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1 **Title: Effects of *Bacillus* lipopeptides on the survival and behavior of the rosy apple**
2 **aphid *Dysaphis plantaginea***

3
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11
12 **Abstract**

13 Within the framework of biocontrol development, several natural lipopeptides produced by *Bacillus subtilis*
14 show well-documented anti-microbial properties, especially in orchards. However, the number of studies on
15 their putative insecticidal effects remain low despite the growing interest to develop new strategies of
16 orchards pests' control. The rosy apple aphid *Dysaphis plantaginea* is the major aphid pest causing great
17 leaf damage to apple trees. In this study, we submitted young adult aphids to topical application of three
18 different families of lipopeptides, Plipastatin (Fengycin), Mycosubtilin (Iturin), and Surfactin, either
19 separately or as a ternary mixture. Their aphicidal effects were investigated at 1, 2.5 and 5 g/L, both at 1h
20 and 24h after exposure, and their effects on aphid behavior were studied at the 2.5 g/L concentration at 24h
21 after exposure. When delivered alone, lipopeptides displayed contrasted effects varying from no aphicidal
22 activity for Mycosubtilin to a mortality induced even at low concentrations by Surfactin. Surprisingly,
23 locomotor activity of the surviving aphids was only affected by the two least lethal treatments, Mycosubtilin
24 and the ternary mix. Their feeding behavior was only impacted by Surfactin, the most lethal treatment, that
25 unexpectedly increased phloem sap ingestion. The results are discussed in the context of lipopeptides
26 applicability for integrated pest management.

27
28 **Keywords**

29 *Bacillus subtilis* – insecticidal activity – sublethal effects – insect behavior – biopesticides –
30 electropenetrography

31 1. Introduction

32
33 The heavy use of chemical pesticides to achieve crop protection raises growing concerns about their possible
34 adverse effects on biodiversity, environment pollution as well as human health (Lechenet *et al.* 2017). Bio-
35 pesticides, which are living organisms or products derived from them (Thakur *et al.* 2020), are considered as
36 promising alternative products in this context. *Bacillus subtilis* is a commonly used bacterial bio-pesticide
37 agent (Stein 2005), its biocidal activities being considered to mainly rely on cyclic lipopeptides. Due to their
38 low ecotoxicity compared to their chemical counterparts (Deravel *et al.* 2014) and their high
39 biodegradability (Mulligan 2005), lipopeptides represent sustainable promising biocontrol products.

40
41 *Bacillus* lipopeptides are formed of a cyclic peptide linked to a fatty acid chain and are divided into three
42 families : Fengycin (Fengycin, Plipastatin), Iturin (Iturin A, Mycosubtilin and Bacillomycin) and Surfactin
43 (Surfactin, Pumilacidin) (Maksimov *et al.* 2020). Lipopeptides of a given class may differ in terms of the
44 number of carbon atoms fatty acid chain, and the amino acid composition of the cyclic peptide can also be
45 subject to minor modification, determining various isoforms (de Souza *et al.* 2018). Both Fengycin and
46 Surfactin interact with lipid layers, altering the cell membrane in a dose-dependent way, and could induce
47 cell lysis at high concentrations (Ongena and Jacques 2008). Iturin has antifungal properties based on
48 osmotic perturbation due to the formation of ion-conducting pores (Aranda *et al.* 2005), and preferentially
49 interact with sterols of biological membranes (Nasir and Besson 2012)

50
51 These different modes of action at the molecular level engender contrasted biocide activities. Iturin displays
52 the strongest antifungal activity, contrary to Surfactin which shows limited antifungal properties. Regarding
53 lipopeptides herbicidal activity, only one study revealed an effect of Surfactin against *Poa annua* (Guo *et al.*
54 2015). Of all lipopeptides, Surfactin displays the strongest insecticidal activity that can affect different
55 orders such as Homoptera (Yun *et al.* 2013; Guo *et al.* 2015; Yang *et al.* 2017), Lepidoptera (Guo *et al.*
56 2015), and Diptera (Assié *et al.* 2002; Geetha *et al.* 2012).

57
58 All these biocide activities could be exploited to develop new strategies to protect apple orchards, as the
59 cultivated apple *Malus domestica* is one of the most treated fruit crops (Drogué and DeMaria 2012). In
60 Europe, apple orchards are targeted by many pests and diseases. The main threat of apple orchards are
61 pathogens, mainly fungi, such as powdery mildew *Podosphaera leucotricha* and apple scab *Venturia*
62 *inaequalis*, the latter being the most damaging. Fire blight is caused by a bacterium, *Erwinia amylovora*.
63 Lipopeptides have already been shown to be efficient against apple scab (Desmyttere *et al.* 2019). Moreover,
64 *Bacillus* strains producing lipopeptides have shown an ability to fight against fire blight (Mora *et al.* 2015).

65 However, their broad activity spectrum makes them ideal to try and control both orchard pathogens and
66 pests.

67
68 Numerous insect pests also target apple trees. Some induce fruit damage, such as the apple codling moth
69 *Cydia pomonella* or the brown marmorated stink bug *Halyomorpha halys*. Others impair fruit production by
70 damaging the flowers, such as the apple sawfly *Hoplocampa testudinea*, or by ingesting phloem sap such as
71 the aphids *Aphis pomi* and *Dysaphis plantaginea*. Finally, the wooly aphid *Eriosoma lanigerum* damages the
72 trunk of apple trees. However, so far, the effects of lipopeptides remain unknown on apple pests.

