of phenolic acids from in the group of benzoic acid derivatives (gallic acid, *p*-hydroxybenzoic acid, protocatechuic acid, syringic acid) and *trans*-cinnamic acid (chlorogenic acid, α-resorcinol, coffeic acid, *p*-kumaric acid, feluric acid). The leaves of the plant species differed in composition, concentration and proportion of individual compounds (Tab. 15, Fig. 7 a–c).

Eight phenolic acids were identified in control leaves of *C. limon* var. Ponderosa and *N. biserrata*, while seven in *F. benjamina*. Four derivatives of benzoic and *trans*-cinnamic acid were observed in lemon, while in fern and ficus three and two derivatives of benzoic acid, respectively, and all identified derivatives from the group of *trans*-cinnamic acid, were found. *P*-hydroxybenzoic and protocatechuic acids were not identified in ficus leaves, in lemon no α-resorcinol, and in fern no syringic acid were found.

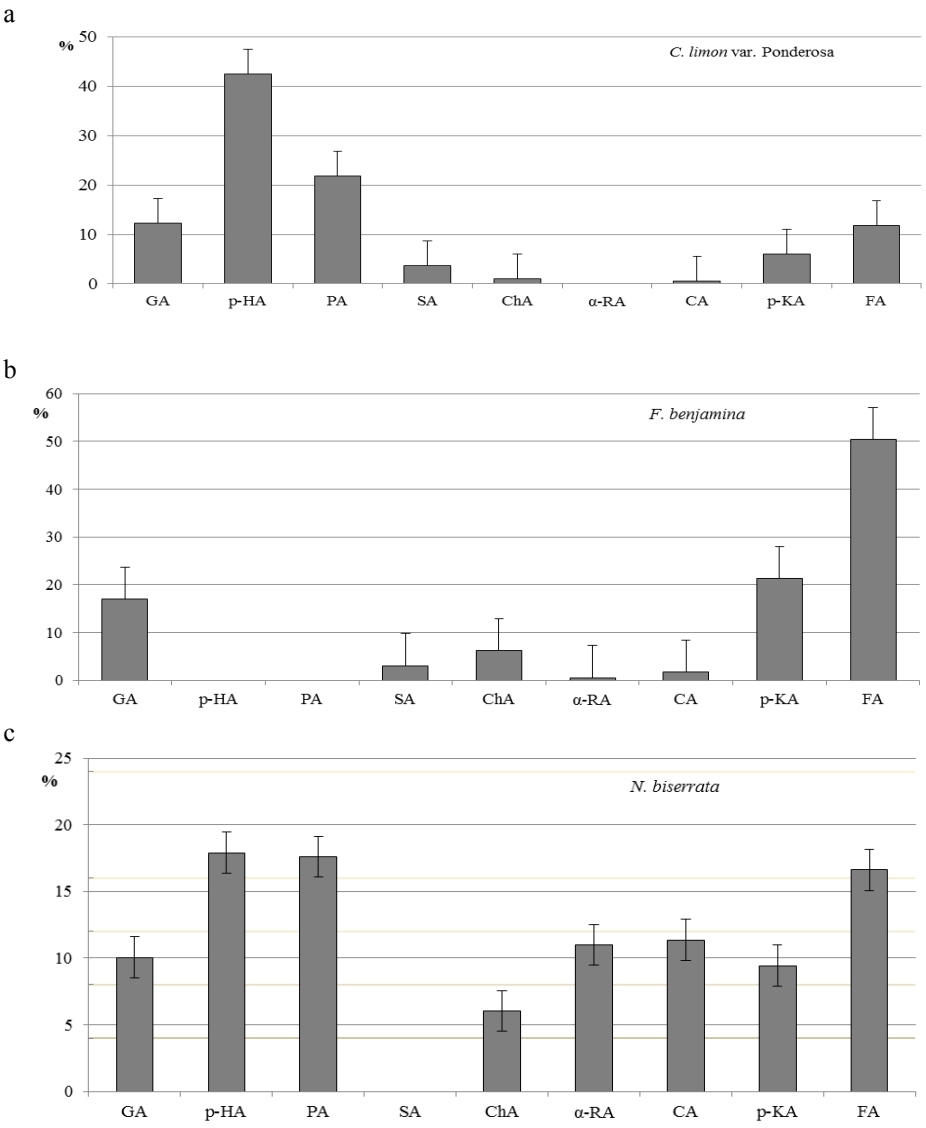
Table 15. Derivatives of benzoic and *trans*-cinnamic acids (µg·g^{-1} dry weight) identified in leaves of three non-infested (control) plant species

Tabela 15. Pochodne kwasu benzoesowego i *trans*-cynamonowego (µg·g^{-1} suchej masy) zidentyfikowane w ekstraktach z trzech gatunków roślin kontrolnych

Host plant Roślina żywicielska	Derivatives of benzoic acid Pochodne kwasu benzoesowego $\bar{x}$ ±SE				Derivatives of *trans*-cinnamic acids Pochodne kwasu *trans*-cynamonowego $\bar{x}$ ±SE				
	gallic acid kwas gallusowy	*p*-hydroxy-benzoic acid kwas *p*-hydroksy-benzoesowy	protocat-echuic acid kwas protokate-chowy	syringic acid kwas syryngowy	chlorogenic acid kwas chloro-genowy	α-resorcinol kwas α--rezorcynowy	coffeic acid kwas kawowy	*p*-kumaric acid kwas *p*--kumarowy	feluric acid kwas ferulowy
Citrus limon var. Ponderosa	0.351 b ± 0.0023	1.214 a ± 0.0150	0.624 b ± 0.005	0.107 a ± 0.0012	0.028 c ± 0.0006	0 c	0.017 b ± 0.0	0.174 c ± 0.0012	0.339 c ± 0.0121
Ficus benjamina	0.241 c ± 0.0003	0 b	0 c	0.043 b ± 0.0001	0.088 b ± 0.0004	0.007 b ± 0.0	0.024 b ± 0.0006	0.303 b ± 0.0004	0.717b ± 0.0011
Nephrolepis biserrata	0.651 a ± 0.0046	1.161 a ± 0.0029	1.141 a ± 0.0081	0 c	0.391 a ± 0.0012	0.713 a ± 0.0092	0.737 a ± 0.0098	0.612 a ± 0.0046	1.077 a ± 0.0017
$F_{(2.6)}$	5003.704				68153.4		5311.128	6607.957	2670.353
$F_{(1.4)}$		12.02	4070.391	3072		5841.047			
P	2.15E^{-10}	0.0256	3.615E^{-7}	6.344E^{-7}	8.53E^{-14}	1.757E^{-7}	1.80E^{-10}	9.34E^{-11}	1.413E^{-9}

Values in columns signed by different letters indicate statistically significant differences at $P \leq 0.01$ (Tukey's *HSD* test)

Wartości w kolumnach oznaczone innymi literami różnią się istotnie dla $P \leq 0{,}01$ (test *HSD* Tukeya)



GA: gallic acid
kwas gallusowy

P-HA: *p*-hydroxybenzoic acid
kwas *p*-hydroksybenzoesowy

PA: protocatechuic acid
kwas protokatechowy

SA: syringic acid
kwas syryngowy

ChA: chlorogenic acid
kwas chloro-genowy

α-RA: α-resorcinol
kwas α-rezorcynowy

CA: coffeic acid
kwas kawowy

P-K A: *p*-kumaric acid
kwas *p*-kumarowy

FA: feluric acid
kwas ferulowy

Fig. 7. Proportion (%) of identified phenolic acids in leaves of control plants *Citrus limon* var. Ponderosa (a), *Ficus benjamina* (b) and *Nephrolepis biserrata* (c)

Rys. 7. Udział (%) zidentyfikowanych kwasów fenolowych w kontrolnych liściach *Citrus limon* var. Ponderosa (a), *Ficus benjamina* (b) and *Nephrolepis biserrata* (c)

In the control leaves of *C. limon* var. Ponderosa, the highest concentration was found for *p*-hydroxybenzoic and protocatechuic acids (Tab. 15). The share of these phenolic acids in lemon leaves constituted about 42.54% and 21.86% of acids pool, respectively (Fig. 7a).

In *F. benjamina* leaves, concentration of identified phenolic acids was the lowest with respect to that observed in *C. limon* var Ponderosa and *N. biserrata* (Tab. 15). Extraordinary high, over 50% share in ficus leaves was noted for ferulic acid. High content was also observed for *p*-coumaric and gallic acids, which constituted 21.3% and 16.9%, respectively, in the pool of identified phenolic acids in the leaves of *F. benjamina* (Fig. 7b).

High concentrations of phenolic acids were observed in *N. biserrata* leaves, however the share of particular phenolic acids identified in fern was not high and did not exceed 20% of total phenolic acids concentration (Fig. 7c). Concentration of phenolic acids in leaves of *N. biserrata* was over 4-fold higher compared to the concentration in leaves of *F. benjamina* and 2-fold with respect to the leaves of *C. limon* var. Ponderosa. Three acids were dominating in the leaves of *N. biserrata*: *p*--hydroxybenzoic, protocatechuic and ferulic (Tab. 15, Fig. 7c). The share of each of them in fern leaves was about 17% of total phenolic acids concentration.

The analysis of correlation demonstrated an existence of linearly negative relationship between the degree of acceptance of the examined host plant and *p*--hydroxybenzoic acid concentration ($r = -0.781$; $P = 0.01$) (Tab. 11). The increased content of *p*-hydroxybenzoic acid in leaves of the studied species affected shortening of pre-reproductive period of *C. hesperidum* ($r = -0.749$; $P = 0.02$). In contrast, the increased content of *p*-hydroxybenzoic acid in leaves of the studied host plant affected elongation of reproduction period of soft brown scale ($r = 0.726$; $P = 0.02$) (Tab. 10). The increase in instar nymphs mortality was also influenced by syringic acid content content ($r = 0.789$; $P = 0.01$) (Tab. 11).

5.3. The response of host plants to *Coccus hesperidum* feeding

5.3.1. Changes in biochemical properties of plants colonized by *Coccus hesperidum*

Significantly higher concentration of sugars (total and reducing), and usually the decrease in other examined primary metabolites concentrations were noted in leaves of plants colonized by *C. hesperidum* (Fig. 8–10).

5.3.1.1. Primary metabolites concentration

Total sugars and reducing sugars

Among the studied plant species, ficus leaves were characterized by significantly higher concentration of total sugars ($F_{5.12} = 926.205$; $P = 4.33E^{-15}$) and reducing sugars ($F_{5.12} = 1584.21$; $P = 1 \cdot 10^{-14}$) in colonized than control plants. The highest, over 23% increase in total sugars content in plants colonized by scale insects compared to control was observed in fern leaves (23.09%;

$F_{1.4}$ = 196.69; P = 0.0002). The similar 9% increase in total sugars content was noted in the leaves from colonized *C. limon* var. Ponderosa (9.54%; $F_{1.4}$ = 99.2; P = 0.0005) and *F. benjamina* (9.50%; $F_{1.4}$ = 39.45; P = 0.003) (Fig. 8).

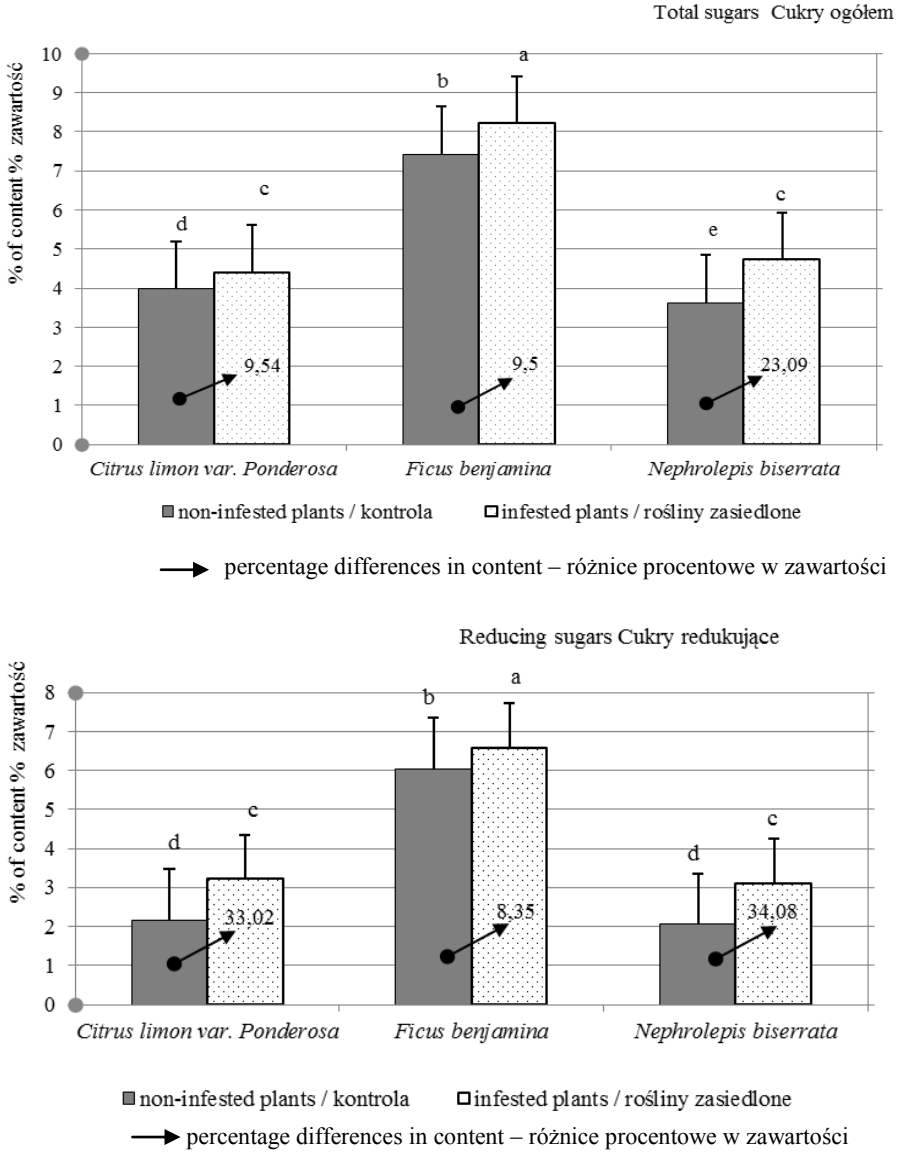
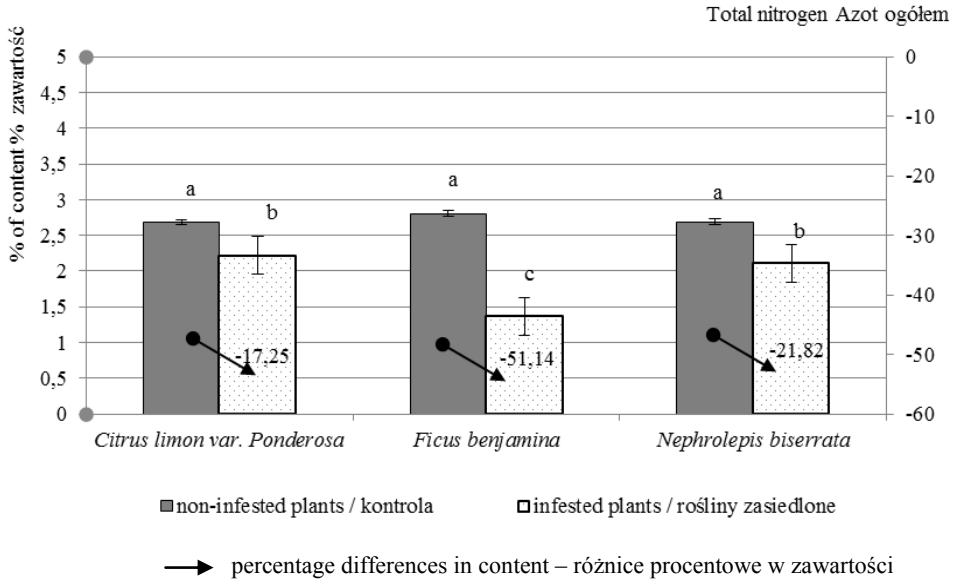
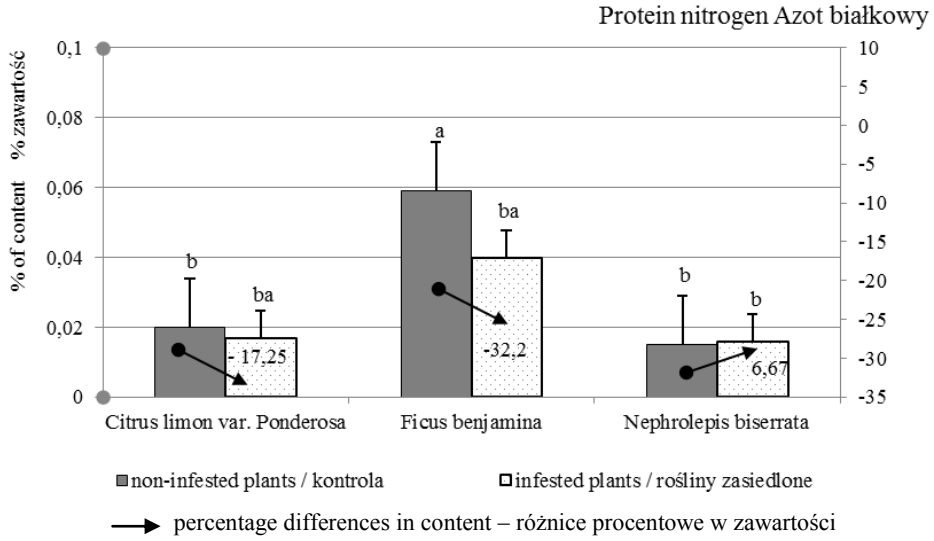


