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The role of biofoam in shielding spittlebug nymphs (Insecta, Hemiptera, Cercopidae) against bright light

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Abstract. 1. Biofoam in spittlebugs has traditionally been seen as a defence against predation and a microclimate that reduces the risks of overheating and drying out. This study addresses the possible role of the foam as a light attenuator.

2. Nymphs exhibit higher mortalities when reared under brighter light (1800–2000 lux) than under less bright light (600–800 lux). At all developmental stages, photoavoidance is strongest when the nymphs are depleted of foam covers. First- and second-instar nymphs appear to be the most vulnerable to exposures by bright light.

3. Smaller bubbles are more effective as light attenuators than are larger ones. As younger instars possess smaller canals from which bubbles are released and, furthermore, exhibit higher concentrations of proteins in bubble liquid, they can produce smaller-sized bubbles and their foams are more effective at reducing light than are those of older nymphs.

4. The findings of this study show that most of the visible and UV radiation is reflected by the foam: transmittance of visible light was 15% at 600 nm and 12% at 350 nm.

5. These results demonstrate that spittlebug foam also possesses properties that render it an effective barrier against potentially damaging solar radiation.

Key words. Insect foam, light avoidance, photoreception, vision.

Background

During their nymphal life stages cercopid insects, commonly known as spittlebugs, cuckoo spit or froghoppers, produce a frothing, soapy white liquid in which to surround themselves. In contrast to the foam of praying mantises, which hardens once the female has laid her eggs in it, that of spittlebug nymphs and of larvae of the tenthredinoid wasps *Blasticotoma filiceti* and *Stauronematus compressicornis* retains its bubbly and delicate consistency for many weeks, during which it is constantly replenished.

In terms of its ecological function, it has long been suggested that spittlebug froth protects the larvae against predators (Henderson *et al.*, 1990) and creates a microclimate that reduces the risks of overheating and drying out (Whittaker, 1970; Dietrich, 2009). The latter suggestions have never seriously been tested, but since a spittebug's mouthparts are firmly achored in the xylem of its host plant, the insect can be expected to receive a constant supply of liquid (Mello *et al.*, 1987), which should make dehydration and overheating less important problems than predation as long as the host plant does not wilt.

Perhaps because of its resemblance to the frothing of snails during physical attack or exposure to noxious chemicals (cf. photo by J. Edwards: http://www.gettyimages.co.uk/detail/photo/forest-snail-generating-defensive-bubbles-to-ward-royalty-free-image/78699443) and because spittlebug froth apparently lacks irritants or repellants (Eisner *et al.*, 2005), it has often been assumed that it acts primarily as a physical defensive shield and deters potential predators such as birds, amphibians, ants and spiders, to name but a few. Moreover, as a variety of parasitoids, such as Pipunculidae and Dryinidae (R. Niedringhaus, pers. comm.), are also known to attack spittlebugs, the foam of the latter could very well represent an effective barrier against them too, even though the conspicuous

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foam might actually increase the chance of being spotted; in fact, a tiny greenish nymph would possibly be less noticeable without the reflective white foam splotch betraying its presence.

For the biofoam of leaf-eating *Stauronematus compressicornis* sawfly larvae, Zhao *et al.* (2009) suggested a role as an antifeeding agent, aimed at reducing feeding on the leaves by *Pseudaletia separata* army worm larvae. However, as spittlebugs do not consume leaf tissue, this explanation should not apply to them. Yamazaki (2017) suggested that the foam's white colour might function as a signal to herbivorous insects and mammals to avoid plants attacked recently. He claimed that the colour 'white' is indicative of reduced plant quality to herbivores.

In the broader context, psyllid larvae ought to be mentioned as well, because their secretions, known as lerps, are also often white and are predominantly thought to protect the delicate insects against desiccation (White, 1970; Sharma *et al.*, 2013). It does seem entirely probable, however, that the lerp also provides protection against UV irradiation on the surfaces of leaves in summer and/or causes herbivores to avoid plants with lerp on them. However, as with spittlebugs, these alternatives to predator avoidance have not been investigated. This paper addresses the issue of a possible role of the spittlebug froth in protecting these insects against harmful effects caused by an exposure to bright light and, in particular, UV radiation.

Essentially, the fragile foamy exudates of spittlebugs are aggregations or clusters of protein and mucopolysaccharide-containing bubbles (Marshall, 1966; Mello *et al.*, 1987), whose surfaces are stabilised and adhere to one another in two ways: three surfaces can merge along an edge, or six surfaces meet at a vertex (Wolf, 1968). Amphipathic molecules adsorb to the gas–liquid interfaces and reduce surface tension. Thus, bubble size depends, first, on the protein and mucopolysaccharide concentrations in the liquid because greater concentrations allow smaller bubbles to be produced, and secondly on the radius of the orifice through which the liquid–air interface bubbles are released. Bubbles, however, will also tend to get larger with time due to air diffusion from small to larger 'cells', a phenomenon known as 'Ostwald ripening' (Kahlweit, 1975).

