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Characteristics of the honeydew excretion process of *Coccus hesperidum* (Linnaeus, 1758) /Hemiptera, Coccoidea/ in different development stages

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Introduction

Honeydew is a substance characteristic for hemipterous of the Hemiptera order such as aphids, scale insects and psyllids. These insects insert their rostrum to the tissues' phloem, taking in plant sap from parenchymal cells or sieve tubes. Scale insects take in nutrients from sieve tube, whereas they remove the excess of water, sugar and some aminoacids in the form of honeydew by means of specially adjusted anal apparatus (BEN-DOV & HODGSON, 1997; KOTEJA 1996a). To satisfy their need for carbohydrates and nitrogen these insects take in large amounts of nutrients. Thanks to a special adjustment of the digestive tract, filter ventricle, they can quickly get rid of unnecessary liquids contained in plant sap. In hemipterons which have filter ventricle there is a twice- and three times more intense excretion of honeydew drops (GAŁUSZKA, 1996). The knowledge on the process of honeydew excretion in scale insects is in general very poor, and the number of publications is based on the methods which use new technologies is scarce (BOGO & MANTLE, 2000). In Poland the process of honeydew excretion was studied by KOTEJA (1981, 1996a). In foreign publications much attention is given to the issue of chemical composition of honeydew (BOGO *et al.*, 1998; BOGO & MANTLE, 2000; BOGO *et al.*, 2001; GRAY 1952; SALAMA & RIZK, 1979; VARSHNEY *et al.*, 1978) and its use by beneficial insects to form honeydew (CROZIER, 1981; SANTAS, 1985; GAŁUSZKA *et al.*, 1996) or to explain the dependencies between the producers of honeydew

and the insects which use it (BACH, 1991; BEGGS, 2001; JAMES *et al.*, 1999; LE RUE & CALATAYUD, 1994; WANG & TANG, 1994).

Coccus hesperidum (Linnaeus, 1758) is one of the honeydew producing scale insects, commonly recorded on ornamental plants cultivated in greenhouses. It is a cosmopolitan species and a typical polyphag observed on many host plant species, one of the most dangerous citrus pest in California, Texas and South Africa (BEN-DOV, 1993). It belongs to difficult species of scale insects in glasshouses which are found in Poland (DZIEDZICKA, 1990). Just like soft scale, they stay on leaves, green parts of shoots and on fruit. The damage caused by single specimens is small, however, when their number is large the leaves become yellow and fall down, there is less fruit and plants are more likely to die. Honeydew is often the first sign of the presence of scale insects on plants which the insect produces in larger amounts in comparison with other species (COPLAND & IBRAHIM, 1985).

The aim of the research was to study the process of honeydew excretion: the activity of honeydew produced and daily activity, duration of particular developmental stages of *C. hesperidum* and physical properties of honeydew.

Material and methods

The analysis of *C. hesperidum* honeydew excretion was carried out on the basis of a method applied by KOTEJA (1981). Three modified daily types of thermohygrographs were applied in the research. In places where paper is put on the thermohygrograph barrel to register temperature and humidity, a x-rayfilm, on which honeydew drops are well preserved, was placed. After each barrel's turnover (about 24 hours) the film were removed and analysed. Over the thermohygrograph barrel which was spinning at a constant velocity, a transparent plastic plate was installed on which plant parts with scale insects were attached from the inside. *Ficus beniamina* L. was used for the experiment (Figs. 1 and 2). Each time 3-5 specimens of soft scales were studied on one thermohygrograph. The experiment was repeated three times, taking for granted that the *C. hesperidum* developmental cycle lasted for about 60 days. The research was carried out in laboratory conditions, for 24 hours. The intensity of honeydew excretion was studied since the first drops of honeydew were observed on plates until they ended for a given specimen. Six fully registered and a dozen shorter paths were selected for analysis. The paths of honeydew excreted by a given specimen were marked on plates with a pen and then the number of drops in each path was counted. The duration of particular developmental stages was assessed on the basis of observations of an uninterrupted process of honeydew excretion. In the experiment it was supposed that in the process of honeydew excretion there would occur intervals which are typical

for the period of moult. Each longer interval that occurred on the plate ended a subsequent developmental stage. The degree of honeydew excretion activity was assessed on the basis of: honeydew excretion activity of a given specimen (average amount of honeydew drops per hour) and daily activity (amount of drops per day).

