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Spittlebug invisibility cloak: experimental tests on the antipredatory effect of the froth of *Philaenus spumarius*

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Abstract

In Europe, the meadow spittlebug *Philaenus spumarius* (L.) (Hemiptera Aphrophoridae) is the main vector of the bacterium *Xylella fastidiosa* Wells *et al.*, the etiological agent of the Olive Quick Decline Syndrome. The froth produced by spittlebug nymphs has a primary function in protecting the insect from dehydration and thermal stress. It is also accepted that the froth protects nymphs from predators, although the underlying mechanism is not completely clear. We investigated such a process using the crab spider *Synema globosum* (F.) and the ant *Crematogaster scutellaris* (Olivier) as model species. Nymphs of *P. spumarius* were divided into two groups, one whose froth was left and one whose froth was removed. The nymphs were then exposed to predators and their survival recorded. The survival of defrothed nymphs was considerably lower than controls with both spiders and ants, though this could be due to increased motility of defrothed nymphs. Moreover, to test the chemical properties of the froth and exclude any physical hindrance effect, *P. spumarius* nymphs and dead *Sarcophaga carnaria* (L.) larvae (maggots) under three different conditions (defrothed, water-coated, and centrifuged froth-coated) were offered to workers of *C. scutellaris*. The survival of the nymphs and ants' bites to both preys were recorded. Again, defrothed nymphs showed a lower survival probability compared to those moistened with water and froth, while no differences were found between these two treatments, suggesting a chemical deterrence or mimicry of the froth. The highest number of ants' bites towards nymphs and maggots was recorded in the defrothed group, while the lowest in the froth-coated nymphs. A significant difference between the water- and froth-coated treatments was only found in nymphs and not in maggots, suggesting the presence of some residual substances on the nymph's integument that could have a deterrent or masking effect. Additionally, our direct observations of ants drinking the froth reinforce chemical mimicry as a more plausible explanation than deterrence. In conclusion, our findings suggest that the froth plays an antipredatory role, at least for predators that use mainly olfactory cues to localise their preys, through a chemical mimicry mechanism.

Key words: Aphrophoridae, biofoam, crab spider, acrobat ant, generalist predator.

Introduction

The meadow spittlebug, *Philaenus spumarius* (L.) (Hemiptera Aphrophoridae), is a polyphagous xylem fluid-feeding species widespread in the Palaearctic and Nearctic realms (Drosopoulos and Remane, 2000; Cornara *et al.*, 2018) where it occurs at different latitudes and altitudes (Yurtsever, 2000). It is also present in the Azores, Hawaii and New Zealand, where it was probably introduced in the second half of twentieth century (Yurtsever, 2000). The common name "spittlebug" refers to the nymphs' habit to develop inside a self-produced froth excreted from the anus. Although spittlebugs have attracted the attention of naturalists for centuries, it is still not fully understood how the froth is produced, what it is composed of and what its exact role is.

The main constituent of spittlebug froth is the liquid derived from xylem sap of the plant on which the insect feeds (Wilson and Dorsey, 1957). Both nymphs and adults are xylem feeders, ingesting large amounts of sap to compensate for the poor nutritional value of this nourishment, and excreting the excess from the anus (Weaver and King, 1954; Wilson and Dorsey, 1957). Nymphs introduce air bubbles into the expelled fluid, by means of telescopic movements of the urites, giving the typical frothy appearance to the spittle mass (Weaver and King, 1954). In addition to the alimentary canal, Malpighian tubules are involved in the production of mucopolysaccharides that probably act as surfactants, giving stability to the froth (Marshall, 1966). Moreover, the Batelli glands, described only in nymphs of species belonging to the Aphrophoridae, seem to produce a lipoid substance that prevents froth desiccation (Marshall, 1965).

