2021 ECOPHYTO
RESEARCH & INNOVATION SYMPOSIUM

ÉCOPHYTO
REDUCE AND IMPROVE THE USE OF PHYTOS

Symposium proceedings

Synthesis report of the webinars presenting the results of the Pesticides 2014, JEVI 2016 and PSPE2 calls for projects




7 October – 16 December 2021

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Document translated from French to English by ABAQUE SAS

Contents

INTRODUCTION

The public is crying out for a reduction in the use of phytopharmaceutical products, which in all cases is an essential part of preserving public health and nature's biodiversity. The successive Ecophyto plans are the embodiment of commitments to reducing the use of phytopharmaceutical products and achieving a pesticide usage policy compatible with sustainable development.

The 2021 Ecophyto Research & Innovation Symposium took the form of a series of 9 weekly webinars presenting and discussing the results of around thirty projects that responded to the 3 calls for projects launched since 2015, concerning:

- Contribution to the progress of biocontrol within the framework of the 'For and On the Ecophyto 2 plan' call for projects (PSPE2);
- The concepts of resistance in terms of pesticides: resisting pests, combating resistance to change in order to reduce risks (Pesticides 2014);
- Development of alternative solutions to the phytopharmaceutical products used in gardens, parks and infrastructures (JEVI 2016).

Research-innovation is an essential lever in achieving these objectives and its importance has been further accented as the various plans have evolved. The "Research & Innovation" axis of the Ecophyto II+ (axis 2), guided by 4 French government ministries (in charge of Agriculture, Environment, Health and Research) with the support of the Research & Innovation Scientific Orientation Committee (CSO R&I), unites and organises the various research-innovation communities around the concept of developing and furthering knowledge and the tools that will be necessary to achieve the objectives set by the plan in terms of reducing the use of phytopharmaceutical products and the risks associated with them.

One major initiative of the Research & Innovation axis will be to launch calls for research-innovation projects and then make the best possible use of their results. The **2021 Ecophyto Research & Innovation Symposium** is therefore aimed at a number of different sectors: research-innovation communities, agricultural and non-agricultural professionals, agricultural supplies manufacturers and professionals, teachers and students, decision-makers and local politicians.

For each webinar session, the results of 2 to 5 projects are presented by their project leaders, followed by participations from one or more expert guests:

- Webinar 1: In search of natural molecules to promote plant health - 7 October
- Webinar 2: Micro-organisms as the defenders of plant health - 14 October
- Webinar 3: The integration and optimisation of biocontrol - 21 October
- Webinar 4: A world of odours - 4 November
- Webinar 5: Macro-organisms: sibling rivalry... - 18 November
- Webinar 6: Resistance as a way to protect crops - 25 November
- Webinar 7: Companion plants for healthy crops and soil - 2 December
- Webinar 8: Technical routes towards zero phyto - 9 December
- Webinar 9: Looking beyond the fields - 16 December

The content of the symposium has been published or posted via a number of different media: on-line posts of the project presentation videos via the dedicated YouTube channel, publication of the webinar brochures on the dedicated EcophytoPIC page, and the project results summaries alongside the main points raised during the webinars in this document.

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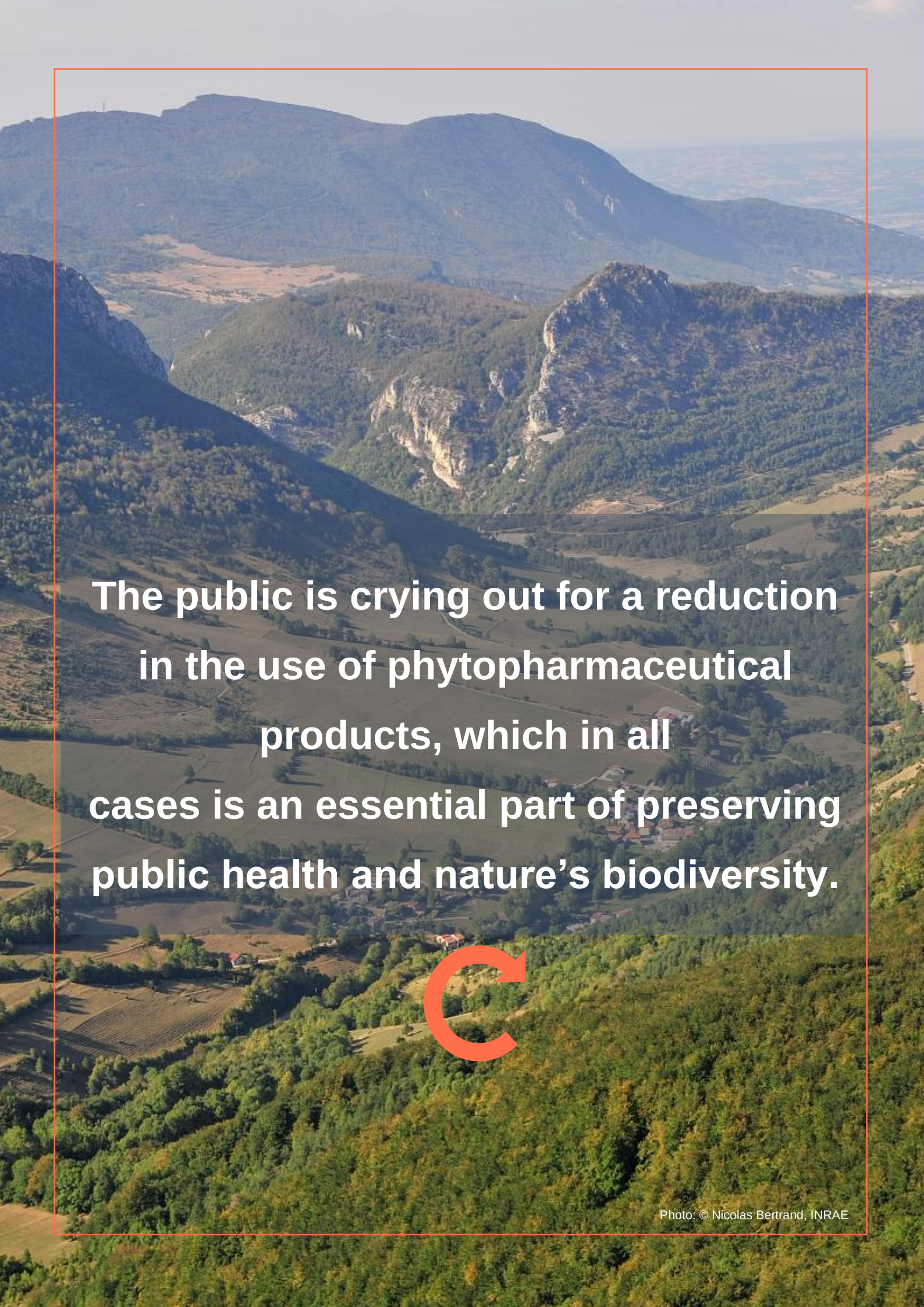


The public is crying out for a reduction in the use of phytopharmaceutical products, which in all cases is an essential part of preserving public health and nature's biodiversity.

Photo: © Nicolas Bertrand, INRAE

GENERAL SYNTHESIS OF THE WEBINARS

Thirty Ecophyto research projects shed light on the future of protecting plants

The 2021 Ecophyto Research & Innovation Symposium took the form of a series of nine webinars, running from 7 October to 16 December 2021, presenting the results of thirty projects. Success, potential, deceptions, questions... A lot has been learnt from these ambitious projects that sought to discover new ways to regulate pest & disease pressure on plants and reduce the use of phytosanitary products and the risks associated with them. They have opened new positive prospects and evoked the clear need to continue consolidating knowledge on the subject and defining ways to apply it.

Three tenders launched since 2015

The thirty projects presented during the 2021 Ecophyto Research & Innovation Symposium were all responses to the three tenders that have been launched since 2015. They concern contributions to progress in biocontrol approaches (For and On the Ecophyto II Plan, PSPE2), the concepts of resistance in terms of pesticides (Pesticides Programme) and the development of alternative solutions to phytosanitary products for gardens, parks and infrastructures (JEVI).

As part of the 2021 Ecophyto II+ Research-Innovation axis symposium, nine webinars were organised between 7 October and 16 December, involving almost 500 participants. Natural molecules, micro-organisms, pheromones, macro-organisms, agronomy practices or service plants... Thirty projects, outlining the future plant protection prospects responding to the three tenders that have been launched since 2015.

Some projects have already met with success and resulting products are actually on the market!

Using living material, such as indigenous or exotic strains of terrestrial or marine fungus, to regulate pest & disease pressure is progressing well as a way to reduce the use of phytosanitary products. A number of the Écophyto projects have demonstrated significant reductions under experimental conditions, such as *MilPomBio* which has reduced fungicide dosages by 40 to 90% by exploring the use of phosphite based solutions to treat potato blight. The *Lipocontrole* project uses lipopeptides to achieve as much as 96% disease protection for wheat or green-house tomato crops. Some experiments have managed to develop pioneering organic alternatives for use on tomato crops, as can be seen with the *Acarosol* project to treat spider-mites affecting solanaceae. Others have even registered and marketed products, as with *OptimPhero* which has developed pheromones for the biological control of the pine processionary and box tree moth.

Furthering solutions and knowledge for tomorrow

In addition to those products that have been marketed, identified or which are currently on trial, all of the projects presented are serving to consolidate knowledge for the agro-ecology of tomorrow. Living material has its own codes, and sometimes springs surprises that can cause solutions to fail completely: the *Attract my fly* project had to deal with this when working on melon fly; they found that using the males as vectors for getting spores of entomopathogenic fungus onto the females of the species in order to control egg production did not meet expectations.

Even when the effectiveness of biocontrol is proven, there is no magic wand. The *DicaBio* project stands as a clear example of this; natural molecules have shown their effectiveness in the laboratory, but the formulations generally require further work before they can achieve the desired objectives in the field. And the same can be said for the *Rhizodia* project, where releases of predator ladybirds were not sufficient to control scale insects on blackcurrant; this meant that further research was required to optimise the use of these biocontrol agents or better define the contexts in which they should be used.

The presented projects are consolidating knowledge for the agro-ecology of tomorrow.