Figure 1. Thermohygrograph used for the experiment

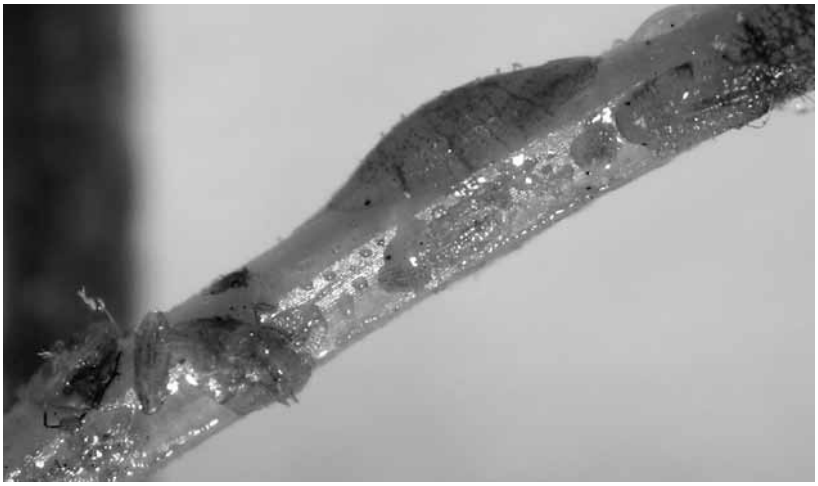


Figure 2. *Coccus hesperidum* on the leaf tail of *Ficus beniamina*

The measurements of the honeydew size and mass were made on the basis of a modified method described in a paper by NISHIDA & KURAMOTO (1963). A fragment of a plant with *C. hesperidum* specimens was placed directly over plate, onto which honeydew drops were falling down. The weigh of honeydew drops was obtained by weighing a plate of a given mass and honeydew drops present on it on an analytical balance. Then the amount of the mass of plate was subtracted from the result. The size of honeydew drop falling on a plate was assessed by means of measuring its diameter using a scaled stereomicroscope. All photographs included in the chapter have been taken by the author.

Results

The duration of subsequent developmental stages which was determined on the basis of observation of the activity of honeydew excretion in particular specimens and intervals that were present there is provided in Table 1.

Table. 1. Mean duration of *C. hesperidum* stages on *F. beniamina* in the observation of honeydew excretion by specimens from 15 full developmental cycles

Developmental stage of scale insect	L_1	$L_1/L_2♀$	$L_2♀$	$L_2♀/♀$	♀	Life cycle longevities
Mean duration (days/hours)	12-21 days	12-21 hours	15-21 days	24,5 hours	20-27 days	49-63 days
Mean	16.5		18		24	56

The *C. hesperidum* life cycle duration which was determined on the basis of analysis of honeydew excretion ranged from 49-63 days. Particular developmental stages lasted respectively: L_1: 12-21 days, $L_2♀$: 15-21 days, ♀: 20-27 days. The intervals in process of honeydew excretion were observed to take place between them, and they lasted respectively: $L_1/L_2♀$ – 28.5 hours and L_2+/ + about 24.5 hours.

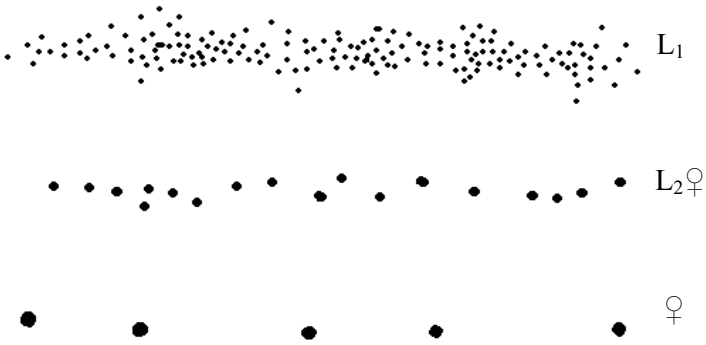
The results of observation of honeydew excretion daily activity by subsequent developmental stages of *C. hesperidum* is presented in Figure 3, 4 and in Table 2. The biggest average daily activity in honeydew excretion was observed in larvae of the first stage, which at the time of their development excreted on average more than 4 drops/ hour. Both larvae of the second stage as well as females excreted less than one drop per hour, 0.94 and 0.71 drop/ hour respectively. The activity of larval stages during a day was higher than in case of mature females and amounted to L_1 – 99.83 drops for $L_2♀$ - 25.5 drops. Females excreted on average more than 17 drops per day (Tab. 2).

Table 2. Mean values of honeydew excretion activity, daily activity, size and weigh of honeydew drops of three developmental stages of *C. hesperidum* on the basis of 10 specimens and 20 honeydew drops in each stage observation

Developmental stage	Average activity of honeydew excretion (drops/hours)	Daily activity (drop/day)	Average size of drops (mm)	Weigh of drops (mg)
L_1	4.16	99.83	0.2-0.3 (0.25)	0.0008-0.0055
L_2♀	0.94	25.55	0.4-0.6 (0.5)	0.01
♀	0.71	17.04	0.4-0.7 (0.55)	0.04

Honeydew excretion by *C. hesperidum* took place by means of producing single drops. A drop diameter ranged from 0.2-0.7 mm. The smallest drop which had the smallest diameter and weigh was excreted by larvae of the first stage. An average drop diameter and weigh was 0.2-0.3 mm and 0.0008-0.0055 mg respectively (Tab. 2). Honeydew drops which were observed on plates and excreted by L_1 were positioned in close proximity, chaotically, rarely along a single line. Specimens in older developmental stages (L_2♀, ♀) excreted drops along a straight line periodically with short intervals in single specimens observed during the day (Fig. 3).