It is widely accepted that the froth protects spittlebug nymphs against desiccation and natural enemies (Whittaker, 1970; del Campo *et al.*, 2011). A thermoregulatory function of the froth has been demonstrated for *Mahanarva fimbriolata* (Stal) (Tonelli *et al.*, 2018) and proposed for *P. spumarius* (Whittaker, 1970; Cornara *et al.*, 2018) and *Poophilus costalis* (Walker) (Sahayaraj *et al.*, 2021). Moreover, some examples of repellence, antibacterial, and antifungal activity of the froth have been reported for various spittlebug species (del Campo *et al.*, 2011; Chang *et al.*, 2019; Tonelli *et al.*, 2019; 2020; Sahayaraj *et al.*, 2021) except for *P. spumarius*. For instance, Whittaker (1970) stated that the nymphs of *Neophilaenus lineatus* (L.) deprived of the froth were significantly more likely found and captured by predators than those with froth. However, despite that a few cases of predation and parasitization on spittlebug nymphs have been recorded, the effective role of the froth in protecting juveniles against predators is not fully understood yet. Observations of entomophagous insects, spiders and harvestmen preying on *P. spumarius* nymphs (Weaver and King, 1954; Yurtsever, 2000) could suggest that the froth is not involved in the protection of the insect against predators. In some cases, the froth seems to be even disadvantageous for nymphs, including those of *P. spumarius*, for example when the froth is used as a clue to locate the prey by some birds (Whittaker, 1970; Weaver and King, 1954) or arthropods (Weaver and King, 1954; del Campo *et al.*, 2011).

Recently, the meadow spittlebug has assumed remarkable importance as a crop pest, being proved as the main vector of the xylem-inhabiting bacterium *Xylella fastidiosa* (Wells *et al.*), the etiological agent of the Olive Quick Decline Syndrome, in Europe (Cornara *et al.*, 2016). For this reason, improving the knowledge about the biology and ecology of *P. spumarius* is crucial. To date, the management of this pest is mandatory in areas where *X. fastidiosa* occurs and nymphs represent the most susceptible stage of the insect (Dáder *et al.*, 2019). Exploring the defensive strategies of *P. spumarius* nymphs could help in planning sustainable and efficient control strategies, as well as in assessing biological control feasibility, using natural enemies and/or entomopathogens.

To evaluate the possible role of *P. spumarius* froth in protecting nymphs from arthropod generalist predators, two model species were used in this study: the acrobat ant *Crematogaster scutellaris* (Olivier) and the crab spider *Synema globosum* (F.), which are commonly found in Mediterranean olive groves (Santini *et al.*, 2011; Benhadi-Marin *et al.*, 2020; Picchi, 2020), where also *P. spumarius* usually live (Elbeaino *et al.*, 2014; Cornara *et al.*, 2016; Antonatos *et al.*, 2019). The aims of this study were to: 1) assess the potential antipredatory effect of the froth produced by *P. spumarius* nymphs; 2) test the chemical effect of the froth as an antipredatory mechanism. We hypothesized that: 1) the froth is an antipredatory trait; 2) the antipredatory mechanism has a chemical rather than a physical nature; and 3) the froth is not chemically repellent but rather it masks the odour of the prey.

Materials and methods

Animal collection

Spittlebugs were collected in the field from a variety of plants (Ranunculaceae, Apiaceae and Fabaceae) and placed on two-months old pot-growing chickpea plants (*Cicer arietinum* L. cultivar "Principe") for at least 48 hours before being used in the experiments, to avoid potential biases due to differences in froth composition. The nymphs were divided into two age classes: class A, including first and second instar nymphs (N1-N2) and class B, comprising third, fourth and fifth instar nymphs (N3-N5), according to their size, colour and presence of wing pads (Yurtsever, 2000).

Adult specimens of *S. globosum* were collected in the field on *Ranunculus* sp. flowers, placed individually in plastic cups and provided with preys *ad libitum* consisting of adults of Mediterranean fruit fly *Ceratitis capitata* (Wiedemann). Before being used in the experiments, spiders were starved for seven days to standardize their conditions (Benhadi-Marin *et al.*, 2020).

