EVALUATION OF THE POPULATION OF THE MEADOW SPITTLEBUG *Philaenus spumarius* IN TUSCAN OLIVE GROVES

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INTRODUCTION

LIFE RESILIENCE is a project co-financed by EU through LIFE programme carried out in Portugal, Spain and Italy, that aims to demonstrate an increase in the sustainability and resistance of the intensive production of olive and almond trees to *Xylella* spreading. This project also addresses the EU priority area of Climate Change Mitigation, mainly in relation to land use and forestry. Last winter an outbreak of *X. fastidiosa multiplex* was identified in the southern part of Tuscany (Mount Argentario), confirming the possibility of a spread of the problem out of the Apulia Region.

Since *Philaenus spumarius* was identified in Apulia as one of the main vectors of the *X. fastidiosa* and EFSA confirmed the importance of controlling this carrier (Di Serio, F., et al. 2019), we carried out samplings in four olive groves of Tuscany to understand the biology and the current status of the local population of the insect and its juvenile forms.

OBJECTIVES

The aims were to monitor the density of the population within four olive groves to understand the pattern of distribution of the insects in Tuscany in relation to the land use of the sourrandings studying the possibility of applying containment measures to prevent the olive quick decline syndrome (OQDS) with sustainable tools.

MATERIALS AND METHODS

Samplings were performed as in Di Serio, F., et al. (2019). in four olive groves of Tuscany (Fig.1 a,b,c,d): SALOV (Pisa), CNR S. Paolina Experimental Farm and Felciaione (Follonica), Il Tombolo intensive olive grove (Marina di Grosseto).

Table 1. Experimental design of the sampling of nymphs and adults.

	Sampling of nymphs	Sampling of adults
Sampling period	March, April and first half of May	from the second half of May
Sample units	• a representative plot of about 1 ha for each olive grove; • a frame of 0,25 mq was thrown 20 times randomly in the plot.	• a representative plot of about 1 ha for each olive grove; • **on ground:** 4 steps and a swept with the sweeping net each step for a total of 4 swept for each point. • **on canopy:** 10 swepts per selected olive tree have been made around the crown.
Data registered	site, date, sampling number, sample (SSUp), percentage of grass cover, grass height (cm), first dominant plant species, second dominant plant species, plant species with spittles, phenology, water stress, spittle position on plant, spittlebug species, number of insects per spittle, their juvenile instar.	Adults have been counted, identified and immediately released. A subsample of insects was stored for sex identification in laboratory

STUDY AREAS

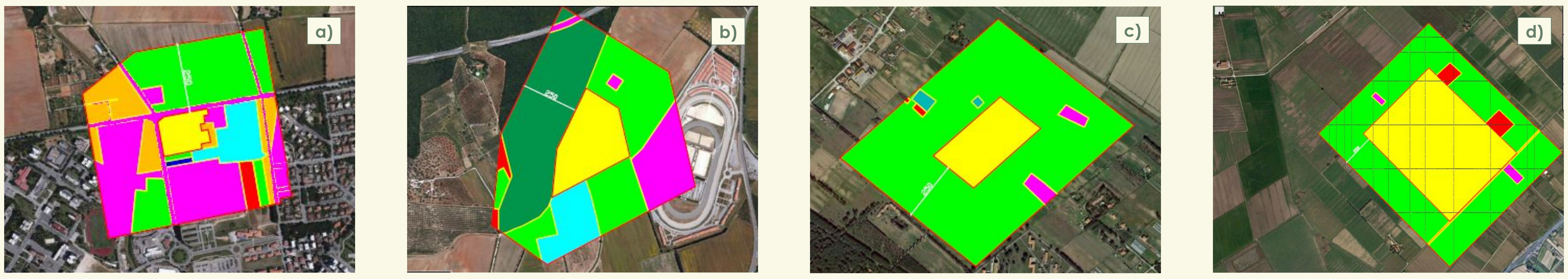

Table 2. Each study areas (olive orchards) are coloured in yellow. The second column represents the colors corresponding to the different land use patterns. Column 3-6 summarize the percentage (%) of land use patterns around the study areas.

		a	b	c	d
Study area	(yellow)	-	-	-	-
Urban area	(magenta)	39,2	14,7	2,5	3,0
Crops	(green)	34,0	34,4	96,0	93,2
Urban green	(orange)	15,4	0,0	0,0	0,0
Orchards	(light blue)	8,7	10,4	0,0	0,0
Other olive groves	(red)	2,2	1,3	0,3	3,8
Vineyards	(blue)	0,5	0,0	0,0	0,0
Wood	(dark green)	0,0	39,2	0,0	0,0
Water	(teal)	0,0	0,0	1,2	0,0

