This is a pre-print version of:

Aznar-Fernández T, Cimmino A, Masi M, Rubiales D & Evidente A, 2018.

Antifeedant activity of long-chain alcohols, and fungal and plant metabolites against pea aphid (*Acyrtosiphon pisum*) as potential biocontrol strategy.

Printed version can be visited at:

Natural Product Research 33(17): 2471-2479,

DOI: 10.1080/14786419.2018.1452013.

Antifeedant activity of long-chain alcohols, and fungal and plant metabolites against pea aphid (*Acyrtosiphon pisum*) as potential biocontrol strategy

Thaïs Aznar-Fernández^{a*}, Alessio Cimmino^b, Marco Masi^b, Diego Rubiales^a, Antonio Evidente^{b*}

^aInstitute for Sustainable Agriculture, CSIC, Av. Menéndez Pidal s/n, 14004, Cordoba, Spain; taznar@ias.csic.es (T.A.), diego.rubiales@ias.csic.es (D.R.)

^bDipartimento Dipartimento di Scienze Chimiche, Università di Napoli Federico II, Complesso Universitario Monte S. Angelo, Via Cintia 4, 80126 Napoli, Italy; alessio.cimmino@unina.it (A.C), marco.masi@unina.it (M.M.), evidente@unina.it (A.E.)

CONTACT T. Aznar-Fernández ✉ taznar@ias.csic.es; A. Evidente ✉ evidente@unina.it

ABSTRACT

Aphids are noxious insect pests of major crops including cereals and legumes. Particularly, pea aphid (*Acyrtosiphon pisum*) causes significant yield and quality losses in pea. Crop protection is largely based on noxious chemical pesticides which have prompted a renewed interest in the discovery of natural products as alternatives to synthetic insecticides. In this study different classes of natural compounds were tested in dual choice bioassays to evaluate their feeding deterrence and mortality effect on pea aphid. High feeding deterrence was produced by some of the compounds, particularly 1-hexadecanol, gliotoxin, cyclopaldic acid and seiridin. On the contrary, aphid mortality was low although significant for 1-heptadecanol, cytochalasin A, 1-nonadecanol and gliotoxin. Phytotoxicity assessment showed low or imperceptible plant damage for cytochalasin A, seiridin and 1-nonadecanol. The results obtained showed the potential of seiridin to be used as an alternative to synthetic insecticides.

KEYWORDS

Pea aphid; long chain alcohols; fungal and plant metabolites; biocontrol

1.Introduction

Pea aphid (*Acyrtosiphon pisum* Harris; Homoptera: Aphididae), from now on abbreviated as PA, is a sucking phloem insect pest that can cause significant yield and quality losses in pea (*Pisum sativum* L.) crop. It has a broad host range, infecting also other legume species such as faba bean, lupin, alfalfa, or lentil (Andarge and Van der Westhuizen 2004; Caillaud et al. 2002; Edward and Singh 2006; Gao et al. 2008; Schwartzberg et al. 2011)). Infestation of the host plants by aphids causes chlorotic damages, curly and wilting leaves, nutrient deficiencies and plant stunting (Goggin 2007). Furthermore, by nailing their stylus, cause an indirect and serious damage acting as viruses' vector.

The widespread and systematic use of chemicals and the use of biocontrol strategies does not provide the required control due to the complex PA life cycle (Blackman and Eastop 2014) and their easy environmental adaptation (Tares et al. 2013; Srinivasan et al. 2014) and risk of development of resistance to pesticides (Vanlerberghe-Massutti and Guillemaud 2007). Nowadays the overuse of chemicals as insecticides is being questioned, regarding the massive environmental damage, their human and animal health risks and application costs (Coats 1994; Schraeder et al. 2010).

In front this scenario, seeking for alternatives to combat this pest is prompted. Recent studies demonstrate the capacity of natural compounds to combat several plant diseases and pests showing low or absent toxicity towards non-target organisms, biodegradability, and in many cases, economical production in comparison to those compounds produced by complete chemical synthesis (Andolfi et al. 2013; Barilli et al. 2017; Prota et al. 2014; Schraeder et al. 2010; Vinale et al. 2017). Moreover the effect of natural substances over the development, survival and/or reproduction of PA has been reported (Sauvion et al. 2004; Golawska et al. 2006; Sadeghi et al. 2009), which reinforce the potential of natural compounds as effective and non-harmful way to generate new biopesticides. A number of compounds have been described as antifeedant or deterrent, turning treated plants unattractive and unpalatable for aphids; or as aphicides, causing pest mortality (Golawska et al. 2008; Golawska and Lukasik 2012; Evidente et al. 2008; Evidente, Andolfi et al. 2009; De Geyter et al. 2012).