Workers of the ant *C. scutellaris* were collected from natural colonies and divided in 20 pseudocolonies, each with about 100 workers, that were acclimated for one week and provided with water *ad libitum* but without food before being used in the experiments to make them more responsive to the tests. The ants were housed in plastic aquaria (10 × 20 × 30 cm) with the walls coated with Fluon (Whitford, Runcorn, UK) to prevent escape.

All animals were kept in the laboratory at ambient conditions (mean room temperature: 25 °C).

Spittlebug mortality after froth removal

To define the duration of experiments, preliminary trials on spittlebug mortality after froth removal (presumably due to dehydration) were carried out. Spittlebug nymphs (N1-N2, n = 20) were removed from their froth on the plant with a soft brush, positioned onto filter paper until the froth was completely absorbed, and introduced into 35 mm Petri dishes closed with Parafilm®, where their status (0 = dead or 1 = alive) was checked every 10 minutes. The first dead nymphs were recorded after 210 minutes (supplemental material figure S1), so that the experiment length was set well below, at 120 minutes. Since N1 and N2 nymphs were considered as more vulnerable than older instars, this experiment duration was evaluated as appropriate for all the bioassays.

Spittlebug mortality when challenged with predators

To test whether spittlebugs' froth acts as an antipredatory trait against generalist predators, we performed arena experiments.

Freshly collected spittlebug nymphs from both age classes (N1-N2 and N3-N5) were divided in two groups: one to which the froth was left (control) and one to which the froth was removed (treatment).

Before the beginning of the experiments, plastic jars with perforated caps and 1.5 ml upside-down Eppendorf® tubes perforated at the bottom were prepared. The basal end of a chickpea cut shoot was then inserted through these perforated containers. For the control group, shoots bearing nymphs wrapped in their froth were used, and the plastic jar (or the tube) contained water, in which the shoot base was immersed, to allow sap transport. For the treated group, nymphs were gently deprived from their froth on the plant with a soft brush and laid onto filter paper to remove froth residuals. The nymph defrothed was then transferred on an unoccupied shoot. In this case, an empty jar (or tube) was used, to prevent sap transport and, therefore, the production of new froth. Occasional froth formed from residual sap was removed with filter paper until no new froth was produced (about 10 minutes).

At the end of this period, each jar or upside-down tube containing the nymph was inserted into the experimental arena hosting the ant pseudocolony or the spider, so that the nymphs were accessible to predators. The status of nymphs (0 = dead or 1 = alive) was checked every 10 minutes for two hours. The experiment was observed for its whole duration to ensure that the observed mortality was in fact due to predation. For each group (age class:treatment) 10 nymphs were tested with spiders, for a total of 40 nymphs, and 20 nymphs per group were tested with ants, for a total of 80 nymphs.

Spittlebug froth deterrence

In this experiment, the potentially deterrent effect of the froth produced by *P. spumarius* nymphs towards *C. scutellaris* was tested. Specifically, the deterrent effect due to the chemical composition of the froth was tested by excluding the confounding effect of the frothy physical state.

Before the beginning of the experiment, froth was collected with a soft brush from lab-reared chickpea plants and from other plants in the field (belonging to the families Ranunculaceae, Apiaceae and Fabaceae). The collected froth was mixed and centrifuged at 448 g for 30 seconds, and the resulting liquid was preserved at −20 °C until used in the experiments.

Nymphs were removed from their froth from the chickpea plants with a soft brush and laid onto filter paper to blot froth residuals. Each nymph was then randomly assigned to one of the following three treatments: nymphs moistened with water (treatment W), nymphs moistened with froth (treatment F), and nymphs without water or froth (treatment N). W and F treated nymphs were wet pouring on them 2.5 µl of either water or froth with a micropipette.