Fig. 1. Maps of land use, elaborated as suggested by Santoiemma et al. (2019). The four olive groves of Tuscany where we performed the samplings of nymphal and adult stages of *P. spumarius:* a) **CNR** S. Paolina Experimental Farm (42°55'58.96''N 10°45'47.34''E); b) **CNR2** Felciaione (42°56'38.64''N 10°46'17.32''E)(Follonica, GR); c) **Marina** «Il Tombolo» intensive olive grove (42°44'6.78''N 10°59'10.00E)(Marina di Grosseto, GR); d) **Pisa** SALOV olive grove (43°48'5.01N 10°21'0.31'')(Pisa, PI).

RESULTS

Sampling of nymphs

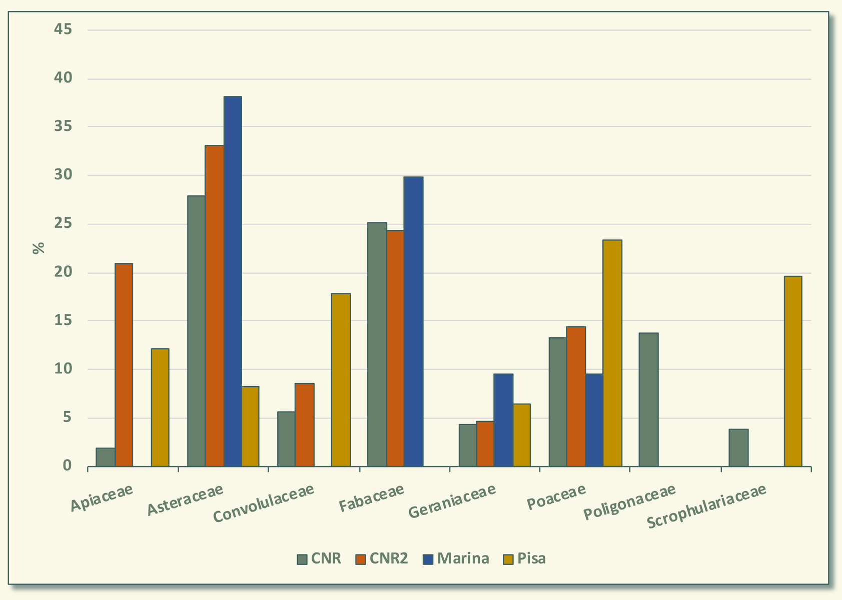

Fig 2. Percentages of the main herbaceous families hosting nymphs of *P. spumarius* in the four study areas.

Fig. 3. Spittle of *P. spumarius* in apical position on a specimen of *Borago officinalis*.

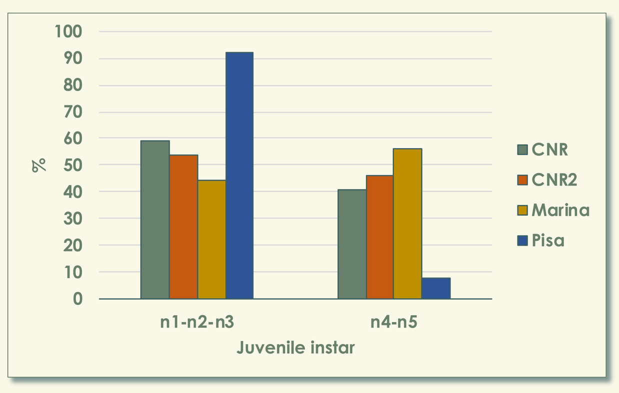

Fig 5. Occurrence of the different phenological life stages (juvenile instars) of nymphs of *P. spumarius* in the same sampling period. The olive grove of Pisa is the northern, so it could explain the presence of mainly early nymphal stage.