The objective of this study was to carry out a first evaluation of the feeding repellence and toxicity to PA of several long chain alcohols (LCOH) and metabolites (derived from different organisms) chosen on the basis of their biological activity, in order to select the most interesting compounds for further analysis, with the long term goal of developing environmentally friendly PA management strategies.

2. Results and discussion

The compounds used to test their antifeedant activity against pea aphid (*A. pisum*) were long chain alcohols (Table S1), namely 1-tetradecanol, 1-pentadecanol, 1-heptadecanol, 1-nonadecanol, 1-eicosanol, 1-hexadecanol, 1-octadecanol, commercially available, while *trans*-9-octadecen-1-ol was obtained from reduction of methyl elaidate as previously reported (Ganassi et al. 2016). The fungal metabolites (Figure 1 and Table S1), as cyclopaldic acid, cytochalasins A and B, gliotoxin, 6-hydroxymellein, papyracillic acid and seiridin were purified, as previously reported, from the culture filtrates of *Seiridium cupressi* (Graniti et al. 1992), *Pyrenophora semeniperda* (Masi et al. 2014), *Neosartorya pseudofischeri* (Masi et al. 2013), *Phoma chenopodiicola* (Cimmino et al. 2013), *Ascochyta agropyrina* (Evidente et al. 2009) and *Seiridium cardinale* (Evidente et al. 1986) respectively. While the only plant metabolite tested, inuloxin A was obtained from the organic extract of *Inula viscosa* (Andolfi et al. 2013).

The results of ANOVA for number of aphids feeding for treatment, reported in Table S2, revealed a significant effect of time (T) followed by compound (CO) and concentration (C), with lower influence of CO x C and C x T interactions. Due to the high influence of T on the amount of feeding aphids, we proceeded to calculate the greatest evaluation time for aphid feeding, being significantly higher 24 hai (67.5). Therefore data of FRI and mortality were analyzed 24 hai. Dual choice assay between segments of leaves painted with control solution vs distilled water, showed no significant differences for aphid feeding preference ($P > 0.05$).

Concerning to FRI, ANOVA with CO and C as fixed factors (Table S3), revealed significant differences for CO ($P < 0.05$) (Figure 2). The most repellent compound was **3**, a LCOH, (FRI 67.6) at 1mM, followed by metabolites **16** (54.0), **11** (48.5) and **12** (47.6), the last two at 0.5 mM (Figure 2). On the contrary compounds **4** and **6**, both LCOH, were phagostimulants (-71.4 and -50.43, respectively) at the higher concentration used (1 mM) with little effect at 0.5 mM.

Regarding aphid mortality, it increased with time for all treatments being maximal at 24 hai (Figure S2). ANOVA for mortality 24 hai, with CO and C as fixed factors (Table 1), revealed significant differences for CO ($P = 0.001$) with lower influence for CO x C interaction and no significant C influence. In spite of significant differences, aphid mortality was in general low, ranging from a maximum of 4.3% for metabolite **4** to 0.6% for compound **12**, both at 1 mM. Data from FRI and mortality for compound and concentration are shown in Table 3. There was no correlation between FRI and mortality for each metabolite ($r = 0.17$; $P = 0.08$).

Concerning to the phytotoxic effect of compounds, no damage was observed in the controls showing nine of the compounds imperceptible leaf damage. ANOVA, carried out with the compounds that showed perceptible LD, showed significant differences for CO ($P = 0.001$), but not

for C neither leaf surface part (Lsp) ($P>0.05$). As Lsp showed no significant interactions with metabolites, values shown in Table 2 are the global mean of the normalized area under phytotoxic progress curve (nAUPPC) of the segments inoculated with the same metabolite for each concentration. The compounds with higher phytotoxicity were: gliotoxin (nAUPPC 261 and 223 for 0.5 and 1 mM, respectively), cyclopaldic acid (109 and 79, respectively) and NND (93 and 72, respectively), showing papyracillic acid LD only under 1mM (29).