In spittlebugs, pairs of spiracles, i.e. ventrally located openings of the tracheal system, periodically liberate air bubbles into a liquid from the anus. This liquid contains 99.30–99.75% water and the aforementioned essential foam-producing proteinaceous ingredients from the Malpighian tubules (Ziegler & Ziegler, 1957). Oscillations of the spittlebug's abdomen can be observed (see Video S1) during the bubble-generating process when a nymph, partially submerged by liquid, entrains air bubbles into the liquid from a duct, formed by the extensions of the abdominal tergites and frequently referred to simply as the 'canal' (Guilbeau, 1908; Whittaker, 1970).

Spittlebug foams, however, are not completely optically transparent and therefore might have an optical role to play; after all, for many years the deleterious effects of UV radiation have been known (Beard, 1972) and it was recently shown that even visible light is damaging for insects (Hori *et al.*, 2014). Because of the tightly packed gas bubbles in the foam and the multitude of gas–liquid interfaces separating adjacent bubbles, any light striking and entering the spittle will be subject to reflection and refraction. The scattering of light, giving foam its white colour, can be exploited to develop quantitative probes of both structure and internal dynamics of foam generally (Durian *et al.*, 1991); however, this is not an aspect we will address in this paper.

As spittlebug foam ages, it can be expected that light transmission through it increases. Does all this matter to the spittlebug inside its foam? Keskinen and Meyer-Rochow (2004), working with the species *Philaenus spumarius*, showed that spittlebug nymphs have compound eyes which react sensitively to light–dark changes. The corneae of the nymphs are only approximately one-third as thick as those of the adults and UV radiation can lead to damaged photoreceptors in insects (Meyer-Rochow *et al.*, 2002). It was recently suggested (Meyer-Rochow, 2015) that in addition to its assumed role in deterring predators and preventing desiccation, the spittlebug's foam might also serve to protect spittlebug nymphs (often feeding on plants in bright sunlight) against excessive UV radiation. The lack of dark pigmentation in the nymphs, their considerably thinner and almost transparent integument when compared with that of the adults, adds further support to the notion that nymphs might be particularly vulnerable to bright light generally, and its UV component in particular. A protective shield could therefore be useful.

The rice spittlebug *Callitettix versicolor* is a species taxonomically closely related to *P. spumarius*. Its eye structure is similar to *P. spumarius* (Jia & Liang, 2015) and all aspects of lifestyle and biology have been studied (Chen & Liang, 2012). These two spittlebugs were selected to examine some properties of foam and to test the nymph's developmental and behavioural responses to light of different intensities. We focused on the light-avoidance behaviour of the nymphs in relation to foam bubble size and distribution and we investigated characteristics of the foam's interstitial liquid itself. In addition we determined the diffuse reflectance and transmittance of visible and UV radiation through the foam produced by the nymphs of *P. spumarius*.

The aim of this study has been to show that one role of spittlebug froth, hitherto neglected or overlooked, is to protect the larva against harmful radiation. We approached this question by monitoring the behaviour and survival of spittlebug nymphs under a range of light intensities and by examining the foam's physical, optical and chemical characteristics.

Material and methods

Insects

Nymphs of *Callitettix versicolor* were obtained and reared with minor modifications for spittle mass collection and behavioural assays as described by Chen and Liang (2012). Rice seedlings in plastic boxes (length 50 cm, width 40 cm, height 20 cm), with 10–15 nymphs in each crisper, were used and placed in an artificial climate chamber (GP-01; Huangshi Hengfeng Medical Instrument Co., Ltd., Hubei, China) under LD 14:10 h conditions, at a temperature of 27 ± 1 °C, light intensity of 1800–2100 lux (measured using a digital light meter: DT-8809A, CEM; Shenzhen Everbest Machinery Industry Co., Ltd., Guangdong, China) and RH of 70 ± 5%. The plastic boxes were surrounded with four layers of black cloth to create a light intensity of 80–110 lux.

Light avoidance assay

Behavioural responses of *C. versicolor* nymphs to different light intensities were recorded with and without foam. Nymphs were randomly selected on the first day of each instar. Nymphs of each instar were transferred to glass Petri dishes (diameter 15 cm; containing a layer of moist cotton covered by a piece of filter paper to provide a moist environment) and reared with 10–15 rice seedlings apiece. The nymphs were given 2 days to stabilise and to produce foams to cover themselves with. Thereafter, the foams were removed using a 1-ml glass syringe and the nymphs were allowed to remain for 10 min. Those that had not moved were selected as candidates for testing, the remainder being discarded. The selected nymphs were then exposed to 1.5-cm-wide circular light spots with intensities of 1000, 2000, 3000, 4000 and 5000 lux. An avoidance response was scored when nymphs moved away from the light spot within the first 30 s of illumination. At the same time, nymphs with foam covers were tested as controls. Light was delivered from a halogen lamp (KL 1500 LCD; Zeiss, Oberkochen, Germany). In order to exclude the heat influnce of illumination, temperature was monitored at the site of nymphal exposure with a thermocouple thermometer (DT-613, CEM; Shenzhen Everbest Machinery Industry Co., Ltd., Guangdong, China) to ensure that there was no temperature change during the tests (Table S1). The assay was carried out in a dark room to prevent the influence of residual light. Each nymph was tested once, but 30–50 nymphs of each instar were tested and the percentages of positive responses were recorded. A two-tailed Fisher exact test was performed to establish statistical significance.