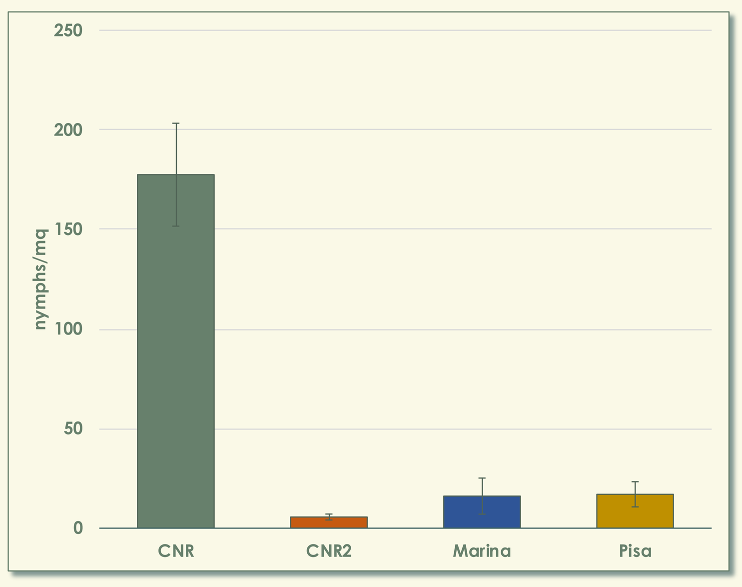

Fig 4. Density per m² of nymphs of *P. spumarius* in the four study areas (±SD): in the olive grove of CNR-IBE S. Paolina in Follonica the density is the highest and in the other CNR-IBE olive grove (CNR2) the density is the lowest although they are located only 1 Km apart.

Fig. 6. Plastic frame used to sample spittles and nymphs of *P. spumarius* in study areas.

Sampling of adults

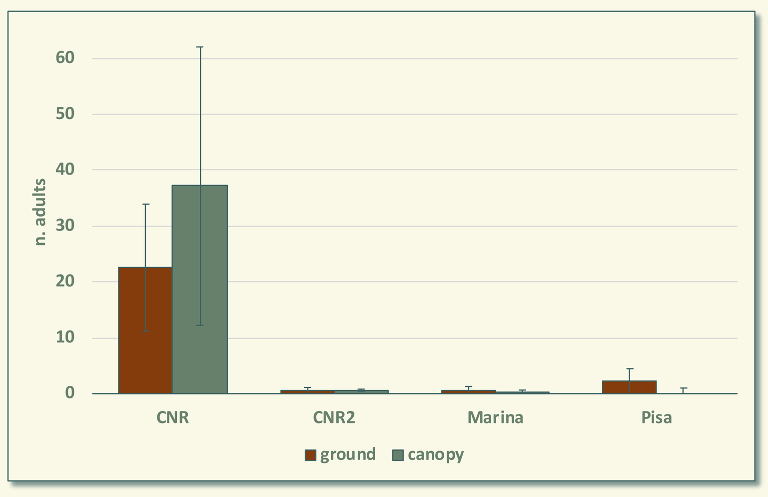

Fig 7. Number of adults (±SD) of *P. spumarius* sampled with an entomological sweep net both on the tree canopies and the ground. As for the nymphs, the highest number of specimens were collected in the olive grove of CNR-IBE S. Paolina. The abundance on single tree was not calculated because the trees were to small to carry out 10 swepts each, as suggested in Serio et al., 2019.

Fig. 8. Adult specimens of *P. spumarius* sampled with a swept in S. Paolina olive grove and collected into a vial for counting.

Fig. 9. Close-up of a yellow glue trap used to verify the presence and the abundance of adults of *P. spumarius* in the four study areas.

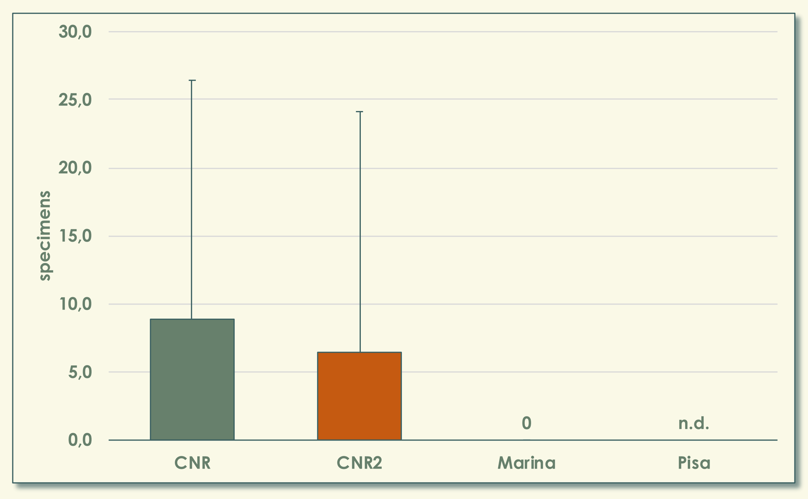

Fig 10. Mean number (±SD) of the adults of *P. spumarius* collected on the yellow glue traps positioned on five olive trees in each study area.