Pea aphid control is based on chemical insecticide worldwide. In a search of alternative natural products with potential applications in future management strategies we identified several compounds belonging to different classes of natural substances, causing significant feeding deterrence and/or small levels of mortality. 1-Tetradecanol, 1-pentadecanol, 1-hexadecanol, 1-heptadecanol, 1-octadecanol, *trans*-9-octadecenol, 1-nonadecanol and 1-eicosanol (**1-8**, Table S1) are long chain alcohols, cytochalsins A and B (**9** and **15**, Figure 1 and Table S1) are [24]oxa-14 cytochalasans, 6-hydroxymellein (**10**, Figure 1 and Table S1) is a trisubstituted isocoumarin, cyclopaldic acid (**11**, Figure 1 and Table S1) is a pentasubstituted isobenzofuranone, seiridin (**12**, Figure 1 and Table S1) is a 3,4-dialkylfuran-2-one, inuloxin A (**13**, Figure 1 and Table S1) is a germacrane sesquiterpene, papyracillic acid (**14**, Figure 1 and Table S1), is a pentasubstituted 1,6-dioxaspiro[4.4]non-3-en-2-one and gliotoxin (**16**, Figure 1 and Table S1) is substituted diketopiprazine.

Cyclopaldic acid and seiridin at 0.5 mM and 1-hexadecanol and gliotoxin at 1 mM, showed high aphid feeding deterrence. Metabolites studied caused in general little aphid mortality being significantly higher for 1-hexadecanol, cytochalsin A both at 0.5 mM and 1-heptadecanol and gliotoxin at 1 mM. Some of these caused significant phytotoxicity on faba bean leaves, but cytochalsin A, seiridin, 1-nonadecanol and 1-hexadecanol were not phytotoxic, reinforcing their value as future biopesticides.

Feeding deterrence and mortality increased markedly from 4 hai, being maximal at 24 hai, indicating that activity of compounds over PA should be evaluated from 4 hai. In addition, for both traits the concentration of the compounds used was the least influential, which reinforce the activity of the compounds used.

Generally all tested compounds showed high or subtle values of deterrence feeding with positive FRI values. However, several compounds displayed negative FRI values, showing phagostimulant activity. Among them highlighted two LCOHs (**4** and **6**) under the higher concentration (1mM). This could be explained because LCOHs have been described as signal molecules in several insects groups ([Ganassi et al. 2016](#)), displaying under higher concentrations greater influence on aphids, such as for 1-hexadecanol (**3**) and 1-heptadecanol (**4**) by showing the

maximum level of feeding deterrence and feeding attraction activity at 1 mM. Same conjecture could explain the greater mortality 24 hai in the LCOHs metabolites. Among the most outstanding LCOH was 1-hexadecanol, which showed at 1 mM a great combination of high FRI and mortality, but performed a moderate sAUPPC value on leaf damage (LD), which discourages its use for biocontrol under these concentrations in faba. On the other hand 1-heptadecanol (**4**) showed at 1 mM the lowest FRI (indicative of highest feeding attraction) and the highest mortality along all the tested compounds (4.3%) and negligible LD. Nevertheless even when some compounds caused significantly higher aphid mortality of PA, actual values are low to have a biological role in control unless the compound also caused high FRI. For this reason 1-nonadecanol (**7**), inducing the second highest mortality (3.6%), but with lower FRI and intermediate LD, has not been considered.

Four fungal metabolites were identified having deterrence activity. These were gliotoxin (**16**), cytochalasin A (**9**), cyclopaldic acid (**11**) and seiridin (**12**). Gliotoxin is a mycotoxin with several biological activities such as antiviral, immunomodulatory (Tuch et al. 1988), antifungal (Coleman et al. 2011) and its ability to deter *Aedes aegypti* mosquito bite (Masi et al. 2017). Gliotoxin showed the highest feeding deterrence and mortality although was also the most phytotoxic, limiting its value as bio pesticide. Cytochalasin A (cytos = cell, chalasis = relaxation) is a mold metabolite, which was also recently re-isolated from the solid culture of the seed pathogen *Pyrenophora semeniperda* (Masi et al. 2014). It has been described as a disruptor of the actin cytoskeleton (Kuo and Lampen 1975), inhibitor of spore germination and hyphal tip growth on some fungus and inhibitor of sugar uptake and growth on some yeasts, as well as recently described as biting deterrent to *Aedes aegypti* (Masi et al. 2017). Cytochalasin A showed little deterrence although was the one showing highest mortality in spite of little phytotoxicity. Still, as although significant, the levels of mortality were too low as for 1-heptadecanol to justify its use in PA control. In contrast, cyclopaldic acid and seiridin showed high deterrence. Both of them have been described as deterrents against the mosquito *Aedes aegypti* (Cimmino et al. 2013) while cyclopladic acid has been described as inhibitor of *Uromyces pisi* and *Puccinia triticina* growth (Barilli et al. 2017). Cyclopaldic acid was phytotoxic on faba bean, but seiridin was not. For this reason seiridin is a better option to conduct a biocontrol strategy. Seiridin originated from a micro-fungus that causes a lethal canker disease on cypress and other related conifers and it is known also for its low antibacterial activity (Sparapano and Evidente 1995).

