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Improving the observation technique of the wettability of greenhouse whitefly (*Trialeurodes vaporariorum* Westwood)

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Keywords: wetting properties, contact angles, greenhouse whitefly, *Trialeurodes vaporariorum*

ABSTRACT

The hydrophobic properties of insects surface protect their organisms from wetting by water. Thanks to these properties pest may reduce wetting by spray droplets. The aim of this work was to improve observation techniques of wetting the greenhouse whitefly (*Trialeurodes vaporariorum* Westwood) after the application of spray droplets. The study confirmed the usefulness of the tilting stage with built-in flat mirror in evaluation of wetting properties of *T. vaporariorum*. The presence of abundant epicuticular waxes and dorsal microstructure may explain the hydrophobicity of pupae and larvae. As the surface tension of the liquid decreased the wetting of the insect increased. Measurement of the contact angles after spontaneous deposition of droplets on pupa of *T. vaporariorum* has not produced satisfactory results due to the shape of the dorsal surface.

Udoskonalenie techniki obserwacji zwilżenia mączlika szklarniowego (*Trialeurodes vaporariorum* Westwood)

Słowa kluczowe: zwilżenie, kąty przylegania, mączlik szklarniowy, *Trialeurodes vaporariorum*

STRESZCZENIE

Hydrofobowe właściwości powierzchni owadów zapewniają ochronę ich organizmów przed zwilżeniem przez wodę. Dzięki tym właściwościom szkodniki roślin uprawnych mogą zmniejszać zwilżenie przez krople rozpylonej cieczy. Celem pracy było udoskonalenie techniki obserwacji zwilżenia mączlika szklarniowego (*Trialeurodes vaporariorum* Westwood) po naniesieniu kropli poprzez opryskiwanie. Badania potwierdziły przydatność wykonanego stolika pochylanego z wbudowanym zwierciadłem płaskim w ocenie zwilżenia *T. vaporariorum*. Obecność obfitych wosków kutykularnych oraz mikrostruktura grzbietowej części puparium i larw mogą wyjaśniać przyczyny ich hydrofobowości. Wraz z obniżeniem napięcia powierzchniowego cieczy zwiększało się zwilżenie owada. Wyznaczenie kątów przylegania cieczy po spontanicznym osadzeniu kropli na puparium *T. vaporariorum* nie przyniosło jednak zadowalających rezultatów z uwagi na kształt powierzchni grzbietowej.

1. INTRODUCTION

Wetting the horizontal, flat, smooth, uniform surface can be fairly easily measured and very precisely characterized [1]. A number of measurement methods was developed, which are now the basis for determining the contact angles (angles of adhesion) and surface tension [2, 3]. However, in the case of biological material things may work differently. The surface of the structures building the outer covering of organisms is heterogeneous, enriched by the presence of cuticular elements and covered with epicuticular waxes. Such conditions make it difficult, sometimes impossible, to measure the wetting. The surface of insects is one of the best examples of this phenomenon. Many insect species are harmful and are routinely controlled by spraying. Animals and plants also use similar mechanisms protecting them against wetting. Epicuticular waxes, micro and nanostructure of the surface play a fundamental role in their hydrophobicity [4, 5]. Improving knowledge about the behavior of a drop of liquid with different physicochemical properties on the surface of the body of harmful insects would provide valuable information to refine the application technique of plant protection products. The greenhouse whitefly (*Trialeurodes vaporariorum* Westwood) was the object of our study because of its nymphs sedentary characteristics and due to this pest's great economic importance in greenhouse production of both vegetables and ornamental plants. The aim of the study was the improvement of observation of wetting using a tilting stage with built-in flat mirror and measurement of the contact angles on the surface of the greenhouse whitefly nymphs after application of droplets by spraying.