Pheromone balls.
Photo: © Nicolas Bertrand, INRAE

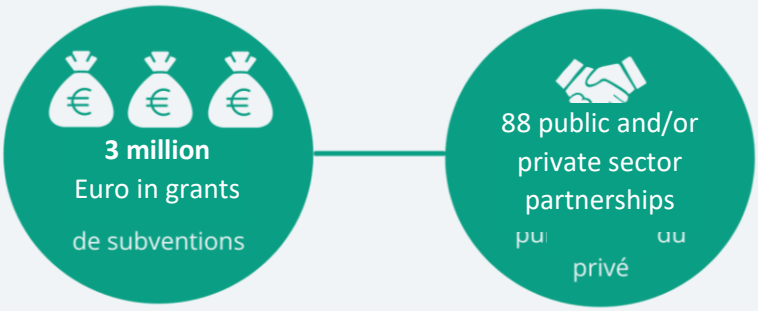
Finding an idea that works under laboratory conditions is not the end of the story, you need to take it out into the field.

Optimise and adjust agricultural practices

If the use of living material appears to be one viable alternative to phytosanitary products, instances of direct substitution, where one solution directly replaces another - like for like, remain very rare. The objective is therefore more about trying to create new solutions that can be integrated into developing general strategies, where the chemical ingredient is no longer the only response. Some products are clearly taking this approach, such as the *Biobot* project, which treats grey mould, caused by the Botrytis fungus, by combining a biocontrol approach with a preventive approach. Others highlight the benefits of service plants to both shelter and encourage the reproduction of auxiliaries, as in the *Pro-Bio-Taupin*, *Acarosol*, *Fleur* and *MacroPlus* projects, or to combat soil parasites, as with the *Serum* and *Biocou* projects.

This combined approach is gaining ground, but many cases still require experimental confirmation to prove their viability. This is another dimension which will need to be taken into consideration in research: that an idea that can be applied in the laboratory is not the end of the story. For many, the presented projects already have one foot in the real world, and often over a number of sites and a number of years to gain a broader view. For example, the *RegPuc* and *Ecoverger* projects propose a number of levers that can be integrated into technical routes to reduce fruit tree pest infestations, or the *Das-Revi* project which studies the possibility of using new disease resistant varieties of grape vines.

Some important figures about these research projects



Collective thinking

Some projects presented during the symposium also question spatial dimensions by looking at the impacts of phytosanitary products on the landscape as a whole and the levers that will need to be implemented to manage such impacts (such as the *Rescape* project). They have even taken the approach further to involve everyone concerned, such as farmers and gardeners, in a participative approach to find suitable alternative real-world solutions to meet phytopharmaceutical product use and impact reduction objectives, as is the case for the *Resyst*, *Trajectoires* and *Althercol* projects.

> *The various Ecophyto plans consolidate the commitments undertaken to respond to this obligation and achieve a sustainable degree of plant protection product use.*

Social issues, widespread distribution

The reduction of the use of phytosanitary products and the restriction of the risks associated with them is a subject that concerns the whole of society. Some projects have clearly raised the question of how acceptable biocontrol solutions really are for farmers... and also a general public that is developing an increasing curiosity of how their food is actually produced. The **2021 Ecophyto Research & Innovation Symposium** is not just aimed at the research-innovation communities but also at a wider public: agricultural and non-agricultural professionals, industrial and agro-supplies businesses, teachers and students, decision-makers and local politicians. The results will be interpreted on a number of different levels: from a purely scientific perspective, with articles published in peer reviewed academic journals, and on a broader scale through written summaries and videos on the projects published on a dedicated Youtube channel. All useful information can be found on the EcophytoPIC web-site.

The Ecophyto II+ Research-Innovation axis

The reduction of the use of phytosanitary products is a major issue for society as a whole, as is the need to improve the health of the soil and all living things. The various Ecophyto plans consolidate the commitments undertaken to respond to this obligation and achieve a sustainable degree of plant protection product use, by reducing their use and restricting the associated risks The Ecophyto II+ plan “Research & Innovation” axis will involve different research communities working together to produce and complete the knowledge and tools necessary to achieve these objectives. It is lead by the ministries of Agriculture and food, Ecology transition, Solidarity and healthcare as well as the Innovation and research in higher education; it also benefits from the expertise of the Research & Innovation Scientific orientation committee (CSO RI).

Agricultural landscape of the ZAPVS, cultivated field and bordering hedge, Rescape Project. *Photo: © Céline Pelosi, INRAE*

Spanish fly, auxiliary insect, predator. *Photo: © Nicolas Bertrand, INRAE*

Webinar 1

In search of natural molecules to promote plant health

Arabidopsis Thaliana plants in the greenhouses of the Jean-Pierre BOURGIN institute. Versailles, 30 September 2021. *Photo: © Nicolas Bertrand, INRAE*

Session programme

▶ **DICABIO**	Evaluation of Dicaffeoylquinic acid as a natural Biocontrol substance (PSPE2)	Myriam Siegwart (INRAE Avignon)
▶ **LIPOCONTROLE**	The search for new lipopeptides that could be used as biopesticides, by screening a collection of Pseudomona (PSPE2)	Philippe Jacques (University of Liège)
▶ **DESHERBAL**	Development and study of the effectiveness of allelopathic substances to improve weed control for particularly resistant species (JEVI 2016)	Claire Richard (Clermont - Ferrand Chemistry Institute)

EXPERT GUEST: Cédric Bertrand, University of Perpignan, Head of the Chemistry Department, President of the PO²N Group, President of the Biocontrol and Integrated Biological Protection Academy, "Sharka" Chair of the UPVD Foundation, Scientific Director of AkiNaO

You can see the complete recording of this session on the symposium's YouTube channel

Biocontrol, prospects and specifics for a number of natural substances

The DicaBio, Lipocontrole and Desherbal projects present the potentials and opportunities raised by natural molecules for the conception of biocontrol products... while also highlighting certain specific characteristics of these substances, which will need to be made clear well in advance before the full potential of these solutions can be realised.

What do peach-trees, the Bacillus and Pseudomonas bacteria and the Mediterranean Rock Rose (Cistus) have in common? They have all inspired projects that apply a single logic: the use of natural molecules to develop biocontrol solutions which could become viable alternatives to synthesised phytosanitary products as well as being respectful of the environment and human health. The results of these three projects, DicaBio, Lipocontrole and Desherbal respectively, have raised promising prospects for the Ecophyto 2+ Research-Innovation plan.

For all three projects, the rapidly biodegradable nature of the raw materials under consideration is worthy of some note. The active ingredients in question, diCT and diCQ acids for DicaBio, lipopeptides for Lipocontrole or terpenes for Desherbal, all bear this characteristic. Xavier Reboud, Ecophyto 2+ axis 2 coordinator, insists on this difference to a very large number of synthesised products.

Combating resistance

Another projected, but as yet unproven, advantage of these biocontrol solutions is their potentially long action durations when compared to synthesised products which often seem to facilitate increased pest resistance instead. This is especially the case for the aphids targeted by DicaBio. According to the researchers, the two identified substances, diCT and diCQ, currently present little risk of resistance compared to synthesised pesticide products. Only one of the aphid clones tested has proven to be unaffected. “A better knowledge of the origin of this phenomenon will help us to identify how these substances work, and achieve a better understanding of the risk of aphids adapting to them”, says Myriam Siegwart, researcher at INRAE Avignon.

“Synthesised chemical products are now forbidden for garden use.”

In the case of Lipocontrole, this is beginning to be taken into account and University of Liège professor, Philippe Jacques explains that he has “high hopes” that the resistance mechanisms of the fungus pathogens targeted by his project will only be able to activate “very slowly”, meaning that the solution will have a long-term action in protecting large-scale crops either in complement or as a substitute to chemical fungicides.

Need to work on the formulation

For their part, Desherbal highlights the visual and highly educative nature of certain plant interactions. For this project, terpenes produced by a number of different plant species were tested for their capacity to restrict the germination of weeds at the bases of trees and in flower beds. This has been done in partnership with the town of Aubière (Puy de Dôme) in its town hall conservation gardens and the local school courtyard. “Synthesised chemical products are now forbidden for garden use” reminds Claire Richard, researcher at the Clermont-Ferrand Chemistry Institute. “The town council is very interested by our work, and plans to continue and broaden the scope of the trials. “

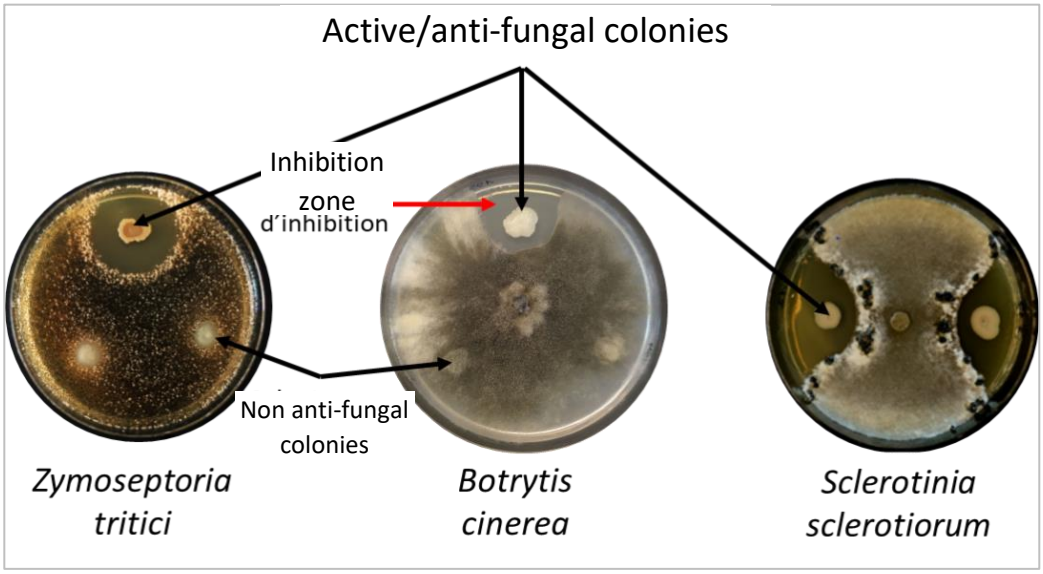
> *We need to identify the pests, weeds or pathogens that the product could be used to control... but we also need to know about the "non-target" species.*

The three scientists' experiences have also led them towards work orientations specifically related to the nature of the substances they are working on. For example, researchers need to remain objective when encouraging results are produced under laboratory conditions and concentrate on finding the best way to use the new molecules *in-situ*. The DicaBio consortium is hoping that a "micro-emulsion" approach will resolve the very low penetration factor of diCT and diCQ in the plant tissues they are being used to protect, they will need to find an industrial partner to help them achieve this. Whereas for Desherbal, the actions of the terpenes present in Leylandii cypress needles has been tested as a concentrate or a mulch, to try to achieve a "very significant" reduction in the germination and growth rates of the clover species selected as control subject. For Lipocontrole, the finalisation of a formulation able to guarantee the conservation and potency of lipopeptides remains one element that still requires more work.