Figure 3. Part of paths of honeydew by *C. hesperidum* three developmental stages (L_1, L_2♀, ♀)

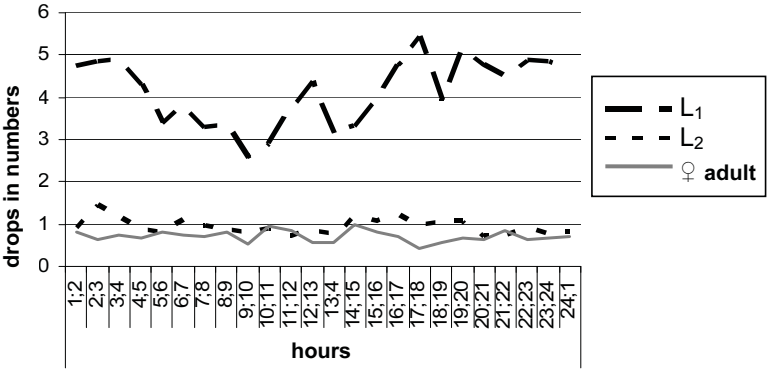


The diameter of honeydew drops excreted by these insects ranged between 0.4-0.6 mm for L_2♀. Average weight of a single drop was respectively L_2♀ = 0.01 mg, ♀ = 0.04 mg (Tab. 2).

In all the observed developmental stages an increase in the activity of honeydew excretion in the afternoon and at night, followed by a decrease in the morning (Fig. 4).

The highest values of average activity of honeydew excretion during the day for the youngest larvae of the first stage was observed between 5 pm and 6 pm (5.42 drops/hour). A significant decrease in the activity of honeydew excretion was recorded between 9 am and 10 am (2.98 drops/hour). After 7 pm the value of activity of honeydew excretion of L_1♀, remained on a high level until 3 am (about 5 drops/hour). Analysing average activity of the larvae of the second stage the smallest value during the day was reached between 11 am and 12 pm (0.69 drops/hour), and the maximum value was reached between 2 am and 3 am (1.43 drops/hour). Average activity of females was quite low and did not exceed 1 drop per hour. The lowest value of activity of honey dew excretion of this developmental stage was observed between 5 pm and 6 pm (0.44 drops/hour), and the maximum between 2 pm and 3 pm (0.98 drops/hour) (Fig. 4).

Figure 4. Mean honeydew excretion in three developmental stages of *Coccus hesperidum* on *Ficus beniamina* during a day



Conclusion and discussion

C. hesperidum is a parthenogenetic species, according to different authors it is described as both oviparae and viviparae (Tereznikowa, 1981) or viviparae (Tereznikowa, 1954-1956; Copland & Ibrahim, 1985; http://www.sel.barc.usda.-gov). Also in the presented studies no males were observed, females were parthenogenetic and ovoviviparous. The fecundity of a female, according to different authors ranges from 70 to 1000 larvae (Dingler, 1923; Tereznikowa, 1981). According to Copland & Ibrahim (1985) there is a large degree of mortality among the larvae of this species, reaching over 80%, influenced mainly by the host plant and other factors (humidity). Drowning of the larvae in honeydew which remains on the plant is another cause of larvae mortality which has been