73
74 The purpose of the current work was to evaluate the aphicidal activity of lipopeptides on the rosy apple
75 aphid (RAA) *D. plantaginea*, the major aphid pest of the cultivated apple *M. domestica* in Europe, Maghreb,
76 and North America (Qubbaj *et al.* 2005). This aphid species develops at the apex of branches and/or on the
77 most recently developed leaves, where it feeds on sap drawn from the phloem. RAA saliva secretion in
78 phloem causes leaf-rolling and impairs shoots growth, provoking great yield loss. For the present work, the
79 effects of different lipopeptides were tested at three different concentrations and compared on *D.*
80 *plantaginea*: Plipastatin (Fengycin), Mycosubtilin (Iturin) and Surfactin (Surfactin), separately or in a
81 ternary mixture as they may display synergic effects (Deravel *et al.* 2014). Among the three main routes of
82 exposure (inhalation, ingestion, contact), topical contact was selected for our bioassays as the most plausible
83 in a context of field spraying. The efficiency of a bio-pesticide should also take into account its sublethal
84 effects (Franca *et al.* 2017), defined as modifications of the behavior or fitness of individuals who survived
85 exposure to a toxic compound (Haynes 1988). The sublethal effects of lipopeptides were investigated at a
86 defined concentration on *D. plantaginea* locomotor behavior using a target arena, and feeding behavior via
87 the electropetrography (EPG) technique.

88 89 **2. Materials and methods**

90 **2.1. Insects and plants**

91
92 A single colony of *D. plantaginea* (Hemiptera: Aphididae) was used. Aphids were sampled as a population
93 in spring 2018 from an apple tree at the Agrocampus Ouest orchard that had never been treated by
94 pesticides, and kindly provided by INRAE (Angers, France) (Philippe Robert, personal communication).
95 The aphid population was mass reared without differentiating individual aphid clones on *M. domestica* cv.
96 “Jonagold” plants obtained by *in vitro* multiplication (Druart 1997). Pots containing three host plants (90 ×
97 90 × 70 mm) were placed in a Plexiglas cube (50 cm). Mass rearing and all the experiments were performed
98 in growth chambers under 20±1°C, 60±5% RH, and a 16:8 L:D cycle.

100 Synchronized first instar nymphs were obtained by placing parthenogenetic adult females on plants for 24
101 hours, after which all the females were removed. The larvipaused nymphs were reared on the plants inside
102 Plexiglas aerated boxes (36 x 24 x 14 cm) for ten days, then used as young RAA adults for the experiments.

104 **2.2 Lipopeptides production and purification**

106 Lipopeptides were produced in shacked flasks by different strains of *B. subtilis* using modified Landy media
107 according to Coutte *et al.* (2010a) for Plipastatin and Surfactin, and according to Béchet *et al.* (2013) for
108 Mycosubtilin. Briefly, Surfactin were produced by the *B. subtilis* BBG131 strain (Coutte *et al.* 2010a),
109 Plipastatin by the *B. subtilis* BS2504 strain (Ongena *et al.* 2007) and Mycosubtilin by the *B. subtilis*
110 BBG125 strain (Béchet *et al.* 2013). After the production step, the lipopeptides were purified and extracted
111 using ultrafiltration process with four steps of sequential diafiltration according to Coutte *et al.* (2010b). At
112 the end of the process, lipopeptides were freeze dried. Purity of the lipopeptides powders were evaluated
113 using UPLC method as described just below. Isoforms composition of the different lipopeptides produced
114 were already analysed by mass spectrometry and published (Mejri *et al.* 2017). Surfactin isoforms are
115 composed with different fatty acid chain length from C₁₂ to C₁₆ carbons, Mycosubtilin with fatty acid chain
116 of C₁₅ to C₁₈ and Plipastatin with saturated and unsaturated fatty acid chain of C₁₄ to C₁₈.

118 Lipopeptides solutions were prepared by solubilizing powder at 20 °C in a 5 % dimethylsulfoxide (DMSO)
119 and 0.2 % Heliosol (ActionPin, France) stirred solution at the concentrations of 1, 2.5 and 5 g/L. The
120 concentrations were chosen according to Yun *et al.* (2013). The control solution was made from ultrapure
121 water also in 5 % DMSO and 0.2 % Heliosol stirred solution. Addition of Heliosol is justified by the fact
122 that it is an adjuvant very often used for the treatment of different crops and especially for apple trees. The
123 lipopeptides powders and the lipopeptides solutions (in 5 % DMSO and 0.2 % Heliosol) were analyzed by
124 reverse phase high-performance liquid chromatography using a C18 column on Acquity UPLC system
125 (Waters, Milford, MA, USA) according to Guez *et al.* (2021) for Surfactin and Mycosubtilin. The same
126 equipment was used for Plipastatin powder and solutions analysis, except that the elution was performed
127 using a gradient of acetonitrile/water/trifluoroacetic acid from 45/55/0.1 to 55/45/0.1 (v/v/v) for 30 min.
128 Purified lipopeptides (Surfactin, Fengycin and Iturin A) supplied by Sigma Aldrich (Saint Louis, USA) were
129 used as standards.

130 The three lipopeptides (Plipastatin, Mycosubtilin, Surfactin) were tested alone (P, M, S, respectively) and in
131 a ternary mixture (PMS, 33% v/v of each lipopeptide solution).