Further bioassays are needed to clarify the effectiveness of this metabolites as bio pesticide under field conditions, as the dose of compound received in this assays is much higher than would be expected from a sprayed surface and to discern if they are specific, or could also damage mammals or other insects, in order to produce healthy commercial bio pesticides. It would be also

desirable to carry out EPG studies (Goławska 2007) to obtain more in-depth information. In addition obtain natural phytotoxic products is advantageous as having great variety of carbon skeleton and different functionalities are able to overcome the resistance that could be developed against synthetic pesticides.

In addition some fungal and plant metabolites are known as phytotoxins but it is important to take into account that many toxins are not selective, being able to cause different toxic effects both on host and non host plants (Evidente et al. 2011; Cimmino et al. 2015) as seen in our results (Table 2). For this reason, the phytotoxicity was again evaluated on faba bean and interestingly, we found significant feeding deterrence in seiridin, with not phytotoxicity for the crop. The identification of a natural compound potentially repellent with no phytotoxic effects is a breakthrough to control PA, for its potential application to aphid management in legume crops. The adverse effects of synthetic pesticides prompted the search for natural compounds with antifeedant and/or mortal activity on pea aphid. Seiridin (12, Figure 1 and Table S1) highlighted with significant deterrence and imperceptible leaf damage.

These results showed the potential of seiridin to be used as alternative to synthetic insecticides for the control of crop pathogens of economic importance as PA. Moreover, in this study gliotoxin, cyclopaldic acid and 1-hexadecanol compounds performed an interesting PA deterrence, which suggest them for future studies, in order to decrease or eliminate its leaf damage, and thus be able to take advantage of its powerful deterrent and mortal activity.

3. Experimental

This section is available as supplementary materials.

4. Conclusions

The antifeedant activity of some long-chain alcohols, and fungal and plant metabolites was evaluated against pea aphid (*A. pisum*) with the aim to find a new potential biocontrol strategy to avoid the use of chemical pesticides. When tested in dual choice bioassays, to evaluate their feeding deterrence and mortality effect on pea aphids, high feeding deterrence was produced by 1-hexadecanol, gliotoxin, cyclopaldic acid and seiridin while aphid mortality was significant for 1-heptadecanol, cyclochalasin A, 1-nonadecanol and gliotoxin. Furthermore, the phytotoxicity assessment showed low or imperceptible plant damaged for cyclochalasin A, seiridin and 1-nonadecanol. These results showed the potential of seiridin to be used as an alternative to synthetic insecticides. However, further studies are needed in order to evaluate its practical application.

Acknowledgements

Spanish Ministry of Economy and Competitiveness FPI program and projects AGL2014-52871-R and AGL2017-82907-R co-financed by FEDER and EU-FP7-LEGATO (grant agreement no. 613551) are acknowledged. Antonio Evidente is associated with “Istituto di Chimica Biomolecolare del CNR”, Pozzuoli, Italy.

Disclosure statement

No potential conflict of interest was reported by the authors.

ORCID

Thaïs Aznar-Fernández: 0000-0001-6108-6555

Alessio Cimmino: 0000-0002-1551-4237

Marco Masi: 0000-0003-0609-8920

Diego Rubiales: 0000-0001-9644-8616

Antonio Evidente: 0000-0001-9110-1656

References

- Andarge A, Van Der Westhuizen MC. 2004. Mechanisms of resistance of lentil *Lens culinaris* Medikus, genotypes to the pea aphid *Acyrtosiphon pisum* Harris (Hemiptera: Aphididae). *Int J Trop Insect Sci.* 24:249-254.
- Andolfi A, Zermane N, Cimmino A, Avolio F, Boari A, Vurro M, Evidente A. 2013. Inuloxins A-D, phytotoxic bi- and tri-cyclic sesquiterpene lactones produced by *Inula viscosa*: potential for broomrapes and field dodder management. *Phytochemistry* 86:112-120.
- Barilli E, Cimmino A, Masi M, Evidente M, Rubiales D, Evidente A. 2017. Inhibition of early development stages of rust fungi by the two fungal metabolites cyclopaldic acid and epi-epoformin. *Pest Manag Sci.* 73:1161-1168.
- Blackman RL, Eastop VF. 2014. Aphids on the world's plants: An online identification and information guide. Published online at <http://www.aphidsonworldsplantsinfo>.
- Caillaud MC, Boutin M, Braendle C, Simon JC. 2002. A sex-linked locus controls wing polymorphism in males of the pea aphid, *Acyrtosiphon pisum* (Harris). *Heredity* 89:346-352.