2. EXPERIMENTAL

2.1 The design of the tilting stage with built-in flat mirror

The basic tool in the observation of the insect's surface was a reflected light microscope. It was assumed that the basis for such observation would be a microscope of typical construction with the lens set in the vertical axis. SteREO Discovery.V12 with the control module EMS-3, AxioVision Software Rel. v.4.8.2 and camera AxioCam ERC 5 were used for this purpose. This microscope set enabled automatic image scaling and measurement. The vertical optical axis of microscope was redirected perpendicularly to enable observation of the droplet's shape. For this purpose, we applied a high quality exterior mirror which is normally used in the optical systems for redirection of the rays forming the image (Fig. 1). A flat mirror was set at a 45° angle to the objective. The specimen holder was placed on the stage. Various locations and shapes of the objects (leaves, insects) required an especially designed stage to

ensure its tilt relative to the central axis and to allow setting the image of the object in line with the observations of droplet shape. The movable specimen holder and the tilting stage allowed to adjust the position of the specimen in such a way that the base of a droplet was parallel to the horizontal line. In Figure 2 the positions of the tilting stage and specimen holder with attached cover glass in various orientations of the insect on the leaf are marked. Tilting stage with specimen holder and built-in flat mirror placed in a base of SteREO Discovery.V12 microscope is shown in Figure 3.
Adapting of the SteREO Discovery.V12 microscope to research was performed at the Optical Devices Service.

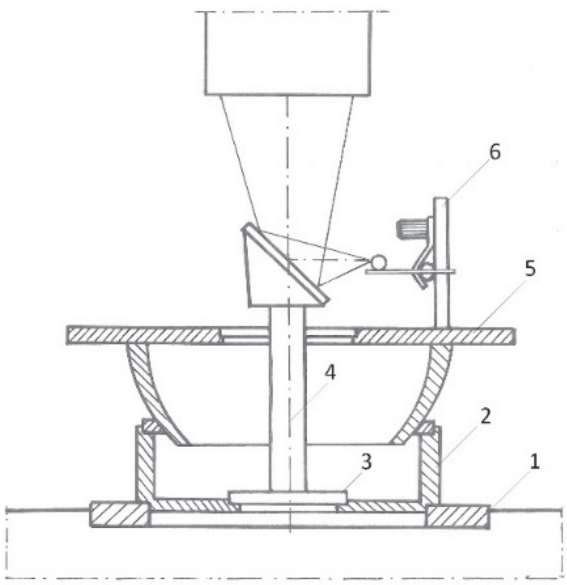

Figure 1 The general schematic diagram of the tilting stage with a specimen holder and a built-in flat mirror:
1 – base stage ring; 2 – tilting stage handle; 3 – flat mirror base; 4 – mirror handle; 5 – tilting stage; 6 – the specimen holder

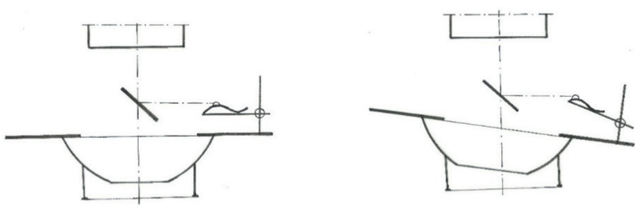

Figure 2 Operating diagram of a tilting stage with a handle and a built-in flat mirror used for positioning of the objects and observing their surface perpendicular to the vertical axis of the optical system of the SteREO Discovery.V12