CONCLUSIONS

- The herbaceous coverage presented a great variety among the sampled olive groves, but nymphs live mainly on species of *Asteraceae*, *Apiceae* and *Fabaceae*.
- CNR olive grove, with the highest diversity of land use along its edges, presented the most abundant population (177,5 nymphs/m² ± 25,84; mean and SD). Although the CNR2 olive grove is located within 1 Km and showed a similar species diversity, the population is significantly less dense (5,6 nymphs/m² ± 1,5; mean and SD).
- The samplings of adults, both by swepts and by glue traps, highlighted that the population of CNR had the highest density, as demonstrated with the previous samplings on nymphs population. The number of insects into the yellow traps was always lower compared to the number captured by the sweep net especially after the summer period.
- This preliminary study indicates that a deep attention must be posed on the olive groves located near areas of high differentiation use of the soil and that a containment strategy must be performed focusing on the nymphal stage of *P. spumarius*.

REFERENCES
Di Serio, F., et al. (2019). Collection of Data and Information on Biology and Control of Vectors of *Xylella fastidiosa*. EFSA Supporting Publications, 16(5).
Santoiemma, G., Tamburini, G., Sanna, F., Mori, N., & Marini, L. (2019). Landscape composition predicts the distribution of *Philaenus spumarius*, vector of *Xylella fastidiosa*, in olive groves. Journal of Pest Science, 92(3), 1101-1109

LIFE RESILIENCE

Partners:

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Journal of Pest Science

Efficacy of sustainable products to control juveniles of Philaenus spumarius (Hemiptera, Aprophoridae) L., main European vector of Xylella fastidiosa

--Manuscript Draft--

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Abstract:	The detection of the bacterial pathogen Xylella fastidiosa in Italy turned the meadow spittlebug, Philaenus spumarius, into a serious key pest for its crucial role in transmitting the bacterium. Since no effective methods to remove the bacterium from infected plants have been discovered yet, controlling insect vectors is the only effective strategy to prevent X. fastidiosa's spread. Nymphal stages of P. spumarius develop inside froths on weeds and natural soil coverage of olive groves or other crops which can be threatened by the bacterium. Within the frame of the LIFE Resilience project funded by European Commission, aimed at the implementation of practical sustainable methods of vector control, different products have been tested to control nymphs of P. spumarius growing on Rumex crispus in the natural coverage of an olive grove. Sulfur, soft soap, Beauveria bassiana, sulfur plus B. bassiana, and pyrethrum have been sprayed on 100 spittles in a split block design and compared to the control. B. bassiana proved to be effective in significantly reducing both the number of spittles and nymphs of P. spumarius. To a lesser extent, also soft soap reduced significantly the overall number of nymphs in comparison to the control. These findings will broad the sustainable integrated pest management of P. spumarius by offering to olive growers or other stakeholders more control tools to reduce X. fastidiosa spread.

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Firenze, November 3rd, 2019

To Prof. Michael Traugott
Editor-in-chief
Journal of Pest Science

Dear Prof. Traugott,

I am pleased to submit an original research article entitled "Efficacy of sustainable products to control juveniles of *Philaenus spumarius* (Hemiptera, Aprophoridae) L., main European vector of *Xylella fastidiosa*" by Claudio Cantini, Letizia Poggioni, Anita Nencioni and Patrizia Sacchetti. We would like to have the manuscript considered for publication in the *Journal of Pest Science*.

The manuscript refers to a field trial aimed at evaluating different sustainable products as potential insecticides to control the nymphal stages of *Philaenus spumarius*, considered as the main vector of the quarantine bacterium *Xylella fastidiosa*. The research was undertaken for the urgent necessity to reduce, and hopefully arrest, the spread of this disease that threatens olive groves as well as other agricultural crops and not-cultivated plants. Sustainable products have been preferred since the research was funded by the EU LIFE Resilience project which is devoted to the protection of olive crops with low impact methods.

To our knowledge, the tested products have not been previously evaluated as insecticides on *P. spumarius*, moreover the entomopathogenic fungus *Beauveria bassiana* has yielded promising results, therefore the paper may result interesting for future practical applications, mainly for organic olive growers. Finally, due to the lack of information about sustainable products to control *P. spumarius*, as well as for the economic importance of the disease, we submit the manuscript for publication as a "Rapid communication".

The manuscript is original, it has neither been published, accepted nor is under consideration for publication elsewhere. We have no conflicts of interest to disclose. All authors have approved the manuscript and agree with its submission to the *Journal of Pest Science*.