Light influence on the growth and foam production of nymphs

The influence of illumination on the growth of the *C. versicolor* nymphs was tested by rearing them under different light intensities. Plastic boxes containing nymphs were surrounded with four layers of black cloth, one layer of black cloth, and no black cloth at all, to create three kinds of light intensity: 80–110 lux (low light intensity, LI), 600–800 lux (medium light intensity, MI) and 1800–2100 lux (high light intensity, HI). Nymphs of all five instar stages (50–75 nymphs each) were exposed to the three different light intensities. Developmental durations and survival rates of each instar were recorded.

Third-instar *C. versicolor* nymphs that moulted on the same day were reared under three light intensities (LI, MI and HI) as described earlier. The foams produced by nymphs were collected after 72 h using a 1-ml glass syringe and weighed using an electronic balance. Fifteen to 20 foams produced at each light intensity were analysed by one-way ANOVA with the least significant difference test.

Measurement of the diameter of bubbles and canals

On the first day of each instar, *C. versicolor* nymphs were randomly selected and reared with rice seedlings in Petri dishes under LI and MI light. After 3 days, the foams produced by nymphs of the various instars were photographed together with a scale using a digital camera (Canon EOS 60D) coupled with a macro photo lens (Canon MP-E 65 mm). The foam and bubbles in the foams were outlined using PHOTOSHOP CS6 software. The images then were processed using the IMAGEJ program coupled with the 'Analyze Particle' tool to measure the diameters of the bubbles. Inferences based on the bulk distributions of the surface bubbles have been shown to allow reliable estimates of average foam bubble sizes (Cheng & Lemlich, 1983; Drenckhan & Hutzler, 2015). The values were grouped and presented as bubble size distribution. The diameters of 500–600 bubbles (from five to 10 foams) from each instar were measured.

In order to measure the diameter of the foam-releasing canals, nymphs were selected randomly on the third day of each instar and were photographed together with a scale. The images were processed using the IMAGEJ program to measure the diameters of the canals. The canal diameters of 10 nymphs of each instar were measured.

Foam surface-active substances and surfactant activity

The foams produced by *C. versicolor* nymphs were collected on the third day of each instar and stored at −80 °C until use. Foam liquid was obtained by drainage or gentle centrifugation. Total protein concentrations of foam liquid of each instar were determined by conventional 'Bradford assay'/Coomassie blue methods (Bradford, 1976), using bovine serum albumin (BSA) as standard. Total carbohydrate concentrations were determined using the phenol/sulphuric acid method calibrated with glucose. Five to 10 foams from each instar were measured.

The equilibrium surface tension of the interstitial liquid of the foam was measured using a interfacial tension meter (DCAT 11; DataPhysics Instruments GmbH, Filderstadt, Germany), using the Wilhelmy-plate method with a roughened platinum plate, interfaced with a computer for automatic data recording at room temperature (24 ± 0.2 °C). The platinum plate was cleaned between each reading by rinsing with deionised water and flaming to remove residual deposits. Because it is difficult to collect enough foam interstitial liquid secreted by nymphs of each instar for the surface tension measurement, the foam liquid was collected without distinguishing between the instars of nymphs and was accumulated together.

In order to compare the surface activity of the proteins in the foam fluid produced by the nymphs with other proteins, the BSA was selected as the control protein. In addition, Ultrapure water of resistivity 18.25 MΩ cm was used to dilute the foam fluid for the purpose of investigating the surface activity of foam protein at different concentrations (dilutions of 1/2, 1/4, 1/6, 1/8, 1/10, 1/20, 1/50, 1/100, 1/1000, and 1/10 000 of the foam fluid were measured). Samples of foam liquid or dilutions were allowed to equilibrate for 5 min before measurements of surface tension were taken in triplicate.

Optical properties of Philaenus spumarius *foam*

For this part of the investigation, common *P. spumarius* fourth- to fifth-instar nymphs were collected from the field (Sevilla, Spain) and maintained in a rearing chamber at 23–18 °C (day

