Ecotoxicology and Environmental Safety 226 (2021) 112840

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

Effects of *Bacillus* lipopeptides on the survival and behavior of the rosy apple aphid *Dysaphis plantaginea*

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ARTICLE INFO

Edited by Dr R Pereira

Keywords:
Bacillus subtilis
Insecticidal activity
Sublethal effects
Insect behavior
Biopesticides
Electropenetrography

ABSTRACT

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

1. Introduction

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

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

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

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https://doi.org/10.1016/j.ecoenv.2021.112840
Received 19 July 2021; Received in revised form 23 September 2021; Accepted 26 September 2021
Available online 4 October 2021

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

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

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

2. Materials and methods

2.1. Insects and plants

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

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

2.2. Lipopeptides production and purification

Lipopeptides were produced in shacked flasks by different strains of *B. subtilis* using modified Landy media according to Coutte et al. (2010a) for Plipastatin and Surfactin, and according to Béchet et al. (2013) for Mycosubtilin. Briefly, Surfactin was produced by the *B. subtilis* BBG131 strain (Coutte et al., 2010a), Plipastatin by the *B. subtilis* BS2504 strain (Ongena et al., 2007) and Mycosubtilin by the *B. subtilis* BBG125 strain (Béchet et al., 2013). After the production step, the lipopeptides were purified and extracted using ultrafiltration process with four steps of sequential diafiltration according to Coutte et al. (2010b). At the end of the process, lipopeptides were freeze dried. Purity of the lipopeptides powders were evaluated using UPLC method as described just below. Isoforms composition of the different lipopeptides produced were already analyzed by mass spectrometry and published (Mejri et al., 2017). Surfactin isoforms are composed with different fatty acid chain length from C_{12} to C_{16} carbons, Mycosubtilin with fatty acid chain of C_{15} to C_{18} and Plipastatin with saturated and unsaturated fatty acid chain of C_{14} to C_{18}.

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

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

2.3. Aphid exposure to lipopeptides

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

2.4. Aphid survival and mortality

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

2.5. Aphid locomotor activity

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) (Fig. 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 s ("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 s. Results were analyzed for the responding adults (nC = 37; nP = 32; nM = 33; nS = 27; nPMS = 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 to the Giga-8 DC-EPG amplifier. The recordings were carried out continuously for eight hours during the photophase. Acquisition and analysis of the EPG waveforms were performed using the EPG Stylet+ daq softaware (EPG Systems, www.epgsystems.eu). Parameters from the recorded waveforms were calculated using the EPG-Calc 6.1.7 software (Giordanengo, 2014). They were based on different EPG waveforms (Tjallingii and Esch, 1993) corresponding to total stylet probing activity within plant tissues, sustained phloem sap ingestion (duration > 10 min) and xylem sap ingestion. A total of 32 plants were used for the EPG records that were obtained for the following numbers of aphids: nC = 28; nP = 20; nM = 22; nS = 26; nPMS = 28.

2.7. Statistical analysis

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

3. Results

3.1. Mortality

Overall, most aphids remained alive following exposure to lipopeptides at 1 and 2.5 g/L except for those exposed to Surfactin (Fig. 2). Regarding the immediate mortality, Surfactin at 1 and 2.5 g/L concentrations induced significant aphid mortalities at 1 h compared to the control (Firth's penalized logistic regression, $p = 0.004$ and $p < 0.001$, respectively) of *ca.* 25% and *ca.* 40%, respectively. At the 5 g/L concentration, compared to the control, Plipastatin, Surfactin and the PMS