Fig. 8. Content of total and reducing sugars in control and infested by *Coccus hesperidum* host plants. Values signed by different letters indicate statistically significant differences at P ≤ 0.01 (Tukey's *HSD* test)

Rys. 8. Zawartość cukrów ogółem i redukujących w roślinach kontrolnych i zasiedlonych przez *Coccus hesperidum*. Wartości oznaczone innymi literami różnią się istotnie przy P ≤ 0,01 (test *HSD* Tukeya)


Total nitrogen Azot ogółem
% of content % zawartość
5
4,5
4
3,5
3
2,5
2
1,5
1
0,5
0
0
-10
-20
-30
-40
-50
-60
a
b
a
c
a
b
-17,25
-51,14
-21,82
Citrus limon var. Ponderosa
Ficus benjamina
Nephrolepis biserrata
non-infested plants / kontrola
infested plants / rośliny zasiedlone
percentage differences in content – różnice procentowe w zawartości


Protein nitrogen Azot białkowy
% of content % zawartość
0,1
0,08
0,06
0,04
0,02
0
10
5
0
-5
-10
-15
-20
-25
-30
-35
b
ba
a
ba
b
b
- 17,25
-32,2
6,67
Citrus limon var. Ponderosa
Ficus benjamina
Nephrolepis biserrata
non-infested plants / kontrola
infested plants / rośliny zasiedlone
percentage differences in content – różnice procentowe w zawartości

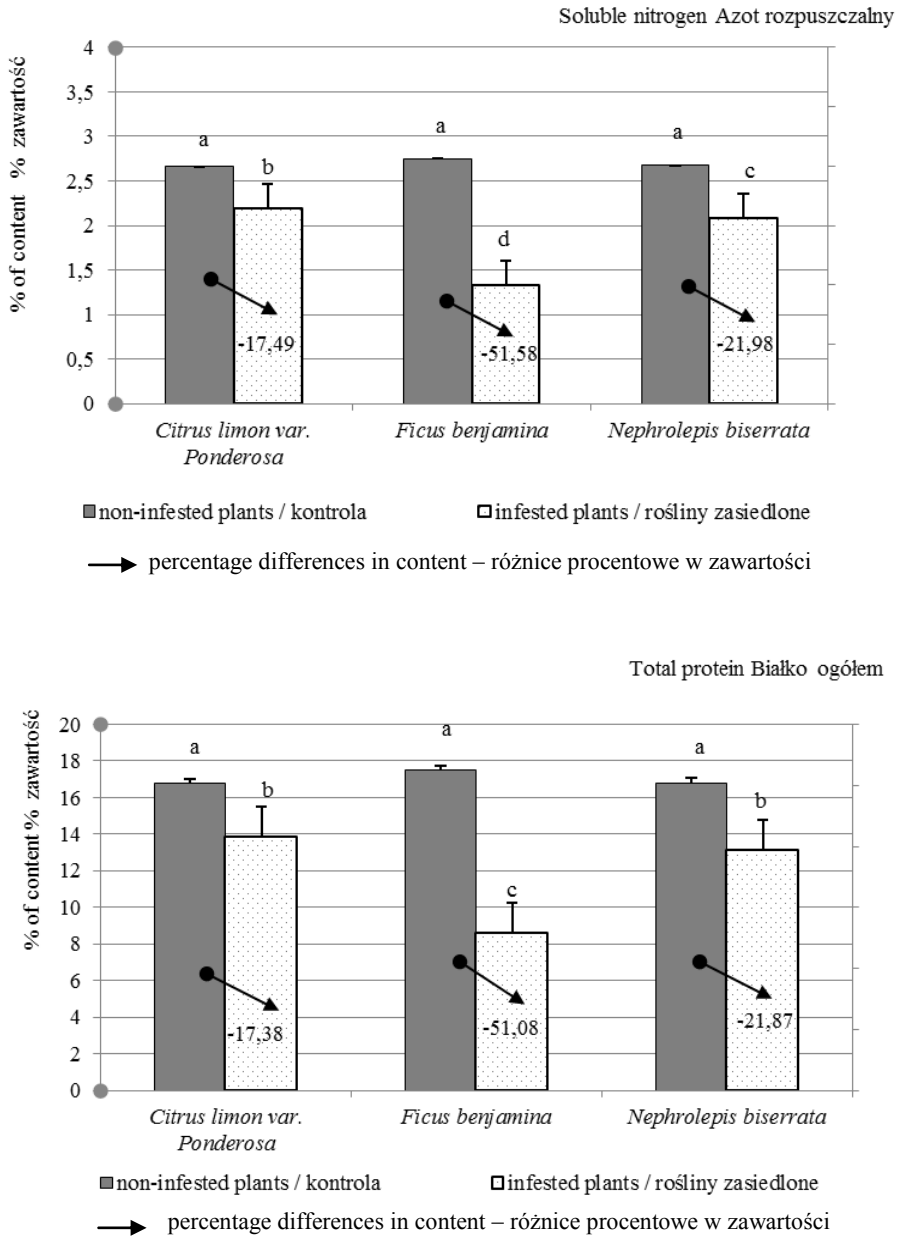
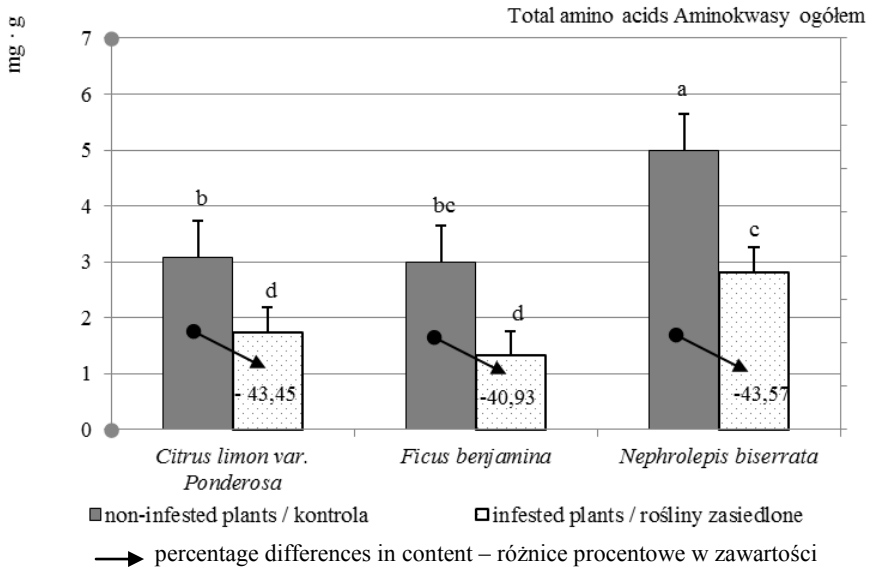
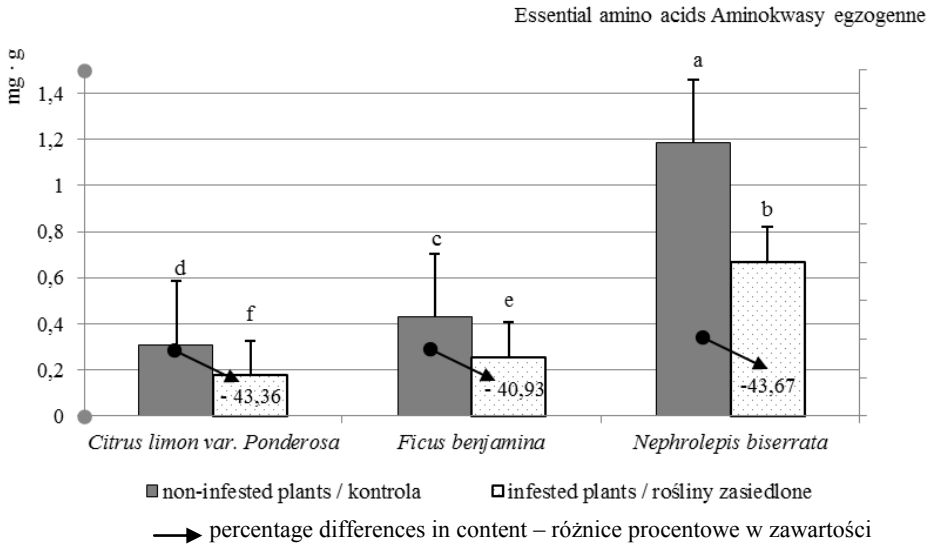


Fig. 9. Content of nitrogen (total nitrogen, protein nitrogen, soluble nitrogen) and total protein in control and infested by *Coccus hesperidum* host plants. Values signed by different letters indicate statistically significant differences at $P \leq 0.01$ (Tukey's *HSD* test)

Rys. 9. Zmiany w zawartości cukrów azotu (ogółem, białkowego i rozpuszczalnego) i białka ogółem w roślinach kontrolnych i zasiedlonych przez *Coccus hesperidum*. Wartości oznaczone innymi literami różnią się istotnie przy $P \leq 0,01$ (test *HSD* Tukeya)


Total amino acids Aminokwasy ogółem
mg . g
7
6
5
4
3
2
1
0
b
d
be
d
a
c
- 43,45
-40,93
-43,57
Citrus limon var. Ponderosa
Ficus benjamina
Nephrolepis biserrata
non-infested plants / kontrola
infested plants / rośliny zasiedlone
percentage differences in content – różnice procentowe w zawartości


Essential amino acids Aminokwasy egzogenne
mg . g
1,4
1,2
1
0,8
0,6
0,4
0,2
0
d
f
c
e
a
b
- 43,36
- 40,93
-43,67
Citrus limon var. Ponderosa
Ficus benjamina
Nephrolepis biserrata
non-infested plants / kontrola
infested plants / rośliny zasiedlone
percentage differences in content – różnice procentowe w zawartości

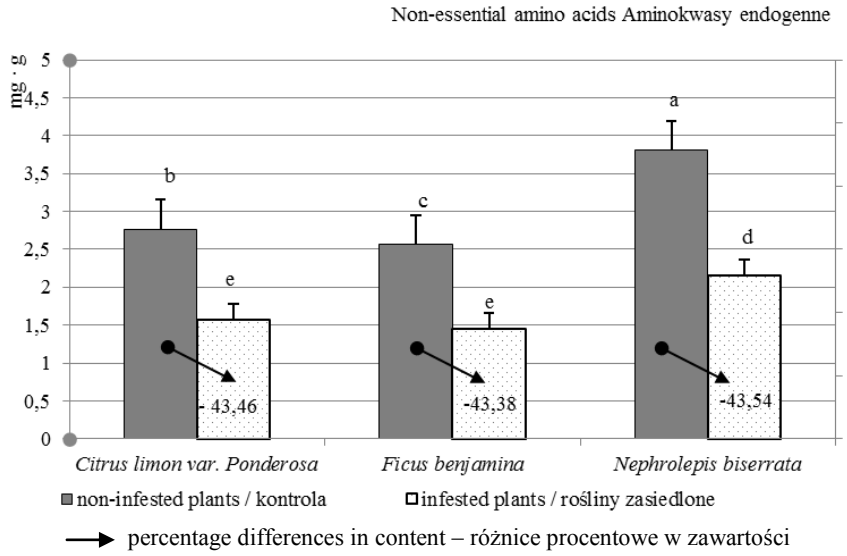


Fig. 10. Content of amino acids (total, essential and non-essential) in control and infested by *Coccus hesperidum* host plants. Values signed by different letters indicate statistically significant differences at $P \leq 0.01$ (Tukey's *HSD* test)

Rys. 10. Zawartość aminokwasów (ogółem, egzogennych i endogennych) w roślinach kontrolnych i zasiedlonych przez *Coccus hesperidum*. Wartości oznaczone innymi literami różnią się istotnie przy $P \leq 0{,}01$ (test *HSD* Tukeya)

The content of reducing sugars in infested plants was the highest in ficus leaves. The lowest increase in the content was noted as a result of scale insects feeding on the leaves of *F. benjamina* (8.35%; $F_{1.4} = 56.24$; $P = 0.001$). The considerably lower content of reducing sugars in infested leaves was found in lemon and fern leaves as compared to ficus. The similar, over 30% increase in reducing sugars content was found in the infested *C. limon* var. Ponderosa (33.02%; $F_{1.4} = 303.59$; $P = 0.0006$) and *N. biserrata* (34.08%%; $F_{1.4} = 207.29$; $P = 0.001$) leaves compared to control plants (Fig. 8).

Nitrogen (total, protein, soluble) and total protein

High, over 51% lower total nitrogen concentration was noted in the infested leaves of *F. benjamina* compared to control leaves (51.14%; $F_{1.4} = 1650.893$; $P = 2.183E^{-6}$). Lower decrease in this metabolite concentration was noted in the leaves of *C. limon* var. Ponderosa (17.25%; $F_{1.4} = 9800$; $P = 6.243E^{-8}$) and *N. biserrata* (21.82%; $F_{1.4} = 276.571$; $P = 0.00007$) infested by scale insects (Fig. 9). The strong decrease in soluble nitrogen content compared to control was observed in infested leaves of *F. benjamina* (51.58%; $F_{1.4} = 964.276$; $P = 6.408E^{-8}$). Lower decrease in this metabolite concentration was noted in the leaves of *C. limon* var. Ponderosa (17.49%; $F_{1.4} = 8561.42$; $P = 8.179E^{-8}$) and *N. biserrata* (21.98%; $F_{1.4} = 286.14$; $P = 0.00007$) (Fig. 9).

The changes in protein nitrogen level in infested leaves of species studied compared to the level in the control leaves, were statistically not significant (Fig. 9).

The changes in total protein content were analogous to changes of total and soluble nitrogen in leaves of the examined plant species (Fig. 9). The decrease in that metabolite concentration under the effect of soft brown scale feeding was the highest in the infested leaves of *F. benjamina* (51.08%; $F_{1.4}$ = 1629.485; P = 2.250E^{-6}) compared to the level noted in colonized leaves of *C. limon* var. Ponderosa (17.38%; $F_{1.4}$ = 6961.119; P = 1.237E^{-7}) and *N. biserrata* (21.87%; $F_{1.4}$ = 287.6746; P = 0.000071).