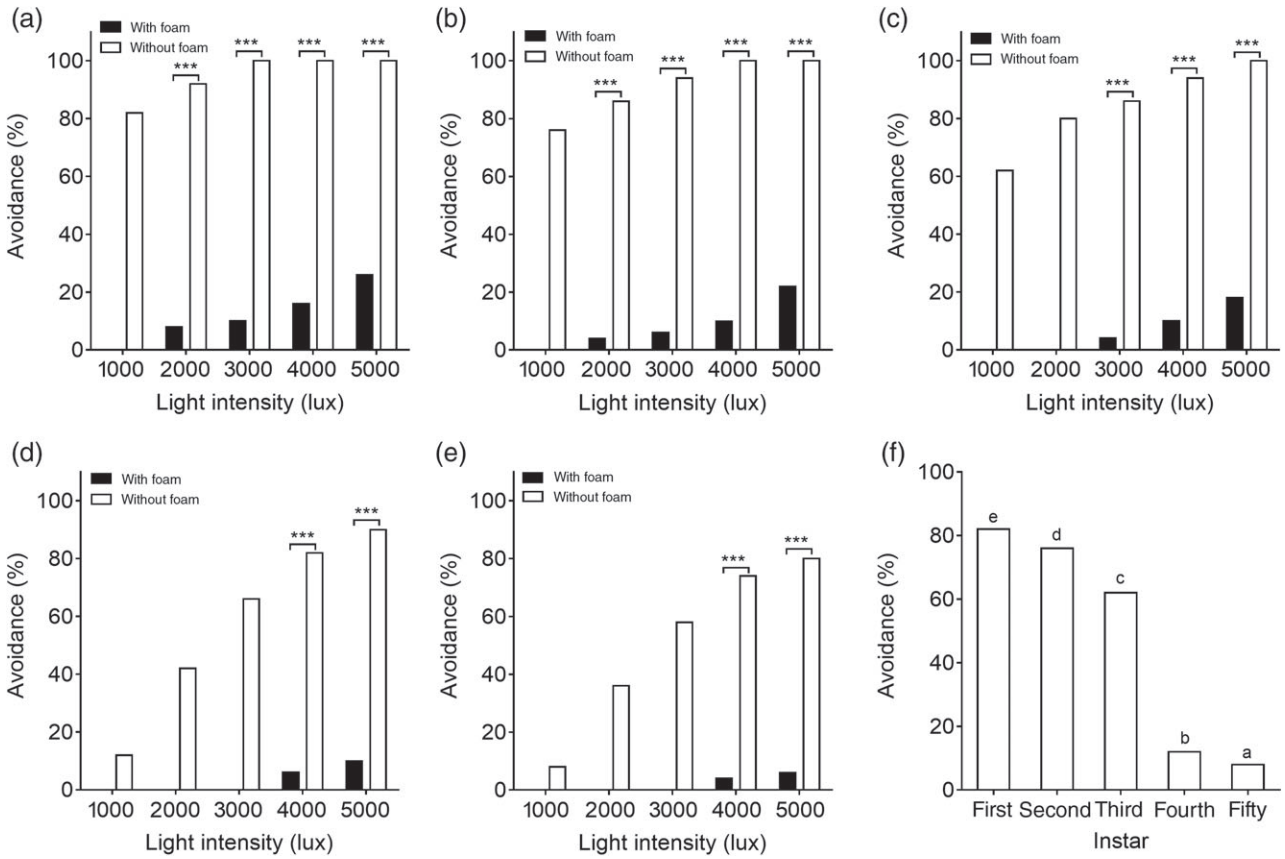

Fig. 1. Light avoidance reaction of spittlebug nymphs. (a) First instar; (b) second instar; (c) third instar; (d) fourth instar; (e) fifth instar; (f) nymphs of each instar without foam at identical light intensity (1000 lux). The absence of a vertical column for a particular condition indicates that photoavoidance was zero. ***$P < 0.0001$, based on the two-tailed Fisher exact test. There were significant differences between the avoidance of each instar with different characters ($P < 0.05$, Mann–Whitney *U*-test). Between 30 and 50 nymphs were tested under each condition.

and night) and LD 14:10 h on *Sonchus oleraceus* plants until analysis. Diffuse transmittance and reflectance data of the foam produced by *P. spumarius* nymphs were obtained for the range of 300–1100 nm wavelengths at the CSIC Torres Quevedo Institute (Madrid, Spain) using a double monochromator Lambda 900 UV/Visible/NIR spectrophotometer (PerkinElmer Life and Analytical Sciences Ltd., Shelton, Connecticut). Diffuse transmittance and reflectance were measured by filling a quartz cell cuvette, 1 cm wide, with foam from fourth- to fifth-instar nymphs with the help of a disposable pipette. Measurements lasting less than 5 min were made immediately after filling the cuvette before the foam bubbles had a chance to disintegrate.

Results

Light avoidance of C. versicolor *nymphs*

Nymphs exhibited increased photoavoidance reactions at higher light intensities. When nymphs without foam covers were tested at five levels of light intensity, low light intensities caused the least strong photoavoidance reactions of all nymphal instars. When nymphs of different instars in the presence of their foam cover were tested at the same five levels of light intensity, they all exhibited less obvious photoavoidance reactions than those that had been tested earlier under the same light intensities, but without their foam shelters (Fig. 1a–e). The most sensitive specimens occurred in the first-instar group, in which approx. 30% showed a photoavoidance reaction, but only to an exposure with the brightest light. No reactions at all were seen to lights with lower intensities or in nymphs of the other instars at any intensity.

As the nymphs aged from first to fifth instar, photoavoidance decreased under identical light intensities (Fig. 1f). These tests demonstrate that *C. versicolor* nymphs sense the light, are able to distinguish different light intensities and seek to avoid exposing themselves to excessive light.