Ten *C. scutellaris* workers were placed into 9 cm diameter Petri dishes with Fluon coated walls to acclimatize to experimental conditions. After one hour, we placed one spittlebug nymph into the Petri dish and video recorded the experiment for the following 30 minutes. The status of the nymph at the end of the experiment (0 = dead or 1 = alive) as well as the number of ants' bites towards the nymph were registered. Only nymphs attacked by ants were scored as "preyed". For each treatment, 20 replicates were performed, for a total of 60 replicate tests.

Table 1. Results of generalized linear model (GLM) selection for spittlebug survival using Akaike information criterion (AIC).

Predictor	d.f.	AIC
Null Model	1	150.26
Predator	2	148.81
Treatment	2	94.93
Age class	2	150.34
Predator + Treatment	3	91.41
Predator + Age class	3	148.83
Treatment + Age class	3	93.71
Predator + Treatment + Age class	4	89.88

To discriminate if the deterrence was caused by the froth only or by other deterrent/unpalatable substances on the tegument of spittlebug nymphs, we repeated this latter experiment using larvae (maggots) of the dipteran *Sarcophaga carnaria* (L.) as bioassays. Maggots have been chosen as alternative preys because they are highly appreciated by ants, either dead or alive. Maggots were washed with water to remove any detritus and killed by freezing as their high motility could interfere with ants' behaviour. Maggots defrosted at ambient temperature were then offered to ants (one for each Petri dish) following exactly the same protocol described above.

Statistical analyses

The spittlebug survival (alive or dead at the end of the experiment) was assessed applying generalized linear models (GLM with binomial distribution) using predator, age class and treatment as predictors. Models of different complexity (from the null to the full model) were evaluated using Akaike Information Criterion (AIC) and the best one was also tested through a χ^2 test. Then, using the best predictors found with GLM selection, Kaplan-Meier curves were drawn and the difference between them was tested applying the Mantel-Haenszel test.

For the deterrence experiment, nymph survival (alive or dead at the end of the experiment) was assessed with binomial GLMs using the treatment as predictor, and the model was tested through a χ^2 test, followed by Tukey tests as multiple comparisons. Moreover, the number of ants' bites to nymphs and maggots were separately tested with a GLM (family = quasipoisson) using the treatment (levels: N, W, F) as predictor, and the model was tested employing a χ^2 test, followed by Tukey tests as multiple comparisons.

All statistical analyses were performed using the software R (R Core Team 2020, version 4.0.5) applying the packages 'survival' (Therneau and Lumley, 2015) and 'multcomp' (Hothorn *et al.*, 2016).

Results

The best GLM was the one considering the predator, the treatment, and the age class as predictors (table 1). However, the predator (spiders or ants) and the age class of the nymphs (class A, N1-N2 or class B, N3-N5) had no significant effect (table 2), therefore they were not considered in the subsequent analyses. The model showed that control spittlebugs died significantly less frequently than the treated ones (table 2).

Table 2. Results of generalized linear model (GLM) on spittlebug survival at the end of the experiment as a function of predator (spiders or ants), treatment (treatment: froth removed, control: froth not removed), age class (class A, N1-N2 or class B, N3-N5).

	d.f.	Deviance	Residual d.f.	Residual deviance	P (> χ^2)
Null model			119	148.26	
Predator	1	3.45	118	144.81	0.06
Treatment	1	59.40	117	85.41	1.288 e-14 ***
Age class	1	3.53	116	81.88	0.06

