

# Journal of Pest Science

## Efficacy of sustainable products to control juveniles of *Philaenus spumarius* (Hemiptera, Arothoridae) L., main European vector of *Xylella fastidiosa* --Manuscript Draft--

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<b>Abstract:</b>	<p>The detection of the bacterial pathogen <i>Xylella fastidiosa</i> in Italy turned the meadow spittlebug, <i>Philaenus spumarius</i>, 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 <i>X. fastidiosa</i>'s spread. Nymphal stages of <i>P. spumarius</i> 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 <i>P. spumarius</i> growing on <i>Rumex crispus</i> in the natural coverage of an olive grove. Sulfur, soft soap, <i>Beauveria bassiana</i>, sulfur plus <i>B. bassiana</i>, and pyrethrum have been sprayed on 100 spittles in a split block design and compared to the control. <i>B. bassiana</i> proved to be effective in significantly reducing both the number of spittles and nymphs of <i>P. spumarius</i>. To a lesser extent, also soft soap reduced significantly the overall number of nymphs in comparison to the control. These findings will broaden the sustainable integrated pest management of <i>P. spumarius</i> by offering to olive growers or other stakeholders more control tools to reduce <i>X. fastidiosa</i> spread.</p>	



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DIPARTIMENTO DI SCIENZE  
E TECNOLOGIE AGRARIE,  
ALIMENTARI, AMBIENTALI E FORESTALI

Firenze, November 3<sup>rd</sup>, 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, Aphrophoridae) 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,

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2

3 **Efficacy of sustainable products to control juveniles of *Philaenus spumarius* (Hemiptera, Aprozhoridae) L., main**

4 **European vector of *Xylella fastidiosa***

5

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16

17 **Abstract**

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

31

32 **Keywords**

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

34

35 **Key message**

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

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

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

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

41

42 **Introduction**

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

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

68 Among potential control products some microbial agents might be evaluated since occasionally different fungi have  
69 been reported as natural enemies, such as *Beauveria bassiana* and *Fusarium oxysporium* (Di Serio et al. 2019).  
70 *B. bassiana* is an entomopathogenic fungus which germinates when gets in contact with the insect cuticle. Then this  
71 fungus penetrates through the insect cuticle reaching the inner body where it produces lethal toxins. The fungus requires  
72 high relative humidity to germinate so that the microhabitat of the froth produced by spittlebugs could be a suitable  
73 substrate for its proliferation.

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

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

80

## 81 **Materials and methods**

### 82 **Experimental setup**

83 The experiment was carried out within a four-hectares olive orchard located in Follonica, Italy (42° 55' 59.75"N 10°  
84 45' 51.16"E). The study area shows a typical Mediterranean climate, with mild winter and hot summer; the mean  
85 annual temperature is 16°C and the average annual rainfall is 655 mm (Brilli et al. 2016).

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

95

### 96 **Tested products**

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

106 Evaluation of the effects of different treatments has been carried out after 7 days from their application. All the tagged  
107 plants were cut at the base and brought to the laboratory for spittle assessment. Since dead insects were not found inside  
108 the spittles, the effects of the treatment were evaluated counting the number of spittles per plant, the number of nymphs

109 per spittle as well as the insect stage (including newly emerged adults inside the spittles). Statistics was performed using  
110 the ANOVA procedure of Systat 11 package (Systat Software Inc. Richmond, CA, USA). The effect of the treatments  
111 compared to both negative and positive control was evaluated by Tukey's mean pairwise comparison test. Pearson's chi-  
112 squared test was applied to assess the difference in distribution within classes of the juvenile forms counted in the  
113 spittles in relation to each treatment.

114

## 115 **Results**

116 Observations on botanical composition of the soil coverage showed a predominance of dicotyledons (73% of the total  
117 plants) mostly represented by *Calendula* spp. (23%), *Vicia* spp. (14%) and *Rumex* spp (23%). Counting of the spittles  
118 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  
119 day of the treatment nearly all the *P. spumarius* (94%) checked inside their spittles were fourth and fifth instar nymphs.  
120 After 7 days from the spraying the nymphs checked inside the spittles of the treated plants did not appear substantially  
121 different from those present on the untreated plants (sprayed with water), except for some that showed a brownish color  
122 or low vitality symptoms. Most of the insects were in the fourth or fifth nymphal stage and the distribution within  
123 classes did not show significant difference among treatments (Table 2).

124 The plants sprayed with sulfur showed a slight increase in the number of spittles (Fig. 1a). The treatment with *B.*  
125 *bassiana* produced a significant reduction ( $p = 0.034$ ) in the number of spittles compared to the control lowering up to  
126 60% the presence of foams on the stems. The effect of the treatment with the fungus was even more effective on the  
127 number of nymphs (Fig. 1b). Only 18% of the nymphs were estimated to survive after the treatment with a highly  
128 significant difference respect to the negative control ( $p = 0.001$ ). The treatment with *B. bassiana* was also effective  
129 when mixed with sulfur ( $p = 0.015$ ), but the sulfur alone did not produce significant effect. Also the soft soap reduced  
130 significantly ( $p = 0.015$ ) the overall number of nymphs in comparison to the control. Both pyrethrum and sulfur reduced  
131 the number of nymphs present after the treatment but the reduction was not statistically significant. All the treatments  
132 (Fig. 1c) reduced the mean number of nymphs per spittle compared to the control with  $p$  ranging between 0.003 (*B.*  
133 *bassiana*) and 0.038 (pyrethrum).