133 **2.3. Aphid exposure to lipopeptides**

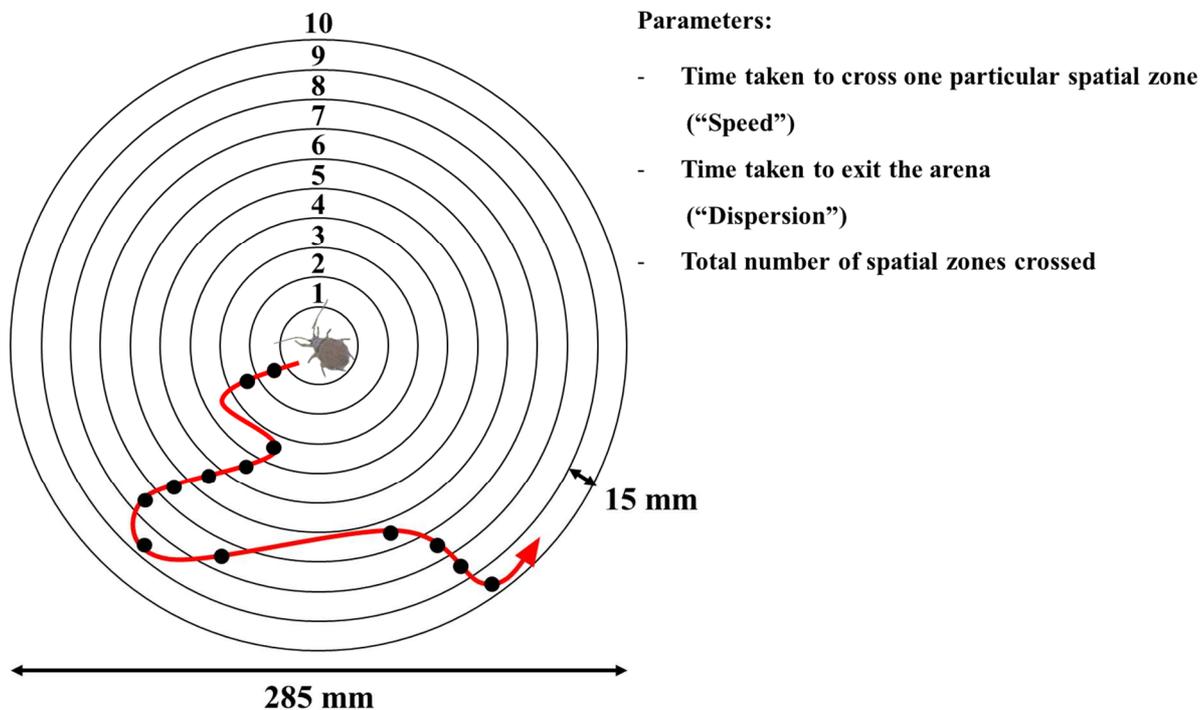
135 Aphicidal effects are classically evaluated at 24h after topical exposure (Yun *et al.* 2013; Yang *et al.* 2017),
136 but can also be immediate (Najar-Rodriguez *et al.* 2007). Therefore, the aphicidal activity of lipopeptides
137 topically applied on aphids was evaluated at two different time scales, 1 hour and 24 hours. Using an
138 Eppendorf micropipette (0.1–2.5 μ l), 0.2 μ l of lipopeptides solution or control solution was applied topically
139 onto the abdomen of each young aphid adult. After topical application, that was performed in the middle of
140 photophase, aphids were left for one hour in the Petri dish in which they had been treated, in order to allow
141 for the solution to penetrate their cuticle. They were then transferred on an artificial diet (Febvay *et al.* 1988;
142 Down *et al.* 1996) for 24 hours before being used for the behavioral and physiological bioassays. All the
143 experiments were conducted at $20 \pm 1^\circ\text{C}$, $60 \pm 5\%$ relative humidity.

144 145 **2.4. Aphid survival and mortality**

146
147 One purpose of this experiment was to evaluate the effect of lipopeptides on aphids mortality at two
148 different time scales. For each lipopeptide and the ternary mix, three different concentrations were tested (1,
149 2.5 and 5 g/L) and applied onto aphids by topical contact as described above. One hour after topical
150 application (*i.e.* before transferring aphids onto an artificial diet), the mortality at 1 h (here defined as the
151 mortality occurring within one hour following the exposure) was first assessed as the number of dead aphids
152 recorded at 1h after exposure. At twenty-four hours after topical application, the mortality was calculated as
153 the total number of aphids recorded dead both at 1h and 24h after exposure. Based on the results for
154 aphicidal activities, the concentration of 2.5 g/L was selected to study the sublethal effect of aphids exposure
155 to lipopeptides on their locomotor and feeding behavior.

156 157 **2.5. Aphid locomotor activity**

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Figure 1. Bioassay set-up used, adapted from Chesnais *et al.* (2020), to record dispersion behavior and locomotor activity of aphids in a paper arena covered with a squared glass plate. Red line: example of an aphid path observed on the arena in 300 sec; black dots: number of spatial zones in which the aphid had entered. The “speed” parameter was defined as the average duration of the time (in sec.) taken to cross one particular spatial zone. The time taken to exit the arena (in sec.) was considered as a “dispersion” parameter.