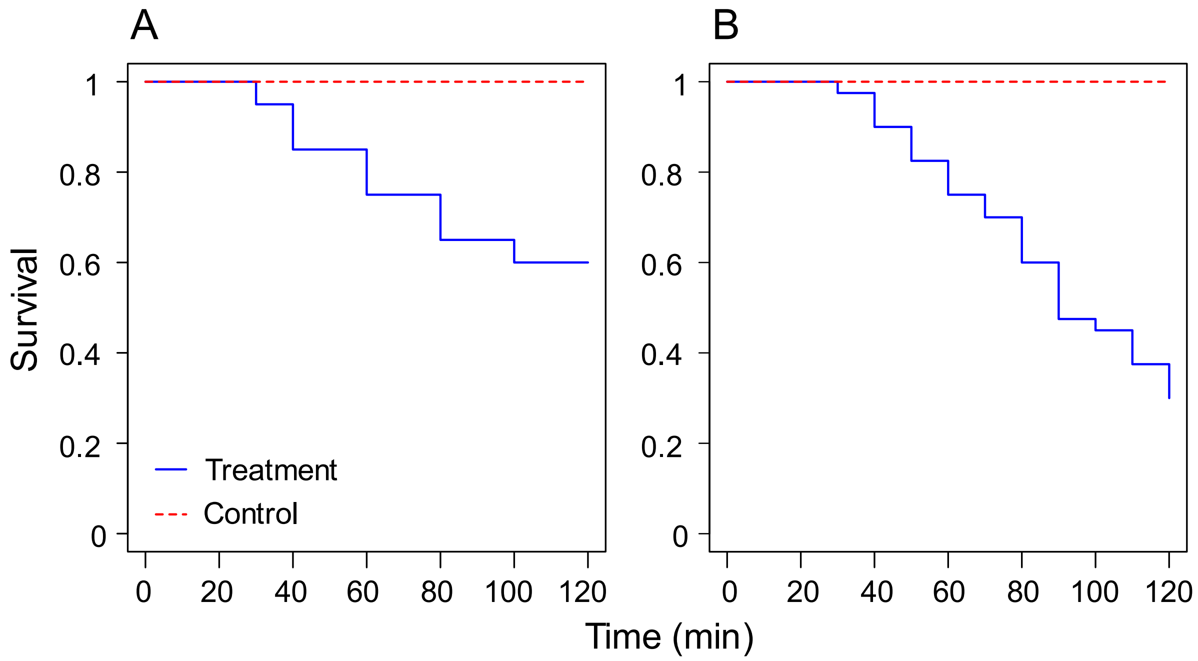

Figure 1. Kaplan-Meier curves showing the survival probability of spittlebugs as a function of time and treatment. (**A**) spittlebugs challenged with spiders, (**B**) spittlebugs challenged with ants (treatment: froth removed, control: froth not removed).

At the end of the arena experiments, no control spittlebugs died, while for the treated group 8 out of 20 (40%) and 28 out of 40 (70%) died in presence of spiders and ants, respectively (figure 1), and the difference between the two Kaplan-Meier curves was statistically significant (spiders: $\chi^2 = 9.8$, d.f. = 1, $P < 0.01$; ants: $\chi^2 = 44.4$, d.f. = 1, $P < 0.001$).

At the end of the deterrence experiment, 16 out of 20 (80%) nymphs of the treatment N, while only 2 (10%) in the treatment W and none in the treatment group F were predated. The effect of the experimental treatment was highly significant (table 3) and, in particular, the number of survivors of group N was significantly lower than either those of treatments W and F ($P < 0.001$ for both), while no difference was observed between treatments W and F ($P = 0.82$).

The number of bites inflicted to the spittlebug nymphs and maggots differed among the three treatments (table 4). In particular, for spittlebugs, the number of bites was higher in the group N compared to treatments W and F ($P < 0.001$ for both), but fewer bites were recorded in F-treated than W-treated nymphs ($P < 0.01$; figure 2A). Similarly, in the bioassays with maggots, a significantly lower number of bites were inflicted toward the treatment F compared to the treatments W and N ($P < 0.001$), but no difference was found between the treatments W and C ($P = 0.12$; figure 2B).

Discussion and conclusions

Spittlebugs are generally affected by a low predation rate (Whittaker, 1965; Biedermann, 2003), although there is evidence of predation by generalist spider or insect species

Table 3. Results of generalized linear model (GLM) on spittlebugs survival at the end of the experiment as a function of treatment (treatments: moistened with water, moistened with froth, moistened with nothing).

	d.f.	Deviance	Residual d.f.	Residual deviance	$P (> \chi^2)$
Null model			59	74.92	
Treatment	2	33.96	57	40.96	4.224 e-08 ***

Table 4. Results of generalized linear models (GLMs) on ants' bites inflicted to spittlebug nymphs and *S. carnaria* maggots as a function of treatment (treatments: moistened with water, moistened with froth, moistened with nothing).