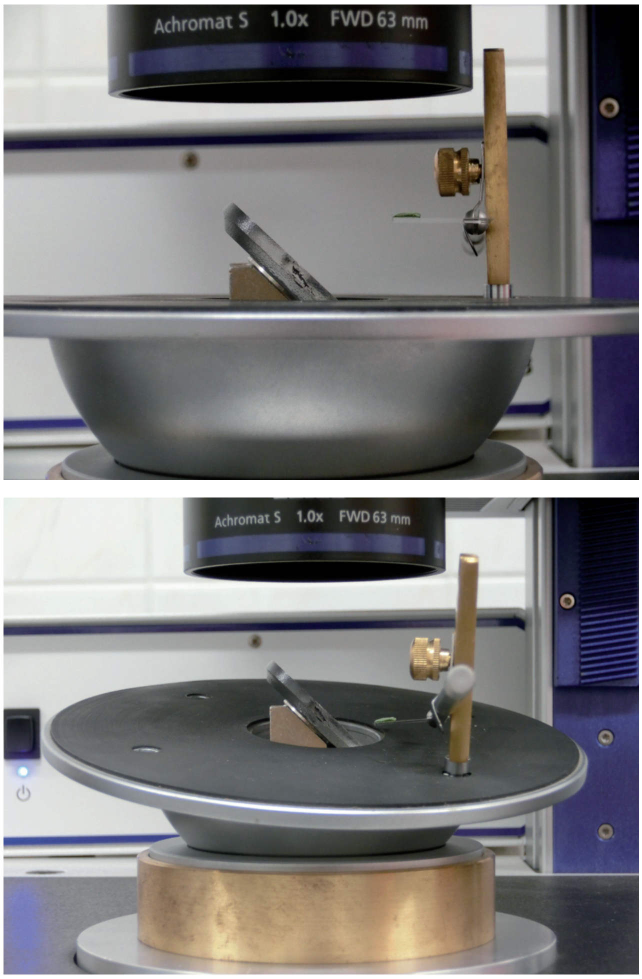

Figure 3 Tilting stage with a handle and built-in flat mirror in selected positions

2.2 Experimental material, observations and measurements

Greenhouse whitefly (Hemiptera, Sternorrhyncha) was used as an insect model in this study, and specifically its nymphal instars fixed to the leaf surface. Tests were also carried out on the surface of a tomato leaf (*Solanum lycopersicum*), which was used for raising the greenhouse whitefly, as well as on parafilm and glass. Parafilm is a standard medium for measurement of the contact angles and surface tension in the examinations of the liquid in plant protection sprays [6]. The series of evaluations of wetting properties was performed on either the surface of insects raised on the leaf or after nymphs were transferred to the adhesive tape. The leaf was cut at the smallest possible distance from the nymphs and the small segment was stuck to the tape on the edge of the cover glass that was placed in the specimen holder for microscopic examination. In the case of last nymphal instar particular individuals were also transferred to the adhesive tape.

This enabled desired positioning of nymphs as well as observation of several individuals at the same time. The observations were performed on 5 individuals within one replication and each treatment consisted of 3 replications.

The following aqueous solutions were used as treatments: deionized water, aqueous solutions of sodium lignosulphonate (0.1%-2%), Glucopon 650 EC (0.1%), Marlowet R 40 (0.1%), Silwet Gold (0.04%). The application of the stream of droplets was carried out by a manual atomizer at a distance of 10-15 cm from the cover glass with the specimen. To improve the visibility of a droplet sodium fluorescein at a concentration of 0.01% or beetroot juice concentrate at a concentration of 2% were added. In the case of sodium fluorescein the illuminator was equipped with a blue exciter filter and the microscope with an interference barrier filter for $\lambda_{max.}$= 525 nm. The halogen illuminator with two optical fibers, shaped in either direction, was used for illuminating the object. Before spraying, the focus was adjusted on one or on several insects in the field of observation. Then objective was moved away automatically to a fixed position and the mirror was covered. After spraying, the objective automatically returned to a fixed position, the cover was removed from the mirror and the focus was adjusted to the droplets shape on an insect. Then magnification was optionally corrected and eventually a picture was taken. The duration of these operations prior to photoshoot was anywhere from 15 to 60 seconds.

The measurements of contact angles and droplet sizes were carried out manually based on standard software features of AxioVision Rel. v.4.8.2. The correctness of measurement of the contact angle was verified based on comparative studies carried out on parafilm using tensiometer KSV and software necessary to perform automated measurements. Comparative studies were carried out in the Department of Agronomy of University of Life Sciences in Poznań.

The documentation of the dorsal surface microstructure of greenhouse whitefly was performed under a scanning microscope Hitachi TM 3000.