- Cimmino A, Andolfi A, Zonno MC, Avolio F, Berestetskiy A, Vurro M, Evidente A. 2013. Chenopodolans A-C: Phytotoxic furopyrans produced by *Phoma chenopodiicola*, a fungal pathogen of *Chenopodium album*. *Phytochemistry* 96:208-213.
- Cimmino A, Andolfi A, Avolio F, Ali A, Tabanca N, Khan AK, Evidente A. 2013. Cyclopaldic acid, seiridin, and sphaeropsidin A as fungal phytotoxins, and larvicidal and biting deterrents against *Aedes aegypti* (Diptera: Culicidae): structure-activity relationships. *Chem Biodivers.* 10:1239-1251.
- Cimmino A, Masi M, Evidente M, Superchi S, Evidente A. 2015. Fungal phytotoxins with potential herbicidal activity: chemical and biological characterization. *Nat Prod Rep.* 32:1629-1653.
- Coats JR. Risks from natural versus synthetic insecticides. 1994. *Annu Rev Entomol.* 39:489-515.
- Coleman JJ, Ghosh S, Okoli I, Mylonakis E. 2011. Antifungal activity of microbial secondary metabolites. *PLoS One.* 6:e25321.
- De Geyter E, Smagghe G, Rahbé Y, Geelen D. 2012. Triterpene saponins of *Quillaja saponaria* show strong aphicidal and deterrent activity against the pea aphid *Acyrtosiphon pisum*. *Pest Manag Sci.* 68:164-169.
- Edwards OR, Singh KB. 2006. Resistance to insect pests: What do legumes have to offer? *Euphytica* 147:273-285.
- Evidente A, Andolfi A, Cimmino A. 2011. Fungal phytotoxins for control of *Cirsium arvense* and *Sonchus arvensis*. *Pest Technol.* 5:1-17 (and references therein cited).
- Evidente A, Andolfi A, Cimmino A, Ganassi S, Altomare C, Favilla M, De Cristofaro A, Vitagliano S, Sabatini MA. 2009. Bisorbicillinoids produced by the fungus *Trichoderma citrinoviride* affect feeding preference of the aphid *Schizaphis graminum*. *J Chem Ecol.* 35:533-541.
- Evidente A, Berestetskiy A, Cimmino A, Tuzi A, Superchi S, Melck D, Andolfi A. 2009. Papyracillic acid, a phytotoxic 1, 6-dioxaspiro [4, 4]nonene produced by *Ascochyta agropyrina* var. *nana*, a potential mycoherbicide for *Elytrigia repens* biocontrol. *J Agric Food Chem.* 57:11168-11173.
- Evidente A, Randazzo G, Ballio A. 1986. Structure determination of seiridin and isoseiridin, phytotoxic butenolides from culture filtrate of *Seiridium cardinale*. *J Nat Prod.* 49: 593-601.
- Evidente A, Ricciardiello G, Andolfi A, Sabatini MA, Ganassi S, Altomare C, Favilla M, Melck D. 2008. Citrantifidiene and citrantifidiol: bioactive metabolites produced by *Trichoderma citrinoviride* with potential antifeedant activity toward aphids. *J Agric Food Chem.* 56:3569-3573.