proved in research (Ben-Dov, 1997). *C. hesperidum* occurs in all the zoogeographical regions. In the tropics and greenhouses it can develop all year long. The number of generations in a year depends on a climate zone; in Israel 6 generations were observed (Bodenheimer, 1951a; Avidov, Harpaz 1969) in the USA 3-5 (Ebeling, 1959); in Azerbaijan 3-4 (Tereznikova, 1981). The period of development from a larva to imago according to different authors depends on external factors and takes on average about 2 months (Metcalf, 1962; Borchsenius, 1957). The temperature can either shorten or prolong this period (Annecke, 1959; Metcalf, 1962). On the basis of observations on the activity of honeydew excretion it was stated that the *C. hesperidum* life cycle length in laboratory conditions lasts from 49 to 63 days. According to different authors, in the studied scale insect species there are three larval stages prior to the adult female stage, while separate the second and third stages differ from one another only by means of body size (Annecke, 1959; http://www.sel.barc.usda.gov.). However, these works do not specify clear differences in morphology or anatomy of particular stages. Saakyan-Baranova (1964), Annecke (1966) and Tereznikova (1981) provide data on the presence of two larval stages in *C. hesperidum*, whereas Tereznikova (1981) remarks a difference in the size of a young female and an adult giving birth to larvae. Saakyan-Baranova (1964) provides detailed differences in the structure of particular stages. The author's own observations did not provide a sufficient answer to these discrepancies. On the basis of honeydew paths left on x-rayfilm, it is clear that two larval stages and adult females mark their activity. On few initial paths from which the observation of the first larval stage started a short period of honeydew excretion can be recorded. It is difficult to be noted down because of the mobility and mortality of young larvae. It lasts from 1 to 3 days and ends with a 27-hour-long interval in honeydew excretion. On the basis of these few observations it is difficult to consider this period in the larva's life as a separate stage, especially since after the analysis of microscope slides no clear differences in the anatomy of observed specimens were recorded. A short initial period of honeydew excretion of the youngest larva may result from the initial excretion of substances gathered in the larva's body still during the embryo stage, before it started to actively take in nutrients. An insect excretes honeydew with a certain delay in relation to the beginning of nutrient intake, hence this period may be reflected by an interval in honeydew excretion by L_1. Between periods of honeydew excretion in every developmental stage there were also intervals in the process of honeydew excretion which were interpreted as a period of moulting. Before them there was a gradual fall in the honeydew excretion activity until it completely disappeared. After the insect entered a new developmental stage and started to take in nutrient, the activity gradually increased.

The research showed that during the development of *C. hesperidum* there are some changes in the honeydew excretion process. As the following stage

takes place, the size and mass of the excreted honeydew drops increase, but the honeydew excretion activity and total daily activity decrease. The greatest honeydew excretion activity was observed in the afternoon and at night, whereas it decreased in the morning and around midday. The highest honeydew excretion activity was typically observed in the first larval stage, and the smallest in adult females. When analysing the daily activity, also larvae of the first stage excreted the greatest number of honeydew drops during a day. During the period of moulting some intervals in the process of honeydew excretion ranging from 24.5-28.5 hours were observed. In their research, NISHIDA & KURAMOTO (1963) observed a decrease in the intensity of honeydew excretion which followed the increase in the number of *Dysmicoccus neobrevipes* (Hemiptera; Coccoidea; Pseudococcidae).

The above results and those presented in the study are alike, but they differ from the results of observations on aphids in which the size of drops and activity of honeydew excretion increased along with the developmental stages (MITTLER 1958; MITTLER & SYLVESTER 1961).

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Charakterystyka procesu spadziowania *Coccus hesperidum* (Linnaeus, 1758) /Hemiptera, Coccoidea/ w różnych fazach rozwoju osobniczego

Streszczenie

Spadź jest substancją charakterystyczną dla pluskwiaków z rzędu *Hemiptera* – mszyc, czerwców i miodówek. Badania prowadzono na jednym z gatunków spadziujacych pospolicie notowanym na ozdobnych roślinach uprawianych pod osłonami, *Coccus hesperidum* L. z rodziny *Coccidae*. Uszkodzenia powodowane przez pojedyncze osobniki tego gatunku sa niewielkie, jednakże przy dużej liczebności populacji zauważalne jest żółknięcie i opadanie liści, zmniejszenie owocowania i wigoru roślin. Często pierwszym objawem obecności tego czerwca na roślinach jest wydalana spadź, którą produkuje w większych od innych gatunków ilościach. W doświadczeniu analiza aktywności spadziowania *C. hesperidum* przepro-

wadzona została w oparciu o metodę stosowaną przez Koteję (1981). Długość trwania poszczególnych stadiów rozwojowych określano na podstawie obserwacji nieprzerwanego procesu wydalania spadzi. Stopień aktywności spadziowania określano na podstawie: aktywności spadziowania danego osobnika (średnia ilość kropli spadzi na godzinę) oraz aktywności dobowej (liczba kropli na dobę).

Przeprowadzone badania wykazały, że w czasie rozwoju osobniczego *C. hesperidum* następują zmiany w procesie spadziowania. Wraz z kolejnym stadium rośnie wielkość i masa wydalanych kropli spadzi lecz maleje aktywność spadziowania oraz aktywność dobowa. Największa aktywność spadziowania obserwowana była w godzinach popołudniowych i nocnych, natomiast malała w godzinach rannych i południowych. Najwyższą aktywnością spadziowania charakteryzowało się pierwsze stadium larwalne, a najmniejszą samice. Analizując aktywność dobową, również larwy pierwszego stadium wydzielały największą liczbę kropli spadzi w ciągu doby. W okresie linienia obserwowano przerwy w procesie spadziowania trwające średnio od 24,5 do 28,5 godzin. Długość cyklu życiowego *C. hesperidum* określona na podstawie analizy spadziowania zawierała się w przedziale od 49 do 63 dni.

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