Prey		d.f.	Deviance	Residual d.f.	Residual deviance	$P (> \chi^2)$
Spittlebug nymphs	Null model			59	853.51	
	Treatment	2	622.66	57	230.85	< 2.2 e-16 ***
S. carnaria maggots	Null model			59	171.91	
	Treatment	2	61.30	57	110.61	6.397 e-08 ***

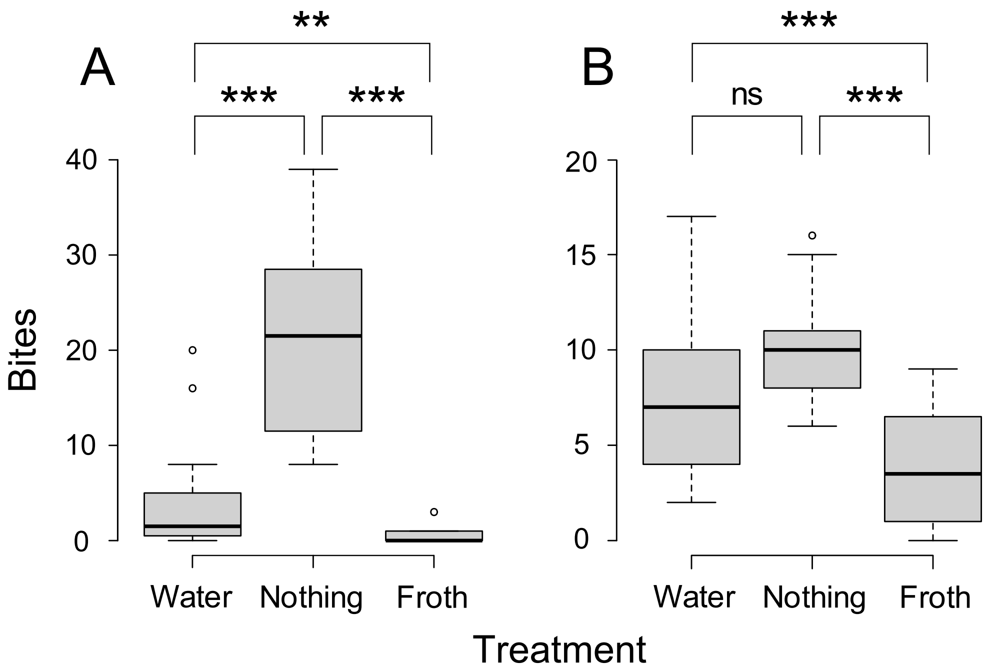

Figure 2. Number of ants' bites inflicted to spittlebug nymphs (**A**) and *S. carnaria* maggots (**B**) for the different treatments (treatments: moistened with water, moistened with froth, moistened with nothing). Significance levels: ns = not significant, ** = P < 0.01, *** = P < 0.001.

(Harper and Whittaker, 1976; Callan, 1980), including ants (Hewitt and Nilakhe, 1986; Sujii *et al.*, 2002; 2004), as well as by specialized predators (Weaver and King, 1954; del Campo *et al.*, 2011). The froth produced by spittlebug nymphs has been usually associated with a thermoregulatory and dehydration-preventing function (Cornara *et al.*, 2018), acknowledging also an antipredatory role, among others (Whittaker, 1970).

