COLLEGE VEGETAL
DOCTORAL ANIMAL, ALIMENT
PAYS DE LA LOIRE MER, ENVIRONNEMENT

# PhD PROPOSAL FOR THE DOCTORAL SCHOOL « Végétal, Animal, Aliment, Mer, Environnement »

## GENERAL INFORMATION

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| **Thesis title:** Involvement of auxiliary proteins in the sensitivity of *Acyrthosiphon pisum* aphids to insecticides: potential targets in crop pest control strategies based on RNA interference. |
| **Acronym of the project: ATRAp (Auxiliary proteins Targeted by RNAi to control Aphid)** |
| **Disciplinary field 1:** Cellular and molecular biology, biochemistry<br>**Disciplinary field 2:** Neurosciences |
| **Speciality:** Biochemistry, cellular and molecular biology |
| **Three keywords**: RNA interference, insects, auxiliary proteins |
| **Registration establishment:** University of Angers |
| **Research unit:** Functional Signalling of Ion Channels and Receptors (SiFCIR) laboratory |
| **Name of the thesis director HDR (Accreditation to supervise research) required:**<br>RAYMOND Valerie<br>**Email address of the thesis director:** valerie.raymond@univ-angers.fr<br>**Name of the thesis co-supervisor (if applicable):** GOVEN Delphine<br>**Email address of the thesis co-supervisor (if applicable):** delphine.goven@univ-angers.fr |
| **Contact(s) (mailing address and E-mail):**<br>Laboratoire SiFCIR, USC INRAE 1330 SFR 4207 QUASAV<br>UFR Sciences, Université d'Angers<br>2 bld Lavoisier<br>F-49045 ANGERS Cedex 01<br>valerie.raymond@univ-angers.fr |
| ☒ **Doctoral school contest**<br>☐ **Interview**<br>☐ **Other (specify):** |

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# SCIENTIFIC DESCRIPTION OF THE PhD PROJECT

## Socio-economic and scientific context: (10 lines)

There are currently more than 5,000 species of aphid in the world, around a hundred of which are crop pests. Leguminous crops represent the 2nd largest agricultural production in the world. The pea aphid *Acyrthosiphon pisum*, our study model, which colonises many legume crops, is responsible for hundreds of millions of dollars in losses every year. With so few active substances currently authorised for crop protection and with the increasing emergence of resistant insects leading to a loss of efficacy of insecticide treatments, it is necessary to move towards agro-ecological control of insect pests and to develop alternative control strategies. In insects, neuronal nicotinic receptors (nAChRs) are the targets of different families of insecticides. The nAChRs interact with a number of proteins known as auxiliary proteins. Although these auxiliary proteins have been shown to play an important role in the function of nAChRs in vertebrates, little data is available on these proteins in insects.

## Assumptions and questions (8 lines)

Studies carried out in insects suggest that auxiliary proteins could modify the sensitivity of nAChRs to different insecticides. In this project, we hypothesise that these auxiliary proteins could be a key target for the development of new control strategies against *A. pisum*. One of the strategies is based on the use of RNA interference targeting these auxiliary proteins. This strategy will be tested both on unexposed aphids and on aphids exposed to a low dose of flupyradifurone, one of the insecticides still used against this pest. By using interfering RNA targeting auxiliary proteins, we will be able to determine 1) whether these proteins are involved in aphid sensitivity to insecticides, and 2) whether these interfering RNAs can be used to control aphids effectively.

## The main steps of the thesis and scientific procedure (10-12 lines)

The project is divided into 3 tasks:

❶ Initially, the quantification of mRNA expression of auxiliary proteins will be undertaken by qPCR in *A. pisum*. The interfering RNAs (RNAi) targeting the auxiliary proteins of interest will then be designed and synthesised.

❷ The RNAi directed against these key proteins will then be tested *in vivo* in aphids and their efficacy as biocontrol agents will be assessed by measuring aphid mortality. To assess the role of auxiliary proteins in the sensitivity of insects to insecticides targeting the cholinergic system, mortality tests will be carried out on aphids treated with RNAi of interest and exposed to an insecticide.

❸ Finally, after determining the changes in the expression of auxiliary proteins induced by the exposure of aphids to a sublethal dose of flupyradifurone, the use of RNAi directed against these auxiliary proteins of interest will be undertaken, on the one hand, to link the variations in the expression of auxiliary proteins to the loss of sensitivity of aphids to flupyradifurone and, on the other hand, to restore the sensitivity of *A. pisum* to flupyradifurone and thus bypass the resistance developed by the insect to this insecticide.

## Methodological and technical approaches considered (4-6 lines)

A bioinformatic analysis will be carried out in collaboration with Dr A. Jones (Oxford, UK) to identify all the pea aphid auxiliary proteins.

Molecular biology techniques will be used to quantify the transcripts of the auxiliary proteins (RT-qPCR), to clone the genes of interest and to synthesise the RNAi (*in vitro* transcription).

Toxicological tests will be used to determine the mortality rate of aphids following the various treatments (RNAi, insecticides, RNAi + insecticides).