A very broad field of research

This part of the research process highlights the advantage of collaborating with formulation specialists, especially phytosanitary product manufacturers. This also serves as a reminder that, in spite of the conclusions and knowledge gained, all three of these projects still require further research work. None of them are ready to be registered for commercialisation. "That's the way thing go when you are innovating from the ground up," says Xavier Reboud. "The relatively long period of time between the start of a project and its possible market launch means that one should never delay launching such a research program. "

The selectivity of the substances being studied is one of the things that remains to be evaluated before considering any potential registration for commercialisation. In other words, their operational scope: what species will be affected? This is not a minor issue. We need to identify the pests, weeds or pathogens that the product could be used to control... but we also need to know about the "non-target" species, what you could call the potential collateral damage. DicaBio has made a start by establishing that diCQ is not toxic to bees. At the same time, the project leaders are planning a deployment on other targets beyond aphids, including certain fungal diseases affecting wheat, using diCT in particular. As for Lipocontrole, the performances measured under greenhouse conditions concern wheat and tomato crops, and have lead to disease protection levels of up to 96 %. However the planned applications are broader, the project hopes to extend to cover septoria leaf blotch on wheat, sclerotinia stem rot on rapeseed and grey mould on vines and tomatoes. The toxicological risks to other organisms are currently being evaluated by another project, Ceres (2020-23). And finally, Desherbal warns that it still remains to be proven as to whether terpenes from Leylandii Cypress needle mulch are totally harmless to soil-life. Other terpene rich plants are still to be identified and tested.



Laboratory tests on the anti-fungal activity of P. syringae bacteria. Anti-fungal bacteria inhibiting fungal growth (inhibition zone). *The* LIPOCONTROLE *project.*
Photo: © Alexandre Bricout, University of Lille

▶ **The views of Cédric Bertrand,** professor at the University of Perpignan

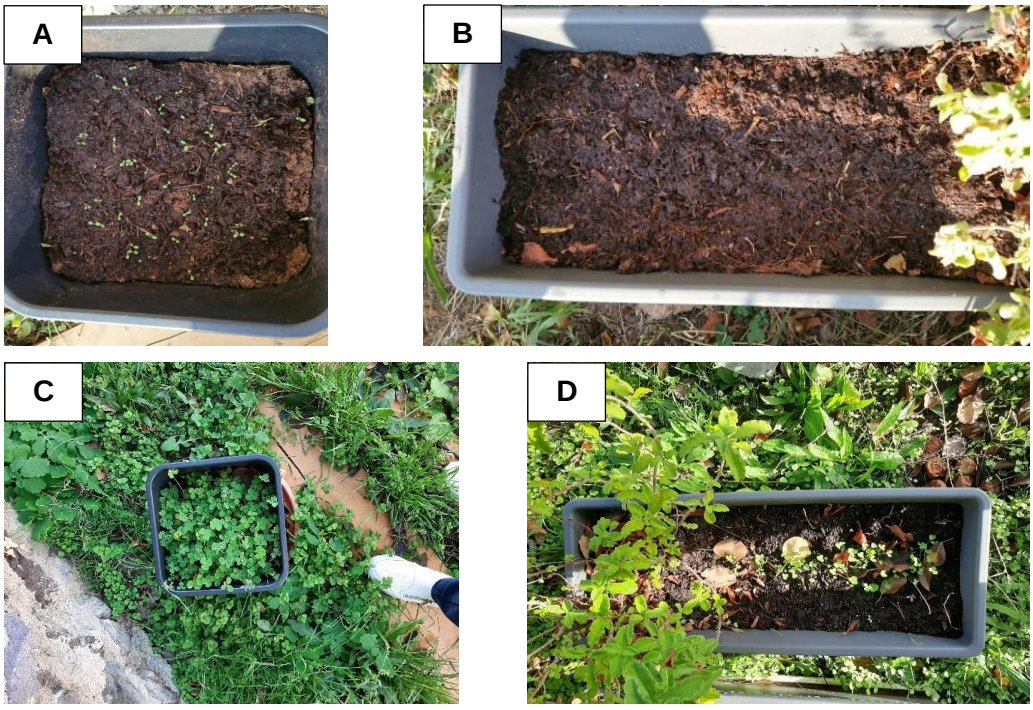
"Sourcing the raw materials is a question which must not be overlooked!"

The guest expert for this webinar, Cédric Bertrand, confirms the trends raised by the various accounts. "Remember that it all comes from observing nature! The bio-active ingredients we are talking about here are chemical mediators which allow the plant to interact with the surrounding micro and macro-organisms. They use a number of operational methods to play a protecting role for the plant: fighting off bioagressors (insects, fungus, weeds, etc.) or stimulating its defences."

> *"It all comes from observing nature!"*

However, he used another project, *Folivarde,* to warn about the danger of just shifting the problem over to other crops The plant from which the project's main active ingredient is taken, *Dittrichia viscosa,* grows in waste grounds. "To be able to set up a large-scale production, you need to immediately ask the following question: are we able to harvest enough *Dittrichia viscosa* in the existing waste grounds, or will special farms need to be set up?" In this case, he mentions another important point: ensure that the production of this *Dittrichia viscosa* does not need need synthetic phytosanitary products, in this case the problem would just be shifted from the protected crop to the one that is being grown to produce the natural molecules to protect it.

And then there is the question of regulations: how can they be adjusted to allow this set of tools to be put on the market, whilst also allowing for the environmental impact that these molecules might have in relation to their scope of activity? The current commercial registration processes are long and complex, especially for complex extracts even if they can be used at lower doses than synthetic products. In the case of the *Folivarde* project "the first ecological screening processes, started with five plants back in 2010 but the 'race' from laboratory work to field trials may not finally result in commercial registration before 2024."



Effects of rock rose on the germination and growth of clover. A: control, 9 days after seeding; B: pot with cystus planted on right, 9 days after seeding; C: control, 5 months after seeding; D: pot with cystus plant on the right, 5 months after seeding. DESHERBAL project. *Photo: © Claire Richard, ICF/CNRS*

Webinar 2

Micro-organisms as the defenders of plant health

Fungus strains grown in petri dish. Some of these micro-organisms are used to protect plants from disease or other bioaggressors. INRAE, Sophia-Antipolis. Photo: © Nicolas Bertrand, INRAE

Session programme

▶ **BIOTI-VIGNE**	Biotisation of young nursery-grown grapevines to prevent trunk diseases (PSPE2)	Marc Fermaud (INRAE Bordeaux)
▶ **Systemyc**	Conception of crop-growing SYSTEms based on the use of MYCorrhization for the biocontrol of earth-bound pests that damage tomato crops (PSPE2)	Marie Chave (INRAE Antilles-Guyane)
▶ **NABUCO**	New Bio-Marine Agents Used for Biological Control (Nouveaux Agents Bio-Marins Utilisables en Contrôle biologique) (PSPE2)	Thomas Guillemette (Université d'Angers)
▶ **AttractMyFly**	Development of attractors and entomopathogenic fungus entomovection processes to control melon fly *Bactrocera cucurbitae* (PSPE2)	Laurent Costet (CIRAD)

EXPERT GUEST: Bruno Le Cam Research Director at INRAE Angers, coordinator of the ENFIN! project (ANR EcoPhyto Maturation) - Development of a new plant protection concept applied to apple scab disease

You can see the complete recording of this session on the symposium's YouTube channel

Micro-organisms and biocontrol, a research field full of promise and surprises

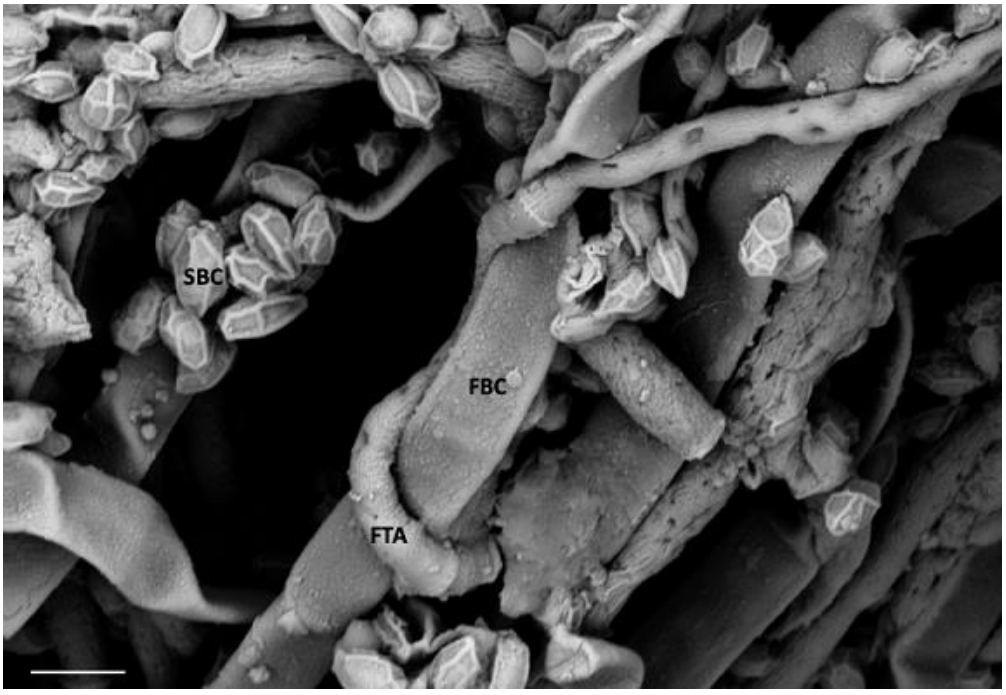
The use of micro-organisms as biocontrol agents raises many interesting prospects for the reduction of the use of phytosanitary products. The Ecophyto research projects that are working in this field must never lose sight off the fact that: living materials have their own codes, which are far from linear and sometimes full of surprises. This is a summary of some of the, sometimes unexpected, lessons learned by Ecophyto projects.