To study the sublethal effect of exposure to lipopeptides (2.5 g/L) on the locomotor behavior of *D. plantaginea* young adults, dispersion behavior and speed of aphids exposed to the control or lipopeptides solutions were monitored on a target arena according to the methodology of Chesnais *et al.* (2020) (Figure 1). Each aphid was individually deposited in the center of the test arena (285 mm diameter) consisting of 10 concentric circles (“spatial zones”) spaced 15 mm apart and covered by a transparent glass plate that was cleaned every five recordings with ethanol then water. The arena was placed between four white foam cardboard “walls” (45 cm high) to avoid external stimuli. For each aphid, the time taken (in sec.) to cross a given spatial zone for a maximum of 300 sec (“speed” parameter), the time taken (in sec.) to exit the arena (“dispersion” parameter) and the total number of spatial zones crossed were determined. The test was completed (i) if the aphid crossed the 10th spatial zone and left the arena, or (ii) at the end of the 300 sec. Results were analyzed for the responding adults ($n_C=37$; $n_P=32$; $n_M=33$; $n_S=27$; $n_{PMS}=38$).

2.6. Aphid feeding behavior

The aim of this experiment was to study the sublethal effect of aphid adults exposure to lipopeptides (2.5 g/L) on their feeding behavior. Electrical penetration graphs (EPG) (Tjallingii 1985) were obtained by connecting individual aphids placed on the abaxial side of a leaf of an individual plant inside a Faraday cage

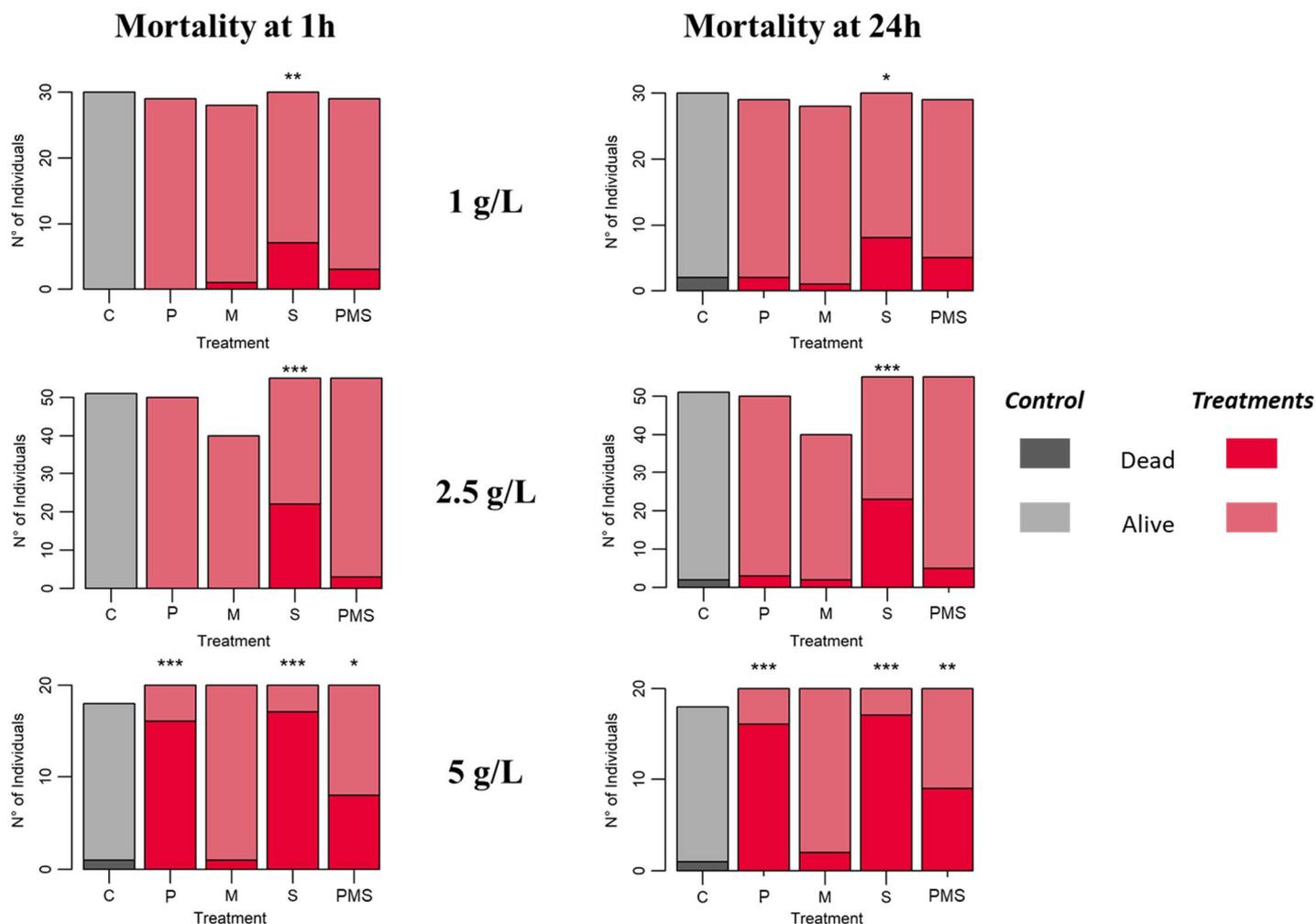
183 to the Giga-8 DC-EPG amplifier. The recordings were carried out continuously for eight hours during the
184 photophase. Acquisition and analysis of the EPG waveforms were performed using the EPG Stylet+ daq
185 software (EPG Systems, www.epgsystems.eu). Parameters from the recorded waveforms were calculated
186 using the EPG-Calc 6.1.7 software (Giordanengo 2014). They were based on different EPG waveforms
187 (Tjallingii and Esch 1993) corresponding to total stylet probing activity within plant tissues, sustained
188 phloem sap ingestion (duration > 10 minutes) and xylem sap ingestion. A total of 32 plants were used for the
189 EPG records that were obtained for the following numbers of aphids: $n_C=28$; $n_P=20$; $n_M=22$; $n_S=26$;
190 $n_{PMS}=28$.