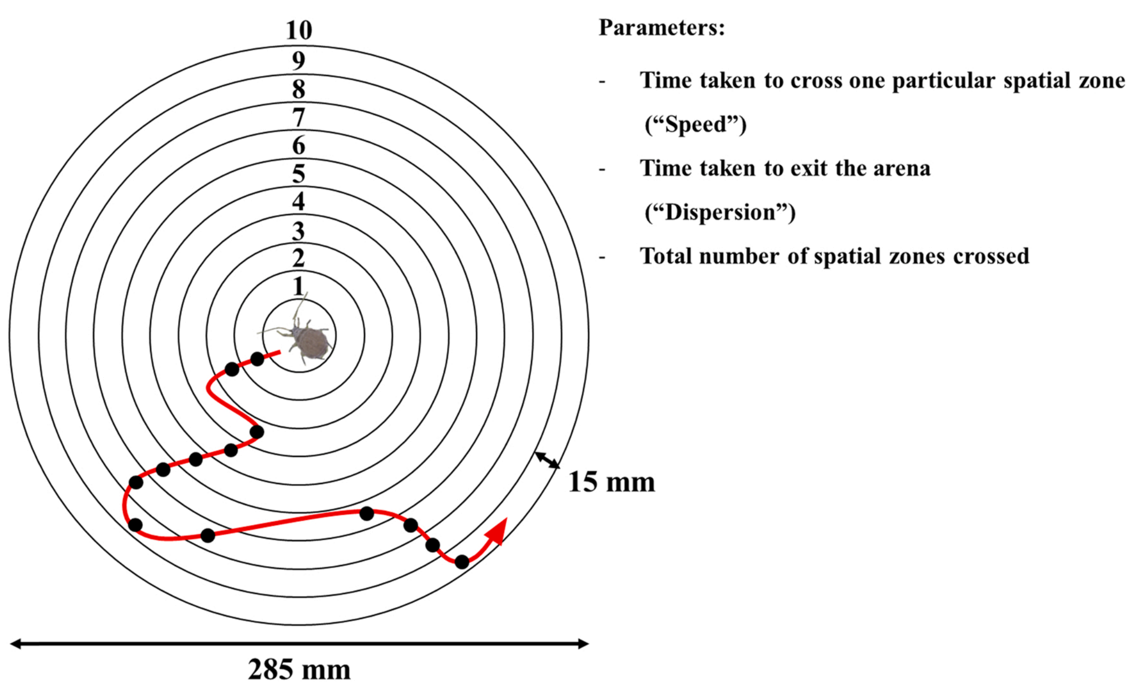

Fig. 1. Bioassay set-up used, to record dispersion behavior and locomotor activity of aphids in a paper arena covered with a squared glass plate. Red line: example of an aphid path observed on the arena in 300 s; black dots: number of spatial zones in which the aphid had entered. The "speed" parameter 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. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
adapted from Chesnais et al. (2020).