1+1 does not always equal two

The Bioti-vigne project has managed to reveal the potential of a strain of *Pythium oligandrum*, this fungus is likely to be able to protect vines from trunk diseases. It is effective in protecting against two major pathogens known to cause these 'diseases'. The project focuses mostly on the potential interest of bringing the vines into contact with this strain at a nursery level, long before the first signs of the diseases appear. The key factor is ensuring that the *Pythium oligandrum* remains effective over the long term. "The vine sees it as both an ally and a potential aggressor", says Marc Fermaud, INRAE researcher. It doesn't exactly roll out the red carpet, but nor does it try to eliminate it, so the *Pythium* can hang around for a while." And another piece of good news: the very good protection levels achieved on grafted plants with the two most common root-stock varieties used in France and other wine-growing countries. However *Pythium oligrandrum* does not currently have a synergistic effect with other bacteria strains with demonstrated high biocontrol potential. "Intuitively speaking we are thinking of complementarity, but our tests have not revealed any additional affects: the advantages of each individual strain are not cumulated when different strains are combined", says Marc Fermaud.

"Can marine fungus save the apple?"

"The Mer Molécules Santé laboratory in Nantes has a library of 900 strains!"



Trichoderma atroviride filament wrapping and parasiting filaments of the phytopathogen agent Botrytis cinerea. Scale: 10 µm. FBC: filament of *B. cinerea* ; FTA: filaments of *T. atroviride* ; SBC: spores of Botrytis cinerea. Nabuco *project Photo © Franck Bastide, IRHS*

Vine with Esca, the initial symptoms of the disease are a discolouration (yellow and red) of the leaves. Over the more or less long-term, the disease will cause the grape vine to wither and die. Château Couhins, Villenave d'Ornon, Gironde, FRANCE, 21 June 2018.
Photo: © Nicolas Bertrand, INRAE

Solutions from the sea

Can marine fungus save the apple? This rather surprising concept is producing encouraging results.

"New bio-marine active ingredients used for biological control"; this is what the project's acronym, Nabuco, stands for and that speaks for itself. The idea, not necessarily the first thing that would come to mind, involves looking for fungus in the marine ecosystem that can be used to protect crops on dry land. "Marine biodiversity is very rich, we are synthesising molecules many of whose properties have yet to be identified" says Thomas Guilllemette, researcher at the University of Angers. "The Mer Molécules Santé laboratory in Nantes has a library of 900 strains! ". Nabuco focuses its work on 50 marine strains of *Trichoderma*. According to the criteria analysed by the project, four of these strains have revealed a potential for cultivation and high degrees of antagonist activities far superior to the fours strains currently on the market. Nabuco has also isolated twelve secondary metabolite extracts from these fungus. There is potential there too. One of these metabolites, and the strain it comes from, is being tested as a preventative treatment of stems and leaves without using any wetting agent, at this point, and already achieving protection levels of around 25% for septoria leaf blotch on wheat and apple scab disease. Could these results raise the interest of a commercial producer?

Males, not always the best vectors for getting at the females

Melon fly does a great deal of damage to cucurbitaceae on the Island of Réunion. Most of the damage comes from the females when they lay their eggs. However the existing pheromone traps only work for males... The Attract my fly project originally aimed to use the males to transport *Beauveria hoplocheli* spores to the females, this being a proven pathogen affecting the female fly. The principle is as follows; capture the males, contaminate them, then release them so that they transmit the spores to the females during mating. Unfortunately "The results are below expectations", says Laurent Costet, researcher at Cirad. "The females are only affected if they mate within 24h of the male being contaminated." and the transmission of *Beauveria* using only this approach will doubtless never be enough. The most promising leads involve finding a way to trap the females. Attract my fly has field tested a number of kairomones that have been singled out as interesting under laboratory conditions. This combination will be applied as part of a known deployment strategy perspective under the name "attract and kill".

Of micro-organisms... and men

Two projects, one of which has reached its finality and the other that has only just started, highlight one of the success criteria for micro-organism-based biocontrol solutions: the human factor!

> *The 50 farmers involved... noted that some of their existing habitual practices also encouraged mycorrhizal symbiosis*

Systemyc concentrates on interactions between plants and soil micro-organisms, such as mycorrhizal symbiosis. Whilst the technical aspects were evidently part of the research scope, the "social acceptance" dimension of any potential solutions would also have to be taken into account. A collective educational plan alternating workshops and surveys provided a means to approach the complexity and incertitude of the mycorrhizal processes. "The 50 farmers involved discovered some of the phenomena, which helped them to comprehend these invisible mechanisms... but they noted that some of their existing habitual practices also encouraged mycorrhizal symbiosis and this helped some of them to feel better about their working methods", says Marie Chave, INRAE researcher. A version of this educational plan will be made available for agricultural training programmes.

The *Enfin!* project (2022-2024) combines two patented INRAE inventions for the treatment of *Ventura inaequalis*, which causes apple scab rot. The first of these aims to force this fungus to reproduce with non-pathogenic strains, such that its offspring become non-virulent when they return the following spring. As well as reducing symptoms, this will significantly reduce the spread of this pathogen. The second patent involves applying the same non-pathogenic strains on the apple trees in spring in order to generate protective immunity in the trees. "The acceptability of this kind of solution is an integral part of the project," says Bruno Le Cam, scientific director (INRAE). "Not just for farmers but for consumers and society as well. We will try to identify the potential hurdles in order to outline ways to remove them where necessary. These limitations are doubtless not only related to technical, regulatory or economic issues. "

> *The acceptability of this kind of solution is an integral part of the project...Not just for farmers but for consumers and society as well.*

Female melon fly affected by *Beauveria hoplocheii.*
Attract my fly project.
Photo: © Brice Derepas, CIRAD

Webinar 3

The integration and optimisation of bio-control

a) Fields of various lettuce and chicory crops b) Field of new potatoes on the isle of Noirmoutier. *Photo: © Jean-Marie Bossennec, INRAE*
c) Monitoring ground beetle populations. *Photo: © Christophe Maitre, INRAE*

Session programme

▶ **MilPomBio**	Potato blight: identification and development of biocontrol products for integrated crop protection (PSPE2)	Marie Turner (UMR Vegenov)
▶ **BIOLIM**	Innovative biocontrol techniques for the management of crop damaging land molluscs: Looking for an evaluation methodology suited to their ecophysiology (PSPE2)	André Chabert (ACTA)
▶ **PRO-BIO-TAUPIN**	Evaluation of biocontrol solutions for dealing with wireworm damage (PSPE2)	Jean-Baptiste Thibord (ARVALIS)
▶ **BIOBOT**	Optimisation, including the global and integrated management, of biocontrol solutions for the treatment of grey mould on grapevines (PSPE2)	Nicolas Aveline (INRAE Bordeaux)

EXPERT GUESTS: Anthony Ginez, Experiments officer at APREL

Thomas Pressecq, PhD student and researcher at the INRAE, working on a thesis entitled “Développer des outils d’aide à la décision pour optimiser l’usage du biocontrôle” (developing decision-making support tools for the optimisation of the use of biocontrol)

You can see the complete recording of this session on the symposium’s YouTube channel

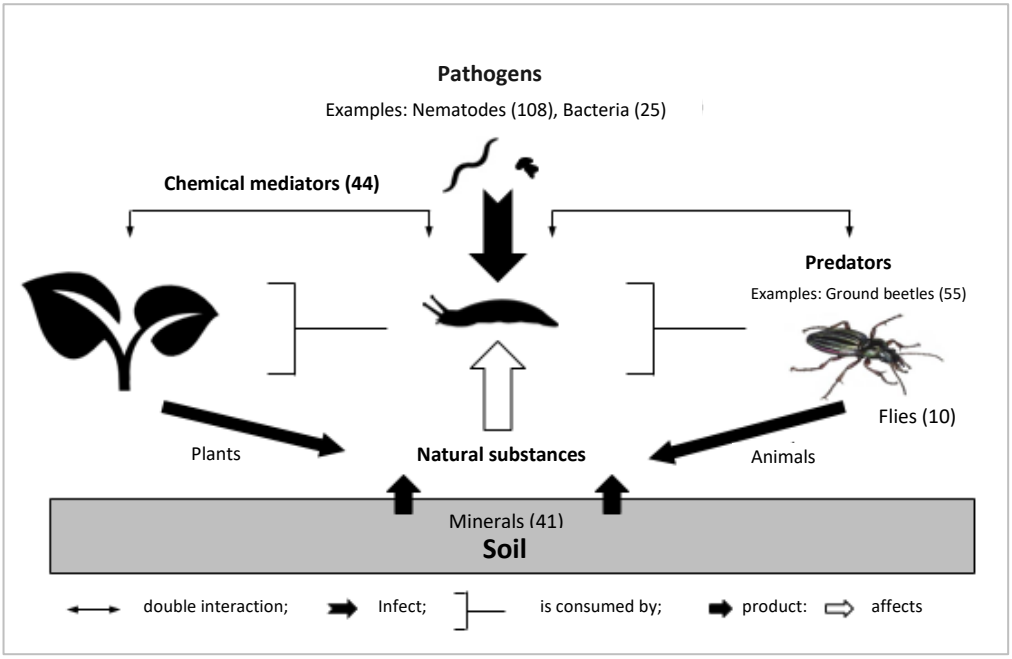
Biocontrol, a lever to be integrated into a combination of solutions

There's no magic wand. If biocontrol appears to be viable alternative to synthetic phytosanitary products, instances of direct substitution, where one solution replaces the other, like for like, remain very rare. The objective is therefore more about trying to create new solutions that can be integrated when developing generalised strategies, where the chemical ingredient is no longer the only response. Here is a summary of some of the levers available to optimise the integration of biocontrol agents.

> *For some varieties, the use of fungicides can be reduced by up to 90% without affecting production*

Combining biocontrol with species selection to reduce dosages of synthetic phytosanitary products

The MilPomBio project aims to identify which solutions, out of a list of 42 already existing or preliminary stage biocontrol products, show the most potential for potato blight control. The most promising ones will be tested in the field after the completion of a laboratory experimentation phase. "We have identified some phosphite-based solutions as promising," says Antoine Menil, researcher for Vegenov. We have tested them in combination with different doses of a traditional fungicide, whilst also monitoring the genetic factor, each variety has its own degree of sensitivity to blight." This is one way of proposing more applied solutions. In this way, it has been seen that the dosage of traditional fungicide can be significantly reduced, but not uniformly across all varieties. For some, the use of fungicides can be reduced by up to 90% without affecting crop production. For others, this figure is closer to a 40% reduction in fungicide use.