- Ganassi S, Grazioso P, De Cristofaro A, Fiorentini F, Sabatini MA, Evidente A, Altomare C. 2016. Long chain alcohols produced by *Trichoderma citrinoviride* have phagodeterrent activity against the bird cherry-oat aphid *Rhopalosiphum padi*. *Front Microbiol.* 7:297.
- Gao LL, Klingler JP, Anderson JP, Edwards OR, Singh KB. 2008. Characterization of pea aphid resistance in *Medicago truncatula*. *Plant Physiol.* 146:996-1009.
- Goggin FL. 2007. Plant-aphid interactions: molecular and ecological perspectives. *Curr Opin Plant Biol.* 10:399-408.
- Goławska S. 2007. Deterrence and toxicity of plant saponins for the pea aphid *Acyrtosiphon pisum* Harris. *J Chem Ecol.* 33:1598-1606.
- Golawska S, Leszczynski B, Wieslaw O. 2006. Effect of low and high-saponin lines of alfalfa on pea aphid. *J Insect Physiol.* 52:737-743.
- Golowska S, Lukasik I. 2012. Antifeedant activity of luteolin and genistein against the pea aphid, *Acyrtosiphon pisum*. *J Pest Sci.* 85:443-450.
- Goławska S, Łukasik I, Leszczyński B. 2008. Effect of alfalfa saponins and flavonoids on pea aphid. *Entomol Exp Appl.* 128:147-153.
- Graniti A, Sparapano L, Evidente A. 1992. Cyclopaldic acid, a major phytotoxic metabolite of *Seiridium cupressi*, the pathogen of a canker disease of cypress. *Plant Pathol.* 41:563-568.
- Kuo SC, Lampen JO. 1975. Action of cytochalasin A, a sulfhydryl-reactive agent, on sugar metabolism and membrane-bound adenosine triphosphatase of yeast. *Biochim Biophys Acta.* 389:145-153.
- Masi M, Cimmino A, Tabanca N, Becnel JJ, Bloomquist JR, Evidente A. 2017. A survey of bacterial, fungal and plant metabolites against *Aedes aegypti* (Diptera: Culicidae), the vector of yellow and dengue fevers and Zika virus. *Open Chem.* 15:156-166.
- Masi M, Meyer S, Cimmino A, Clement S, Black B, Evidente A. 2014. Pyrenophoric acids B and C, two new phytotoxic sesquiterpenoids produced by *Pyrenophora semeniperda*. *J Agric Food Chem.* 62:10304-10311.
- Prota N, Bouwmeester HJ, Jongsma MA. 2014. Comparative antifeedant activities of polygodial and pyrethrins against whiteflies (*Bemisia tabaci*) and aphids (*Myzus persicae*). *Pest Manag Sci.* 70:682-688.
- Sadeghi A, Van Damme EJ, Smagghe G. 2009. Evaluation of the susceptibility of the pea aphid, *Acyrtosiphon pisum*, to a selection of novel biorational insecticides using an artificial diet. *J Insect Sci.* 9:1-8.

- Sauvion N, Hubert C, Febvay G, Rahbé Y. 2004. Effects of jackbean lectin (ConA) on the feeding behaviour and kinetics of intoxication of the pea aphid, *Acyrtosiphon pisum*. Entomol Exp Appl. 110:31-44.
- Schrader KK, Andolfi A, Cantrell CL, Cimmino A, Duke SO, Osbrink W, Wedge DE, Evidente A. 2010. A survey of phytotoxic microbial and plant metabolites as potential natural products for pest management. Chem Biodivers. 7:2261-2280.
- Schwartzberg EG, Böröczky K, Tumlinson JH. 2011. Pea aphids, *Acyrtosiphon pisum*, suppress induced plant volatiles in broad bean, *Vicia faba*. J Chem Ecol. 37:1055.
- Sparapano L, Evidente A. 1995. Studies on structure-activity relationship of seiridins, phytotoxins produced by three species of *Seiridium*. Nat Toxins 3:166-173.
- Srinivasan DG, Abdelhady A, Stern DL. 2014. Gene expression analysis of parthenogenetic embryonic development of the pea aphid, *Acyrtosiphon pisum*, suggests that aphid parthenogenesis evolved from meiotic oogenesis. PLoS One 9:e115099.
- Tares S, Arthaud L, Amichot M, Robichon A. 2013. Environment exploration and colonization behavior of the pea aphid associated with the expression of the foraging gene. PLoS One 8:e65104.
- Tuch BE, Lissing JR, Suranyi MG. 1988. Immunomodulation of human fetal cells by the fungal metabolite gliotoxin. Immunol Cell Biol. 66:307-312.
- Vanlerberghe-Massutti F, Guillemaud T. 2007. Les résistance des pucerons aux insecticides. Biofutur 279:27-30.
- Vinale F, Nicoletti R, Lacatena F, Marra R, Sacco A, Lombardi N, d'Errico G, Diglio MC, Lorito M, Woo SL. 2017. Secondary metabolites from the endophytic fungus *Talaromyces pinophilus*. Nat Prod Res. 31:1778-1785.