3. RESULTS AND DISCUSSION

Adapting the microscope based on the applied solution enabled the correct measurement of contact angles on a flat surface of glass and parafilm. The results were verified using tensiometer and KSV software. Additionally, tensiometer enabled measurement of contact angles and surface tension as a function of time. The newly created technical device allowed for measurement of contact angle for a droplet with a diameter smaller than 500 µm, which is a diameter of a droplet suitable for atomization of spray liquid (Fig. 4).

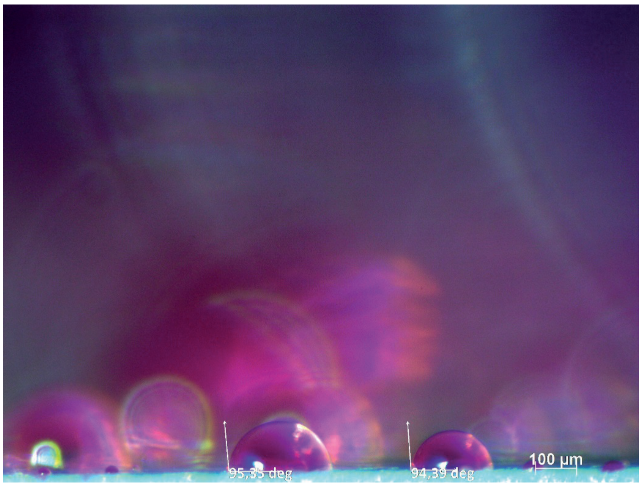

Figure 4 Contact angles of a droplets of a solution of sodium lignosulphonate at a concentration of 0.1% on parafilm

In studies conducted on larvae and pupae of greenhouse whitefly it was assumed that the surface of the dorsal part of an insect would be an expected place of droplets deposition during spraying (Fig. 5). In the postembryonic development of greenhouse whitefly, insects in the second, third and fourth instar nymph are devoid of legs and do not move. The first instar nymph are moving very slowly, therefore instar nymphs of this species are good material for in vivo research.

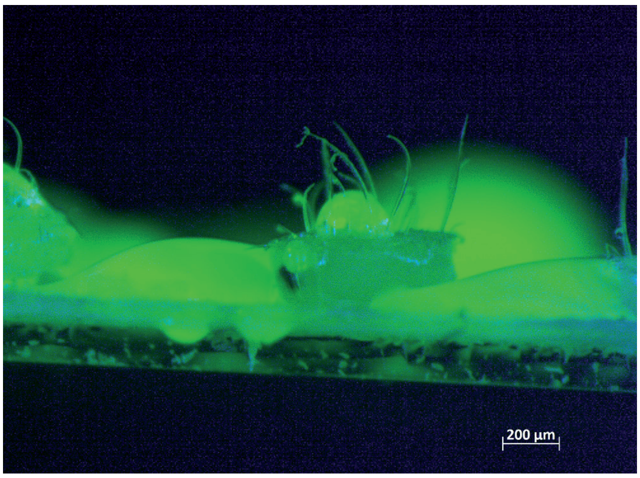

Figure 5 Pupa of greenhouse whitefly with a droplet of deionized water

Despite the large number of droplets formed during the spraying, the efficiency of an appropriate size droplet landing on the central part of the insect's the dorsal shell was very low, and it was estimated at less than 12% in the case of pupae. The probability of the right insect being hit by a droplet was related to the size of a nymph and increased with successive stages. In light of the low hitting efficacy we abandoned observation of individuals in their natural position on the leaf in favor of observing the nymphs placed on the adhesive tape (Fig. 6). The attempts of larvae transfer did not give good results because of the very frequent defects and deformities of their bodies. The larvae are small in sizes. According to Goszczyński [7] the average length of the insects in the first larval stage is 0.29-0.31 mm, 0.37-0.4 mm in the second, 0.5-0.54 mm in the third, and pupa is 0.7-0.74 mm.