134

## 135 **Discussion**

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

140 1994; CABI 2012). In our opinion, the spittles formed on these soaring plants represented a homogeneous sample,  
141 avoiding biases due to plot density variation, host plant effect, spittle position. Moreover, the *R. crispus* erect plants,  
142 after being accurately sprayed with a hand sprayer, facilitated to assess the effects of the tested products on *P.*  
143 *spumarius* nymphs.

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

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

166 Effects of pyrethrum treatment differed greatly from those produced by synthetic pyrethroids such as deltamethrin  
167 tested with encouraging results both against nymphs and adults in previous trials (Dongiovanni et al. 2018a;  
168 Dongiovanni et al. 2018b, Dáder et al. 2019). In our experiment natural pyrethrum did not highlight significant  
169 reduction of the number of nymphs and spittles. The unsatisfactory effect of the pyrethrins might be related to the lower  
170 persistence and effectiveness respect to pyrethroids and to the protective action of the froth against different

171 insecticides, as already reported in field and laboratory trials against *P. spumarius* nymphs (Jones and Barratt 1990). As  
172 recently reported, the effect of natural pyrethrum might be enhanced by adding the synergic piperonyl butoxide (Dáder  
173 et al. 2019). On the contrary, the spittle probably created more suitable conditions for the germination and development  
174 of *B. bassiana* that performed the most effective action in our experiment.

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

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

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

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

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

193

#### 194 **Author contributions**

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

198

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201

202 **Compliance with ethical standards**

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

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

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

207

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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. <i>Beauveria bassiana</i>	Naturalis	Biogard	Insecticide and acaricide; spores of <i>Beauveria bassiana</i> , ATCC74040 strain, 0.0185 g (Concentrated Suspension)	2.0 mL/L
E. <i>B. bassiana</i> + 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

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**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).

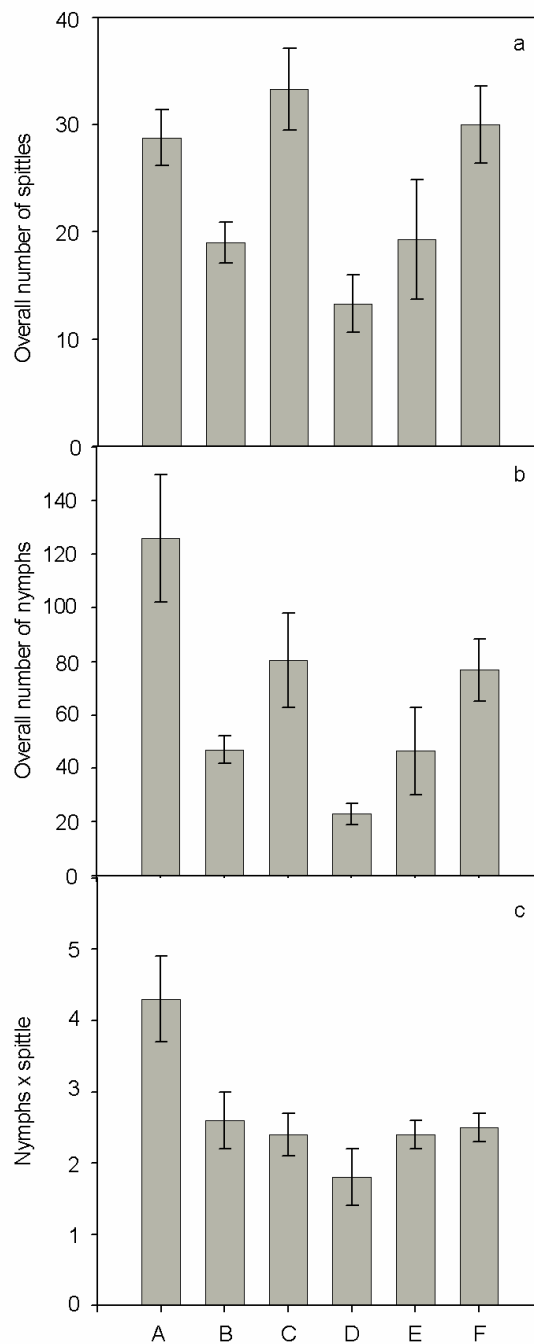
treatment	N2-N3	N4-N5	adult	$\chi^2$
A. Control	0.03	0.91	0.05	-
B. Soft soap	0.08	0.87	0.05	0.09 ns
C. Sulfur	0.01	0.87	0.12	0.11 ns
D. <i>Beauveria bassiana</i>	0.02	0.94	0.03	0.01 ns
E. <i>Beauveria bassiana</i> + sulfur	0.02	0.96	0.02	0.02 ns
F. Pyrethrum	0.02	0.92	0.05	0.00 ns

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280 Figure caption

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



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