Amino acids (total, essential, non-essential)

Scale insects feeding on the leaves of three plant species caused a significant, over 40% decrease in the concentration of all examined amino acids groups compared to the concentration in leaves of non-infested by soft scales (Fig. 10).

Fern leaves were characterized by highest level of the total, essential and non-essential amino acids concentration in the leaves of control plants and those infested with *C. hesperidum* in comparison to lemon and ficus. In turn, similar level of total amino acids was noted in control and colonized leaves of *C. limon* var. Ponserosa and *F. benjamina* (Fig. 10). Scale insects feeding affected significant decrease in the total amino acids content in all host plant leaves (*C. limon* var. Ponserosa; 43.45%; $F_{1.4}$ = 130.11; P = 0.001, *F. benjamina*: 40.93%, $F_{1.4}$ = 2357.48; P = 0.00001, *N. biserrata*: 43.57%; $F_{1.4}$ = 4111.53; P = 3.544E^{-7}).

Among lemon and ficus, the higher content of essential amino acids before and after colonization by scale insects was noted in *F. benjamina* leaves (Fig. 10). In turn, concentration of non-essential amino acids was significantly higher in the leaves of control *C. limon* var. Ponserosa plants. Scale insects feeding caused similar, about 43% decrease in these amino acids content and their similar content in colonized leaves of lemon (43.46%; $F_{1.4}$ = 326.14; P = 0,00005) and ficus (43.38%; $F_{1.4}$ = 21.82; P = 1.256 E^{-6}). Brown soft scale feeding on fern leaves led to a decrease of essential amino acids content (43.67%; 51947.33; P = 2.223E^{-9}) as well as non-essential amino acids content (43.54%; $F_{1.4}$ = 2743.6; P = 7.952E^{-7}).

5.3.1.2. Secondary metabolites concentration

Phenolic acids

The highest concentration of phenolic acids among the examined plant species was found in the leaves of *N. biserrata*, while the lowest in the leaves of *F. benjamina* (Fig. 11). The changes in phenolic acids concentration were observed in the leaves infested with *C. hesperidum* ($F_{5.12}$ = 3452.56; P = 1·10^{-14}). Decrease in these secondary metabolites concentration was noted in infested ficus leaves (38.75%; $F_{5.12}$ = 220.07; P = 0.0001). The decrease was stronger than in fern leaves (10.69%; $F_{5.12}$ = 109.51; P = 0.0004), however, an increase in the concentration was found in the leaves of *C. limon* var. Ponderosa (18.62%; $F_{5.12}$ = 504; P = 0.00002).

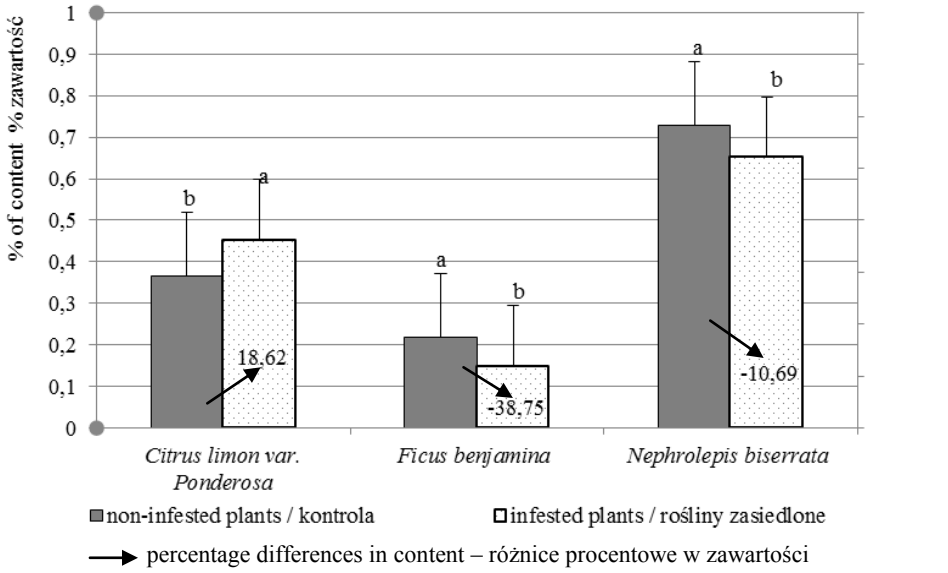
Separation of plant extracts allowed an identification and determination of concentration of phenolic compounds observed in scale insect infested leaves of *C. limon* var. Ponderosa, *F. benjamina* and *N. biserrata* (Tab. 16). Identified phenolic acids present in the leaves of control plants were also observed after colonization with scale insects.

Significant increase in concentration of six out of eight phenolic compounds were observed in the leaves of *C. limon* var. Ponderosa colonized by *C. hesperidum,* compared to the lemon control leaves (Tab. 16). Over 2-fold increase (compared to the control) in chlorogenic acid concentration was observed as a result of the scale insects feeding. Over 50% increase in protocatechuic and syringic acids content was noted. An increase in caffeic, gallic and *p*-coumaric acids content by 35.3%, 45.3% and 22.4%, respectively, was also found in damaged lemon leaves compared to control. Decrease in ferulic acid content resulting from scale insects feeding was noted, and its concentration in leaves infested with *C. hesperidum* decreased nearly 30% compared to the control (Tab. 16). Proportion of identified acids in control and infested lemon leaves were variable (Fig. 12a).

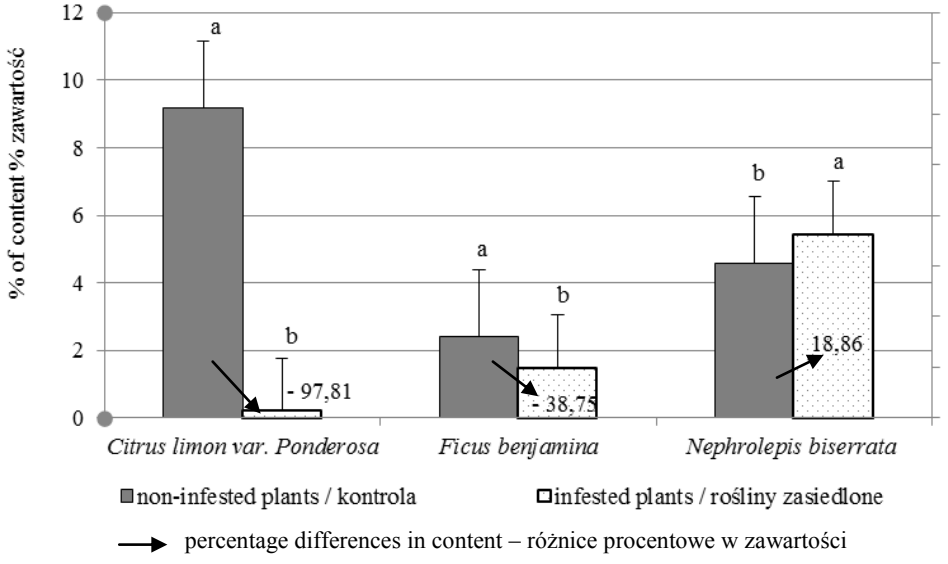
The lowest proportion of identified acids in control and infested leaves were characterized by caffeic acid and chlorogenic acid, while *p*-hydroxybenzoic acid and protocatechuic acid were found in the highest proportion of total phenolic acids concentration (Fig. 12a).

A significant decrease in the content of four identified phenolic acids, and increase in the content of three of those acids was noted in the least scale insects colonized leaves of *F. benjamina* compared to the control leaves (Tab. 16). In turn, proportion of phenolic acids in ficus leaves resulting from scale insects feeding increased for five phenolic acids, and decreased for two phenolic acids with respect to control leaves (Fig. 12b). Over 6-fold concentration decrease (84.4%) (in comparison to control) of ferulic acid was noted as a result of scale insects feeding on ficus leaves. High, 4-fold decrease in infested *F. benjamina* leaves was also noted for syringic acid (74.41%), which contribution in phenolic acids pool in infested ficus leaves decreased over 62% (Fig. 12b). High, over 2-fold increase in α--resorcinol acid content was found in ficus leaves infested with the insects compared to the control (128.57%) (Tab. 16). Proportion of this phenolic acid demonstrated the highest, over 4-fold, increase compared to the control. The content of gallic and caffeic acids decreased of 20%, however share of these acids increased in the leaves of infected plants (about 30%). As a result of scale insects feeding on ficus leaves, about 40% increase in chlorogenic acid concentration (39.77%) and *p*-coumaric acid was noted (38.94%), for which also over 2-fold increase in percentage share in infested leaves was found in comparison to the control.

Phenolic acids Kwasy fenolowe



Tannins Garbniki



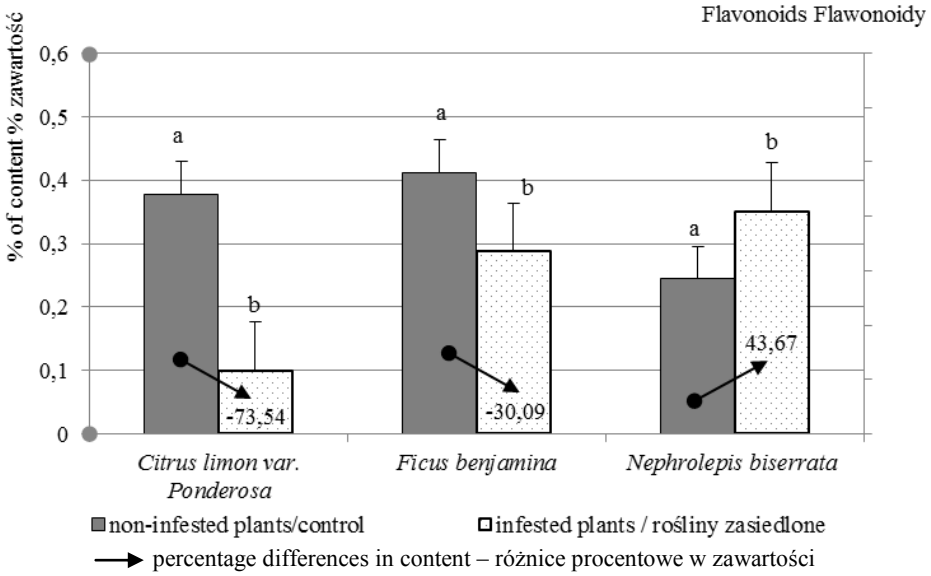


Fig. 11. Changes in the concentration (%) of secondary metabolites in non-infested and infested by *Coccus hesperidum* plant species *Citrus limon* var. Ponderosa, *Ficus benjamina*, *Nephrolepis biserrata*. Values in columns signed by different letters are statistically different at $P \leq 0.01$ (Tukey's *HSD* test)

Rys. 11. Zmiany stężenia (%) metabolitów wtórnych w roślinach kontrolnych i zasiedlonych *Citrus limon* var. Ponderosa, *Ficus benjamina*, *Nephrolepis biserrata* przez *Coccus hesperidum*. Średnie oznaczone tymi samymi literami nie różnią się istotnie przy $P \leq 0,01$ (test *HSD* Tukeya)

The concentration and proportion of identified phenolic acids in control and infested *N. biserrata* leaves varied (Tab. 16, Fig. 12c). The insects feeding cause the decrease in the content of six phenolic acids and the increase in the content of two these metabolites in infested leaves of fern in comparison to control (Tab. 16). Over 6-fold decrease in concentration of chlorogenic acid (83.63%) and about 2-fold decrease of α-resorcinol acid (44.8%) were observed. The high decrease in concentration, over 25%, resulting from *C. hesperidum* feeding were noted for gallic and caffeic acid. The highest, over 38% increase in *p*-coumaric acid content was noted in fern leaves infested with the insects in comparison to the control. The content of protocatechuic acid also increased in the infested leaves in comparison to control (9.11%). The highest decrease in the proportion of chlorogenic acid and α-resorcinol acid was noted in fern infested leaves. The highest increase (about 58%) in the proportion of identified phenolic acids in the infested fern leaves compared to the control, was found for *p*-coumaric acid (Fig. 12c).

Table 16. Derivatives of benzoic and *trans*-cinnamic acids (μg·g^{-1} dry weight) identified in leaves of three non-infested and infested by *Coccus hesperidum*

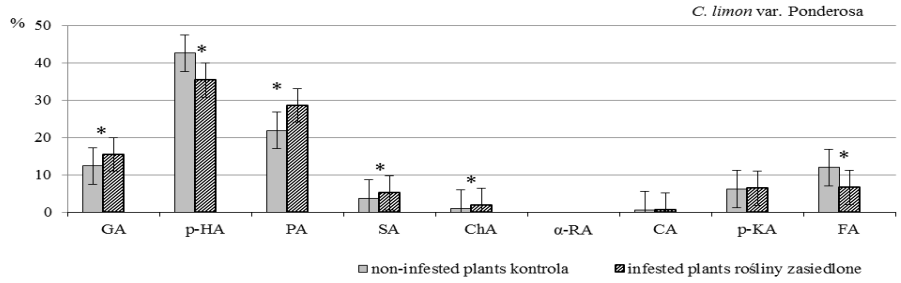
Tabela 16. Pochodne kwasu benzoesowego i *trans*-cynamonowego (μg·g^{-1} suchej masy) zidentyfikowane w ekstraktach z trzech gatunków roślin kontrolnych i zasiedlonych przez *Coccus hesperidum*

Host plant Roślina żywicielska	Series Seria	Derivates of benzoic acid Pochodne kwasu benzoesowego $\bar{X}$ ±SE				Derivatives of *trans*-cinnamic acid Pochodne kwasu *trans*-cynamonowego $\bar{X}$ ±SE				
		gallic acid kwas gallusowy	*p*-hydroxy- benzoic acid kwas *p*-hydroksy-benzoesowy	protocat-echuic acid kwas protokate-chowy	syringic acid kwas syryngowy	chlorogenic acid kwas chloro-genowy	α-resorcinol kwas α--rezorcynowy	coffeic acid kwas kawowy	*p*-kumaric acid kwas *p*--kumarowy	feluric acid kwas ferulowy
Citrus limon var. Ponderosa	non-infested plants kontrola	0.351 d ± 0.0023	1.214 a ± 0.0150	0.624 d ± 0.0006	0.107 b ± 0.0011	0.028 e ± 0.0006	0 e	0.017 c ± 0.072	0.174 f ± 0.0011	0.339 d ± 0.0121
	infested plants rośliny zasiedlone	0.510 b ± 0.0035	1.171 a ± 0.0208	0.941 c ± 0.0064	0.169 a ± 0.0029	0.057 d ± 0.0011	0 e	0.023 c ± 0.0012	0.213 e ± 0.0012	0.224 e ± 0.0040
Ficus benjamina	non-infested plants kontrola	0.241 e ± 0.0006	0 c	0 e	0.043 c ± 0.0016	0.088 c ± 0.0001	0.007 d ± 0.002	0.024 c ± 0.0004	0.303 d ± 0.0006	0.717 c ± 0.0016
	infested plants rośliny zasiedlone	0.194 f ± 0.0021	0 c	0 e	0.011 d ± 0.0183	0.123 b ± 0.0029	0.016 c ± 0.0013	0.019 c ± 0.0006	0.421 c ± 0.0052	0.109 f ± 0.0035
Nephrolepis biserrata	non-infested plants kontrola	0.651 a ± 0.0046	1.161 a ± 0.0029	1.141 b ± 0.0081	0 e	0.391 a ± 0.0013	0.713 a ± 0.0092	0.737 a ± 0.0098	0.612 b ± 0.0046	1.077 a ± 0.0017
	infested plants rośliny zasiedlone	0.484 c ± 0.0014	1.078 b ± 0.0011	1.245 a ± 0.0029	0 e	0.064 d ± 0.0	0.394 b ± 0.0012	0.551 b ± 0.0012	0.845 a ± 0.0023	1.038 b ± 0.0021
$F_{5.12}$ $F_{3.8}$ P		3997.18 $1{\cdot}10^{-14}$	19.39 0.0005	2614.14 $2.65E^{-12}$	2035.17 $7.21E^{-12}$	5489.37 $1{\cdot}10^{-14}$	5202.79 $1.69E^{-13}$	5489.04 $1.37E^{-13}$	7076.33 $1{\cdot}10^{-14}$	5670.67 $1{\cdot}10^{-14}$