Influence of light on growth of C. versicolor *nymphs and foam production*

When the nymphs were reared under LI and MI, the durations of each instar were similar. However, when the nymphs were reared under HI, the durations of each instar increased significantly in comparison with the durations of nymphs reared under

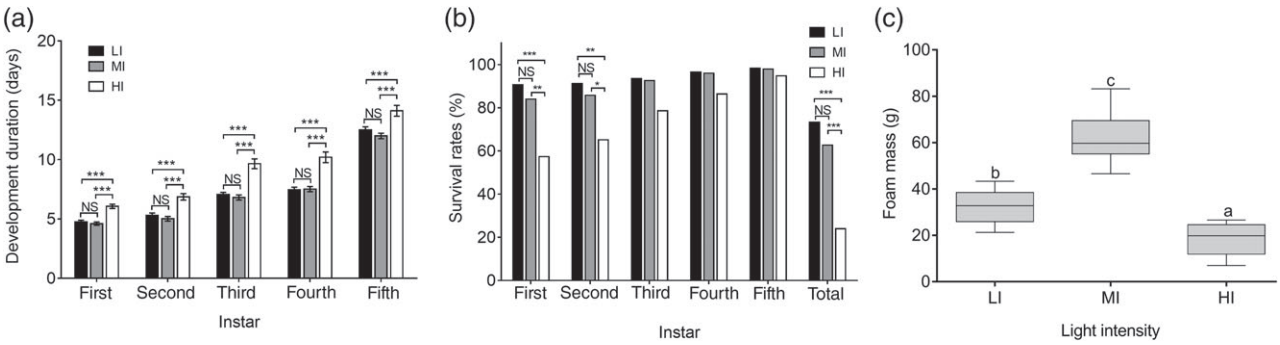

Fig. 2. Influence of light intensity on developmental duration (a), survival rate (b), and foam production (c) of nymphs reared under low light intensity (80–110 lux, LI), medium light intensity (600–800 lux, MI) and high light intensity (1800–2100 lux, HI) conditions. For (a), ***$P < 0.0001$, one-way ANOVA followed by a least significant difference (LSD) post-test; for (b), *$P < 0.05$, **$P < 0.01$, ***$P < 0.0001$, NS, not significant according to two-tailed Fisher exact test. For (c), there are significant differences between columns with different letters ($P < 0.01$, one-way ANOVA with LSD post-test). Error bars indicate standard error. Between 50 and 75 nymphs were tested under each condition.

LI and MI intensities (Fig. 2a). The survival rates of nymphs of each instar that were reared under LI and MI were also similar, but in nymphs reared under HI, survival rates of all instars decreased. The total survival rates of nymphs that were reared under LI, MI and HI were 62%, 58% and 24%, respectively (Fig. 2b). The prolonged developmental duration and decreased survival rate of nymphs under HI indicated that high light intensity had a severely negative effect on growth and viability of the nymphs.

Third-instar nymphs produced most foam under MI illumination. Under LI, the amount of foam was less, but surprisingly, the amount of foam produced under HI was even less than that produced under LI (Fig. 2c).

Bubble size distribution and canal diameter of C. versicolor *nymphs*

The mean bubble diameters of foams increased with the increase of nymph instars under both LI and MI. Moreover, nymphs of each instar reared under LI produce foams with larger bubbles than those reared under MI (Fig. 3a).

The mean diameters of the canals of the five successive instars, involved in the creation of the bubbles, were 0.040, 0.086, 0.164, 0.241 and 0.318 mm, respectively (Fig. 3b). This shows that younger nymphs with higher protein-containing liquids at their disposal, as well as canals of considerably smaller diameters than those present in older instars, possess the anatomical as well as physiological endowments necessary to produce smaller bubbles.

Concentrations and surfactant activity of proteins in the foam's interstitial liquid of C. versicolor

The first-instar nymphs possessed foam liquids with the highest concentration, i.e. 296.5 ± 10 μg ml^{-1}. Concentrations produced by nymphs of the second, third, fourth and fifth instars were 245.4 ± 10, 189.7 ± 10, 148.6 and 157.4 ± 10 μg ml^{-1}, respectively (Fig. 4a).

The foam liquid produced by nymphs of *C. versicolor* exhibited strong surface properties and reduced the surface tension of water to a degree much greater than the control protein (BSA), (Fig. 4b). At total protein concentrations above 30 μg ml^{-1}, corresponding to a six-fold dilution of foam fluid, surface tension drops sharply from the value of pure water (about 73 mN m^{-1}) to about 43 mN m^{-1} at the concentrations of about 178 μg ml^{-1}, present in the combined foam liquids of nymphs of all instars. The protein concentration of the mixed foam fluid approximated the concentration of the foam liquid produced by third-instar nymphs.

Reflectivity and light transmission of foam produced by P. spumarius *nymphs*

Our reflectivity measurements of foams collected from fourth- and fifth-instar nymphs demonstrated that across a range of 300–1100 nm wavelengths most of the visible light and UV radiation is reflected by the foam (Fig. 5a). Transmittance of visible and UV radiation was always below 15% and reached 12% at 350 nm wavelength because of the high reflectivity of the foam (Fig. 5b).