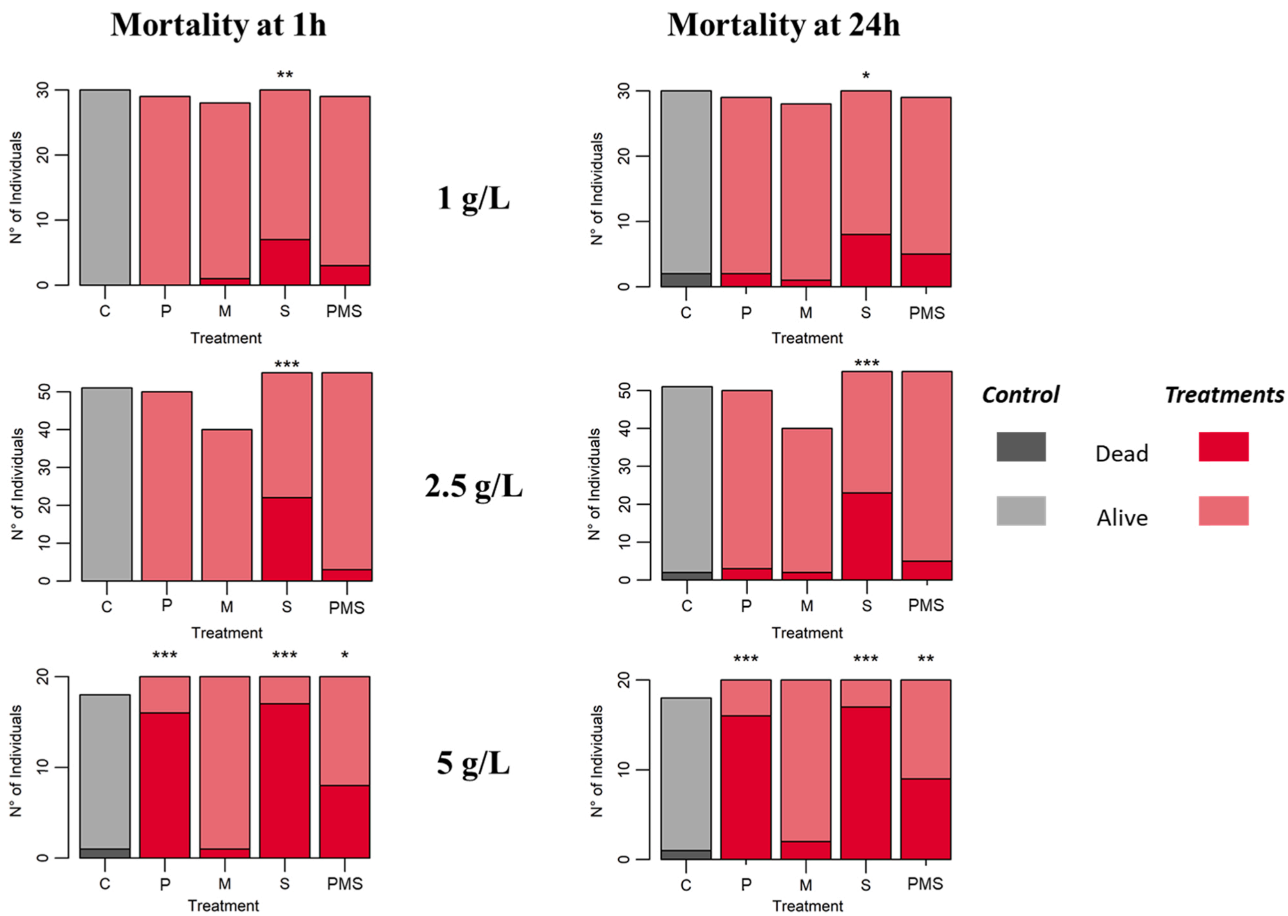

Fig. 2. Number of dead and alive *D. plantaginea* adults at two different time scales following topical exposure (1 h and 24 h) to lipopeptides at three different concentrations (1, 2.5 and 5 g/L). C: Control; P: Plipastatin; M: Mycosubtilin. S: Surfactin; PMS: Ternary mixture of Plipastatin, Mycosubtilin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

ternary mix induced a significant aphid mortality (Firth's penalized logistic regression, $p < 0.001$, $p < 0.001$ and P = 0.013, respectively). Plipastatin and Surfactin induced *ca.* 80% of aphid mortality, and *ca.* 40% of the aphid exposed to the PMS ternary mix were dead at 1 h. Exposure to lipopeptides resulted in mortalities at 24 h that were similar to those measured at 1 h, suggesting that most of aphid mortality had occurred within the first hour following topical exposure. Because most treatments induced mortalities above 50% or more at the 5 g/L concentration, this concentration was excluded to test for sublethal effects on surviving aphids. Except for Surfactin (S) that was globally lethal whatever the concentration, for all the other treatments (P, M, PMS) both the 1 g/L and 2.5 g/L concentrations induced mortalities in adults that were not significantly different compared to controls and could therefore be considered sublethal as defined by Desneux et al. (2007). To carry out all behavioral bioassays, we selected the greatest concentration among those two, that is 2.5 g/L.

3.2. Locomotor behavior

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

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

	Control	Plipastatin	Mycosubtilin	Surfactin	PMS ternary mix
Number of aphids	*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 (***)**

Means ± sem; Locomotor items were compared two by two with the control using a permutation test (*: p < 0.05; ***: p < 0.001). Italic: number of individuals; bold: significant differences between a given treatment and control.