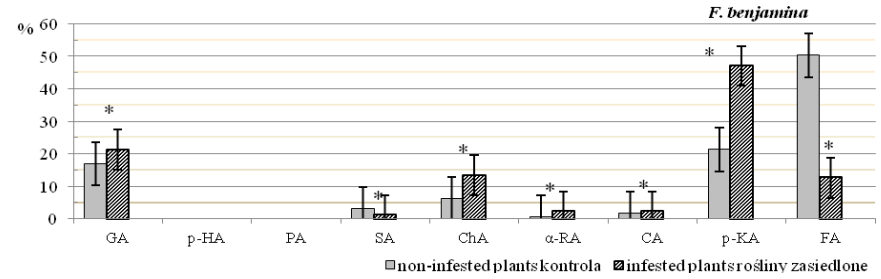
Values in columns signed by different letters indicate statistically significant differences at $P \leq 0.01$ (Tukey's *HSD* test)

Wartości w kolumnach oznaczone innymi literami różnią się istotnie dla $P \leq 0{,}01$ (test *HSD* Tukeya)

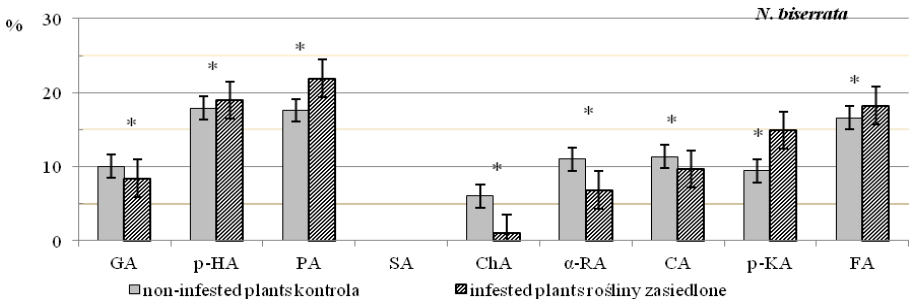
a



b



c



GA: gallic acid kwas gallusowy	P-HA: *p*-hydroxybenzoic acid kwas *p*-hydroksybenzoesowy	PA: protocatechuic acid kwas protokatechowy
SA: syringic acid kwas syryngowy	ChA: chlorogenic acid kwas chloro-genowy	α-RA: α-resorcinol kwas α-rezorcynowy
CA: coffeic acid kwas kawowy	P-K A: *p*-kumaric acid kwas *p*-kumarowy	FA: feluric acid kwas ferulowy

Fig. 12. Proportion of phenolic acids identified in non-infested and infested by *Coccus hesperidum* leaves of host plants (a – *Citrus limon* var. Ponderosa, b – *Ficus benjamina*, c – *Nephrolepis biserata*). Values for each fenolic acids signed by "*" are statistically different at $P \leq 0.01$ (t-Test)

Rys. 12. Udziale procentowy zidentyfikowanych kwasów fenolowych w roślinach kontrolnych i zasiedlonych przez *Coccus hesperidum* (a – *Citrus limon* var. Ponderosa, b – *Ficus benjamina*, c – *Nephrolepis biserata*). Średnie dla poszczególnych kwasów oznaczone „*" różnią się istotnie dla $P \leq 0{,}01$ (Test t)

Tannins, flavonoids

Significant differences in tannins level ($F_{5.12}$ = 3452.56; P = 110^{-14}) were observed in the leaves of plants infested with *C. hesperidum* in comparison to the control (Fig. 11). Very high decrease in these metabolites concentration in infested leaves of *C. limon* var. Ponderosa (97.81%; $F_{1.4}$ = 1631.56; P = 2.245E^{-6}) and slightly lower in the leaves of *F. benjamina* (38.75%; $F_{1.4}$ = 28.71; P = 0.005) were noted in comparison to the control. An increase in tannins level was only observed in the infested leaves of *N. biserrata* (18.86%; $F_{1.4}$ = 295.84; P = 0.00006) with respect to the control (Fig. 11).

Significant differences in flavonoids content were also found in the leaves of plants colonized by *C. hesperidum* in comparison to the control ($F_{5.12}$ = 38592.5; P = $1 \cdot 10^{-14}$) (Fig. 11). Over 70% decrease in these metabolites concentration (73.54%; $F_{1.4}$ = 63232.36; P = 1.500E^{-9}) was noted in the infested lemon leaves, and it was higher than in *F. benjamina* (30.09%; $F_{1.4}$ = 27676.8; P = 7.831E^{-9}). Over 40% increase in flavonoids content in the leaves colonized by scale insects with respect to the control was found in fern (43.67%; $F_{1.4}$ = 52164.5; P = 2.205E^{-9}).

5.3.2. Changes in the physiological processes of host plants

5.3.2.1. Physiological state of control plants

In order to determine the influence of *C. hesperidum* colony size on the changes in antioxidant enzymes activity in host plants, five classes of scale insects density were distinguished for *C. limon* var. Ponderosa and *N. biserrata,* and three classes for *F. benjamina*.

Table 17. Physiological state of non-infested plants. Malondialdehyde content and level of antioxidant enzymes activity in un-infested leaves of host plants

Tabela 17. Stan fizjologiczny roślin kontrolnych. Poziom zawartości aldehydu malonowego i aktywność enzymów antyoksydacyjnych w liściach roślin kontrolnych

Host plant Roślina żywicielska	Malondialdehyde (nmol · g^{-1} fm) Aldehyd malonowy (nmol g^{-1}św. m.)	Mean value of antioxidant enzymes activity Średnia aktywność enzymów antyoksydacyjnych (U·ml^{-1})	
		ascorbate peroxidase peroksydaza askorbinianowa	quaiacol peroxidase peroksydaza gwajakolowa
Citrus limon var. Ponderosa	34.48 a ± 0.635	0.7 a ± 0.087	76.8 a ± 1.6
Ficus benjamina	2.81 c ± 0.338	0.12 b ± 0.009	43.79 b ± 1.553
Nephroplepis biserrata	4.63 b ± 0.133	0.09 b ± 0.023	0.09 c ± 0.005
$F_{2.9}$ P	2300.82 6.41E^{-13}	42.458 0.0002	893.192 3.75E^{-8}

Values in columns signed by different letters indicate statistically significant differences at P ≤ 0.01 (Tukey's *HSD* test)
Wartości w kolumnach oznaczone innymi literami różnią się istotnie dla P ≤ 0,01 (test *HSD* Tukeya)

An analysis of control plants state demonstrated an occurrence of differences in the value of examined parameter between different plant species, which was confirmed by statistical analysis (Tab. 17).

The control leaves of *C. limon* var. Ponderosa were characterized by the highest content of ascorbate and quaiacol peroxidase with respect to *F. benjamina* and *N. biserrata* leaves. The level of ascorbate peroxidase in the leaves of *C. limon* var. Ponderosa was nearly 8-fold higher compared to APX in fern leaves, and 6-fold compared to the level in ficus leaves. The content of quaiacol peroxidase in lemon leaves was over 850-fold higher compared to fern, and about 2-fold compared to ficus leaves (Tab. 17).

In turn, the lowest level of malondialdehyde was observed in the control leaves of *F. benjamina* compared to other host plants. The highest content of MDA was noted in lemon leaves. It was over 12-fold higher than in ficus, and about 7,5-fold than in fern (Tab. 17).

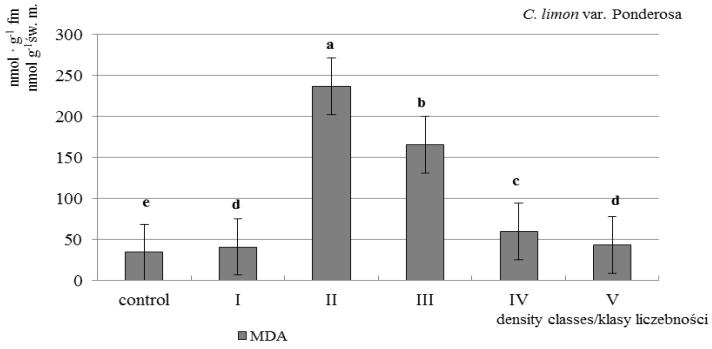
5.3.2.2. The state of cell membranes in host plants infested by scale insects

A significant increase in malondialdehyde content (compared to the control) ($F_{5,12} = 4523.31$; $P = 1 \cdot 10^{-14}$) was observed in the leaves of *C. limon* var. Ponderosa as a result of plants colonization by scale insects. Feeding of small number of *C. hesperidum* individuals on lemon leaves (I class) caused over 19% increase in MDA content (Fig. 13a). Next increase in scale insects individuals number on the leaves in II class (11–30 *C. hesperidum* individuals per leaf) affected the strongest, ca. 7-fold, increase in the value of examined parameter in comparison to the control. In the leaves of plants infested with 31 to mass abundance of *C. hesperidum* individuals (III, IV, V classes) the MDA level decreased, but was still higher compared to the control (Fig. 13a).

In the leaves of *F. benjamina* infested with scale insects in I class (up to 10 individuals per leaf) the malondialdehyde level increased compared to the control (Fig. 13b). Next increase in scale insects individuals number on the leaves (II class; 11–30 individuals per leaf, III class; 31–50 individuals per leaf) caused the strong, over 3-fold increase in MDA content. Statistical analysis confirmed significant differences in MDA content in ficus leaves affected by an increasing scale insects number ($F_{2,6} = 21.279$; $P = 0.001$) (Fig. 13b).

Small number of scale insects feeding on *N. biserrata* leaves (I class) caused 4% increase in MDA content (Fig. 13c). Next increase in soft scale insects individuals number on the leaves in II class (11–30 individuals per leaf) resulted 1,7-fold increase in malondialdehyde level. In the leaves of plants infested with 31 to 50 and 51 to 100 *C. hesperidum* individuals on average (III and IV classes) the level of MDA in fern leaves decreased, but was still maintained on high, over 40% higher level compared to the control. The level of MDA increased significantly as a result of mass scale insects feeding (V class) on the fern leaves.

a



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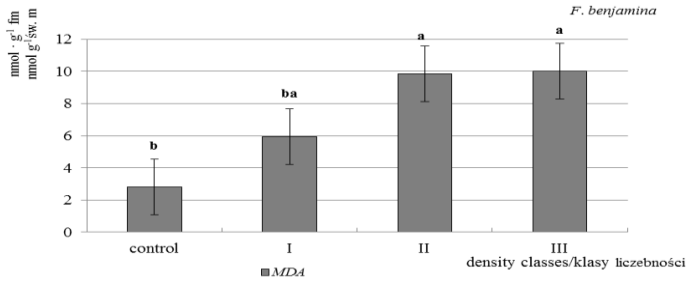

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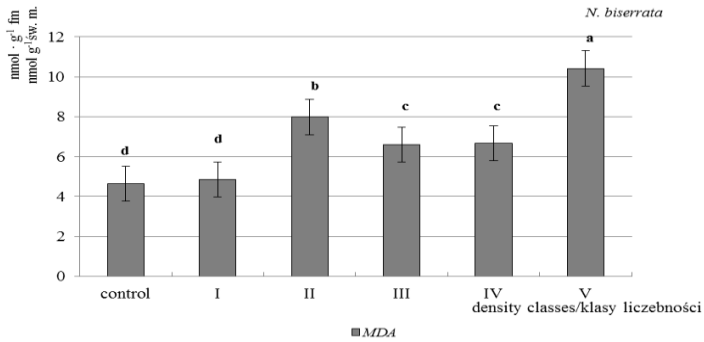


Density classes: control – non-infested plants; I class – up to 10 individuals per leaf; II class – from 11 to 30 individuals per leaf; III class – from 31 to 50 individuals per leaf; IV class – from 51 to 100 individuals per leaf; V class – over than 100 individuals per leaf

Klasy liczebności: kontrola – rośliny nie zasiedlone; klasa I – do 10 osobników na liść; klasa II – od 11 do 30 osobników na liść; klasa III – od 31 do 50 osobników na liść; klasa IV – od 51 do 100 osobników na liść; klasa V – powyżej 100 osobników na liść

Fig. 13a–c. Malondialdehyde content in the leaves of *Citrus limon* var. Ponderosa, *Ficus benjamina* and *Nephrolepis biserrata* in relation to the number of *Coccus hesperidum*. Values signed by different letters indicate statistically significant differences at $P \leq 0.01$ (Tukey's *HSD* test)

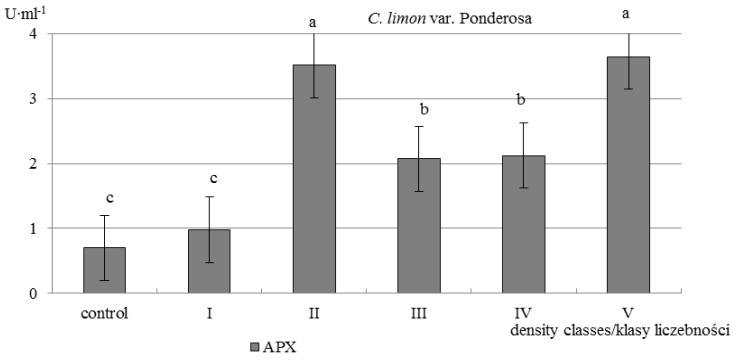
Rys. 13a–c. Zawartość aldehydu malonowego w liściach *Citrus limon* var. Ponderosa, *Ficus benjamina* i *Nephrolepis biserrata* w odniesieniu do liczebności *Coccus hesperidum*. Wartości oznaczone innymi literami różnią się istotnie przy $P \leq 0,01$ (test *HSD* Tukeya)

Feeding of mass abundance of scale insects on *N. biserrata* leaves affected the highest, over 2-fold increase in malondialdehyde content compared to the control. Statistical analysis confirmed significant differences in MDA content in fern leaves affected by an increasing scale insects number ($F_{5,15} = 114.55$; $P = 2.12E^{-11}$).

5.3.2.3. The activities of antioxidant enzymes

An analysis of physiological state of plants infested by scale insects demonstrated that *C. limon* var. Ponderosa, *F. benjamina* and *N. biserrata* differently reacted to an increasing abundance of insects feeding on them (Fig. 14–16). Feeding

a



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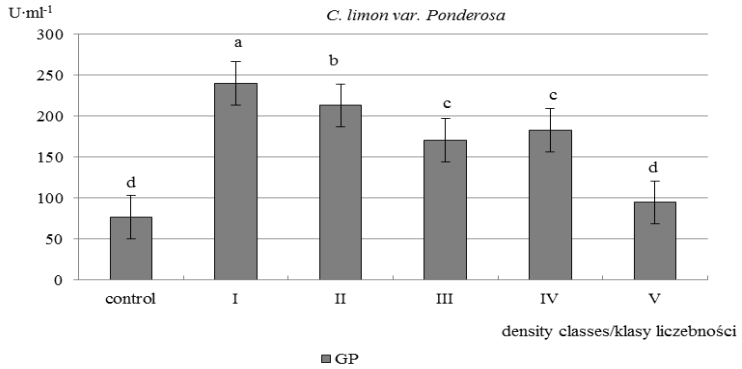


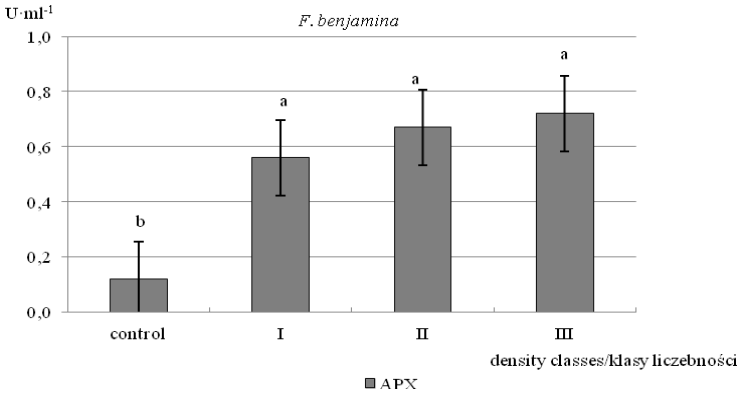
Density classes: control – non-infested plants; I class – up to 10 individuals per leaf; II class – from 11 to 30 individuals per leaf; III class – from 31 to 50 individuals per leaf; IV class – from 51 to 100 individuals per leaf; V class – over than 100 individuals per leaf.