We therefore hope you will consider our manuscript suitable for publication.

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We look forward to hearing from you at your earliest convenience.

Best regards,

Patrizia Sacchetti

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Efficacy of sustainable products to control juveniles of *Philaenus spumarius* (Hemiptera, Aprophoridae) L., main European vector of *Xylella fastidiosa*

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Abstract

The detection of the bacterial pathogen *Xylella fastidiosa* in Italy turned the meadow spittlebug, *Philaenus spumarius,* into a serious key pest for its crucial role in transmitting the bacterium. Since no effective methods to remove the bacterium from infected plants have been discovered yet, controlling insect vectors is the only effective strategy to prevent *X*. *fastidiosa*'s spread. Nymphal stages of *P*. *spumarius* develop inside froths on weeds and natural soil coverage of olive groves or other crops which can be threatened by the bacterium. Within the frame of the LIFE Resilience project funded by European Commission, aimed at the implementation of practical sustainable methods of vector control, different products have been tested to control nymphs of *P*. *spumarius* growing on *Rumex crispus* in the natural coverage of an olive grove. Sulfur, soft soap, *Beauveria bassiana*, sulfur plus *B*. *bassiana*, and pyrethrum have been sprayed on 100 spittles in a split block design and compared to the control. *B*. *bassiana* proved to be effective in significantly reducing both the number of spittles and nymphs of *P*. *spumarius*. To a lesser extent, also soft soap reduced significantly the overall number of nymphs in comparison to the control. These findings will broad the sustainable integrated pest management of *P*. *spumarius* by offering to olive growers or other stakeholders more control tools to reduce *X*. *fastidiosa* spread.

Keywords

Meadow spittlebug, natural coverage, olive grove, sustainable control, *Beauveria bassiana*

Key message

Sustainable control methods to control *Xylella fastidiosa* vectors are urgently needed.

Juveniles of insect vectors are settled on herbaceous weeds and do not spread the disease.

Sulfur, soft soap, *Beauveria bassiana* and pyrethrum have been tested to control *P*. *spumarius* nymphs growing on *Rumex crispus*.

B. *bassiana* proved to be effective in significantly reducing both the number of spittles and nymphs of *P*. *spumarius*.

Introduction

Xylella fastidiosa is a Gram-negative plant pathogenic bacterium that causes severe damage to several economically important crops, included olive trees. The species is genetically variable, having developed five subspecies with tens of strains (Almeida and Nunney 2015) which cause significant diseases to an increasing list of host plant (EFSA 2015) so that it is considered as a quarantine plant pest worldwide. Recently this pathogen destroyed hundreds of hectares of olive orchards in Apulia representing a serious threat to the cultivation of olive trees in the whole Mediterranean Basin.

In May 2019 the European Food Safety Agency (EFSA) Plant Health Panel (PLH) stressed the importance of implementing control measures, such as controlling the insects that are known to transmit the pathogen. Recent studies identified in *Philaenus spumarius* Linnaeus (Hemiptera: Aphrophoridae) the main vector of the pathogen in Apulia olive orchards (Saponari et al. 2014; Cornara et al. 2017). The adults of this spittlebug feed on the xylem-sap of many plant species and can transmit *X. fastidiosa* through their mouthparts: bacterial cells, after being sucked together with the sap from an infected plant, adhere to the walls of the insect foregut and they are subsequently released when the insect feeds on a new plant. Spittlebug nymphs colonize herbaceous plants and shrubs producing a whitish froth which protect them from dehydration and predators. They complete their growth developing through five stages before adult emergence (Biedermann and Niedringhaus 2009). To reduce indirectly the spread of *X. fastidiosa* is crucial to control the population density of *P. spumarius*, since no effective methods to remove the bacterium from infected plants have been discovered yet (EFSA News 2019). Moreover, controlling *P. spumarius* during preimaginal development appears to be particularly favorable and effective because nymphs are quite stable and do not spread the bacterium. The implementation of practical sustainable methods of vector control is one of the main goals of the LIFE Resilience project recently financed by the European Commission (www.liferesilience.eu). Synthetic, broad spectrum insecticides which are recommended in Italy to control the vector (Ministero delle Politiche Agricole Alimentari e Forestali 2018) cannot be applied selectively and can be harmful to beneficial insects such as honeybees (Blacquière et al. 2012) besides their use as spray is not allowed in organic farming. In a study aimed at testing different insecticides on *P. spumarius* some neonicotinoids and pyrethroids proved a significant reduction of the number of nymphs and spittles on the treated vegetation (Dongiovanni et al. 2018b). To control this vector natural substances such as orange essential oil, kaolin and zeolite have been also tested with questionable results (Dongiovanni et al. 2018c).