Biotic and abiotic relations of slugs with their environment. The number of listed publications (between 1992 and 2018) for each biocontrol method is shown here in brackets.
Image: © André Chabert, ACTA

> *As far as slugs are concerned, it is quite difficult to separate agricultural practices from biocontrol implementation, whatever the selected solution!*

Integrating biocontrol into technical routes and associating "service plant" crops

Biolim has set its sights on slugs. Again, the objective was to establish a range of potential biocontrol leads to deal with this pest: natural substances, predators or "service plants", such as using repulsive mustards as intermediate crops. Each biocontrol agent has its strengths and weaknesses but André Chabert, researcher at ACTA, tells us that one common denominator has been identified: "As far as slugs are concerned, it is quite difficult to separate agricultural practices from biocontrol implementation, whatever the selected solution! ". So performances need to be evaluated in terms of global coherency.

In the case of Pro-Bio-Taupin, the project's initial spectrum was also very broad. To deal with wireworm, a highly damaging beetle larva against which chemical solutions are becoming increasingly rare and ineffective, researchers have explored a number of possibilities, such as natural substances containing glucosinolate. "Large quantities are needed for it to work", says Jean-Baptiste Thibord, researcher at Arvalis. One interesting approach would be to cultivate plants that produce these substances directly in the field, either before or during the cultivation of the crop to be protected." Today the intermediate crop concept is more advanced than that of companion plant associations. In all cases, these strategies require farmers to adjust their practices and crop-rotation methods.

Grey slug on a corn leaf. BIOLIM project. *Photo: © André Chabert, ACTA*

Wire-worm, beetle larvae
Photo: © Serge CARRE, INRAE

Grey mould on grapes. BIOBOT project.
Photo: © Nicolas Aveline, IFV

Avoid damage through epidemic-surveillance and prevention

André Chabert, Biolim project leader, insists that the presence of pests in fields must be analysed in detail. “Detectors and traps allow us to survey slug populations and they are our first ally in crop protection, including biocontrol! “

In wine growing, grey mould, caused by the Botrytis fungus, reduces harvests and grape quality. For the Biobot project leaders, the 14 currently approved biocontrol solutions propose an interesting alternative. The project aimed to define the best ways to integrate biocontrol into grapevine cultivation practices. For Nicolas Aveline, what they have learnt is its complementarity with preventive measures. “One of them involves reducing the number of leaves on the vine to air the fruit and reduce the conditions that favour fungus proliferation. As well as a direct effect on the pathogen, this practice also has the advantage of making it easier to apply biocontrol products onto the grapes”, he explains.

“It is essential to activate more than one lever”

▶ **The views of Anthony Ginez,** experiments officer at Aprel

Anthony Ginez works at the Provençal vegetable research and experimentation association (APREL) on the protection of sheltered crops. For him, biocontrol must be used as part of a combined approach since its effectiveness is much less clear-cut than that of chemical solutions. “In my work, it is essential to activate more than one lever. Biocontrol is therefore combined with physical pest-control barriers such as nets, an active biodiversity that promotes the presence of auxiliaries and, as a last resort, phytosanitary products.” Sometimes he even combines biocontrol... with biocontrol! “For example, with the strawberry aphid we combine lacewing and ladybirds, as predators, with potassium salts of fatty acids, as a natural substance with an insecticide action that does not have a negative impact on the auxiliaries. “

DST to support of biocontrol

Farmers often use Decision Support Tools (DST) to protect their crops. This technical term covers various ways to guide farmers in their practices. And biocontrol is no exception to this, the Ecophyto projects integrate them frequently in their work. In the case of MilPomBio, a DST is also used for the chemical treatments, Mileos in this case, it is used to define the real necessity and the right time to apply. Biobot has used the “Ciliberti index” to orient the best possible fungicide applications and has improved it for use with biocontrol. In other cases, specific DSTs have been developed, such as Deci Control which is currently at the prototype stage with the Provençal vegetable research and experimentation association (Aprel) for use in market gardens. “At the moment we are mostly working on a local scale, but the aim is to program the tool for nationwide use.” says Ginez, Experiements officer at APREL.

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Webinar 4

A world of odours

Isomate ®: pheromone diffuser in an integrated or organic fruit tree orchard. *Photo: © Jean-Charles Bouvier, INRAE*

Session programme

▶ **SEMIOTRAP**	Development of a biocontrol trap to survey and monitor the palm borer moth, *Paysandisia archon* (JEVI 2016)	Johann Fournil (M2i)
▶ **OPTIM'PHERO**	Optimising Pheromones and Transposing the results obtained with the pine processionary to other insects, such as pest lepidoptera in Non-Agricultural Zones (PSPE2)	
▶ **AM&BAS**	Agro-messages and treatment of insect pests in seedling production and large-scale crops (PSPE2)	Brigitte Frérot iEES Paris

EXPERT GUESTS: Emmanuelle Jacquin-Joly, Research Director at INRAE – iEES Paris

Philippe Lucas, Research Director at INRAE – iEES Paris

Denis Thiery, Research Director at INRAE – UMR SAVE

You can see the complete recording of this session on the symposium's YouTube channel

Pheromones and kairomones, a chemical control solution from nature

We are all surrounded by odours which can attract us, disgust us or even guide us... And its the same for pest insects! Sexual confusion has been a genuine success in French vineyards, research on using pheromones and kairomones to deal with crop-damaging pests is leading to a number of promising prospects. In this vast scientific and technical field, the Semiotrap, Optim'Phero and Am&Bas projects are shedding light on these intriguing prospects.

You can do anything with odours: trap, restrict mating or keep pests away from crops! This is what has been revealed by the Semiotrap, Optim'Phero and Am&Bas projects which are part of axis 2 of the Ecophyto plan. Whilst sexual confusion is now in use in 17% of French vineyards, chemical signals, especially pheromones and kairomones, are becoming increasingly promising solutions for biocontrol, with one particular major advantage: odours are environmentally friendly.

"The teams have managed to identify, define and synthesise the pheromone to conclusively demonstrate its power of attraction over males and females"

"We have already received two commercial registration approvals and have therefore been able to put two new products on the market"

Identify, formulate and observe the pheromone's capacity to attract

The Semiotrap project, originially set up to protect the heritage palm-trees of the Mediterranean coastlines from damage caused by *Paysandisia archon*, has made some major advances for biocontrol methods in general. "We had a large number of scientific obstacles to get around: would it be possible to define, synthesise and use a pheromone to trap this insect? If yes, how do we use it? How do we apply it? What kind of trap will we need to make for this insect?" says Johann Fournil of M2i. The results have been a genuine success, the teams have managed to identify, define and synthesise the pheromone to conclusively demonstrate its power of attraction over males and females. But one of the specific things about the insect, a large hovering moth, is that it has turned out to be highly resistant to traps. "We considered two types of trap: electrical and net", he continues. None of the solutions were effective since the moth resists electrical charge and does not fly into the net." Research will continues in 2022-2023 to find a suitable trap and market the system.

Paint-ball and sprayers for sexual confusion, some solutions have already been put on the market

However, Optim'Phero is currently already on the market! "At the end of this Ecophyto project, we received two commercial registration approvals and have therefore been able to put two new products on the market", says Johann Fournil, M2i being also a partner for this project. This time pheromones are being used to hinder the reproduction of two major pests that cause both environmental and physical damage in France and Europe: the pine processionary and the box tree moth. The research work has allowed us to develop effective solutions. "For the pine processionary, we achieved a significant reduction of 78% in the population of the moths with a product action period running from June right through to September. For the Box tree moth, we have combined our product with a larvicide and this has resulted in dividing the population of moths by a factor of ten." The project team has taken things even further with the development of two new application method innovations. For the Box tree moth, the pheromones are applied with a hand sprayer, and for the Pine processionary, the researchers came up with the idea of... paint-ball! The solution is produced as a gel and packed into biodegradable balls, made exclusively from natural raw materials, which can be easily fired onto the tree using special launchers.

Paysandia archon adult with wings spread.
Photo: © Jean Drescher, INRAE

Pine processionary caterpillar.
Photo: © Olivier Bertel, INRAE

Kairomones are the new selection criteria for cultivated plant varieties

In the world of odours, the Am&Bas project is interested in another type of chemical message, the kairomone. Whilst pheromones are used to communicate within a single species, kairomones communicate between different species to cause the pest's behaviour to change. They are frequently emitted by plants to attract insects, they can also be used to trap them or alter their egg-laying behaviour. This is what the project team decided to base their work on, they have demonstrated the effectiveness of a kairomone in dealing with two pests affecting large-scale crops: the *Tychius aureolus* weevil on alfalfa and the European corn borer, *Ostrinia nubilalis*. Even if suitable trap designs remain elusive, the identification of this kairomone is opening up some very wide ranging genetic selection prospects, as Brigitte Frérot of the INRAE, explains: "The cultivated varieties are all very attractive! They produce the same volatile compounds that interest the pest insects. This data could orient selection towards resistant varieties that do not produce this kairomone." The Am&Bas project has therefore provided a solution that can concentrate egg-laying at a distance from the crops or trap large numbers of these two pests. It also opens up research prospects where kairomone production can become a plant variety selection criterion.

▶ **The views of Emmanuelle Jacquin-Joly and Philippe Lucas,** INRAE research directors

"Machine Learning and 3D modelling are accelerating chemical signal discoveries that could be useful for biocontrol"

Attract, repel, block. These are the three main types of chemical signals that the INRAE staff working under Emmanuelle Jacquin-Joly are looking for. The researchers use a genuine innovation to identify them: the reverse chemical ecology approach: "we work on the insect's olfactory receptors, then we work our way back to the behaviour to study the effectiveness of the chemical signal for biocontrol." This approach is based on divergences between the olfactory receptors: each family of insects has its own receptors, which are also very different to those of vertebrates. In this way the treatment is targeted and there is no collateral damage to auxiliaries. "Once the desired receptor has been identified, we use it to screen the volatile molecules *in vitro* and *in silico*, and then we model its 3D structure in order to see which molecule will be be able to correspond with its geometry and therefore produce a response," she explains. "It was quite difficult to use this method before because we couldn't do 3D modelling: but this is now possible thanks to cryogenic electron microscopy!" The coupling of the chemical signal and receptor is then checked experimentally, and then the responses of the pest insect were tested. "With this method, between 60 and 80% of the tested molecules turn out to be active", says the research director. Philippe Lucas, who is also an INRAE research director, uses the PheroSensor project as an example to explain another application of olfactory receptors; the project was set up to develop bio-inspired sensors based on insect receptors which detect the presence of pest insects without capturing them since they can detect the pheromones that they emit into the environment. "To test out the concept, we are concentrating on 3 pests that cause considerable economic damage: the red palm weevil, the fall armyworm and the African cotton leaf worm" he explains.