Klasy liczebności: kontrola – rośliny nie zasiedlone; klasa I – do 10 osobników na liść; klasa II – od 11 do 30 osobników na liść; klasa III – od 31 do 50 osobników na liść; klasa IV – od 51 do 100 osobników na liść; klasa V – powyżej 100 osobników na liść.

Fig. 14. Activities of ascorbate peroxidase (a) and quaiacol peroxidase (b) in the leaves of *Citrus limon* var. Ponderosa in relation to the number of *Coccus hesperidum*. Values for enzymes activity signed by different letters indicate statistically significant differences at $P \leq 0.01$ (Tukey's *HSD* test)

Rys.14. Aktywność peroksydazy askorbinianowej (a) i peroksydazy gwajakolowej (b) w liściach *Citrus limon* var. Ponderosa w odniesieniu do liczebności *Coccus hesperidum*. Wartości oznaczone innymi literami różnią się istotnie przy $P \leq 0{,}01$(Test *HSD*)

a



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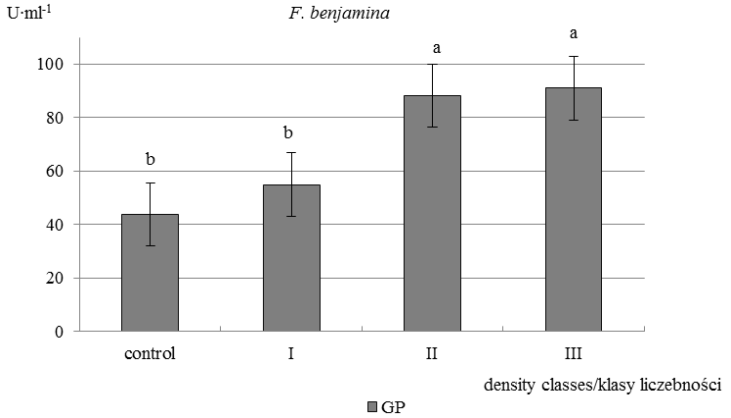


Density classes: control – non-infested plants; I class – up to 10 individuals per leaf; II class – from 11 to 30 individuals per leaf; III class – from 31 to 50 individuals per leaf; IV class – from 51 to 100 individuals per leaf; V class – over than 100 individuals per leaf

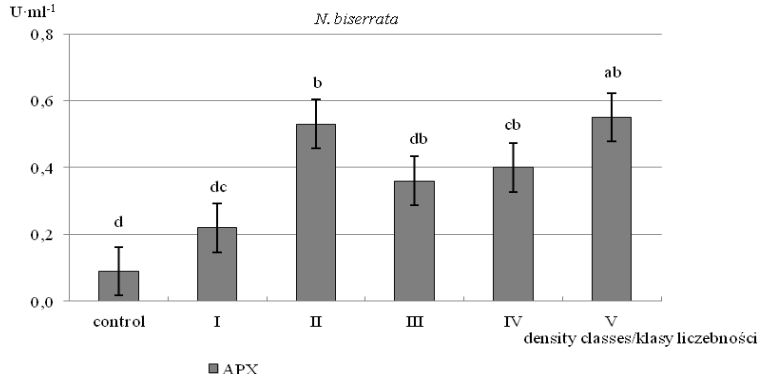
Klasy liczebności: kontrola – rośliny nie zasiedlone; klasa I – do 10 osobników na liść; klasa II – od 11 do 30 osobników na liść; klasa III – od 31 do 50 osobników na liść; klasa IV – od 51 do 100 osobników na liść; klasa V – powyżej 100 osobników na liść

Fig. 15. Activities of ascorbate peroxidase (a) and quaiacol peroxidase (b) in the leaves of *Ficus benjamina* in relation to the number of *Coccus hesperidum.* Values for enzymes activity signed by different letters indicate statistically significant differences at $P \leq 0.01$ (Tukey's *HSD* test)

Rys. 15. Aktywność peroksydazy askorbinianowej (a) i peroksydazy gwajakolowej (b) w liściach *Ficus benjamina* w odniesieniu do liczebności *Coccus hesperidum.* Wartości oznaczone innymi literami różnią się istotnie przy $P \leq 0{,}01$ (test *HSD* Tukeya)

of variable abundance of scale insects on the leaves of all plant species affected the changes in APX (*C. limon* var. Ponderosa: $F_{5.12} = 374.154$; $P = 9.74E^{-13}$, *F. benjamina*: $F_{3.8} = 22.3524$; $P = 0.0003$, *N. biserrata*: $F_{5.15} = 13.702$; $P = 0.00003$) and GP level (*C. limon* var. Ponderosa: $F_{5.12} = 360.98$; $P = 1.21E^{-8}$, *F. benjamina*: $F_{3.8} = 22.374$; $P = 1.388E^{-6,}$ *N. biserrata*: $F_{5.15} = 127.85$; $P = 9.51E^{-12}$) compared to the control.

a



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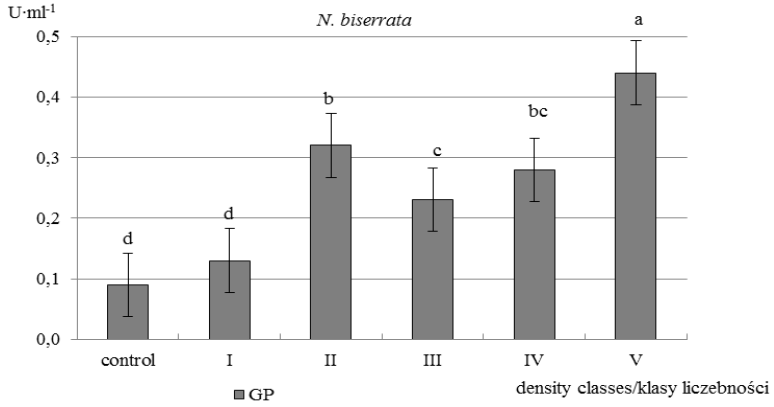

Density classes: control – non-infested plants; I class – up to 10 individuals per leaf; II class – from 11 to 30 individuals per leaf; III class – from 31 to 50 individuals per leaf; IV class – from 51 to 100 individuals per leaf; V class – over than 100 individuals per leaf

Klasy liczebności: kontrola – rośliny nie zasiedlone; klasa I – do 10 osobników na liść; klasa II – od 11 do 30 osobników na liść; klasa III – od 31 do 50 osobników na liść; klasa IV – od 51 do 100 osobników na liść; klasa V – powyżej 100 osobników na liść

Fig. 16. Activities of ascorbate peroxidase (a) and quaiacol peroxidase (b) in the leaves of *Nephrolepis biserrata* in relation to the number of *Coccus hesperidum*
Values for enzymes signed by different letters indicate statistically significant differences at $P \leq 0.01$ (Tukey's *HSD* test)

Rys. 16. Aktywność peroksydazy askorbinianowej (a) i peroksydazy gwajakolowej (b) w liściach *Nephrolepis biserrata* w odniesieniu do liczebności *Coccus hesperidum*. Wartości oznaczone innymi literami różnią się istotnie przy $P \leq 0{,}01$ (test *HSD* Tukeya)

Small number of scale insects feeding on *C. limon* var. Ponderosa leaves resulted in about 40% increase in an activity of ascorbate peroxidase in I class (up to 10 individuals per leaf) (Fig. 14a). Next increase in an abundance of *C. hesperidum* feeding on lemon leaves (II class: 11–30 individuals per leaf) caused the strong over 5-fold increase in APX level compared to the control. In

the leaves of lemon infested with scale insect individuals in III and IV classes, the activity of analyzed enzyme in lemon leaves decreased but was still maintained on higher level compared to the control plants. Feeding of mass abundance of scale insects on lemon leaves (V class) resulted in the highest, over 5-fold increase in the activity of ascorbate peroxidase compared to the control.

Feeding of small number of scale insects on *C. limon* var. Ponderosa leaves caused the highest, over 3-fold increase in GP activity in I class (up to 10 individuals per leaf) (Fig. 14b). In the leaves of plants infested with 11 to 30 *C. hesperidum* individuals on average (II class), an activity of analyzed enzyme in lemon leaves decreased, but was still maintained on high, 2.7-fold higher level compared to the control plants. Next increase in scale insects individuals number on the leaves (III class; 31–50 individuals, IV class; 51–100 individuals) caused over 2-fold increase in the peroxidase level compared to the control. Feeding of mass scale insects abundance on lemon leaves (V class) caused about 23% increase in GP activity compared to the control leaves.

The activity of ascorbate peroxidase in ficus leaves in subsequent two first classes of density was about 5-fold higher than the activity of the enzyme in the control leaves (Fig. 15a). Maximum level of APX in the leaves of *F. benjamina* was observed in III class, where the strongest, 6-fold higher APX activity, compared to the control was also observed.

The 1.4-fold higher in GP activity was observed in the leaves of *F. benjamina* infested with sparse individuals of *C. hesperidum* (I class) compared to the values in the leaves of control plants (Fig. 15b). The GP activity in the plants infested with higher number of individuals maintained on the high level in II and III classes.

The lowest level of ascorbate peroxidase and quaiacol peroxidase was noted in the leaves of *N. biserrata* (Tab. 17). Both enzymes exhibited similar tendency in a response to the increasing abundance of coccids on the leaves (Fig. 16a, b). In the leaves of *N. biserrata,* the strongest, significantly different compared to the control, 6-fold higher content of APX was noted in II and V classes (Fig. 16a). The 3.5-fold higher in GP activity in infested fern leaves was observed in II class (11 to 30 individuals per leaf) compared to the values in the leaves of control plants. Maximum level, 5-fold higher GP activity compared to the control, was observed in V class (mass infestation) (Fig. 16b). Lower level in activity of examined enzymes compared to II and V classes was observed in fern leaves in III (31–50 individuals per leaf) and IV (51–100 individuals per leaf) density classes. However, the activity of APX and GP in these classes was still maintained on the higher level compared to the control (Fig. 16a, b).

6. DISCUSSION

6.1. Effects of host plant on scale insects behaviour

The plant species analyzed belong to common hosts of *C. hesperidum,* however their reactions on insects feeding observed in the study varied. The plants exhibited various manners of susceptibility to scale insects and none of the examined species demonstrated a complete resistance to soft brown scale.

Among the plant species studied, ficus appeared to be the plant of the lowest degree of susceptibility with respect to *C. hesperidum*. Unfavorable (antibiotic) influence of *F. benjamina* on demographic and morphometric parameters (body size) as well as the feeding behaviour and honeydew excretion of C. *hesperidum* compared to other host plants was demonstrated in the study. *C. limon* var. Ponderosa was suitable and the most abundantly colonized by *C. hesperidum* plant species. On that plant the insects reached sexual maturity within a shorter time and were characterized by an increased reproduction. Soft brown scale individuals feeding on lemon were characterized by the higher size of the body, longer time of feeding in phloem and the higher honeydew excretion rate and daily excretion, as compared to individuals from *F. benjamina*. Feeding preference of *C. hesperidum* concerning lemon corresponds with numerous worldwide literature reports [Annecke 1969, Argyriou 1970, Chatterjee et al. 2000, Yasnosh et al. 2005, Lopes et al. 2008, Papadopoulou 2012]. The scale insects were less abundant on *N. biserrata,* however they found suitable conditions for development on the fern, which was reflected in the range of parameters comparable to individuals from lemon. Positive influence of fern as a host plant is also confirmed by low mortality of larval stages and high share of females and older instars in the age structure of the soft brown scale colony on this host species.

Demographic parameters are usually used in determination of plant suitability by the insects and degree of plants resistance. The rate of aphis development is examined the most often in the literature [Dąbrowski 1988; Ciepiela 1990, Zeng et al. 1993, Legrand and Barbosa 2000, Gantner 2007, Kordan et al. 2008, Goławska 2010, Kot and Kmieć 2012], and more rarely in scale insects [Erkiliq and Uygun 1997, Polat et al. 2010]. One of more important effects of host plant on insects is their fecundity reduction and increased mortality of instar nymphs [Awmackand and Leather 2002, Goławska 2010]. According to Awmackand and Leather [2002] host plant quality affects the fecundity of herbivorous insects

both at the individual and the population scale. As demonstrated by numerous authors, the fecundity of *C. hesperidum* females and mortality of nymphs is influenced mainly by host plant species and external conditions [Dingler 1923, Tereznikowa 1981, Copland and Ibrahim 1985]. An effect of host plant species on the demographic parameters was confirmed in the present study. Copland and Ibrahim [1985] found high, over 80%, mortality among brown soft scale nymphs, which was also observed for instars feeding on *F. benjamina* and *C. limon* var. Ponderosa in the present study. The lowest fecundity in this present study was also noted for *C. hesperidum* females feeding on ficus, which together with the highest mortality of instar nymphs from this plant species resulted in the lowest *F. benjamina* population of the scale insects. The highest share of first-instar nymphs and the lowest of females were observed in scale insects age structure on ficus, with respect to those noted on lemon and fern. In turn, Erkiliq and Uygun [1997] observed a strong influence of host plant species on the longevity and fecundity of *Pseudaulacaspis pentagona* (Hemiptera; Diaspididae), however with a slight effect on development time of this species. Boyero et al. [2007] explains differences in females fecundity noted for two species of scales *Cornuaspis beckii* (Newman) and *Parlatoria pergandii* Comstock as the influence of varietal variation in orange trees the scale insects fed on. The differences presented in this study concerned the duration of pre-reproductive (pre-oviposition) period, which was elongated for individuals feeding on *F. benjamina*, and shortened for scale insects from *C. limon* var. Ponderosa and *N. biserrata*. Such differences were also reflected in a time of one generation development. As observed by Golan [2009] the time varied depending on scale insect host plant species. Scale insects feeding on ficus leaves were characterized by the elongated pre-reproductive (pre-oviposition) period, shortened reproductive (oviposition) period, the lowest daily fecundity and the highest nymphal mortality.