192 **2.7. Statistical analysis**

193
194 All statistical analyses were performed using the R software version 3.6.2 (The R Foundation,
195 <https://www.r-project.org/>). For all the parameters studied, results obtained for each treatment were
196 compared with the ones obtained for the control. A Firth's penalized logistic regression with the package
197 *logistf* (Heinze *et al.* 2020) was used to evaluate the effect of topical exposure to lipopeptides on aphid
198 mortality. The impact of lipopeptides on aphid locomotor and feeding behaviors was assessed carrying out a
199 permutation test (5000 replicates) to test for the significance of the differences of the means of each
200 parameter between controls and treated aphids.

202 **3. Results**

203 **3.1. Mortality**



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206

207 Figure 2. Number of dead and alive *D. plantaginea* adults at two different time scales following topical exposure (1h
 208 and 24h) to lipopeptides at three different concentrations (1, 2.5 and 5 g/L). C: Control; P: Plipastatin; M:
 209 Mycosubtilin. S: Surfactin; PMS: Ternary mixture of Plipastatin, Mycosubtilin and Surfactin. Mortality induced by
 210 each treatments was compared with the control using the Firth's penalized logistic regression (*: $p < 0.05$; **: $p < 0.01$;
 211 ***: $p < 0.001$)

212

213 Overall, most aphids remained alive following exposure to lipopeptides at 1 and 2.5 g/L except for those
 214 exposed to Surfactin (Figure 2). Regarding the immediate mortality, Surfactin at 1 and 2.5 g/L
 215 concentrations, induced significant aphid mortalities at 1h compared to the control (Firth's penalized logistic
 216 regression, $p = 0.004$ and $p < 0.001$, respectively) of *ca.* 25% and *ca.* 40 %, respectively. At the 5 g/L
 217 concentration, compared to the control, Plipastatin, Surfactin and the PMS ternary mix induced a significant
 218 aphid mortality (Firth's penalized logistic regression, $p < 0.001$, $p < 0.001$ and $P = 0.013$, respectively).
 219 Plipastatin and Surfactin induced *ca.* 80% of aphid mortality, and *ca.* 40% of the aphid exposed to the PMS
 220 ternary mix were dead at 1h. Exposure to lipopeptides resulted in mortalities at 24h that were similar to
 221 those measured at 1h, suggesting that most of aphid mortality had occurred within the first hour following
 222 topical exposure. Because most treatments induced mortalities above 50% or more at the 5 g/L

223 concentration, this concentration was excluded to test for sublethal effects on surviving aphids. Except for
224 Surfactin (S) that were globally lethal whatever the concentration, for all the other treatments (P, M, PMS)
225 both the 1 g/L and 2.5 g/L concentrations induced mortalities in adults that were not significantly different
226 compared to controls and could therefore be considered sublethal as defined by Desneux *et al.* (2007). To
227 carry out all behavioral bioassays, we selected the greatest concentration among those two, that is 2.5 g/L.

228 **3.2. Locomotor behavior**

229

230 **Table 1: Sublethal effects of lipopeptides (2.5 g/L concentration) on the locomotor behavior of exposed *D. plantaginea* adults**

231

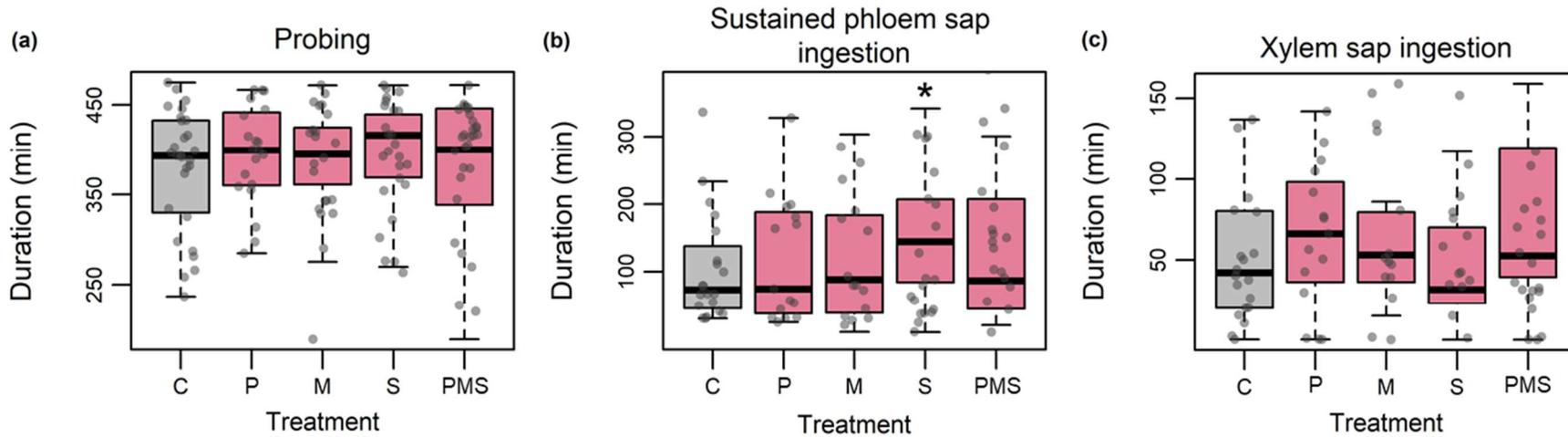
	Control	Plipastatin	Mycosubtilin	Surfactin	PMS ternary mix
<i>Number of aphids</i>	37	32	33	27	38
Speed (Time in sec. to cross one spatial zone)	10.34 ± 0.84	9.58 ± 0.64	8.33 ± 0.47 (*)	10.06 ± 0.96	11.58 ± 0.82
Dispersion (Time in sec. to exit the arena)	111.00 ± 9.76	106.29 ± 7.47	96.90 ± 7.13 (*)	116.89 ± 11.44	123.21 ± 10.44 (*)
Total number of spatial zones crossed	10.78 ± 0.34	10.59 ± 0.47	10.48 ± 0.35	10.74 ± 0.59	13.07 ± 0.69 (***)