> *"In this way the treatment is targeted and there is no collateral damage to auxiliaries"*

▶ **The views of Denis Thiery,** INRAE Research Director

Sexual confusion in grapevines: avoid the white zones!

When it comes to reducing the use of phytosanitary products, wine-growing is a major issue. “Grapevines represent 3% of French agricultural land for 20% of the national use of phytosanitary products”, explains Denis Thiery, INRAE Research Director. Using pheromones to provoke sexual confusion is becoming one of the leading organic farming techniques thanks to its 80 to 90% success rate, but it does have a few limitations: pest insects tend to mate at the end of the day when pheromone concentrations are quite low, the summer treatment period extended to the end of September, effectiveness depending on the pest population levels, difficulties in hot or windy climates, pheromones absorption by leaf cuticles. Denis Thiery proposes three possible ways of dealing with these limitations: “You have to avoid the white zones, where pheromone concentrations are too low, and use modern diffusion methods, such as the Puffer ®, instead of passive distribution methods. We also need to gather a lot more data on pheromone concentrations using sensors placed directly in the fields.“

Tychius aureolus, the alfalfa seed weevil.
Photo: © Serge CARRE, INRAE

Larva of the European corn borer (Ostrinia nubilalis L.) inside a corn stem.
Boigneville (91720). *Photo: © Pascal Thiebeau, INRAE*

Webinar 5

Macro-organisms: Sibling rivalry...

Adult *Perillus bioculatus* (two spotted stink bug) feeding on *Leptinotarsa decemlineata* (Colorado beetle) eggs. *Photo: © Jeanne Daumal, INRAE*

Session programme

▶ **Rhizodia**	Regulation of scale insect populations by releasing the scale-eating ladybird, *Rhizobius lophantae* (PSPE2)	Maria-Marta Fernandez (CTIFL)
▶ **BIOCCYD**	Biological control of the codling moth, *Cydia pomonella* (BIOControl of CYDia pomonella) (PSPE2)	Nicolas Borowiec (UMR ISA)
▶ **ACAROSOL**	Biological control of red spider mite on Solanaceae (PSPE2)	Marie-Stéphane Tixier (SupAgro)

EXPERT GUEST: Cécilia Multeau, Biocontrol Innovation and partnership officer – INRAE

Marc Kenis, Invasion ecology risk analysis manager – CABI

You can see the complete recording of this session on the symposium's YouTube channel

Managing auxiliary populations to regulate pests naturally

Finding the right balance between pests and auxiliaries in order to to naturally and sustainably regulate pest pressure on agricultural crops. Population dynamics are at the heart of organic pest control methods involving macro-organisms. The approaches of these three projects, Rhizodia, Acarosol and Bioccyd, range from introducing new species to increasing existing population levels; they are producing results that are going beyond just reducing the Treatment Frequency Index (TFI). They are defining the criteria for a highly effective agro-ecology.

Using auxiliary macro-organisms (invertebrates, insects, spider-mites or nematodes) highlights the potential for naturally regulated pest populations. The question of achieving a balance between auxiliary and pest populations is central to organic treatment, regardless of whether this involves increasing the population levels of an already existing species or introducing new species.

Rhizodia investigated the overwintering of auxiliaries

The Rhizodia project sheds a lot of light on this issue as it concerns the biological control of scale insects on black-current crops. “Black-current farmers have been subjected to pest damage for the last 15 years, this has quite often forced them to tear up whole fields of plants”, says Maria-Martha Fernandez of the Inter-professional Fruit and Vegetable Technical Centre (CTIFL). The project teams have identified the *Rhizobius Iophantae* ladybird as a means to deal with this scale insect, this ladybird species is relatively easy to raise in large numbers, which is an essential criteria for biological control methods. There was a twofold objective: establish whether regulation can be effective and reduce operating costs. “The release programs represented between 20 and 45% of gross income, that’s a big investment!” she insists. With two ladybird release operations, 6,000 individuals, at the stage where the scale insect larvae are colonising the plant, results where not conclusive. “We saw populations drop by 69% during the first year, but the pest would return by the third year, with a population explosion exactly in the same spot where we had originally released the ladybirds”, explains Maria-Martha Fernandez. And most worrying of all, the ladybirds had completely disappeared from the field, which raised the question of how they overwintered... After a number of other attempts, modifying the locations and dates of the ladybird releases, researchers failed to achieve promising treatment effectiveness results. Research work continues and could now be redirected towards the identification and raising of new parasitoids for dealing with scale insects.

> *“There was a twofold objective: establish whether regulation can be effective and reduce operating costs.”*

The benefits of companion plants as homes to auxiliary populations

The Acarosol project seeks to deal with spider mite on solanaceae, especially *Aculops lycopersici* on tomato crops, it has successfully identified a promising predator from the Phytoseiidae family, *Typhlodromus (Anthoseius) recki*. “So far, all of the tests we have done with spider mite from this family have ended in failure”, explains Marie-Stéphane Tixier, professor at Montpellier SupAgro. The structure of the tomato plant itself hinders the development and dispersion of these predator spider-mites and consequently their consumption of their prey.” When identifying and collecting *Typhlodromus (Anthoseius) recki*, researchers noticed that this spider mite is naturally present in tomato crop fields. But they also discovered another particularity: the populations naturally occupy mint plants. So they decided to release the auxiliaries by placing branches of mint next to the tomato plants, in order to offer an environment that would facilitate the development and dispersion of populations. This was a good bet! Results confirmed the effectiveness of the auxiliary and that this was finally the

first viable organic alternative for dealing with *Aculops lycopersici*. And maybe more, since as a general predator, *Typhlodromus (Anthoseius) recki* could turn out to be effective in dealing with other pests. This project also highlighted the benefits of developing companion plants in agriculture for use as reservoir habitats for auxiliaries.

> *The possibility of introducing Mastrus ridens into France opens up a good perspective for reducing treatment frequency indexes*

A new sustainable management eco-systemic service for dealing with the codling moth

Biocontrol methods for reducing the damage caused by the codling moth, *Cydia pomonella*, on apples do already exist but their performance is quite limited. The Bioccyd project managers therefore decided to add to these techniques by testing two new biological control methods, one using an already present parasitoid of the *Trichogramma* genus and the other involving the introduction and acclimatisation of a tropical parasitoid, *Mastrus ridens*. Whilst the first of these turned out to be of little interest, with low collection rates and no exclusivity for the codling moth as the target, the second seems to show more promise. "This parasitoid has already been introduced in other countries to control *Cydia pomonella*", explains Nicolas Borowiec of the INRAE. The researchers completed additional experiments to confirm the specificity of this parasitoid and received the necessary authorisations for introducing *Mastrus ridens* into the country and then to release it in agricultural fields around Avignon. It was quite an obstacle course! "The possibility of introducing Mastrus ridens into France opens up a good perspective for reducing treatment frequency indexes" he exclaims. Its permanent establishment and natural dispersion would lead to a sustainable regulation of codling moth populations. If this works, we would have a new eco-systemic service that would be free of charge for growers."

▶ **The views of Marc Kenis,** researcher at the CABI research centre in Switzerland

"Organic pest control through acclimatisation involves major long-term economic and ecological benefits"

Traditional or acclimatisation-based biological pest control methods involve the introduction of an exotic predator to re-establish the natural regulation of a pest insect. "Since 1889, there has been 6,164 introductions to combat 692 different pest insects", explains Marc Kenis, researcher at the CABI. A well-established biological pest control programme provides constant control of the pest insects and very high economic benefits: during the 1990s the cost/benefit ratio of biological pest control methods for the cassava mealybug in Africa was 1 for 200 to 500. And this increases over time as the predator continues to control the pest!" The benefits are also ecological, they safeguard plant populations, often crops, from certain death: this was the case with the ladybirds that saved rubber trees on the Isle of Sainte-Hélène by naturally controlling mealybug populations. But there are also risks, notably negative effects on the surrounding biodiversity, but the strengthening of evaluation protocols and the biologal study of auxiliary species have significantly reduced this. "Generally speaking, a parasite will not eliminate a non-target species because this will provoke an imbalance between prey and the biological control agents", he says. "Once the pest insect population begins to fall then that of the predator will do the same. "

> *A well-established biological pest control programme provides constant control of the pest insects and very high economic benefits*

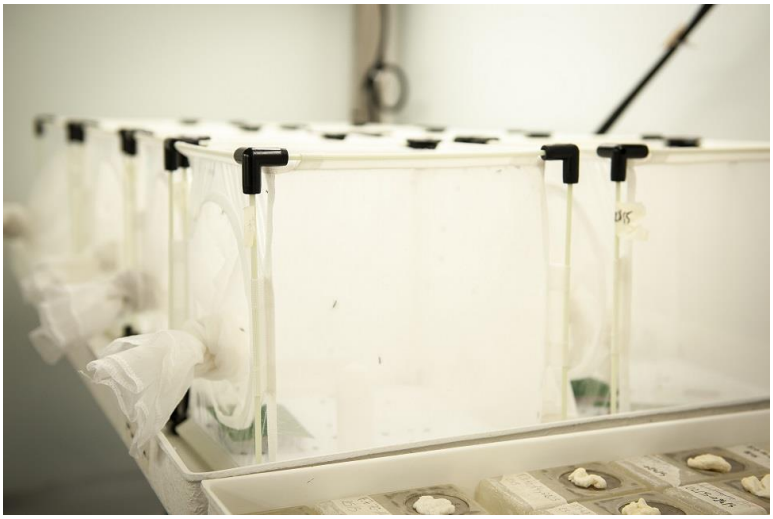
▶ **The views of Cécilia Multeau,** Biocontrol Innovation and partnerships officer at INRAE

Macro-organisms are a dynamic sector!