In the presented study, the differences occurred between the degree of acceptance of plant species in free choice test and the conditions which are provided by the plant to insects for their further development. The lemon was the plant species of the lowest degree of acceptance by mobile scale insects instar nymphs in the free choice test, but the most abundantly colonized as a result. The insects were abundant on *N. biserrata,* also willingly accepting it from the beginning. The species of the highest acceptance (free choice test) was *F. benjamina*, however it turned out the plant that in small number was colonized by scale insects. Unsuitable conditions created by ficus for *C. hesperidum* resulted in distinctly lowered dynamics of scale insects population development, and in the lowest abundance on it, compared to other plant species. The differences observed in acceptance of plant species during free choice tests and various rates of development of scale insects colonizing lemon, ficus and fern, suggest that plant properties play a significant role in the discussed scale insect–host plant interactions, by antixenosis (lack of acceptance) and antibiosis mechanisms. One of the first barriers which the insects face during plant colonization are morphological-

anatomical properties of the plants, which together with physiological and biochemical properties determine structural resistance of the plants based on mechanism of lack of acceptance (antixenosis) and antibiosis. Morphological-anatomical structure of the leaves is considered as one of the factors differentiating structural resistance of the plants to various species of mites and insects [Dąbrowski 1988, Walker 1988, van Lenteren and de Ponti 1990, Bernays and Chapman 1994, Lei et al. 2001, Kiełkiewicz-Szaniawska 2003] including scale insects [Kaushik et al. 2012]. The literature concerning probing and feeding behaviour of insects with chewing and sucking mouthparts, points at the special role of leaves structure in plants acceptance and their colonization [van Lenteren and de Ponti 1990, Walker 1988, Lei et al. 2001]. According to Koteja [1996a, b], the choice of permanent feeding site by scale insects is to a high degree conditioned by food abundance, but also topographical conditions and plant ontogenesis. Scale insects, unlike closely related aphids, insert their proboscis once on their life cycle and remain sedentary for the rest of their life [Kaushik et al. 2012]. According to numerous authors, leaf thickness is considered as one of the factor preventing feeding of numerous groups of Hemiptera [Niraz et al. 1982, Campbell et al. 1986, Walker 1988, Leszczyński 2001, Gantner 2007]. The occurrence of physical barriers like thorns, spines, and trichomes, epicuticular wax films and wax crystals, preventing *C. hesperidum* from feeding, was not observed in the present study on the leaves of plant species. Comparison of leaves structure with their acceptance by scale insects mobile stages demonstrated that there is a relationship between preliminary plant choice by scale insects, and leaf blade thickness in main nerve and its vicinity (100 μm behind the main nerve). *F. benjamina* species, the most willingly accepted by *C. hesperidum* instar nymphs in free choice test, was characterized by the thinnest leaves in the area of main nerve, while the least accepted *C. limon* var. Ponderosa was characterized by the thickest leaves in the main nerve and its vicinity. In view of literature data, an increase in plants acceptance by some Hemiptera species is affected by large number of stomata, through which these insects may introduce stylets in epidermis and to phloem [Niraz et al. 1982, Gantner 2007]. In this study, the highest number of stomata was noted in the leaves of *C. limon* var. Ponderosa the most abundantly infested by scale insects, however statistical analysis did not confirm a significance of this relationship. No relationship between acceptance degree and abundance of scale insects colonizing the plant, and phloem distance from lower leaf side which was infested by insects, was noted, either. Kaushik et al. [2012] in their study concerning *Kerria lacca* (Kerr) (Hemiptera, Tachardiidae (Kerridae)) found that phloem distance did not play any significant role in determination of host plant choice by this scale insect species. However Calatayud et al. [2001] observed strict positive relationship between the most often localization of *Phenacoccus herreni* (cassava mealybugs) in the vicinity of leaf main nerve and phloem sap availability. High abundance of scale insects colonizing lemon compared to ficus and fern may be the cumulative effect of such features

of leaves structure as the least distance of phloem from lower leaf side, the thinnest epidermis of lower leaf blade and numerous stomata. However, these relationships were not confirmed statistically, and are not reflected in the results obtained for the leaves of other two plant species. It may be concluded based on the own results, that the features of leaves anatomical structure do not play crucial role in *C. hesperidum* interactions with the plants. Earlier, Renard [1999] basing on observations of *Phenacoccus manihoti* behaviour, did not confirm any considerable role of physical properties of host plants for scale insects from Pseudococcidae family.

The analysis of EPG parameters also confirmed postive effect of *C. limon* var. Ponderosa and *N. biserrata* on the feeding of scale insects. Females on lemon and fern started plant tissues penetration faster, penetrated peripheral tissues shorter and spent longer time feeding in phloem compared to individuals on *F. benjamina*. Similarity of feeding phases observed for *C. hesperidum* to analogical models observed in mealybugs and aphids was demonstrated. However, the waveform G related for xylem tissue penetration which was occasionally demonstrated for mealybugs [Calatayud et al. 1994b, 2001, Calatayud and Rü 2006, Cid and Fereres 2010, Huang et al. 2012] and is usually noted in aphids [Tjallingii 1988, Gabryś et al. 1997, Urbańska et al. 2002, Wróblewska et al. 2012], whiteflies [Lei et al. 2001] and leafhoppers [Trębicki et al. 2012] as well as waveform F related to derailed stylet mechanics, for the first time observed for *P. solenopsis* [Huang et al. 2012] were not found during *C. hesperidum* feeding.

Shorter time of epidermis and mesophyll tissues penetration, as well as an elongation of feeding time in phloem, were noted on lemon and fern compared to *F. benjamina*, in the present study. It was earlier demonstrated in broad bean varieties attractive for *Aphis fabae* concerning plants susceptibility to Hemiptera feeding, that the feeding time in phloem is considerably longer and the time of peripheral tissues penetration is shorter [Cichocka et al. 1999]. Wróblewska et al. [2012] and Kordan et al. [2008] basing on the lack of penetration, sporadically occurring phloem phase and short periods of feeding during stylet penetration, determined low susceptibility of selected species of lupine to *Acyrthosiphon pisum*. Sparse observations of scale insects feeding conducted on representatives of Pseudococcidae family confirmed the occurrence of relationship between the values of EPG parameters, mainly availability of phloem sap, and plants susceptibility to scale insects [Calatayud et al. 1994]. On unsuitable plants species, the insects face difficulties in phloem sap ingestion during tissues penetration, which are usually caused by antixenosis (lack of preference) [Calatayud et al. 1994b]. Presumably, the longest time of *C. hesperidum* in peripheral tissues of ficus (C waveform), where large amounts of secondary metabolites were found, would have negatively affected nymphs mortality and demographical parameters of this scale insect species, mainly via antixenosis. However, these observations were not confirmed statistically.

The influence of host plant species on the parameters determining the honeydew process in *C. hesperidum* was proved in the study. The individuals settled on *F. benjamina* were characterized by the lowest honeydew daily excretion and excretion rate compared to scale insects feeding on lemon and fern. In turn, lemon and fern profitably the scale insects honeydew daily excretion and excretion rate. The size of honeydew droplets observed in this study demonstrated a tendency to increase with individual development of the insects, irrespective of host plant species. Golan [2008a] in the study concerning daily and individual changes in the process of scale insects honeydew excretion, demonstrated an increase in size and weight of honeydew droplet for subsequent developmental stages and a decrease in honeydew excretion rate. As observed by Nishida and Kuramoto [1963], the number of honeydew droplets excreted by *Dysmicoccus neobrevipes* (Hemiptera; Pseudococcidae) decreased with insect's age. Different results are described in the studies concerning the aphid honeydew excretion, where the number and size of honeydew droplets excreted by them increased with insects individual development, while honeydew daily excretion was not changed with obtaining full maturity by the females [Mittler 1958; Mittler, Sylvester 1963]. This relationship is connected to metabolism, nutrition, food intake–amount of food consumed decreases when scale insects reach maturity. Aphids, after reaching sexual maturity, continue feeding, while in scale insects food intake is inhibited as sexual maturity is obtained [Nishida and Kuramoto 1963]. In this study, mature *C. hesperidum* females, compared to second instar–nymphs, were characterized by a decrease in honeydew daily excretion which was probably caused by bionomic specificity, e.g. less intense plant sap collection.

Differences observed in this study concerning insect behaviour occurring under the influence of host plant species prove that these relationships were mainly conditioned by nutritional value of host plant tissues. It was demonstrated in numerous studies that plants ability for protection against herbivores depends on quantitative and qualitative chemical composition of host tissues (constitutional resistance) and on the ability of mobilization of specific metabolic reactions deteriorating herbivore feeding or limiting nutritional usefulness of host tissues (induced resistance) [Karban and Baldwin 1997, Leszczyński 2001, Agrell et al. 2003, Despres and David 2007, Sempruch 2008].

As reported by Dąbrowski [1988], Karban and Baldwin [1997] and other authors [Srivastava and Auclair 1971, Lawton 1982, Leszczyński 2001, Wright 2003, Gantner 2007, Patra et al. 2008], the initial choice of plants by herbivores is mainly determined by concentration of nutrients and allelocompounds observed in the plant. The results presented point at unequivocal relationship between concentration of primary metabolites and an initial choice of the plant species by *C. hesperidum*.

A significant influence of primary metabolites on selection of host plants and further development of *C. hesperidum* was observed in this study. It was proved using an analysis of correlation, that the highest attractiveness of *F. ben-*

jamina during plant selection by *C. hesperidum* nymphs resulted from relatively high concentration of total and reducing sugars and total proteins, as well as total, protein and soluble nitrogen in the leaves. Concurrently, with high sugars content, ficus leaves contained the least amount of total and non-essential amino acids, and relatively low content of essential amino acids. The studies conducted by other authors demonstrate a significant role of sugars and ratio of sugars to nitrogen compounds as feeding stimulators during the selection of host plant by the insects [Niraz et al. 1987, Ciepiela 1990, 1991, Harborne 1997, Oleszek et al. 2001, Gantner 2007, Sempruch 2010]. The leaves of *F. benjamina*, which in the free choice test were selected by nearly half population of *C. hesperidum* nymphs, contained about 2-fold and 3-fold higher amounts of total sugars and reducing sugars, respectively, compared to *C. limon* var. Ponderosa and *N. biserrata*. Kiełkiewicz-Szaniawska [2003] observed a correlation between an increase in attractiveness of tomato leaves during colonization by red spider mites and higher concentration of total sugars and non-reducing sugars in the plant. In the study on acceptance of *Cedrus* by *Exophthalmus jekelianus* (White) (Coleoptera: Curculionidae) Wright et al. [2003] also observed that the leaves with higher content of fructose, galactose and saccharose were more willingly colonized by beetles. Ciepiela [1990, 1991] and Gantner [2007] reported in their studies that high content of total sugars with low total nitrogen content was one of the main factors conditioning plants antibiosis towards aphis. The qualitative composition of sugars, which is known to affect feeding preferences of scale insects and influence their growth and development, was not examined in this study. However it was observed in the previous study by Golan and Najda [2011] that predominant sugar composition in the leaves of *F. benjamina* was an arabinose. This sugar is included in some glucosides and bacterial polysaccharides. Presumably, negatively affects scale insects development on ficus by influencing the high mortality observed in the present study. The influence of sugar content in plant tissues on the length of pre-reproductive and reproductive periods in scale insects was documented. It was found that the high content of total sugars and reducing sugars in the leaves of the plant species caused the elongation of pre-reproductive period in the insects and the shortening of the reproductive period. An increased plant acceptance by *C. hesperidum* nymphs was observed at higher ratio of total sugar to total nitrogen, however analysis of correlation did not confirm a significance of this relationship. In turn, the influence of these mutual proportions on honeydew excretion rate and honeydew droplet size was proved. The influence of higher content of total sugars on shortening of saliva secretion time and sieve element salivation (E1 waveform) was observed.

Beside sugars, equally important role in interactions between plants and insects is played by various nitrogen forms [Karley et al. 2002, Kusano et al. 2007, Sempruch 2010]. A positive influence of an increased content of essential amino acids on the abundance of scale insects was demonstrated in this study. The size of scale insects body was positively affected by the increased content of all ami-

no acids forms (total, essential and non-essential ones). In turn, a decrease in the content of all amino acids forms (total, essential and non-essential ones) caused an increase in *C. hesperidum* nymphs mortality. Calatayud et al. [2002] observed an inhibition in *Phenacoccus herreni* development with lowered content and incomplete composition of amino acids in the plants being the food source of scale insects. Karley et al. [2002] observed the changes in quality and amount of amino acids disturbing host plants acceptance by herbivores. In aphids and mealybugs, certain amino acids act synergistically with sugars, mainly sucrose as phagostimulants [Srivastava 1987, Calatayud et al. 2002]. The shortening of reproductive period of *C. hesperidum* was affected by an increased level of nitrogen (total and soluble) as well as total proteins in the leaves of the plant species. Earlier Gantner [2007] showed that an increased content of total and protein nitrogen resulted in an increase in reproduction rate and in a rate of multiplication of the size of *Myzocallis coryli* Goetze population with shortened time of generation development.

High level of antixenosis and antibiosis with respect to herbivores results mainly from the presence of specific secondary metabolites in the leaves, responsible for the suppression of growth rate, development and possibly reproduction [Dąbrowski 1988, Leszczyński 2001, Gantner 2007, Goławska 2010]. Among the allelocompounds, the highest significance in constitutive resistance to arthropods is attributed to phenolic compounds including phenolic acids, tannins and flavonoids [Montlor 1991, Calatayud et al. 1994a, Leszczyński et al. 1996, Bi et al. 1997, Leszczyński 2001, Eleftherianos et al. 2005, Calatayud and Rü 2006, Gantner 2007]. It was proved in the present study based on an analysis of correlation that limited acceptance of *C. limon* var. Ponderosa leaves by *C. hesperidum* nymphs in free choice test resulted from high concentration of tannins in its leaves. The tannins might discourage insects to feeding, and negatively affect development and fecundity as well as increase insect mortality [Schultz and Baldwin 1982, Feeny 1970, Harborne 1997, Czerniakowski 2006]. Numerous examples of negative correlation between the content of phenolic compounds in plant tissues and the abundance of herbivorous Hemiptera have been described in the literature, and many of these studies concern aphids [Miles 1978, Cole 1984, Cichocka et al. 1999, Ciepiela and Chrzanowski 1999, Leszczyński 2001], while those considering scale insects are sparse [Fernandes et al. 2011]. The literature available lacks of information concerning an influence of secondary metabolites on scale insects development [Fernandes 2011]. In the case of aphids, an increased content of tannins causes an elongation in the time of generation development and a decrease in the rate of these Hemiptera population development [Oleszek et al.1992, Goławska 2006, 2007, Goławska et al. 2006, Gantner 2007, Goławska and Łukasik 2009]. The relationship between the high degree of *F. benjamina* acceptance by *C. hesperidum* instar nymphs and low phenolic acids and tannins content in the leaves was demonstrated in this study. However, this relationship was not confirmed by significant correlations.

In turn, a positive relationship between the length of pd waveform duration and flavonoids content was proved. Flavonoids also caused an increase in instar nymphs mortality. Such a result may suggest that relatively higher concentrations of these metabolites act in a deterrent way on scale insects, discouraging them from feeding, as well as in toxic way increasing their mortality. Divergent results concerning the role of flavonoids in plant–herbivore interactions, may be found in the literature. The results on the relationships and the influence of flavonoids content on mites and some insect species are similar to those already reported in the literature [Wrubel and Bernays 1990, Kiełkiewicz-Szaniawska 2003]. Gantner [2007] observed a positive effect of flavonoids on the attractiveness of *Corylus* L. varieties for *Myzocallis coryli* in the study concerning European hazel resistance to aphids. In contrast, Calatayud et al. [1994a, b] did not observe any influence of flavonoids level in the leaves of various cassava varieties on the feeding behaviour and development of mealybugs. They demonstrated the occurrence of strong relationship between the level of phenolic acids in the leaves of cassava and feeding parameters of *Phenacoccus manihoti*. These authors proved that on the less preferred cassava variety with a high phenolic acid level in its extracellular fluids, the mealybugs spent the longest time in searching for the phloem. Plant phenolics were regarded as one of the important defenses against insects in various studies [Berbehenn and Martin 1994, Berbehenn et al. 1996, Henn 1997]. However, their specific mode of action is not yet clearly known [Rattan 2010]. No influence of total phenolic acids content in the plant species tissues on *C. hesperidum* was demonstrated in present study. However, a negative influence of particular phenolic acids was documented. Chromatographic analysis showed differences in composition, concentration and proportion of particular phenolic acids in control leaves of the plant species. Based on an analysis of correlation, it was proved in this study, that the degree of plant species acceptance by scale insects, and pre-reproductive period length of *C. hesperidum* were negatively affected by higher concentration of *p*-hydroxybenzoic acid. However, the increase in content of *p*-hydroxybenzoic acid caused an increase in the length of the soft brown scale reproductive period. The increased content of syringic acid also caused an increase in *C. hesperidum* nymphs mortality. Both acids were highly concentrated in the leaves of control *C. limon* var. Ponderosa plants strongly inhibiting lemon leaves acceptance by nymphal stages in the free choice test, shortening pre-reproductive period, elongation of reproductive period and high mortality of nymphs feeding on lemon during the study. Concurrently, high content of *p*-hydroxybenzoic acid in the control leaves of *N. biserrata* and syringic acid absence caused a decrease in an acceptance, shortening pre-reproductive period, elongation of reproductive period and very low mortality of nymphs. However, no presence of *p*-hydroxybenzoic acid was noted in ficus which was the most willingly accepted by soft brown scale instar nymphs during the free choice test. The content of syringic acid negatively affected the instar nymphs mortality.