Among potential control products some microbial agents might be evaluated since occasionally different fungi have been reported as natural enemies, such as *Beauveria bassiana* and *Fusarium oxysporium* (Di Serio et al. 2019).

B. bassiana is an entomopathogenic fungus which germinates when gets in contact with the insect cuticle. Then this fungus penetrates through the insect cuticle reaching the inner body where it produces lethal toxins. The fungus requires high relative humidity to germinate so that the microhabitat of the froth produced by spittlebugs could be a suitable substrate for its proliferation.

Within the frame of the LIFE Resilience project, a field trial aimed at controlling nymphal stages of *P. spumarius* using low impact products has been carried out. *Beauveria bassiana* was tested in comparison with other products recommended in integrated pest management such as potassic soap, sulfur, and pyrethrum. The aim of the work was to evaluate if any of the tested products could affect to some extent the development of the nymphs favoring the control of

P. spumarius populations. The final purpose was to improve low impact control strategies which could be applied by olive growers.

Materials and methods

Experimental setup

The experiment was carried out within a four-hectares olive orchard located in Follonica, Italy (42° 55' 59.75"N 10° 45' 51.16"E). The study area shows a typical Mediterranean climate, with mild winter and hot summer; the mean annual temperature is 16°C and the average annual rainfall is 655 mm (Brilli et al. 2016).

The olive grove, with natural soil coverage, was managed under traditional dry farming practices by the National Research Council of Italy (CNR). The monitoring method proposed by EFSA for macrocosm (Di Serio et al. 2019) was used to assess presence and number of spittles in the sampling unit, the botanical composition of the natural soil coverage and the phenological stage of the herbaceous plants. Plant identification was based on field guides and manuals dealing with Mediterranean flora (Pignatti 1982). After that, plants of *Rumex crispus* Linnaeus, at pre-flowering phenological stage, with spittles located only in median and apical position on the stem, were chosen as the most convenient for the experiment for different reasons: abundance in the soil coverage, high presence of nymphal stages and plant shape suitability for the planned test. Each plant was labeled with a colored tag also reporting the number of spittles present on the stem (1 or 2).

Tested products

Four products were tested singly plus two of them in combination and compared to the control (Table 1). The experiment design was a split block arranged within a selected 1-ha unit of the olive orchard, divided in four areas (=blocks). In each of the four blocks 150 spittles were labelled: 25 spittles for each of the six treatments. Each treatment was sprayed on 25 spittles in four replicates for a total of 100 spittles. The treatment was performed spraying 1±0.5 mL of the different solutions directly on each spittle using a hand sprayer. The pyrethrum treatment was considered as a positive control, since the effectiveness of contact insecticide, synthetic pyrethroids, had already been tested on *P. spumarius* nymphs (Dongiovanni et al. 2018b). Other 25 spittles in four replicates were tagged, sprayed with tap water and used as negative control. The number of nymphs as well as their developmental stage was assessed by destructive analysis of 100 spittles just before the treatment.

Evaluation of the effects of different treatments has been carried out after 7 days from their application. All the tagged plants were cut at the base and brought to the laboratory for spittle assessment. Since dead insects were not found inside the spittles, the effects of the treatment were evaluated counting the number of spittles per plant, the number of nymphs per spittle as well as the insect stage (including newly emerged adults inside the spittles). Statistics was performed using the ANOVA procedure of Systat 11 package (Systat Software Inc. Richmond, CA, USA). The effect of the treatments compared to both negative and positive control was evaluated by Tukey's mean pairwise comparison test. Pearson's chi-squared test was applied to assess the difference in distribution within classes of the juvenile forms counted in the spittles in relation to each treatment.

Results

Observations on botanical composition of the soil coverage showed a predominance of dicotyledons (73% of the total plants) mostly represented by *Calendula* spp. (23%), *Vicia* spp. (14%) and *Rumex* spp (23%). Counting of the spittles on the plants showed a 43% of them formed in the bottom third position, close to the soil or at the rosette of leaves. The day of the treatment nearly all the *P. spumarius* (94%) checked inside their spittles were fourth and fifth instar nymphs. After 7 days from the spraying the nymphs checked inside the spittles of the treated plants did not appear substantially different from those present on the untreated plants (sprayed with water), except for some that showed a brownish color or low vitality symptoms. Most of the insects were in the fourth or fifth nymphal stage and the distribution within classes did not show significant difference among treatments (Table 2).