Discussion

Negative phototaxis is a common trait among many insects and this study showed that nymphs of the spittlebug *C. versicolor* also exhibited such photoavoidance reaction, the strengths of which depended on the age of the nymphs and on the light intensities to which the insects were exposed. The weaker responses in older nymphs suggested greater tolerance to light or less sensitive eyes, or both.

Survival rates decreased and developmental duration increased when nymphs were reared under high light intensity. It therefore seems that spittlebugs need to compromise between habitats of low light intensity to safeguard normal development, at least during the early stages of development, and bright habitats that are favourable to the plants these insects feed on.

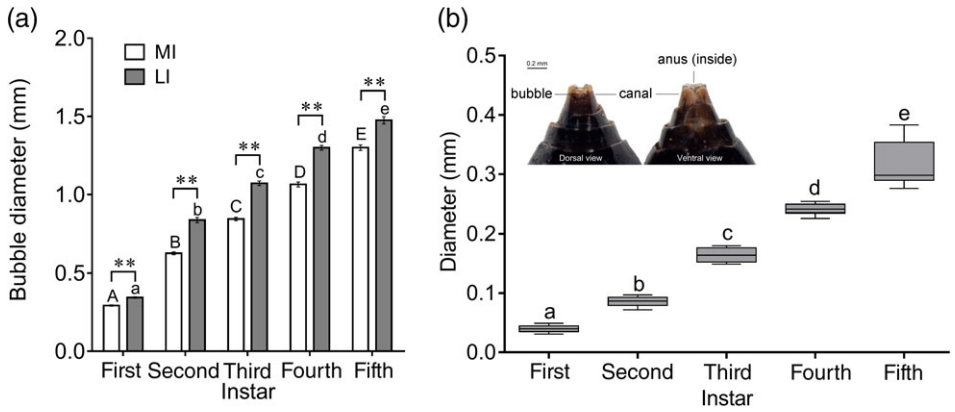

Fig. 3. The arithmetic mean bubble diameters (a) and bubble-releasing canal diameters (b) for each instar. In (a), nymphs were reared under low light intensity (80–110 lux, LI) and medium light intensity (600–800 lux, MI). ***$P < 0.0001$, based on two-tailed unpaired *t*-test. There were significant differences between the diameters with different letters [$P < 0.05$, one-way ANOVA with least significant difference (LSD) post-test]; 500–600 bubbles (from five to 10 foams) were measured for each condition. The inset in (b) shows a bubble-releasing canal of a fifth-instar nymph of *Callitettix versicolor*; there were significant differences between the diameters with different letters ($P < 0.01$, one-way ANOVA with LSD post-test); canal diameters of 10 nymphs from each instar were measured. Error bars indicate standard error. [Colour figure can be viewed at wileyonlinelibrary.com].

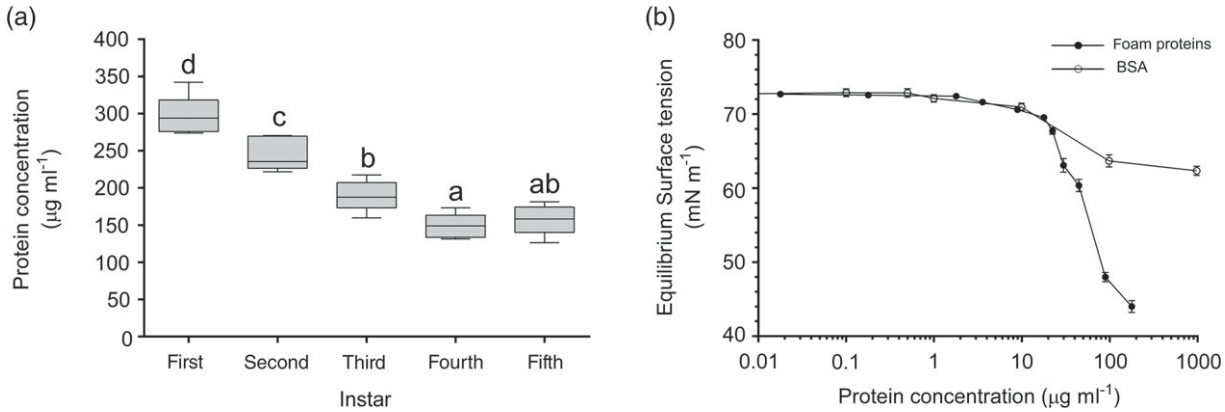

Fig. 4. Surfactant properties of spittlebug foam interstitial liquid. (a) Total protein and carbohydrate concentrations of foam fluid produced by nymphs of each instar; five foams of each instar were measured. (b) Surface tension versus concentration of protein in foam fluid in comparison with a moderate surfactant control protein (bovine serum albumin, BSA). There were significant differences between the concentrations with different characters ($P < 0.01$, one-way ANOVA with least significant difference post-test).