Consistently with what has been reported for other spittlebug species (Nachappa *et al.*, 2006; del Campo *et al.*, 2011), in the first experiment we observed considerably lower survival probability in *P. spumarius* nymphs deprived of froth compared to the control nymphs. This result was consistent either when the nymphs were challenged with spiders or ants. However, this result should be taken cautiously, because it probably owed more to the behaviour of the nymphs than to the predators' ability. Indeed, the nymphs deprived of the froth moved to search (without finding it) a new point in the shoot where to feed and produce new froth (Weaver and King, 1954). Increased motility, however, also increased the probability of nymphs to run into the predators, for example because they fell from the shoot or moved around in the arena. In contrast, the number of intentional attacks by spiders and ants were very low, in line with previous studies (Henderson *et al.*, 1990). Similarly, in the control group, no death occurred at the end of the experiment, because the predators did not engage in direct attacks and the nymphs stationary remained within their froth.

This result partially deviates from the one from Henderson *et al.* (1990), who observed a predation rate of 25% on *P. spumarius* nymphs within their froth by the ant *Formica montana* Weelher. Nevertheless, it should be noted that their experiment lasted 72 hours, much longer than ours, and involved a bigger species and a higher number of workers in the experiments. On the other hand, Nachappa *et al.* (2006) observed that nymphs of *Prosapia bicincta* (Say) in presence of colonies of the ant *Solenopsis invicta* Buren for 24 hours were rarely killed when left within their froth compared to nymphs whose froth was removed. Hewitt and Nilakhe (1986) reported predation by several ant species belonging to the genera *Solenopsis*, *Pheidole* and *Conomyrma* on *Zulia entrerriana* (Berg). In particular, predation occurred on the eggs and newly hatched nymphs before they produced froth. Yet another study found considerably higher number of workers of *S. invicta* on defrothed compared to froth-coated nymphs of *M. fimbriolata* in a cafeteria experiment, and the authors concluded that this was due to chemical repellence of the froth (Tonelli *et al.*, 2019). However, their conclusion seems not fully supported, given that their findings could simply reflect a preference for more readily available prey.

Several authors reported that spittlebugs can be killed by spiders or other arachnids (Weaver and King, 1954; Phillipson, 1960; Harper and Whittaker, 1976; Yurtsever, 2000). For example, the DNA of *P. spumarius* has been found in the gut content of the wolf spider *Alopecosa cuneata* (Clerck) collected in an olive orchard in spring (Lantero *et al.*, 2018), while the spiders *S. globosum* and *Araniella cucurbitina* (Clerck) showed type I and type II functional responses (respectively) towards *P. spumarius* adults (Benhadi-Marin *et al.*, 2020). In Mediterranean ecosystems, *S. globosum* can be commonly observed on flowers of several plant species (Ajuria and Reader, 2014; Benhadi-Marin *et al.*, 2019), often sharing the same plant with the nymphs of *P. spumarius* (personal observation). Nonetheless, no direct attack of *S. globosum* against

P. spumarius juveniles is reported. Crab spiders (Thomisidae), such as *S. globosum*, are ambushing predators that stay motionless on flowers waiting for prey that they capture with their strong front legs (Gertsch, 1939). Therefore, the absence of spider attacks observed in our experiment could be explained both by the effect of the spittlebug froth and the hunting strategy, mainly targeting actively moving prey. However, the froth could be an adaptation to hide the nymph from predators that use visual cues to detect their prey, a strategy adopted also by some hemipteran species (Moss *et al.*, 2006). Further studies involving spiders with different hunting strategies should be carried out to better explore this hypothesis.

Therefore, from the results of the first experiment we cannot conclude that froth has a deterrent effect, as we cannot exclude other possible explanations, such as a physical hindrance by the froth, increased mortality due to increased motility of nymphs when not covered by froth, or even an effect of the hunting strategy. However, this latter aspect seems to be not an issue here, since a lack of aggression towards nymphs was observed also with *C. scutellaris*, which are active predators (Frizzi *et al.*, 2016; Balzani *et al.*, 2020). Moreover, the effect of the age class was not significant, even if this factor, as well as the aggregation rate, could affect the mortality of spittlebug nymphs, as found for *Neophilaenus albipennis* (F.) by Biedermann (2003).