The plants sprayed with sulfur showed a slight increase in the number of spittles (Fig. 1a). The treatment with *B. bassiana* produced a significant reduction (p = 0.034) in the number of spittles compared to the control lowering up to 60% the presence of foams on the stems. The effect of the treatment with the fungus was even more effective on the number of nymphs (Fig. 1b). Only 18% of the nymphs were estimated to survive after the treatment with a highly significant difference respect to the negative control (p = 0.001). The treatment with *B. bassiana* was also effective when mixed with sulfur (p = 0.015), but the sulfur alone did not produce significant effect. Also the soft soap reduced significantly (p = 0.015) the overall number of nymphs in comparison to the control. Both pyrethrum and sulfur reduced the number of nymphs present after the treatment but the reduction was not statistically significant. All the treatments (Fig. 1c) reduced the mean number of nymphs per spittle compared to the control with p ranging between 0.003 (*B. bassiana*) and 0.038 (pyrethrum).

Discussion

The natural soil coverage where the experiment was carried out was composed of several plants which are common in mowed soil coverage olive orchards (Simoes et al. 2014) and that are known as host plants of *P. spumarius* (Cornara et al. 2018). Among herbaceous plants of the natural soil coverage, *R. crispus* was chosen as the target species for the experiment on the basis of its plant structure, mainly for its height which can range from 50 to 150 cm (Fitter and Peat

1994; CABI 2012). In our opinion, the spittles formed on these soaring plants represented a homogeneous sample, avoiding biases due to plot density variation, host plant effect, spittle position. Moreover, the *R*. *crispus* erect plants, after being accurately sprayed with a hand sprayer, facilitated to assess the effects of the tested products on *P*. *spumarius* nymphs.

The product based on *B*. *bassiana* showed a high effectiveness in reducing both the number of nymphs and the number of spittles present on the plants. Products based on this entomopathogenic fungus are used largely to control pests in agriculture, veterinary and forestry (McKinnon et al. 2017) since *B*. *bassiana* is a pathogen that causes diseases in more than 700 species of arthropods, mainly insects (Mascarin and Jaronski 2016). Although this fungus is considered cosmopolitan and polyphagous, environmental conditions are crucial for its effectiveness. In particular, humidity is the most critical abiotic factor for its growth both in laboratory and field conditions (Mascarin and Jaronski, 2016; James et al. 1998). As a consequence, the promising results obtained in our experiment with Naturalis, used alone and mixed with sulphur, could have been favored by the high relative humidity of the spittle microhabitat. As a matter of fact, the froth produced by nymphs is made of a watery fluid given out from the anus (Yurtsever 2000). Our results suggest that microbial products formulated with *B*. *bassiana* could be applied successfully in organic farming, ensuing helpful also in conditions where the mechanical control of preimaginal stages is not workable or inadequate to reduce the populations of *P*. *spumarius*.

The current experiment has underlined that even another sustainable product, the soft soap, resulted valid in controlling nymphs in open field, producing a significant reduction of the overall number of nymphs on the treated plants in comparison to the control. This result could be helpful for olive growers since, up to now, in Italy only a few products have been registered for the control of *P*. *spumarius* in organic farming. As a matter of fact, soft soaps are commonly applied in sustainable farming to control different soft-bodied arthropods, such as aphids, whiteflies and mites, among others, as summarized in Baldwin and Koehler (2007). To our knowledge soft soap has not been tested on Aphrophoridae juvenile stages, while it was tested on another species of the Cicadomorpha group, *Scaphoideus titanus*, although without significant results (Tacoli et al. 2017). However, different surfactants have been tested on *P*. *spumarius* nymphs on dwarf beans proving that some of them can increase the mortality of contact insecticides by breaking down the froth (Jones and Barratt 1990).

Effects of pyrethrum treatment differed greatly from those produced by synthetic pyrethroids such as deltamethrin tested with encouraging results both against nymphs and adults in previous trials (Dongiovanni et al. 2018a; Dongiovanni et al. 2018b, Dáder et al. 2019). In our experiment natural pyrethrum did not highlight significant reduction of the number of nymphs and spittles. The unsatisfactory effect of the pyrethrins might be related to the lower persistence and effectiveness respect to pyrethroids and to the protective action of the froth against different insecticides, as already reported in field and laboratory trials against *P. spumarius* nymphs (Jones and Barratt 1990). As recently reported, the effect of natural pyrethrum might be enhanced by adding the synergic piperonyl butoxide (Dáder et al. 2019). On the contrary, the spittle probably created more suitable conditions for the germination and development of *B. bassiana* that performed the most effective action in our experiment.