The biocontrol macro-organisms market sector is going through a phase of transformation. There are many issues involved: industrial scale production, legislation out of sync with EU legislation and the application of the Biodiversity Convention, the diversification of business models with the creation of insect farms as parts of cooperatives, and the monitoring of unintentional effects to evaluate the interference risks of auxiliaries, especially when exotic macro-organisms are introduced. Cécilia Multeau, Innovation and partnerships officer for the biocontrol sector at INRAE, gives some more information on a few major innovation orientations: “With genetic selection for example, a broad range of characteristics are now being screened such as longevity, or more specifically resistance to thermal stress. As far as mass production is concerned, the digital revolution and automation are having a major impact on processes. There have also been many introduction strategy and auxiliary deployment innovations. Finally, the sterile insect technique, is being deployed dynamically by the collectif TIS, with a transfer potential for new insect species such as *Drosophila suzukii*. “

The seven-spot ladybird (Coccinella septempunctata).
Photo: © *Bastien Castagneyrol, INRAE*

Trichogramma brassicae parasiting the eggs of *Ostriania nubilalis* (european corn borer).
Photo: © Jeannine Pizzol, INRAE



Mastrus ridens production room, a parasitoid of codling moth larvae *(Cydia pomonella)*. INRAE Sophia Antipolis.
Photo: © Nicolas Betrand, INRAE

Webinar 6

Resistance as a way to protect crops

Sunset over the grapevines of the Château Couhins, Villenave d'Ornon, Gironde, FRANCE, 21 June 2018. *Photo: © INRAE*

Session programme

▶ **DAS-REVI**	Socio-technical development and appropriation of plant variety resistance in sustainable wine-growing (Pesticides 2014)	François Hochereau (UMR INRAE SAD-APT)
▶ **MDRisque**	Evaluation of the potential for multi-drug resistance in the wheat septoria leaf blotch agent, *Mycosphaerella graminicola* (Pesticides 2014)	Anne-Sophie Walker (INRAE BIOGER)

EXPERT GUEST: Marie-France Coriot-Costet, Research Director – INRAE UMR SAVE

Mourad Hannachi, Research Officer – INRAE UMR SADAPT

Marie Turner, R&D Quality Control and Plant Health Manager – Vegenov

You can see the complete recording of this session on the symposium's YouTube channel

Crop protection: resistance development challenges

The protection of agricultural crops from the numerous, and often difficult to thwart, biotic threats present in their surrounding environment is becoming a vital challenge for the agricultural sector, often requiring a great deal of invention.

To cope with the frequent episodes of high past and disease pressure on crops, it will be necessary to find effective protective solutions. The intensive use of phytopharmaceutical produces is now being questioned in the light of their impact on human and environmental health. Their repeated application is actually increasing the resistance of certain pests to these products and putting crops under further danger. So there is clearly room for alternatives to synthetic products on this 'battleground'. This is a dynamic system, where natural selection competes with the resources applied by humanity to preserve plant health.

Innovation through the hybridisation of varieties as a lever for combating grapevine diseases

> *These varieties have allowed us to change our approach and reduce our use of phytosanitary products by as much as 90%*

The wine growing and making sector suffers heavily from the effects of pathogen fungi like mildew; this is why the sector is responsible for the consumption of two thirds of all of the fungicides sold in France. This huge quantity could even increase over the years to come according to INRAE researcher, Marie-France Corio-Costet: "The first instance of the resistance of grapevine powdery mildew to QoI type, mitochondrial respiration inhibitor, fungicides was detected in 2008; in just a few years it became almost total and generalised over all of the wine-growing regions of France". The acquisition of fungicide resistance by these pathogens has been caused by mutations that ensure a selective advantage for the survival and reproduction of the resistant strain. Which will then therefore multiply and spread as a result of natural selection. Generally, the first reflex would be to apply more phytosanitary products in the hope of greater effectiveness over these pests. But that just feeds a vicious circle, which means that the only way forwards is to find an alternative solution.

Using resistant grapevine varieties, such as Cabernet-Jura or Souvignier, is therefore a powerful lever in the fight against these diseases; and that merits attention. The DAS-REVI project is therefore committed to identifying the socio-economic benefits and pitfalls related to the cultivation of these hybrid varieties. "These varieties have allowed us to change our approach and reduce our use of phytosanitary products by as much as 90%", says Jérémy Ducourt, Bordeaux region wine grower and a pioneer in the use of these varieties since 2014. These varieties have many advantages: reduced overheads, protection of the environment, easy to grow... All of which are good reasons for taking a decision which might have appeared precarious at first: the wine-making and growing sector has been built on a very tradition-oriented image with a penchant for historic grape varieties, some of which have since become international brands, and a large number of consumer guarantee regulations (AOP and AOC). How to go about sustainably introducing these new varieties into a highly competitive market is still an open question that will be profoundly influenced by the reception of the product's consumers.

A crop protection arms race

> *The more the use is wide-scale, the higher the probability of it being rendered sensitive will increase in just a matter years*

A rather worrying phenomenon has been observed in farmland across France and Europe since 2017: some strains of *Zymoseptoria tritici*, the pathogenic fungus responsible for septoria leaf blotch in wheat, is developing a multi-drug resistance (MDR) to a number of phytosanitary products. Genome rearrangements cause an over-expressed efflux transport system that actively expels a wide variety of fungicides out of the pathogen cells. As a result the cells become less sensitive and continue to attack the crops in spite of the treatments. This recent phenomenon, studied at INRAE by Anne-Sophie Walker and Sabine Fillinger as part of the MDRisque, project, presents a major threat to agricultural productions, since very few solutions currently exists for coming with the phenomena. “The implementation of a surveillance network to monitor and try to limit the large-scale selection of this mutation will be the first step”, says Anne-Sophie Walker, “and the MDRisque project has allowed us to develop awareness of the dangers posed by the MDR mechanism”.

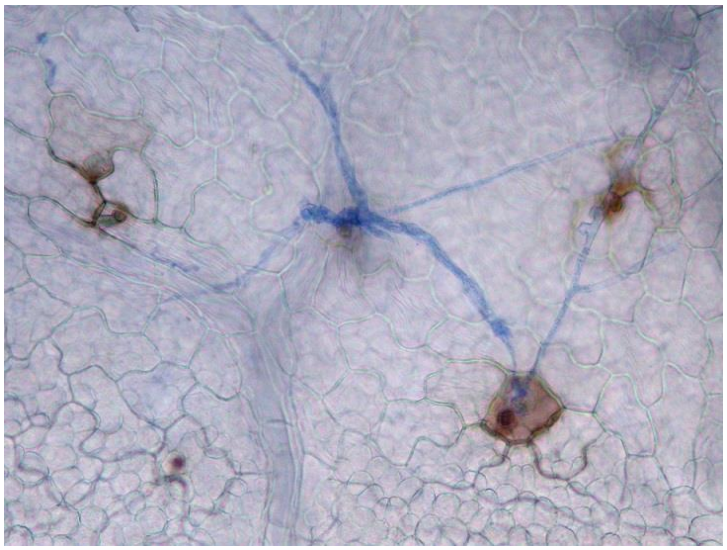
On a wider scale, this resistance acquisition has become an integral part of phytosanitary product management policy, and this will need to be re-assessed according to Mourad Hannachi, Research Officer at INRAE. “What we are seeing now is that as soon as a new disease-resistant variety comes onto the market, a lot of growers are going to use it. And so the more the use is wide-scale, the higher the probability of it being rendered sensitive will increase in just a matter of years” he explains. Since these products have now been subjected to what could be referred to as the “Tragedy of the Commons”; and he suggests that they are treated and managed as such. “In this sense the Commons can be defined as a resource that struggles to remain exclusive and becomes a source of rivalry”. To avoid draining this resource there needs to be a solution: implement the coordination of all of the parties involved in the industry and the farmlands, covering all of its aspects from plant breeding right up to end use. But this new form of management will involve fundamental changes and will certainly require the a genuine political willingness.

Stimulate defences rather than fight the pests and diseases

After the encouraging results raised by RMT Elicitra demonstrating how plants can be partially protected by PDS (Plant Defence Stimulators), a new project, BESTIM (Stimulate plant health in agro-ecological systems), was launched at the start of 2021 with the aim of taking this field of research further, especially in terms of ecological immunity. Marie Turner, R&D, Quality Control and Plant Health Manager at Vegenov, defines this concept as “the external and internal environmental factors within which the plant develops and which contribute to stimulating its immunity system”. For example we could consider that genetics, microbiota, climate conditions or interactions with other species are all factors that will influence plant health. When used alone or in association with other phytosanitary products to improve their effectiveness, PDS could now represent a new plant protection approach, and find numerous real-world applications allowing the agricultural industry to shift towards more sustainable production methods. However this vigorous stimulation of the immunity system must not turn out to have a negative effect on the development and growth of the plants themselves.

Vines under the autumn sun at Château Couhins. Villenave d'Ornon, 21 October 2021. *Photo: © Nicolas Bertrand, INRAE*

Wheat septoria leaf blotch. *Photo: © Frédéric Suffert, INRAE*



Defensive reactions in a sensitive plant treated with a natural defence stimulator (NDS): hypersensitive response of the damaged cells (brown-coloured cells) and deterioration of the mycelium of the parasitic fungus (in blue). Enlargement x 100.
Photo: © TROUVELOT Sophie - Université Bourgogne / INRAE

Webinar 7

Companion plants for healthy crops and soil

Marigold flower. *Photo: © Corine Enard, INRAE*

Wild mustard in flower.
Photo: © Corine Enard, INRAE

Buckwheat flowers. *Photo: © Jean Weber, INRAE*

Session programme

▶ **FLEUR**	Manipulation of the wildflower biodiversity of cereal crop fields (PSPE2)	Joan van Baaren (UMR CNRS ECOBIO)
▶ **MacroPlus**	What techniques could be used to assist the installation of *Macrolophus pygmaeus?* (PSPE2)	Jérôme Lambion (GRAB)
▶ **SERUM**	Disinfecting soils in market gardens (PSPE2)	Ingrid Arnault (University of Tours)
EXPERT GUEST: Christophe Jarry, Director of Jardin Enchanté (Herblay)		

You can see the complete recording of this session on the symposium's YouTube channel

Using companion planting to improve crop and soil health

Phytopharmaceutical products are specifically designed to protect the health of cultivated plants. But they are not the only solution: one interesting alternative could be companion planting which can include hundreds of specific plant species cultivated either before or during cropping periods.