Our results hint at the effectiveness of the foams in protecting the nymphs against excessive amounts of light. Obviously, moderate light intensities were able to stimulate the nymphs to produce more foam, but excessive light led to a lower production, perhaps because it causes physiological damage and an inability of the nymphs to continue producing foam in the usual way. An effect of the light on the host plant supplying the nymphs with the raw material for the foam is a further, as yet untested possibility.

Sensing light intensities must be of importance to spittlebug nymphs of all instars, an observation that supports the conclusion reached earlier by Keskinen and Meyer-Rochow (2004). Although it was briefly mentioned by Mikkola (1995) that spittlebug foam might possibly attenuate light and protect the nymph inside its foam against UV radiation, neither he nor anyone else had demonstrated that the foam, in addition to its other functions (as discussed in the introduction), can play such a role. Besides the well-known damage following exposure to UV and involving lipids, proteins and DNA, owing to free oxygen radicals created by radiation with short wavelengths (Moan & Peak, 1989), it has recently been shown that visible light, too, can have lethal species-specific effects in insects (Hori *et al.*, 2014). How exactly non-UV radiation affects an insect's state of health is, however, still a matter of speculation, but photoproducts have been implicated (Hori *et al.*, 2014). Although data on light transmission characteristics of the foam in the field under natural conditions are still lacking, the fact that identically aged nymphs reared under different light intensities yielded unequal average bubble sizes suggests that nymphs have some control over the sizes of the bubbles they want their foam to consist of. It also suggests that the nymphs utilise the optical properties of their foam covers to attenuate the amount of light reaching them.

The liquid foam is not a static system but instead undergoes complex dynamic processes, which make its macroscopic

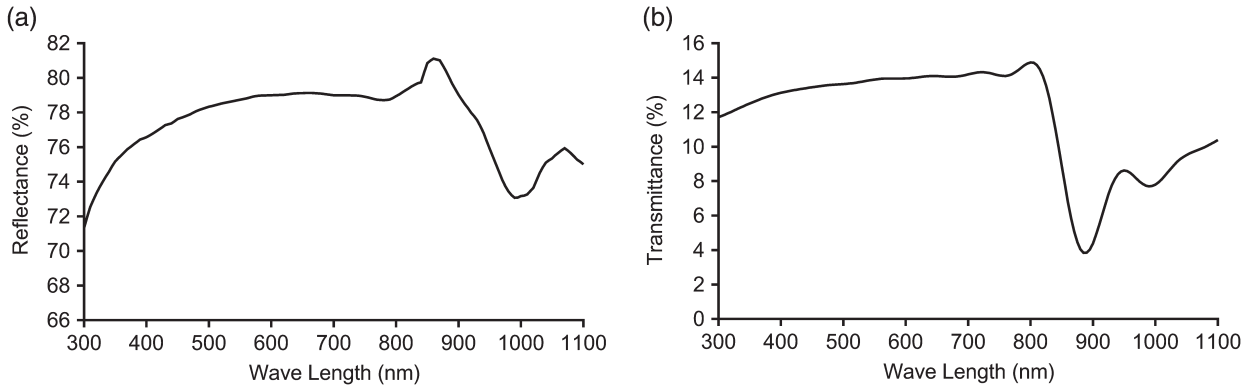

Fig. 5. Reflectance and transmission spectra of foam from fourth-instar (a) and fifth-instar nymphs (b) of *Philaenus spumarius*.

appearance change with time. As time progresses, inter-bubble gas diffusion and bubble coalescences cause bubbles to grow larger (Kahlweit, 1975; Neethling *et al.*, 2005), while under the influence of gravity, liquid drains from the foam and accumulates at the bottom, leaving the top deprived of liquid (Neethling *et al.*, 2005). In the course of these changes, light transmission of liquid foam increases with the ageing of foam, which is why spittlebug nymphs need to constantly produce new bubbles to maintain the required morphological characteristics of the foam.

That an exposure to bright light could possibly alter the properties of the foam both via an effect on the host plant's xylem fluid and directly through photochemical reactions with components of the foam is unlikely, given the minute amounts of stabilising chemicals in the latter, but should not be dismissed altogether. For example, the regulation of foam amounts and bubble sizes under different light intensities has to be important for the nymphs, and in parallel with the reported sensitivity changes of their photoreceptors between night and day, it may in fact lead to day/night differences in foam amounts and compositions. This is something that ought to be looked at in the future.

The apparently greater need of first- and second-instar rather than older nymphs to protect themselves against harmful radiation correlates well with the higher reflectivity of their foams, the greater amounts of proteins in their bubbles, and the smaller average sizes of the latter. It also agrees with the position of the spittlebugs lower down on the plant rather than closer to the top, where exposure to sunlight would be most severe. Although full sunlight may reach 100 000–120 000 lux and our brightest lights (5000 lux) were similar to those of an overcast day, they were more in line with the dimmer light assumed to prevail in the regions of the nymphs between the stems of the food plants. The fact that the intensities used in our experiments produced the reactions we reported suggests that higher light intensities might well have been even more detrimental.