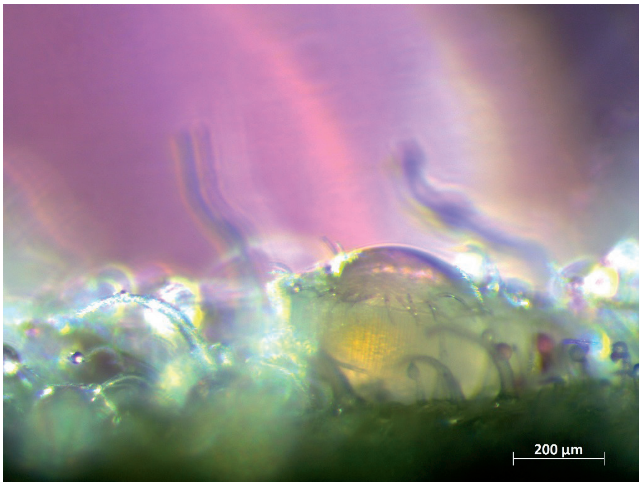

Figure 6 Pupa of greenhouse whitefly devoid of dorsal wax rays with a droplet of aqueous solution of sodium lignosulphonate at concentration of 0.5%, surface tension of 58 mN/m

After spraying the insects, setting of magnifications, focus adjustment and picture acquisition were achieved almost always within a given time of 15 to 60 seconds. The observations showed that the wetting of pupae increased as the surface tension of the liquid decreased (Fig. 5 and 6) and it was maximal after spraying with Silwet Gold solution. The surface tension of the examined solutions ranged from 64 mN/m (sodium lignosulphonate at a concentration of 0.1%) to 22 mN/m (Silwet Gold at a concentration of 0.014%). However, the measurement of contact angle on the surface of pupae after the application of deionized water and liquids of lower surface tensions than water resulted in less replicable results. Sources of measurement error were identified, which were related to the insect morphology. These were mainly the shape of pupae which changed in different phases of its growth and the presence of the dorsal wax rays (Fig. 6). After removing the rays (Fig. 6) a correct indication of the droplet basis was still impossible due to the morphology and the shape of the dorsal surface and the tergite structures (Fig. 8). The main problem was the random deposition of droplets due to the shape of the dorsal shell of a pupae (Fig. 6 and 8). It is possible that use of the sessile droplets method for the contact angle measurements would give better results as it has been shown on the surface of the cuticle of wingless springtails (Collembola) [5]. In the case of the greenhouse whitefly pupae the droplet of less than 0.4 µl should be used. The problems with the correct

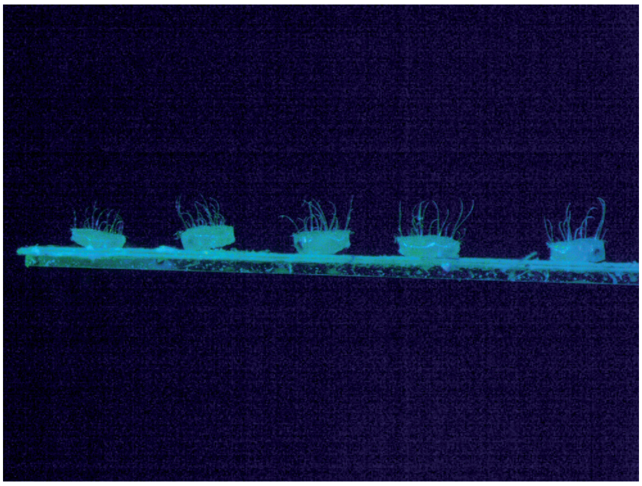

Figure 7 Greenhouse whitefly's pupae seated on the edge of adhesive tape on a cover glass in the specimen handle