A negative influence of particular phenolic acids identified in this study, such as ferulic, caffeic, chlorogenic and *p*-coumaric acids on plants demographic parameters, was demonstrated in studies conducted by numerous authors [Dreyer and Jones 1981, Cole 1984, Leszczyński et al. 1985, Santiago et al. 2005, Chrzanowski et al. 2007, 2010]. The acids may suppress feeding, elongate pre-reproductive period, limit fecundity and increase mortality of these insects. Deterrent or toxic activity of chlorogenic, *p*-hydroxybenzoic, *p*-coumaric and gallic acids on mites were observed by Dąbrowski and Rodriguez [1972]. Leiss et al. [2009] observed an influence of chlorogenic acid on suppression in thrips occurrence on chrysanths. Urbańska et al. [2002] noted the shortening of the probing phase of *Sitobion avenae* under the influence of caffeic and gallic acids. Mortality of Lettuce Root Aphid *Pemphigus bursarius* (L.) and life parameters of butterflies species nymphs *Heliothis zea* (Boddie) were caused by the content of isochlorogenic, gallic and tannic acids in host plants leaves [Duffey and Isman 1981, Cole 1984, Ananthakrishnan et al. 1990]. Leszczyński [1987] observed the influence of ferulic and *p*-coumaric acids on demographic parameters of aphids, mainly their fecundity. The available literature lacks studies concerning an influence of phenolic acids on scale insects behaviour. Only Fernandes et al. [2011] observed a negative influence of chlorogenic acid on *Coccus viridis* nymphs from Coccidae family which stimulated the locomotion of crawlers thus reducing the feeding. Due to small number of publications concerning plants interactions in terms of secondary metabolites content on scale insects, this issue requires further research.

6.2. The response of host plant to scale insects feeding

An increase in total and reducing sugars and a decrease in the content of other examined primary metabolites was noted in the leaves of all plant species studied infested with scale insects. Low, a few percent increase in protein nitrogen content was only noted in the case of fern leaves infested with *C. hesperidum*. The poorest increase in sugars content and the strongest decrease in various forms of nitrogen and proteins, reaching even 50%, was noted in ficus which was the least susceptible to insects feeding. It may be supposed, this reaction based on antibiosis of *F. benjamina*, affecting less profitable food composition for the feeding insects and negatively influencing their growth and development. Considerable accumulation of sugars in infested leaves demonstrated in the present study resulted probably from disorders occurring in plants colonized with scale insects, and especially from the decrease in photosynthesis intensity noted as a result of scale insects feeding [Golan et al. 2013]. According to Tomczyk

[1996], red spider mites (*Tetranychus urticae* Koch) feeding on *Chrysanthemum* caused a decrease in photosynthesis activity and limited sugars transformation into starch, which affected an increase in monosaccharides content in the leaves. Earlier Golan and Najda [2011] observed an increase in the content of glucose, fructose and saccharose in the leaves of the host plants resulting from *C. hesperidum* feeding. Similar reaction to various species of insects and mites feeding was noted by other authors [Hildebrand et al. 1986b, Behle and Michels 1993, Kiełkiewicz-Szaniawska 2003]. Other results may be found in the literature available. Gomez et al. [2006] did not observe any significant influence of feeding and variable aphids abundance on the level of sugars noted in infested leaves of host plants. A decrease in carbohydrates and free amino acids content as a reaction to infestation of leaves by *Rhopalosiphum padi* L. was also observed in bird cherry [Sytykiewicz 2007].

According to Karban and Baldwin [1997] herbivore insects may induce changes in host plant quality. It was demonstrated in the present study that susceptibility of plant species to *C. hesperidum* feeding was modified not only by the constitutive resistance based on qualitative and quantitative chemical composition of host tissues, but also depended on specific reactions stimulating or inhibiting insects feeding or nutritional usefulness of host tissues for herbivores. As demonstrated by numerous authors, the changes in primary metabolites content occurring in the leaves as a result of insect feeding depend on specific host plant species reaction [Bi et. al. 1997, Leszczyński 2001, Agrell et al. 2003, Kiełkiewicz-Szaniawska 2003, Despres and David 2007, Sempruch 2008]. Leszczyński et al. [2001] found that a decrease in the content of free protein essential and non-essential amino acids in the tissues of bird cherry caused that aphids left the primary host and moved to secondary host, the tissues of which contained about 10% higher level of free amino acids. As demonstrated, the lack of deficiency of nitrogen in diet is a source of serious disorders in the course of proper growth and development of the insects [Malinowski 2008, Sempruch 2010], which was also confirmed in this study. Studies concerning the induction of changes in metabolites content in plant tissues affected by scale insects feeding are not very popular [Fernandes 2011]. They seem however to be of a special significance, since as shown by Gonzales et al. [2002] a negative effect of insect feeding on quality of nutrients in the plant, significantly disorders further development of the species of low mobility, including scale insects.

The changes in the content of examined phenolic compounds affected by soft brown scale feeding were found in the plant species. The lemon, which was the most abundantly infested by scale insects, reacted to their feeding with a significant increase in phenolic acids content and strong decrease in the content of tannins and flavonoids in the infested leaves. The least colonized ficus was characterized by the lowest content of phenolic acids and tannins, and the highest level of flavonoids, also in the leaves of the control plants. Its reaction to feeding was a significant decrease in all secondary metabolites level in the in-

fested leaves. Fern leaves' reaction to scale insects feeding was a decrease in phenolic acids content with a concurrent increase in the level of tannins and flavonoids. The result prove a specific response to scale insects feeding of each plant species secondary metabolism. An increase in phenolic compounds concentration is considered to be one of the basic immunological responses of the plants to insects and mites feeding [Tomczyk 2001, Gantner 2007]. In the present study, an increase in total content of phenolic compounds (tannins and flavonoids) resulting from *C. hesperidum* feeding, was only observed in fern leaves. However, in the light of the results, its significance for soft brown scale feeding on *N. biserrata* was not proved. Numerous scale insects were feeding on fern despite the higher tannin and flavonoids content in the leaves of infested plants as compared to the control as well as other infested plants species. Additionally, the scale insects colony feeding on *N. biserrata* were characterized by low nymphal mortality and large body size as compared to individuals feeding on lemon and ficus. Qualitative analysis of these secondary metabolites should be performed in further studies aimed at determination of the role of particular compounds including tannins and flavonoids in interactions between the examined plant species and *C. hesperidum*.

Significant changes in the content of particular identified phenolic acids were noted in the leaves infested with scale insects. It seems that in the case of *C. hesperidum*–host plant interactions, changes specific for each plant observed in the content of particular phenolic acids affecting the scale insects via antibiosis should be taken into account. Strong, 40% increase in *p*-coumaric and chlorogenic acid concentration, and ca. 2-fold increase in α-resorcinol acid content were noted in ficus which was the least susceptible to *C. hesperidum* feeding. Lemon leaves reacted to scale insects feeding with an increase in gallic, protocatechuic, syringic, caffeic and chlorogenic acids contents. The strongest decrease in *C. limon* var. Ponderosa leaves was noted for ferulic acid. The strongest decrease in the content and percentage of chlorogenic and α-resorcinol acids was observed in infested leaves of *N. biserrata.* Despite the high content of identified phenolic acids in the ferns leaves in relation to other plants species, these metabolites did not constitute a barrier to scale insects feeding and development. Presumably, the reason was that the leveled contribution of particular phenolic acids, identified in fern leaves, did not exceed 22%. Soft brown scale feeding on *N. biserrata* spent the most time on phloem sap ingestion. *C. hesperidum* individuals on fern were characterized by the lowest nymphal mortality, the large body dimensions and honeydew daily excretion and excretion rate compared to scale insects feeding on ficus and lemon.

It may be concluded in the view of results that α-resorcinol and chlorogenic acids may play a significant role in an induced resistance of plants to *C. hesperidum*. This is reflected by a strong reaction of ficus involving an increase in the content of mentioned phenolic acids in the leaves infested with scale insects, and considerably weaker reaction in lemon and fern leaves abundantly infested by *C. hesperidum*.

Among benzoic acid derivatives identified in the study, resorcinol acid is characterized by the strongest reducing properties. In turn, as reported by numerous authors, chlorogenic acid may be induced as a result of insects feeding conditioning their resistance [Gueldner et al. 1992, Ellis 1999]. Its negative influence on feeding and mobility of *Coccus viridis* instar nymphs [Fernandes et al. 2011], development of aphids and thrips [Leszczynski et al. 1985, Miles and Oertli 1993, Leiss et al. 2009] as well as the insects from Lepidoptera (*Spodoptera frugiperda*, *Helicoverpa zea*) and Diptera (*Psila rosae*) genus, was proved [Gueldner et al. 1992, Ellis 1999]. Alpha-resorcinol acid was not observed in lemon leaves, and despite 2--fold increase in chlorogenic acid content observed in leaves infested with scale insects, percentage share of this acid in total pool of phenolic acids was low. In fern leaves, strong, 6-fold decrease in the content of chlorogenic acid and 2-fold of α-resorcinol acid was noted, which would have affected insects preferences and additionally assure very good conditions for development on this plant.

As demonstrated in numerous studies [Hildebrand et al. 1986, Felton et al. 1989, Bi and Felton 1995, Bi et al. 1997, Leszczyński 2001] total phenols are considered as antioxidant enzyme substrates, the decrease in the total phenol content of the infested leaf was concomitant with the high activities of peroxidases. Phenolic compounds may be oxidized to quinones, which are characterized by considerably higher toxicity towards the pests by peroxidases which cause formation of hydrogen peroxide and hydroxyl radical. Hydroxyl radical may initiate lipids peroxidation, determined by an increase in malondialdehyde content. An increase in hydrogen peroxide production observed in the plants as a result of biotic stress may activate defense mechanisms in plants, including peroxidases activity increase. The main task of oxidative enzymes is catalyzing and reduction of toxic intermediate products of oxygen metabolism, which prevents damages formation in plant cells [Hildebrand et al. 1986, Felton et al. 1994a, b, Stout et al. 1999, Chaman et al. 2001, Ni et al. 2001, Heng-Moss et al. 2004]. Increased peroxidase levels may enhance the plant ability to tolerate insect feeding. That phenomenon plays an important role in the plant defense system. Peroxidases contribute in detoxication of numerous phenolic compounds as well as in regeneration processes of plant cells, lignification, suberization, somatic embryogenesis, auxin metabolism, wound healing, as well as, defense against pathogens and other biotic and abiotic factors [Hildebrand et al. 1986, Constabel 1999, Hiraga et al. 2001]. Production and destroying of reactive oxygen species (ROS) in plants are of a key significance in plant tolerance mechanism [Hildebrand et al. 1986, Heng-Moss et al. 2004]. In a view of the results, mainly ascorbate peroxidase is responsible for the course of reaction as a response on *C. hesperidum* feeding in *C. limon* var. Ponderosa, *F. benjamina* and *N. biserrata*. The role of ascorbate peroxidase is first of all catalysation of hydrogen peroxide to water and oxygen, while guaiacol peroxidase oxidizes phenolic compounds in the expense of hydrogen peroxide and is considered to be a key enzyme in biosynthesis of lignin [Maffei et al. 2007]. In the present study the changes in APX activi-

ty resulting from scale insects abundance with respect to the level in control plants were usually higher than those demonstrated for GP. An increase in peroxidases activity influenced by feeding of various species of herbivores Cicadellidae and *Tetranychus urticae* on soya, *Aphis gossipi* Glover and *Heliocoverpa zea* (Boddie) on cotton, was noted in numerous studies available in the literature [Hildebrand et al. 1986, Felton et al. 1989, Bi et al. 1997, Tomczyk 2001, Gomez et al. 2004]. On the other hand, Khattab [2007] observed significantly reduced oxidative enzyme activities among them, ascorbate peroxidase while polyphenol peroxidase and oxidase activities were enhanced by aphid infestation. The results of the present study also demonstrated variable activity of APX and GP in plants infested with scale insects. The relationship between the changes in the activity of enzymes analyzed and scale insects abundance was demonstrated for the first time in this study. The highest increase in APX activity in lemon and fern leaves compared to the control was caused by *C. hesperidum* feeding in II (11–30 individuals per leaf) and V (mass occurrence) density classes. An increase in malondialdehyde in II class was accompanied by a strong increase in APX and GP activity, as an enhancement of natural defense mechanisms of the plants (systemic resistance) already observed in I class. It seems that II class (11–30 individuals per leaf) is a critical value for this plant, on which scale insects feeding caused severe damages to cytoplasmic membranes. Chlorosis, local necrosis and leaves falling were observed in II class (11–30 individuals per leaf) on lemon leaves and these symptoms were intensified with an increase in scale insects population abundance on the leaves [Golan, unpublished data]. Hildebrand et al. [1986] showed that increased level of peroxidase activity affected superoxides detoxication and lowered tissues damage compared to susceptible plants. In the leaves of ficus which is the least susceptible to *C. hesperidum*, strong activity of enzymes and high content of malondialdehyde was maintained for all density classes, while in lemon and fern leaves this activity was variable. Antioxidants level and malondialdehyde content increase observed in the present study was probably a reaction of the plants to the increasing level of oxidation stress in the tissues and demonstrated lipids peroxidation. Heng-Moss et al. [2004] observed the changes in peroxidase content, which in resistant plants was maintained on higher level in plants infested with Hemiptera compared to the control, while susceptible plants reacted to insect feeding with a decrease or insignificant changes in its activity. Aslanturk et al. [2011] also recorded that herbivore insect of *Eucalyptus* exerted a significant increase in lipid peroxidation, measured as malondialdehyde, compared with healthy ones. As may be concluded from the literature and results presented, biotic stress stimulate subsequently lipid peroxidation of the cell macromolecules [Baker and Orlandi 1996, Aslanturk et al. 2011]. The increase in lipid peroxidation may be due to the incapability of antioxidants to capture all the active oxygen species produced by this biotic stress [Aslanturk et al. 2011].

Scale insects in abundance from 31 to 100 individuals per leaf (III and IV classes) feeding on the leaves of C. *limon* var. Ponderosa and *N. biserrata*, caused a suppression in strong activity of the enzymes and their maintenance on stable level, however higher than the control. In the leaves of lemon infested with scale insects, the lowest level in MDA content was found as compared to II class (however higher than in the control) was noted in III class (from 31 to 50 individuals per leaf), IV class (from 51 to 100 individuals per leaf) and V class (over than 100 individuals per leaf) was noted. The reaction of lemon leaves on mass infestation by scale insects was the subsequent strong increase in APX activity, decrease in GP activity and decrease in malondialdehyde level. Lemon reaction may be explained by breaking up its resistance which in turn may be cause by the increased stress factor (insect abundance) probably inducing next increase in ROS (reactive oxygen species). It may be supposed, the present results show the degradation of cell membranes and slow apoptosis of the cells damaged as a result of insects attack and hydrogen peroxide generated from oxidation stress. Similar tendency was observed in fern leaves for the changes in both enzymes activity and malondialdehyde content. In turn, renewed increase in malondialdehyde content and both enzymes activation were noted as a result of mass infestation of the leaves with scale insects. These results may suggest that despite numerous population of scale insects feeding on the leaves, *N. biserrata* is able to maintain the balance between reduced and oxidized forms of all biomolecules counteracting biotic stress [Olko and Kujawska 2011]. The literature data [Hildebrand et al. 1986, Heng-Moss et al. 2004] prove that these processes play a key role in plants tolerance mechanism, while the results obtained confirm that thesis. The results of this study prove active, complex, often contrast mechanisms initiated in *N. biserrata* in order to neutralize the results of biotic stress and to enable normal functioning of cells in plants infested by scale insects. The symptoms of scale insects feeding on *N. biserrata* leaves were less visible compared to those observed for lemon (Golan unpublished data). According to numerous authors [Stout et al. 1999, Ni et al. 2001, Chaman et al. 2001, Khattab 2007], the changes in APX activity in plants may be affected by various time of phytophagous feeding. In the study conducted by Kiełkiewicz-Szaniawska [2003], peroxidase (PX) was characterized by higher activity compared to other enzymes in an initial stage of mites feeding on the leaves of less susceptible tomato varieties, however the peroxidase activity weakened after longer time of herbivores feeding on the leaves. Łukasik et al. [2012] observed higher activity of APX in less susceptible triticale varieties compared to susceptible varieties, as a response to aphid feeding. Maximum level of enzyme was observed after 72 hours of aphids feeding. The earliest reaction to herbivore feeding describing an occurrence of induced resistance mechanisms on plants is most often studied [Hildebrand et al. 1986, Felton et al. 1989, Bi et al. 1997, Stout et al. 1999, Chaman et al. 2001, Ni et al. 2001, Tomczyk 2001, Gomez et al. 2004, Khattab 2007]. Recent results of the research demonstrate that defense strategies of nu-

merous plant species to herbivores are just connected with induced resistance. This probably results from the fact, that resistance induction is less costly strategy for the plants compared to constitutive response [Baldwin 1998, Kiełkiewicz-Szaniawska et al. 2010].