Older nymphs with larger bubbles and foams that reflect short-wavelength radiation less well could possibly compensate for these deficiencies by secreting larger amounts of foam or by becoming more resistant to photic damage due to the increased thickness of their integument. There is, however, the problem that instar durations are longer in older than in younger nymphs and that bubbles then have more time to coalesce if they are not regularly replaced (cf., Ostwald ripening; Kahlweit, 1975), giving rise to less effective foam shields. Observations by Biedermann (2003), which showed that nymphs aggregate with up to four individuals per spittle (and are therefore capable not only of producing a much larger foam cover than a single nymph, but also of replacing ageing bubbles more frequently with new ones) lend support to our conclusion that spittlebug foam not only functions as an anti-predator device or microclimate regulator, but also has an optical role to play.

However, given the increases in body size of the older versus the younger nymphs and the fact that the distances from their eyes to the foam surface did not change significantly between the different developmental stages, we might conclude, focusing on vision, that the eyes of the older nymphs become less susceptible to photic damage and require less protection than do those of the younger stages. As, in addition to eyes, many insects have extraocular photoreceptors, be it in the brain (Yoshida, 1979; Hariyama, 2000) or on the larval body wall, as in *Drosophila* (Xiang *et al.*, 2010), light detection and avoidance may not depend solely on the conditions of the eyes. Yet, the existence of such photoreceptors in spittlebug larvae is still to be demonstrated.

Foams are energetically expensive to make and difficult to maintain for organisms (Cooper & Kennedy, 2010). The spittlebug foam liquid is made up of the anal secretion and mucilaginous substance of Batelli glands (Guilbeau, 1908). Although the foam fluid produced by the nymphs of the spittlebug is an anal exudate of similar composition to the juices of the host plant (Whittaker, 1970; Mello *et al.*, 1987), the nymphs need to secrete special components, such as glycoproteins and proteoglycans, to reduce the surface tension of water for sustaining the froth's bubbly texture (Mello *et al.*, 1987; Cooper & Kennedy, 2010; del Campo *et al.*, 2011). The composition and molecular structures of the foam ingredients, and to what extent and how nymphs select, modify and incorporate the proteins and carbohydrate into their foam liquid, are questions still not fully solved. In this study we show that the nymphs of spittlebugs can regulate the concentrations of these essential additives accordingly. The concentrations of proteins decrease and this contributes to an increase in bubble sizes in older nymphs. The surface tensions of the foam liquids produced by first- and second-instar nymphs were smaller than those of the mixed foam liquid. Thus,

the younger nymphs produce foams with smaller-sized bubbles using foam liquid with higher protein concentrations.

The foam fluid produced by nymphs of *C. versicolor* was found to possess a very low surface tension (about 44 mN m^{-1}) at a total protein concentration of about 178 μg ml^{-1}. This helps to overcome the surface tension of water effectively. Although many studies about foam produced by different organisms suggest that proteins in foam liquid play a major role in reducing the surface tension (Cooper & Kennedy, 2010), there could be additional constituents to reduce the surface tension of water (Mello *et al.*, 1987; Ponder *et al.*, 2002; Buxton *et al.*, 2007) in spittlebug foam liquid. Small amounts of cholesterol and fatty acids, for example, have recently been identified in the foam of *Stauronematus compressicornis* by Zhao *et al.* (2009). Whether spittlebug foam varies in relation to the host plant a nymph has been feeding on is still an open question, but any effects on the optical properties of the bubbles are expected to be negligible.

Because different animals produce their frothing secretions by different means, foam amounts and properties, as well as the uses to which they are being put by their producers, are likely to vary. The foamy secretions known from frothing land snails, bubble nests used by certain species of frogs (Fleming *et al.*, 2009) and fish (Linke, 1991), and even the foams of insects, such as those secreted anteriorly (e.g. *Stauronematus compressicornis*) or produced posteriorly (e.g. spittle bugs), may all appear equally chaotic superficially, but they are likely to possess their own unique compositions and properties. In view of our results, it would seem worthwhile to investigate whether the exudates of other organisms also have photoprotective roles. This is particularly true for foams containing eggs and larvae that float on freshwater bodies (Linke, 1991) or seawater (Castilla *et al.*, 2007), where there is no protection from solar radiation.

Our study has shown that the foam of the spittlebug may not only act as a shelter for the nymphs to avoid predation, but also functions as a light regulation system. The nymphs can shield themselves against harmful radiation and, according to their developmental stage and the intensity of the external light, can control the amount of foam bubbles needed to achieve the desired effect of their exudate. That the foam may benefit the nymphs in many other ways as well is not disputed by the current findings.

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Supporting Information

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Table S1. Monitoring of temperature change at the site of nymphal exposure during behavior assays. The temperature was measured with a thermocouple thermometer (DT-613, CEM; Shenzhen Everbest Machinery Industry Co., Ltd., Guangdong, China). To measure the temperature resulting from illumination, the light used in the nymph avoidance assay [as delivered from a halogen lamp (KL 1500 LCD; Zeiss, Oberkochen, Germany), yielding a light spot of 1.5 cm diameter] was focused onto the probe sensor.

Video S1. Bubbles were being produced by fifth instar nymphs of *C. versicolor*.

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