232 Means ± sem; Locomotor items were compared two by two with the control using a permutation test (*: p<0.05 ; **: p<0.01 ; ***: p<0.001).

233

234 Compared to controls, aphids exposed to Mycosubtilin took significantly less time to cross a given spatial zone (permutation test, $p=0.046$),
 235 indicating that their speed was greater (Table 1). Also, they took significantly less time to exit the arena (permutation test, $p=0.048$), highlighting
 236 a greater dispersion compared to controls following Mycosubtilin exposure. On the contrary, aphids exposed to the PMS ternary mix took
 237 significantly more time to exit the arena compared to controls (permutation test, $p=0.004$), suggesting that the PMS ternary mix did reduce aphid
 238 dispersion. Finally, the total number of spatial zones crossed by aphids exposed to the PMS ternary mix was significantly greater compared to
 239 controls (permutation test, $p=0.022$). Plipastatin and Surfactin had no significant effect on aphid locomotion.

240 **3.3. Feeding behavior**



241

242 Figure 3. Feeding behavior parameters of *D. plantaginea* adults topically exposed to the control or the 2.5 g/L lipopeptides solutions. (a) Total duration of
243 probing phase in plant tissue; (b) Total duration of sustained phloem sap ingestion (> 10 minutes); (c) Total duration of xylem sap ingestion. C: Control; P:
244 Plipastatin; M: Mycosubtilin. S: Surfactin; PMS: Ternary mixture of Plipastatin, Mycosubtilin and Surfactin. The asterisks indicate a significant difference
245 between aphids exposed to control and the corresponding lipopeptides solution (Monte-Carlo permutation test on differences in means, *: $p < 0.05$).

246 Compared to the controls, the total duration of probing was not significantly different for each lipopeptide treatment and lasted on average
247 around 380 minutes (Figure 3a). The total duration of sustained phloem sap ingestion was significantly greater for aphids exposed to Surfactin
248 (160.69 ± 7.24 mins) compared to controls (103.92 ± 4.06 mins) (permutation test, $p = 0.040$) (Figure 3b). Finally, although no significant
249 difference could be observed, aphids exposed to Plipastatin, Mycosubtilin and the ternary mixture tended to ingest xylem sap for a greater total
250 duration compared to controls (Figure 3c).

251 4. Discussion

252

253 Our study confirmed that Surfactin is the best insecticidal candidate compared to other
254 lipopeptide families, as they induced mortality in aphids even at the lowest concentration
255 tested. Surfactin is the lipopeptide in which insecticidal effects have been the most researched.
256 They are lethal to Diptera and Lepidoptera by ingestion (Assié *et al.* 2002; Guo *et al.* 2015),
257 and to Diptera and Hemiptera by topical contact (Geetha *et al.* 2012; Yun *et al.* 2013; Yang *et*
258 *al.* 2017). Our study also revealed that at the highest concentration tested (5 g/L) Plipastatin
259 induced *ca.* 70% mortality in the topically exposed aphids. The insecticidal effect of Fengycin
260 was previously described on a Lepidopteran pest, via ingestion (Kim *et al.* 2004). The lack of
261 aphicidal activity we recorded here for Mycosubtilin seems congruent with the study by Assié
262 *et al.* (2002) showing that Iturin incorporated to an artificial diet did not induce mortality for
263 *Drosophila melanogaster* (Diptera). Topical aphicidal properties of lipopeptides could be
264 explained by their capacity to interfere with lipid layers (Ongena and Jacques 2008) and
265 particularly with cuticle molecules such as phospholipids and fatty acids (Puterka *et al.* 2003;
266 Jang *et al.* 2013). This interaction between lipopeptides and cuticle molecules can induce
267 aphid cuticle dehydration, as shown in *Rhopalosiphum padi* after a topical exposure to *B.*
268 *atropheus* surfactants containing the three families of lipopeptides and mainly Surfactin (\geq
269 90%) (Rodríguez *et al.* 2018).