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| **Scientific and technical skills required by the candidate** |
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| The candidate should have skills in bioinformatics, molecular biology and toxicology. Knowledge of insect neurobiology and the mode of action of insecticides would be appreciated. |

## THESIS SUPERVISION

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| **Unit name:**<br>Laboratoire de Signalisation Fonctionnelle des Canaux Ioniques et Récepteurs (SiFCIR) | **Team name:** |
| **Unit director name:**<br>RAYMOND Valerie | **Team director name:** |
| **Mailing address of the unit director:**<br>valerie.raymond@univ-angers.fr | **Mailing address of the team director:** |

**Thesis director**

Surname, first name: RAYMOND Valerie

Position: Professor

Obtained date of the HDR (Accreditation to supervise research): 2010

Employer: University of Angers

Doctoral school affiliation: VAAME

Rate of thesis supervision in the present project (%): 50

Total rate of thesis supervision in ongoing theses (supervisions and co-supervisions) (%): 0

Number of current thesis supervisions/co-supervisions: 0

**Thesis co-supervisor 1 (if applicable)**

Surname, first name: GOVEN Delphine

Position: Associate professor

Accreditation to supervise research ☐ yes ☒ no If yes, date diploma received:

Employer: University of Angers

Doctoral school affiliation: VAAME

Rate of thesis supervision in the present project (%): 50

Total rate of thesis supervision in ongoing theses (supervisions and co-supervisions) (%): 0

Number of current thesis supervisions/co-supervisions: 0

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**Professional status of previous PhD students supervised by both director and co-supervisors (from 5 years)**

*Please provide the following information for each PhD students supervised*

Surname, first name: BANTZ Alexandre

Date of PhD beginning and PhD defence: October 2017 – December 2020

Thesis supervision: RAYMOND Valerie & GOVEN Delphine (co-supervisor)

Professional status and location: looking for a job after ATER position

Contract profile (post-doc, fixed-term, permanent): not applicable

List of publications from the thesis work:

- Bantz A, Camon J, Froger JA, Goven D, Raymond V. (2018). Exposure to sublethal doses of insecticide and their effects on insects at cellular and physiological levels. *Curr Opin Insect Sci.* 30:73-78.

- Jones AK., Goven D., Froger JA., Bantz A., Raymond V. (2021). The cys-loop ligand-gated ion channel gene superfamilies of the cockroaches *Blatella germanica* and *Periplaneta americana*. *Pest Manag. Sci.* 77 (8): 37987-3799.

- Bantz A., Goven D., Siegwart M., Maugin S, Raymond V. (2022). Exposure to a sublethal dose of imidacloprid induces cellular and physiological changes in *Periplaneta americana*: Involvement of α2 nicotinic acetylcholine subunit in imidacloprid sensitivity. *Pestic Biochem Physiol.* 181:105014.

Surname, first name: PILON Alexandre

Date of PhD beginning and PhD defence: September 2018 – July 2022

Thesis supervision: RAYMOND Valerie & GOVEN Delphine (co-supervisor)

Professional status and location: Student in medicine, University of Angers

Contract profile (post-doc, fixed-term, permanent): not applicable

List of publications from the thesis work:

Pilon A., Goven D., Raymond V. (2022) Pharmacological and molecular characterization of the A-type muscarinic acetylcholine receptor from *Anopheles gambiae*. *Insect Mol Biol.* 31(4):497-507.

Surname, first name: LIGONNIERE Sebastien

Date of PhD beginning and PhD defence: October 2020 –December 2023

Thesis supervision: RAYMOND Valerie & GOVEN Delphine (co-supervisor)

Professional status and location: Engineer, Angers

Contract profile (post-doc, fixed-term, permanent): Fixed-term

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List of publications from the thesis work:
Ligonniere S., Raymond V., Goven D. (2023) Use of double-stranded RNA targeting β2 divergent nicotinic acetylcholine receptor subunit to control pea aphid *Acyrthosiphon pisum* at larval and adult stages. *Pest Manag Sci.* 80(2):896-904.

- Ligonniere S., Bantz A., Raymond V., Goven D. (2024) Using RNA interference targeting a nicotinic acetylcholine receptor subunit to counteract insecticide accommodation mechanisms: example of the β1 subunit in the imidacloprid-accomodated american cockroach, *Periplaneta americana*. *J. Pesticide Sci.*49(1):58-64.

**Five main recent publications of the supervisors on thesis subject:**

**Patent:**

Goven D., Raymond V. (2022). Utilisation d'ARN interférents dirigés contre le système cholinergique pour lutter contre les insectes nuisibles. Numéro d'enregistrement : FR2204889.

**Publications**

- Jones AK., Goven D., Froger JA., Bantz A., Raymond V. (2021). The cys-loop ligand-gated ion channel gene superfamilies of the cockroaches *Blatella germanica* and *Periplaneta americana*. *Pest Manag. Sci.* 77 (8): 37987-3799.

- Bantz A., Goven D., Siegwart M., Maugin S, Raymond V. (2022). Exposure to a sublethal dose of imidacloprid induces cellular and physiological changes in *Periplaneta americana*: Involvement of α2 nicotinic acetylcholine subunit in imidacloprid sensitivity. *Pestic Biochem Physiol.* 181:105014.

- Ligonniere S., Raymond V., Goven D. (2023) Use of double-stranded RNA targeting β2 divergent nicotinic acetylcholine receptor subunit to control pea aphid *Acyrthosiphon pisum* at larval and adult stages. *Pest Manag Sci.* 80(2):896-904.

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## THESIS FUNDING

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| **Origin(s) of the thesis funding:** Doctoral Contract Institution |
| **Gross monthly salary:** 2100 euros |
| **Thesis funding state:** Non acquired |
| **Funding beginning date/duration of the thesis funding:** 01/10/2024 (three years) |

**Date:** 15/03/2024

**Name, signature of unit director:**

V. RAYMOND

**Name, signature of team director:**

**Name, signature of thesis project director:**

V.RAYMOND