The slight increase, albeit not significant, in the number of spittles observed in the sulfur and pyrethrum treatments could be explained by the movement of some nymphs out of the original froth due to possible disruption caused by the treatment. Application of sulfur significantly reduced only the number of nymphs per spittle. However, based on our data (see Table 2), sulfur appeared to have increased the number of newly emerged adults as their development was accelerated by the treatment. This observation cannot find any explanation, to our knowledge.

When sulfur was used in mixture with *B. bassiana*, it lowered the effectiveness of the entomopathogenic fungus on spittlebug nymphs.

Our experiment was conducted on fourth and fifth instar nymphs, similarly to field trials carried out in Apulia (Dongiovanni et al. 2018b). However, the action of sustainable products quite likely might be more effective by spraying insects at an earlier developmental stage.

B. bassiana demonstrated a good potential in lowering the population of nymphal stages of *P. spumarius:* the treatment has indeed proved to be effective in significantly reducing both the number of spittles and nymphs in comparison to the control. *B. bassiana* is well-known as an entomopathogenic fungus: moreover, it also can be advantageously used against plant pathogens (Jaber et al. 2018) and recently it was successfully established as endophytic fungi in horse-chestnut leaves after artificial inoculation of saplings (Barta 2018).

Since our promising results for the first time support evidences that a single treatment with *B. bassiana* might produce a significant reduction in the preimaginal population density of *P. spumarius*, in our opinion this microbial product is worth to be included in the integrated control of spittlebugs in olive orchards.

Author contributions

All authors conceived and designed research. CC, LP and AN conducted experiments. CC provided products and tools and analyzed data. All authors contributed to the writing and revising of the manuscript. All authors read and approved the manuscript.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Human and animal rights This article does not contain any studies with human participants or animals (vertebrates) performed by any of the authors.

Informed consent Informed consent was obtained from all individual participants included in the study.

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Table 1 Tested treatments with the respective codes, trade name and firm, commercial product composition, active ingredients, and concentration of the final solution.

Treatment	**Trade name**	**Firm**	**Composition and features of the commercial product**	**Concentration of the sprayed solution (diluted in tap water)**
A. Control	-		Tap water	1000
B. Soft soap	“Sapone molle”	Al.Fe S.r.l., Mantova, Italy	Plant defence enhancer, 0.8% of potassium salts (with 10% K) (water solution)	6 mL/L
C. Sulfur	Zolfo SC	Diachem S.p.A., Bergamo, Italy	Fungicide, 56.09% of pure sulphur (Concentrated Suspension)	1.5 mL/L
D. *Beauveria bassiana*	Naturalis	Biogard	Insecticide and acaricide; spores of *Beauveria bassiana*, ATCC74040 strain, 0.0185 g (Concentrated Suspension)	2.0 mL/L
E. *B. bassiana* + sulfur	Naturalis plus Zolfo SC		Just prepared mixture	1.5 + 2.0 mL/L
F. Pyrethrum	“Piretro Actigreen PFnPE”	Solabiol, SBM Life Science, France	Pyrethrins 2% (Emulsifiable Concentrate)	2 mL/L

Table 2 Frequency distribution of *P. spumarius* specimens (pooled into three developmental groups) found inside spittles seven days after the treatment (Pearson’s chi-squared test, for df=2 limit of significance p=0.05; $\chi^2 \geq 5.99$. ns= not significant).

treatment	N2-N3	N4-N5	adult	χ^2
A. Control	0.03	0.91	0.05	-
B. Soft soap	0.08	0.87	0.05	0.09 ns
C. Sulfur	0.01	0.87	0.12	0.11 ns
D. *Beauveria bassiana*	0.02	0.94	0.03	0.01 ns
E. *Beauveria bassiana* + sulfur	0.02	0.96	0.02	0.02 ns
F. Pyrethrum	0.02	0.92	0.05	0.00 ns

Figure caption

Fig. 1 Effects of six different treatments sprayed on spittles present on *Rumex crispus*: A = tap water; B = soft soap; C = sulfur; D = *Beauveria bassiana*; E = *B. bassiana* and sulfur mixture; F = pyrethrum. Mean values (± SE) have been compared (Tukey's mean pairwise comparison test) for each parameter: a) overall number of spittles; b) overall number of nymphs; c) number of larvae per spittle