270

271 Studies investigating the aphicidal effects of lipopeptides vary in terms of aphid lifestage
272 used, dose applied on aphids, aphid model or mode of exposition (Yun *et al.* 2013; Yang *et al.*
273 2017; Rodríguez *et al.* 2018; López-Isasmendi *et al.* 2019). For a same dose, Surfactin-
274 induced mortality is greater in the literature than in our study. At the two highest
275 concentrations used here (2.5 g/L and 5 g/L), we applied 0.5 and 1 μ g of lipopeptides per
276 aphid, respectively. For Yun *et al.* (2013), the 0.5 and 1 μ g doses induced *ca.* 95 % and 99 %
277 of mortality in aphids, respectively, compared to *ca.* 40 % and *ca.* 85 % in our study. This
278 difference in mortalities could be due to the isomers present in the Surfactin extract used in
279 our study. Indeed, Surfactin displays various isomers that differ in their aphicidal activity, as
280 the LC₅₀ or LC₉₀ can be up to three time higher from one isomer to another (Yang *et al.*
281 2017). The difference in mortalities could also be attributable to the aphid species used.
282 Adults weigh *ca.* 560 μ g in *D. plantaginea* (Denoirjean *et al.* 2021) versus *ca.* 270 μ g in
283 *Myzus persicae* (Baudry *et al.* 2021). In their study Yun *et al.* (2013) used *M. persicae* second

284 instar nymphs that are even lighter compared to adults, which could contribute to the greater
285 mortality observed in this other aphid species compared to our study.

286

287 Finally, we showed that when applied in a ternary mixture, lipopeptides did not display
288 synergistic effects through greater insecticidal activity, contrary to what has been reported for
289 their antifungal activity. Indeed two by two synergy between lipopeptides has been described
290 for numerous fungal pathogens, mostly between Surfactin and Iturin/Mycosubtilin (Deravel *et*
291 *al.* 2014), but also between Surfactin and Fengycin/Plipastatin (Desmyttere *et al.* 2019).

292

293 Our study clearly revealed that lipopeptides treatments induced sublethal effects in surviving
294 aphids by altering adult aphid behavior at the 2.5 g/L concentration used. Concerning the
295 locomotor behavior, aphids exposed to Mycosubtilin exhibited greater speed and dispersion,
296 whereas aphids treated by the PMS ternary mixture had their dispersion reduced compared to
297 controls. Such behavioral impacts have already been credited to the neurotoxic effects of
298 pesticides or surfactants (Drewes *et al.* 1987; Bayley and Baatrup 1996) and some
299 lipopeptides (LePage *et al.* 2005; Velkov *et al.* 2018), whereas other lipopeptides have been
300 reported to exhibit protective effects on animal nervous systems (Park *et al.* 2013). In field
301 conditions, a modification of adult aphids locomotion behavior could impact their potential to
302 colonize new host plants and propagate viruses, and also modulate their probability to
303 encounter natural enemies (Irwin *et al.* 2007).

304

305 Concerning aphid feeding behavior, the duration of total stylet probing activity within plant
306 tissues was not affected by lipopeptides exposure, whatever the treatment. Only Surfactin had
307 a significant impact on aphid feeding behavior. Unexpectedly, at the concentration inducing
308 *ca.* 50% of mortality in Surfactin-treated aphids, phloem sap ingestion revealed to be greater
309 for treated aphids compared to controls. An increased food intake following exposure to
310 pesticides or toxic compounds has been similarly described for rotifers (Liu *et al.* 2017),
311 daphnies (Liu *et al.* 2018, 2019; Li *et al.* 2020) and tadpoles (Semlitsch *et al.* 1995). This
312 phenomenon could be explained by a mechanism of temporary overcompensation for
313 inhibitory challenges, in which excess in physiological processes provides compensation after
314 damaging stress (Calabrese, 2001; Xie *et al.*, 2012). After a potential cuticle dehydration
315 suggested to occur by Rodríguez *et al.* (2018) as a consequence of lipopeptides treatment, we
316 expected that xylem sap ingestion should be greater for treated aphids to overcome hydric
317 stress. Despite a tendency to ingest xylem sap for a longer time, especially for aphids treated

318 by Plipastatin, no significant difference was observed on treated aphids compared to controls,
319 which invalidates our hypothesis.