Our second experiment allowed us to test the chemical effect of the froth by excluding the confounding effect of the physical hindrance (due to the foamy texture of the froth). Predation on defrothed nymphs by *C. scutellaris* was confirmed in almost all replicates, whereas nymphs moistened with water or with centrifuged liquid froth were more rarely predated. These results suggest that the froth could chemically mask the prey odour, or be repellent for ants. Also, it seems that even the defrothed nymphs would continue carrying residuals of some substance that, diluted with water, played a masking or repellent effect. The ants' bite counts provided support to this hypothesis. Indeed, the number of bites was greater in the defrothed group, intermediate in the water-coated group and lower in the froth-coated group. In particular, the ants' response to water-coated nymphs compared to the other treatments seems to confirm the deterrent or masking effect of some residual substance on the nymph integument, as previously suggested by Henderson *et al.* (1990). Our findings support the ones found on another spittlebug species, *Aphrophora cribrata* (Walker), whose froth was analysed and its deterrence tested against the ant *Formica exsectoides* Forel (del Campo *et al.*, 2011). These authors found that *A. cribrata* nymphs coated with froth had a significantly lower mortality compared to the control nymphs, and both the natural froth and the artificially synthesized one had a deterrent effect towards ants in 90% of contacts. The authors concluded that the deterrence cannot be based on volatile chemicals, since the ants did not avoid the froth, but rather is due to some contact-deterrent substance in the froth, as proposed later also by Tonelli *et al.* (2019). While our observations confirmed this, we highlight that the authors did not consider the possibility that the froth could have an odour-masking effect, that seems a better explanation.

Moreover, ants that encountered the froth-coated nymphs were previously observed self-grooming their antennae (Henderson *et al.*, 1990; del Campo *et al.*, 2011; Tonelli *et al.*, 2019). All these authors interpreted this behaviour as a consequence of froth deterrence. We too observed sporadic cases of self-grooming of the antennae in ants after contacting the froth. However, this seemed more related to the need of cleaning the antennae after the contact with a sticky and frothy substance than a true deterrence. On the contrary, during the experiments, we could observe that sometimes the ants drank the froth, especially when it was liquefied, as already documented by a previous paper (Henderson *et al.*, 1990). This strongly reinforces our hypothesis that a chemical mimicry is a more plausible explanation than a chemical deterrence.

The results of maggot bioassays allow to further clarify the role of the froth in protecting nymphs from predators. Indeed, while the number of ants' bites to froth-coated larvae was lower than towards the other treatments, no significant difference was found between the control and the water-coated groups. This finding further supports the idea of a masking effect of froth and corroborates the assumption of residual substances on the spittlebug nymph body, substances not present on the maggot body.

To reduce the spread of *X. fastidiosa* in olive growing areas, research of effective biological control agents against its main vector *P. spumarius* has increased in the last years (e.g. Liccardo *et al.*, 2020; Mesmin *et al.*, 2020). The results of our experiments show that the hunting strategy and sensory ability of predators must be considered in evaluating nymphs' potential biocontrol agents. In particular, our results suggest that generalist predators using chemical cues to locate the prey, such as ants, could not be efficient biocontrol agents for nymphs of the meadow spittlebug. Thus, considering the importance of nymphs' control in the *X. fastidiosa* management (Dáder *et al.*, 2019), further studies aimed at clarifying the antipredatory trait of froth appear to be crucial.

In conclusion, we provided evidence that spittlebugs froth, beyond protecting the nymph from thermal stress and dehydration, could play a role as a multifaceted protection against generalist predators. While its frothy state might represent a physical hindrance also hiding the nymphs from visual predators, like spiders, its chemical composition could prevent detection by predators that rely most on olfactory stimuli, such as ants. Regarding the chemical effect of the froth, our results suggest that froth could contain specific odour-masking substances that hide nymphs from predators, rather than acting as a true deterrent. Further studies on the chemical composition of the froth are needed to clarify the role of this self-produced biofoam in the chemical mimicry of spittlebug nymphs.

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