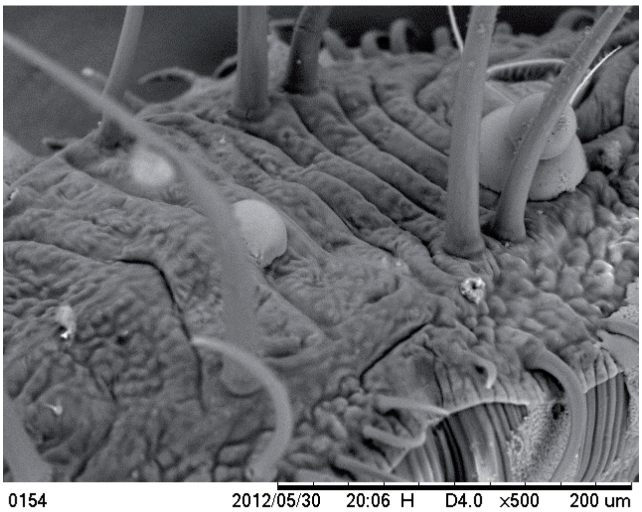

Figure 8 The layout and texture of tergites on the dorsal surface of the greenhouse whitefly pupae

indication of the droplet basis related to the structure of the tergites and their texture are an obstacle to overcome. The defined base line of a droplet may be the result of hollows and hills. Already in 1936 [8] the relationship between roughness and wetting was determined and the surface roughness measurement methods were normalized for applications in materials [9].

The rule describing wetness of rough surfaces states that the hydrophobicity (or hydrophilicity) of the surface is enhanced due to its roughness. On the basis of the observations of microstructure of pupae tergites of *T. vaporariorum* we can conclude, that such microstructure can reduce the percentage of the insect's body that is wetted by water. The epicuticular waxes do play a fundamental role. The results of research carried out on engineering materials indicate that if the scope of local micro-valleys and micro-summits on the surface of the insect is within the range of 5-10 microns then moistening may increase [10]. Figure 9 illustrates that the deionized water droplet was only slightly wetting micro-cavities of thorax of pupa. Hence, tergite texture can increase hydrophobicity and flowing of the liquids with physical properties similar to water. In the case of *T. vaporariorum* nymphal instars such a liquid is an anal excrement which appears several times during the day on the insect's dorsal surface. The cuticule nanostructure also plays a role in wetting. It was studied regarding insects hydrophobicity on their wings [4, 11]. The nanostructure of the greenhouse whitefly surface and its role in protecting the insect before wetting is unknown.

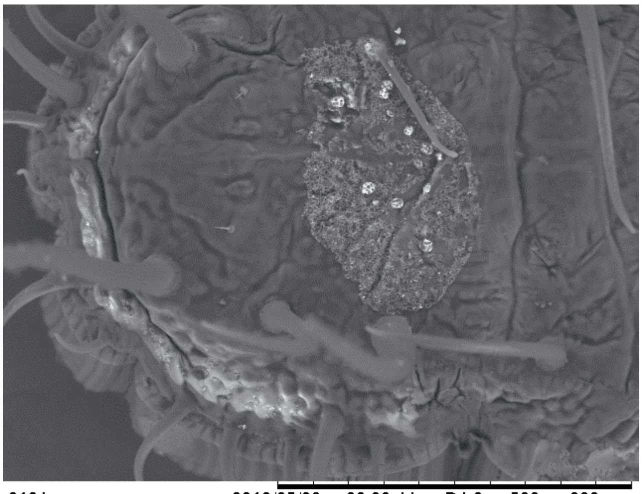

Figure 9 Trace after a water droplet on pupae of greenhouse whitefly indicates that micro-cavities are poorly wetted by water

Despite the failure in measurement of the contact angles, studies have shown that the macrostructure of pupa also reduces the efficiency of wetting of its body by droplets during spraying. Noteworthy is the presence of tall wax dorsal rays on fourth nymphal instar (Fig. 7-10). The appendages are saturated with waxes and droplets can be either deposited whole or broken up on them during spraying (Fig. 10). The greenhouse whitefly is present on the underside of the leaf, so the wax rays are usually directed downwards. Thus, the risk of liquid flowing from the appendages to the body is low. The wax rays are the product of cuticle and do not perform metabolic functions. Accordingly, the pesticides deposited on the wax rays should not affect the viability of the insect. We hypothesize that indicated mechanism may even strengthen the system of greenhouse whitefly resistance in the last nymphal stage against plant protection products applied by spraying.