320

321 On an agronomical point of view, this study confirms the potential of lipopeptides to control
322 aphid populations in the field regarding the insecticidal effects displayed by Surfactin, but
323 also in view of the sublethal behavioral changes recorded in lipopeptides-exposed *D.*
324 *plantaginea* aphids. As natural substances of microbial origin, lipopeptides represent
325 promising biocontrol agents, especially to regulate pathogens in orchards, where lipopeptides
326 mixtures have been shown as efficient as a conventional fungicide to control apple scab
327 (Desmyttere *et al.* 2019). Conventional fungicides may have non-intentional effects that are
328 beneficial regarding crop protection, as they can negatively impact numerous crop insects
329 pests (Chalfant *et al.* 1977; Ledieu and Helyer 1983; Arakawa *et al.* 2008). Because
330 lipopeptides appear like broad-spectrum pesticides, our study suggests they could control the
331 rosy apple aphid *D. plantaginea*, by inducing mortality through the use of Surfactin, and/or by
332 affecting its behavior through the same lipopeptides treatments that would be used to protect
333 apple trees against fungi pathogens. Thanks to their wide insecticidal activity spectrum,
334 Surfactin are known to control a myriad of insect taxa. We confirmed here that Surfactin
335 would be the best aphicidal candidate among lipopeptides. Known for their aphicidal effects
336 against the highly polyphagous aphid *M. persicae* (Yun *et al.* 2013; Yang *et al.* 2017), they
337 may also be responsible of the aphicidal activity of *Bacillus* biosurfactants against the cereal
338 specialist *R. padi* (Rodríguez *et al.* 2018). Our work reveals the applicability of this
339 lipopeptide family to also regulate an orchard specialist aphid pest, the rosy apple aphid. In a
340 context of orchard integrated pest management, it would be interesting to further investigate
341 the insecticidal effects of Surfactin on other apple tree pests such as *C. pomonella*
342 (Lepidoptera), *H. testudinea* (Hymenoptera) and *C. capitata* (Diptera). However, non-
343 intentional effects should also be evaluated as it has been shown that fungicides could
344 negatively affect organisms delivering ecological services such as natural enemies (Sotherton
345 *et al.* 1987; Sutherland *et al.* 2010; Delpuech and Allemand 2011) or pollinators (Johnson *et*
346 *al.* 2010; Belsky and Joshi 2020).

347

348 **5. Conclusion**

349

350 The present work is the first study investigating the lethal effects of the three main *Bacillus*
351 lipopeptide families by testing them individually or in a mixture on an insect, here the rosy
352 apple aphid *D. plantaginea*. Their effects on this orchard pest were contrasted, varying from
353 no aphicidal activity for Mycosubtilin whatever the concentration tested, to an aphicidal
354 activity even at low concentration for Surfactin. Mortality occurred immediately, mainly
355 within the first hour after topical exposure. This study also explores for the first time the
356 sublethal effects of exposure to lipopeptides on insect behavior. Again, the effects recorded
357 largely depended on the lipopeptides tested. Surprisingly, the locomotor activity of the
358 surviving aphids was only affected by the least lethal treatments, *i.e.* Mycosubtilin and the
359 lipopeptide ternary mix. Concerning the feeding behavior, only the most lethal treatment, *i.e.*
360 Surfactin, had an impact by unexpectedly increasing phloem sap ingestion by aphids.

361

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363

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374

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559

How do lipopeptides affect the rosy apple aphid survival and behavior?

Aphicidal effects
(at 1, 2.5 and 5 g/L)



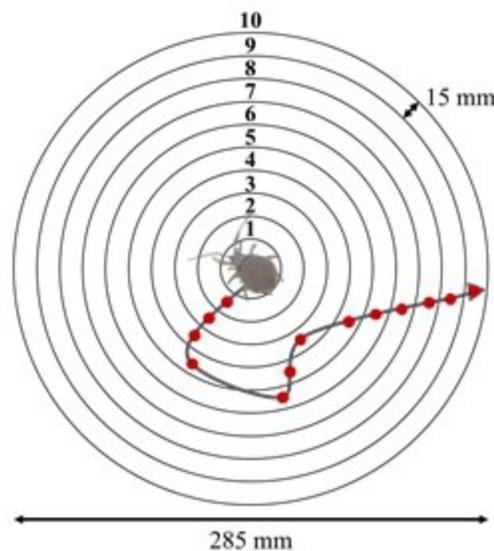
Dysaphis plantaginea

Surfactin: **at all concentrations**
Plipastatin, Ternary mix: **only at 5 g/L**
Mycosubtilin: no effect



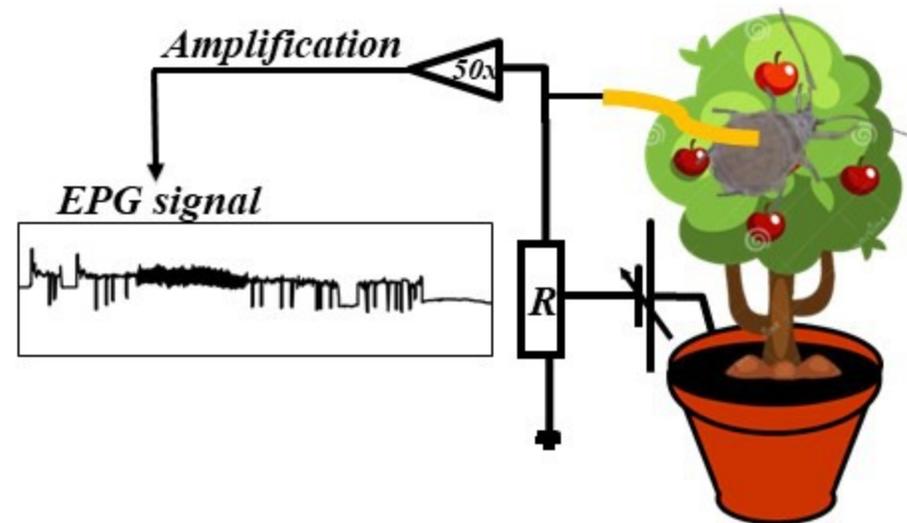
Behavioral modifications
(at 2.5 g/L)

Aphid locomotor activity
(target with 10 zones)



↗ **Dispersion** ↗ **Speed** (Mycosubtilin)
↘ **Dispersion** (Ternary mix)

Aphid feeding behavior
(electropenetrography)



↗ **Duration of phloem sap ingestion** (Surfactin)