In order to provide an unequivocally objective response to this issue and due to small number of publications concerning scale insects, further research is needed to investigate elicitors, components of the plant defense-signaling pathways, and additional metabolic responses that are induced by *C. hesperidum* attack.

7. CONCLUSIONS

1. Interactions between host plants and *C. hesperidum* occur on all relationship levels.

2. The host plant has a crucial influence on morphometric and demographic parameters of *C. hesperidum* and the level of acceptance and colonization by Coccidae. Soft brown scale showed negative values of morphometric and demographic parameters feeding on *F. benjamina*. In contrast, the individuals developing on *C. limon* var. Ponderosa and *N. biserrata* were characterized by higher body size, short pre-reproductive period and elongated reproduction period, as well as high females fecundity, as compared to scale insects feeding on *F. benjamina*. *C. hesperidum* individuals on fern were characterized by the lowest mortality index, and older larval stages as well as females were dominant in the age structure of their colonies.

3. The examined plant species are characterized by various degree of susceptibility to *C. hesperidum* feeding. *C. limon* var. Ponderosa and *N. biserrata*, appeared to be a suitable hosts for soft brown scale on which *C. hesperidum* could develop and expand in number. The low level of susceptibility is characteristic for *F. benjamina*. Ficus turned out to be the lowest abundant plant species as compared to lemon and fern. The low susceptibility of *F. benjamina* to *C. hesperidum* feeding were affected by the low amino acids content influencing the reduction of body dimension and increasing nymphs mortality. The highest content of flavonoids in ficus leaves inhibits the insects feeding and increasing nymphs mortality. The high content of total sugar, reducing sugars and shorter time of feeding in floem affected the elongation of pre-reproductive period. The reproductive period is reduced by the high content of sugars and increased content of protein and nitrogen in ficus leaves.

4. The differences in the level of plants acceptance by *C. hesperidum* are conditioned by their constitutional properties (via antixenosis and antibiosis): leaves structure and mainly biochemical composition. The highest acceptance of *F. benjamina* in the free choice test by 1st-instar nymphs, as compared to lemon and fern, is positively influenced by leaves structure (small thickness of the leaf blade at the main nerve and its vicinity) and mainly biochemical composition (high content of total sugars and reducing sugars, total nitrogen and protein nitrogen as well as total protein, with low content of tannins and phenolic acids).

5. The differences in host plants colonization by scale insects result from an induction via stimulation or limitation of the concentration of substances constitutionally observed in the plants. The intensity and direction of metabolic changes in ficus leaves (high decrease in amino acids content, relatively high content of flavonoids and high increase in the content of α-resorcinol, chlorogenic acids) considerably limited the colonization and development rate of *C. hesperidum,* more effectively than in lemon and fern.

6. Four EPG waveforms were distinguished: 1) C waveform: penetration of peripheral tissues; 2) pd waveform: potential drops; 3) E11 waveform: sieve element salivation 4) E2 waveform: phloem sap ingestion. No waveform G: xylem sap ingestion, and F: derailed stylet mechanics were observed during soft brown scale feeding.

7. The duration of particular feeding phases of *C. hesperidum* was differentiated and depend on plant suitability for insects. Soft scale insects spent the longest time on feeding in plant peripheral tissues and less time on phloem sap ingestion on less susceptible *F. benjamina*. The longest feeding phase in phloem and shortened in peripheral tissues of the plants were found on the more susceptible *C. limon* var. Ponderosa and *N. biserrata.*

8. All development stages of *C. hesperidum* contribute in honeydew excretion process, whereas the highest honeydew excretion rate and daily excretion are characteristic for the first–instar nymphs.

9. The host plant affects an excretion rate and daily excretion of honeydew. The highest number of honeydew droplets in time unit and per day was excreted by the scale insects feeding on fern. Relatively low honeydew excretion rate and daily excretion was noted for scale insects from ficus. The size of the droplet of honeydew excreted depends on the development stage of the insect and its host.

10. *C. hesperidum* feeding affects the changes in the physiological state of its host plants. The kind of scope of these changes depend on host plant species and number of insects feeding on it. The scale insects colony in a number from 11 to 30 individuals per 1 leaf are the threat to the plant resistance mechanisms for which the maximum level of the analyzed physiological parameters are observed.

11. The results may be used in the practice. Monitoring the size and number of honeydew droplets facilitates registration of this pest presence on the plants, identification of its development stage and determination of optimum date of its control. The chemical control of *C. hesperidum* should be conducted when the mean abundance of insects does not exceed 10 individuals per one leaf.

SUMMARY

Interactions between host plants and *Coccus hesperidum* L. (Hemiptera; Sternorrhyncha; Coccidae)

Understanding of complex relationships between the insects and their hosts is one of the main aims of current ecology and plant protection. Although a great number of Sternorrhyncha studies have been carried out on basic plant–insects interactions during the last ten years, interaction between plants and scale insects are poorly described. Therefore the following research aims were accepted in order to determine: 1) the effect of host plants on morphometric, demographic parameters and age structure of *C. hesperidum* developing on various host species; 2) the plant acceptance and colonization by *C. hesperidum* 3) the process of *C. hesperidum* feeding monitored in plant tissues using EPG; 4) honeydew excretion dynamics on various host plant species; 5) the response of *C. hesperidum* to host plant biochemical properties; 6) the effect *C. hesperidum* feeding on biochemical changes of colonized plants; 7) host plant susceptibility to *C. hesperidum* feeding.

The studied material consisted of two-year old plants of *Citrus limon* var. Ponderosa, *Ficus benjamina* and *Nephrolepis biserrata*. For artificial colonization *Coccus hesperidum* a polyphagous pest of ornamental plants was selected. Identification of particular stages of *C. hesperidum* was conducted based on microscopic sliders. The feeding behaviour of soft brown scale was monitored using the technique of EPG [Leszczyński and Tjallingii 1994]. The analysis of *C. hesperidum* honeydew excretion activity was performed following the method by Koteja [1981] modified by Golan [2008a]. The biochemical and physiological analysis of plants material control and infested by scale insects was performed using commonly known methods described in literature.

The study demonstrated significant differences between the time of pre-reproductive period and females average daily fecundity only for *C. hesperidum* feeding on lemon and ficus and over 98% mortality of the first-instar nymphs from *F. benjamina.* During presented study *C. limon* var. Ponderosa was the most abundantly colonized host species. However in the free-choice test nearly 50% of mobile instar nymphs of *C. hesperidum* chosen *F. benjamina* for a suitable host for feeding on. The results of EPG tests proved that females feeding on *C. limon* var. Ponderosa and *N. biserrata* faster started plant tissues penetration,

shorter penetrated peripheral tissues and longer ingested phloem sap compared to individuals from *F. benjamina.* The values of honeydew excretion rate and daily excretion of *C. hesperidum* decreased with insect's age. The leaves of analyzed host plants differed significantly in the content of primary and secondary metabolites. *F. benjamina* leaves contained highest amount of sugars and protein nitrogen but the highest content of essential and non-essential amino acids was characteristic for the control leaves of *N. biserrata*. Among analyzed host plants the highest content of tannins was noted in lemon leaves, when leaves of ficus were characterized by significantly highest content of flavonoids and leaves of fern – phenolic acids. Scale insects infestation caused increase concentration of sugars and usually decrease in other examined primary metabolites concentration in leaves. Also the changes in physiological state of the plants are a result of *C. hesperidum* feeding, changes depend on host plant species and number of individuals feeding on it. Constant high activity of antioxidative enzymes and malondialdehyde level is characteristic for *F. benjamina* which is the least susceptible on soft brown scale feeding. The analyzed parameters changes in the leaves of lemon and fern were the strongest during *C. hesperidum* individuals feeding on the leaves in density class II and V. The threat for resistance mechanisms breaking in these plants is soft brown scale population in a number from 11 to 30 individuals per 1 leaf, for which maximum level of the analyzed physiological parameters is observed. In *C. limon* var. Ponderosa, plants colonization with this number of insects caused the changes in cell membranes permeability leading to their degeneration and plants leaves dying.

The examined plant species are characterized by various degree of susceptibility on *C. hesperidum* feeding. Low degree is characteristic for *Ficus benjamina,* on which a decreased rate in a development of *C. hesperidum* colonies was noted with unprofitable influence of this plant on morphometric, demographic parameters, as well as feeding and honeydew of this scale insect. The studies for the first time have shown the differences in the degree of plants acceptance by *C. hesperidum* are conditioned by their constitutional properties: leaves structure and mainly biochemical composition. The differences in host plants colonization by scale insects result from an induction via stimulation or limitation of the concentration of substances constitutionally observed in the plants. The results obtained may be used in the practice, and especially practical application may be found for the results of the study on honeydew process and on mechanisms of plants resistance on *C. hesperidum* feeding.

STRESZCZENIE

Interakcje pomiędzy roślinami żywicielskimi a *Coccus hesperidum* L. (Hemiptera; Sternorrhyncha; Coccidae)

Zrozumienie złożonych zależności pomiędzy owadami i ich żywicielami jest jednym z głównych celów współczesnej ekologii i ochrony roślin. W ostatnich latach prowadzono wiele badań na temat interakcji owadów z i ich roślinami żywicielskimi, jednakże prace dotyczące czerwców były sporadyczne. W związku z powyższym, w niniejszej pracy zaplanowano kompleksowe badania obejmujące biochemiczne i behawioralne interakcje pomiędzy czerwcami i ich roślinami żywicielskimi. W badaniach wytyczono następujące cele: 1) określenie wpływu roślin żywicielskich na parametry morfometryczne, demograficzne, strukturę wiekową kolonii *C. hesperidum*, 2) określenie zasad wyboru i zasiedlania roślin, 3) opracowanie procesu żerowania *C. hesperidum* na różnych gatunkach roślin żywicielskich, 4) opracowanie procesu spadziowania *C. hesperidum*, 5) określenie reakcji *C. hesperidum* na właściwości biochemiczne roślin, 6) określenie wpływu żerowania misecznika cytrusowego na biochemiczne i fizjologiczne parametry roślin, 7) określenie stopnia podatności roślin żywicielskich na żerowanie *C. hesperidum* na podstawie biochemiczych uwarunkowań interakcji roślina – czerwce.

Materiał do badań stanowiły rośliny *Citrus limon* var. Ponderosa, *Ficus benjamina* i *Nephrolepis biserrata*. Identyfikację stadiów rozwojowych czerwców przeprowadzono na podstawie trwałych preparatów mikroskopowych. Rejestrację zachowania *C. hesperidum* na roślinach monitorowano z zastosowaniem techniki EPG [Leszczyński i Tjallingii 1994]. Analizy procesu spadziowania czerwców wykonywano metodą opisaną przez Koteję [1981] i zmodyfikowaną przez Golan [2008a]. Analizy biochemiczne i fizjologiczne materiału roślinnego wykonywano, opierając się na powszechnie stosowanej w piśmiennictwie metodyce.

Badania wykazały występowanie różnic w wartości analizowanych parametrów demograficznych *C. hesperidum* pod wpływem gatunku rośliny żywicielskiej. Wydłużeniem okresu prereprodukcyjnego, skróceniem okresu reprodukcji, najniższą średnią płodnością dzienną oraz niezwykle wysoką śmiertelnością stadiów larwalnych odznaczały się osobniki *C. hesperidum* żerujące na *F. benjamina*. Porównanie średniej liczby osobników *C. hesperidum* na bada-

nych roślinach wykazało, że *C. limon* var. Ponderosa był gatunkiem najliczniej zasiedlanym, jednakże podczas testu swobodnego wyboru niemal połowa stadiów ruchomych wybierała żerowanie na fikusie. Wyniki testów EPG dowiodły, że samice *C. hesperidum* żerujące na cytrynie i paproci szybciej rozpoczynały penetrację tkanek roślinnych, krócej penetrowały tkanki peryferyjne oraz dłużej pobierały pokarm z floemu w porównaniu z osobnikami z fikusa. Intensywność i aktywność spadziowania czerwców malały wraz z wiekiem owadów. Wyniki analiz chemicznych wykazały, że liście badanych gatunków roślin różniły się istotnie zawartością metabolitów podstawowych i wtórnych. Liście *F. benjamina* zawierały najwięcej cukrów oraz azotu białkowego, natomiast liście *N. biserrata* aminokwasów egzo- i endogennych. W kontrolnych liściach cytryny notowano najwyższą zawartość garbników, fikusa: flawonoidów, natomiast w liściach *N. biserrata*: kwasów fenolowych. Pod wpływem żerowania *C. hesperidum* w liściach analizowanych gatunków roślin obserwowano wzrost stężenia cukrów. Stężenie pozostałych metabolitów pierwotnych spadało w liściach roślin zasiedlonych czerwcami w porównaniu z kontrolą. Zawartość kwasów fenolowych rosła w zasiedlonych czerwcami cytrynach, wzrost zawartości garbników i flawonoidów pod wpływem żerowania czerwców notowano w liściach paproci. Reakcją obronną cytryny na żerowanie czerwców był wzrost zawartości kwasu chlorogenowego, protokatechowego, syryngowego i kawowego. W zasiedlonych liściach fikusa notowano natomiast wzrost zawartości kwasu α-rezorcynowego, chlorogenowego i *p*-kumarowego, a w liściach paproci: kwasu *p*-kumarowego i protokatechowego. Również reakcja fizjologiczna badanych gatunków roślin na zasiedlenie zmienną liczebnością czerwców była zróżnicowana. Maksymalny poziom MDA, APX i GP w liściach *C. limon* var. Ponderosa i *N. biserrata* wystąpił pod wpływem żerowania kolonii czerwców w liczebności od 11 do 30 osobników na jeden liść.

W wyniku prezentowanych badań wykazano, że badane gatunki roślin cechuje różny stopień podatności na żerowanie *C. hesperidum*. Niskim stopniem charakteryzuje się *F. benjamina*, który niekorzystnie oddziaływał na analizowane parametry owadów. W wyniku prezentowanych badań po raz pierwszy wykazano, że różnice w stopniu akceptacji roślin przez *C. hesperidum* warunkowane są budową anatomiczną roślin oraz ich właściwościami konstytutywnymi. Na różnice w zasiedlaniu roślin żywicielskich przez czerwce wpływało natomiast indukowanie substancji występujących w roślinach konstytutywnie. Uzyskane rezultaty mogą być wykorzystane w praktyce, a w szczególności zastosowanie mogą znaleźć wyniki badań nad procesem spadziowania oraz nad mechanizmami odporności roślin na żerowanie *C. hesperidum*.

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