Tilting stage with built-in flat mirror proved to be a valuable tool when photographing live insects and other objects in a variety of positions. Given the importance of the stage in the observation, documentation and measurements, its development also contributed to improving the quality of education of students in subjects related to the plant protection against pests.

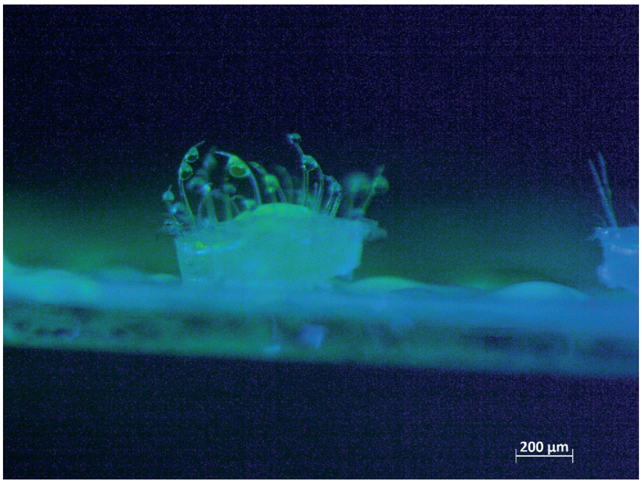

Figure 10 Pupa of greenhouse whitefly with droplets deposited on the dorsal wax rays and the body

4. CONCLUSIONS

Based on the conducted studies, the usefulness of tilting stage with built-in flat mirror was determined for observation of the surface wetting by droplets of sizes common in spraying crops for plant protection. Developed set enabled both observation and characterization of wetting the greenhouse whitefly in nymphal stages. The method of spontaneous droplets deposition used during spraying turned out to be inappropriate for precise measurement of the contact angles on the dorsal surface of the insect.

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REFERENCES

[1] Šikalo Š., Tropea C., Marengo M., Ganić E. N., Spreading of droplets on horizontal surfaces, New and Renewable Technologies for Sustainable Development. Springer US, 2002, 683-692.
[2] Adamson A. W., Chemia fizyczna powierzchni. Warszawa, PWN, 1963, 1-535.
[3] Yuan Y., Lee T. R., Contact angle and wetting properties, Surface science techniques. Berlin, Heidelberg, Springer, 2013, 3-34.
[4] Nguyen S. H., Webb H. K., Mahon P. J., Crawford R. J., Ivanova E. P., Natural insect and plant micro-/nanostructsured surfaces: an excellent selection of valuable templates with superhydrophobic and self-cleaning properties. Molecules, 19, 2014, 9, 13614-13630.
[5] Gundersen H., Leinaas H. P., Thaulow C., Surface structure and wetting characteristics of Collembola cuticles. PloS One, 9, 2014, 2, e86783, doi:10.1371/journal.pone.0086783.
[6] Sobiech L., Idziak R., Woznica Z., Skrzypczak G., Effect of various adjuvants on physicochemical properties and control efficiency of herbicides. Przemysł Chemiczny, 93, 2014, 6, 945-947.
[7] Goszczyński W., Biologiczne podstawy integrowanego zwalczania mączlika szklarniowego (*Trialeurodes vaporariorum* Westw.). Warszawa, SGGW-AR, 1998, 1-124.
[8] Wenzel R. N., Resistance of solid surfaces to wetting by water. Industrial & Engineering Chemistry, 28, 1936, 8, 988-994.
[9] ISO 25178-6 Specyfikacje geometrii wyrobów (GPS) – Struktura geometryczna powierzchni, 2011.
[10] Kubiak K. J., Wilson M. C. T., Mathia T. G., Carval P., Wettability versus roughness of engineering surfaces. Wear, 271, 2011, 3, 523-528.
[11] Sun M., Watson G. S., Zheng Y., Watson J. A., Liang A., Wetting properties on nanostructured surfaces of cicada wings. Journal of Experimental Biology, 212, 2009, 19, 3148-3155.