3.3. Feeding behavior

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

4. Discussion

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

Studies investigating the aphicidal effects of lipopeptides vary in terms of aphid lifestage used, dose applied on aphids, aphid species model or mode of exposition (Yun et al., 2013; Yang et al., 2017; Rodríguez et al., 2018; López-Isasmendi et al., 2019). For a same dose, Surfactin-induced mortality is greater in the literature than in our study. At the two highest concentrations used here (2.5 g/L and 5 g/L), we applied 0.5 and 1 µg of lipopeptides per aphid, respectively. For Yun et al. (2013), the 0.5 and 1 µg doses induced *ca.* 95% and 99% of mortality in aphids, respectively, compared to *ca.* 40% and *ca.* 85% in our study. This difference in mortalities could be due to the isomers present in the Surfactin extract used in our study. Indeed, Surfactin displays various isomers that differ in their aphicidal activity, as the LC_{50} or LC_{90} can be up to three time higher from one isomer to another (Yang et al., 2017). The difference in mortalities could also be attributable to the aphid species used. Adults weigh *ca.* 560 µg in *D. plantaginea* (Denoirjean et al., 2021) ***versus*** *ca.* 270 µg in *Myzus persicae* (Baudry et al., 2021). In their study Yun et al. (2013) used *M. persicae* second instar nymphs that are even lighter compared to adults, which could contribute to the greater mortality observed in this other aphid species compared to our study.

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

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

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

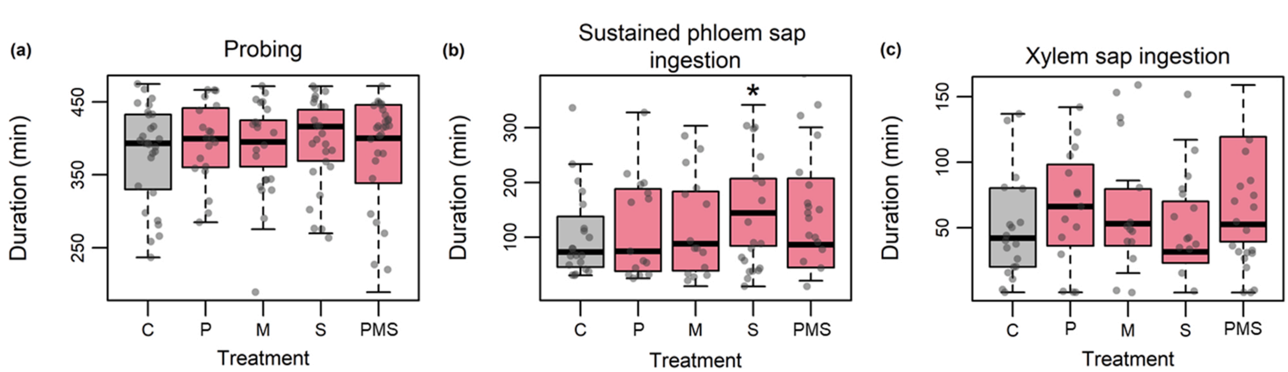

Fig. 3. Feeding behavior parameters of *D. plantaginea* adults topically exposed to the control or the 2.5 g/L lipopeptides solutions. (a) Total duration of probing phase in plant tissue; (b) Total duration of sustained phloem sap ingestion (> 10 min); (c) Total duration of xylem sap ingestion. C: Control;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

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

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

5. Conclusion

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

CRediT authorship contribution statement

Thomas Denoirjean: Conceptualization, Methodology, Formal analysis, Investigation, Writing – original draft; **Géraldine Doury:** Conceptualization, Methodology, Writing – original draft, Supervision; **Pedro Poli:** Formal analysis, Writing – original draft; **François Coutte:** Conceptualization, Investigation, Resources, Writing – original draft, Funding acquisition; **Arnaud Ameline:** Conceptualization, Methodology, Writing – original draft, Funding acquisition, Supervision.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: François Coutte from the University of Lille is also the co-funder of Lipofabrik company which markets mycosubtilin and other lipopeptides from *B. subtilis*.

Acknowledgments

This work was supported by the project PROVERBIO (Protection of orchards by biological control: an adapted selection of auxiliaries) financed by the European Union, under the scope of the FEDER program and INTERREG initiative: Programme 2014 - 2020 INTERREG V-A Belgium - France (France - Wallonie - Vlaanderen). This work was also supported by the funding from the AgriBioPOM research program and from the ALIBIOTECH program funding administered by the Hauts-de-France Region. We thank Philippe Robert (INRAE Agrocampus Ouest, Angers, France) for providing the population of *Dysaphis plantaginea*. Françoise Dubois is thanked for her technical assistance. We thank Marie-Pierre Boley for her help with the administrative tasks. Michel Yvon is thanked for the locomotor behavior set-up, and Andrew Roots for the critical reading of the English.

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