Ecosystems have their own interspecific biological pest and disease control mechanisms which could be exploited for their high crop and soil protection potential. The intelligent management of plant biodiversity would encourage the use of certain species for the beneficial role they can play within a crop ecosystem; this approach is relatively little used at the moment but it could be a potentially powerful lever for a more natural plant health protection policy. What follows is an analysis of these ways of using companion planting to support crop health.

Using the capacity of certain flowers to attract auxiliaries and thus improve crop health

> *"All we need to do now is identify the combination of plant species which will provide the most effective protection"*

We are not the only ones to appreciate colourful scented flowers, insects love them too. The FLEUR project, under the leadership of Joan Van Baaren, CNRS researcher, is concerned with conservation based biological cereal crop pest and disease control techniques. "This involves organising the environment surrounding crop fields to encourage the presence of the natural enemies of crop pests", she explains. More precisely, this project is concentrated on a specific action lever: the installation of flower beds planted with specially chosen species, located such that they can be a suitable home and environment for auxiliaries, providing them with everything they need (such as pollen or nectar) as well as place to find refuge.

All we need to do now is identify the combinations of plant species which will provide the most effective protection. For example, to control the populations of aphids that pass on the Barley Yellow Dwarf Virus (BYDV) in autumn, a combination of buckwheat, cornflower, mustard and broad beans has been found to be particularly effective for cereal crops in Brittany. "Certain limitations must be taken into consideration", states Joan van Baaren. "The flowering seasons need to coincide with the growth and development phase of the crop, the predators you want to attract must not be dangerous to neighbouring crops. We also need to allow for the impact of climate change: some natural enemies are developing increasingly long periods of activity, this could lead to crops being under almost constant threat."

This solution can also be used in greenhouses. Jérôme Lambion, the MacroPlus project manager, has been concentrating on the *Macrolophus pygmaeus* stink bug which is a predator of certain pests that threaten tomato crops, such as spider mite, white fly or the tomato leaf miner, *Tuta absoluta*... *Macrolophus* was already known for its effectiveness and these stink bugs are indeed currently used on crops via one-shot release programs, although these can be quite costly. It would be better to attract them naturally, or ensure that there ate populations near to the crops or inside the greenhouses for long periods of time. "Marigold plants are an ideal host for *Macrolophus*. So we set up beds of marigolds inside and outside the greenhouses". When that was not enough, the researchers also cut the stems of the marigolds so that they could be placed around the bases of the tomato plants and thus increase the active transfer of *Macrolophus* onto the crops that were to be protected. And it works! The spider mite, *Tuta absoluta* and white fly populations dropped off significantly on tomato plants grown near to marigolds. But

> *Biological pest and disease control methods... need to be integrated as part of a global approach, combined with other compatible techniques*

Jérôme Lambion still maintains a certain relativism regarding these results: “Conservation based biological pest and disease control methods are indeed an effective lever for reducing the use of phytosanitary products, but they need to be integrated as part of a global approach, combined with other compatible techniques”. And there is the question of the companion plants own sensitivity to parasites: “Marigolds can be affected by powdery mildew (a fungus), which can make them less appetising for *Macrolophus,* but there is no risk of transmission to the crops since the different plants are not affected by the same species of mildew.

> *I had to commit the whole of my enterprise to this dynamic*

Some real-world applications of this plant protection method are already in place! Christophe Jarry is a horticulturist from the Paris region who has produced 15,000 chrysanthemums over the last 3 years without using any phytosanitary products. “It took a lot of testing to achieve this”, he tells us. “It wasn’t enough to just plant some flower beds in the unused parts of the greenhouse and between the rows of plants, I had to commit the whole of my enterprise to this dynamic. That was a lot of work, requiring training, maintenance and careful monitoring”. He has basically set up insect refuges, blue tit nests, owl nesting boxes, etc. “All of which also play a useful educational role, such that clients can learn about how we are doing all this”. Whilst the initial financial risks were quite high, Christophe Jarry is now deeply satisfied with the results, and intends to extend this plant protection approach to other flower species that he cultivates.

Row of marigold plants next to tomato crops. The MacroPlus project.
Photo: © Jérôme Lambion, GRAB

Using plants to purify the soil

Protecting crops is not just about protecting the plants we are cultivating: soil health is also an essential aspect which must not be neglected. Many pest species, such as the agent that causes bacterial wilt in tropical regions or tomato nematodes, live in the soil and can cause serious damage to crops. These days soils are treated with a synthetic phytosanitary product: Dimethyl disulphide (DMDS). To reduce dependence on such phytosanitary products, the SERUM project has been studying possible alternatives on tomato crops in mainland France and the French Caribbean. “The choice of service plants evidently depends on their power to purify the soil as well as their geographical availability. Our tests have involved species of rattlepod and *Allium* (especially onions), under controlled laboratory conditions and in the field”, explains Ingrid Arnault, manager of the Innophyt University Transfer and Expertise Centre (CETU) of the University of Tours. The idea: the service plant is cultivated on the field that needs to be treated, then cut down and left to break down on the surface of the soil as a “protective” layer (mulching). We can also grind up the service plant and plough it into the soil, or even cultivate the service plant and the crop plant in association. “Indian hemp has produced promising results, significantly reducing the presence of the bacterial wilt agent and tomato nematodes where previously the soil needed to be treated, and this without causing any unwanted side-effects to other insect species populations”. However, using this method to protect crops can only be partial. But we could imagine combining it with an application of DMDS at significantly lower concentrations. But there still remains the fact that this method is quite difficult for producers to apply: for Indian hemp two seeding, growth, cutting and break-down cycles seem to be necessary to sufficiently purify the soil; and this means that the field would be out of productive action for around 200 days. During this period the growers would not be able to grow anything on the fields and this would involve an insupportable loss of earnings for many of them...

Aphidius matricariae feeding of buckwheat inflorescence. The FLEUR project. *Photo: © Bernard Chaubert, INRAE*

Poppies and cornflower (Paris Region).
Photo: © *Jean Weber, INRAE*

C. Spectabilis mulch. The SERUM project.
Photo: © Ingrid Arnault, CETU Innophyt

Webinar 8

Technical routes towards zero phyto

INRAE experimental fields in Versailles. Flax in flower. *Photo: © Nicolas Bertrand, INRAE*

Agricultural machinery working on a grapevines at the Château Couhins vineyard, Villenave d'Ornon, Gironde, FRANCE, 21 June 2018. *Photo: © Nicolas Bertrand, INRAE*

Session programme

▶ **Reg-Puc**	Designing irrigation and fertilisation strategies for controlling green aphid populations in peach tree orchards? (Pesticides 2014)	Marie-Odile Jordan (INRAE PSH)
▶ **ECOVERGER**	Designing low pesticide-use technical routes for orchards that respect current farming restrictions and objectives. An applied modelling approach for peach and mango trees (Pesticides 2014)	Isabelle Grechi (CIRAD UPR HortSys)
▶ **BIOCOU**	Organic disease control for the for the grapevine fanleaf virus: the impact of integrating growing techniques involving intermediate crops and the use of a resistant root-stock on nematode populations; analysis of how nematode-killing ground-cover plants operate (Pesticides 2014)	Olivier Lemaire (University of Strasbourg)
EXPERT GUEST: Thierry Goujon, Director – Terre de Lin Cooperative		

You can see the complete recording of this session on the symposium's YouTube channel

Technical routes towards zero phyto

The implementation of any innovations that involve the slightest dependence on phytosanitary products must integrate the specific restrictions that growers are now facing. We also asked ourselves about the possible contribution of various technical routes involving zero pesticide crop management. The development of alternative methods through scientific work on orchards, vineyards and flax crops must involve a detailed knowledge of each individual plot of land and a constant adaptation to the needs of the crops. If "zero phyto" still seems to be an ideal that we must strive towards; encouraging agricultural levers for the significant reduction of the use of phytosanitary products must involve essential concepts that will need to be mastered and combined for a successful agro-ecological transition.

Dealing with pests in orchards by changing growing practices

> *One idea would be to put together a "tool-box" of alternative approaches*

Green aphid represents a serious threat to orchards, resulting in significant damage to fruit, foliage and stems as well as being a major vector for disease. Marie-Odile Jordan, INRAE researcher, has been working on strategies that seek to regulate green aphid populations through specific agricultural practices. "Preliminary studies have shown that water and nitrates applied to young trees result in increased aphid populations and more severe infestations", she tells us. Consequently, the RegPuc project aims to evaluate the possibility of effectively restricting aphid populations in commercial fruit-tree orchards by changing irrigation and fertilisation practices. This needed to cover the twin conditions of the fluctuating natural infestations of a number of aphid species and the need to guarantee production quality. "The severity of the infestation of a tree depends on the percentage of affected branches and the damage potential of the predominant species of aphid". Consequently, a twofold modulation of water and nitrate input managed to reduce aphid infestations in 2018 and 2019, because this altered the composition of the tree apex, with lower levels of soluble sugars and amino acids. "Weather and the effects of specific tree varieties must also be allowed for: such as the more vigorous and early-fruiting 'Ivory' peach tree variety which is more sensitive to aphids than the 'Conquise' variety". This had produced encouraging results, that should be handled with care: "The effects remain limited and these practices will not suffice in isolation. One idea would be to put together a "tool-box" of alternative approaches, that each grower can adapt to suit the needs of their plantations and the approaches they have already put in place".

> *Some restrictions could also be lifted if information can be shared better*

The ECOVERGER project, under the leadership of Isabelle Grechi, CIRAD researcher, is concentrated on evaluating the feasibility of alternative technical solutions for dealing with the pests and diseases that threaten commercial orchards: brown rot, fruit fly or mango blossom gall midge. Once more, "we needed to allow for all of the technical and economic factors, not forgetting production quality", says Isabelle Grechi. A number of action levers have been identified: bringing the harvest forward to 20 days before full maturity for mangos and synchronising flowering seasons to restrict the sensitivity period for the fruit and the exposure of the blossom to pests; restrict irrigation to achieve slower development, making them less susceptible to infection from brown rot; systematically remove infected fruit to avoid the propagation of pathogen spores; or cover the ground with plastic sheets or ground-cover planting to disturb the ground-level development of the blossom gall midge. "These levers can be effective, and some are quite easy to put in place. Some restrictions could also be lifted if information can be shared better. For example, for early harvesting: contrary to what might be thought, if mango fruit are harvested 20 days before maturity that will only reduce their quality by 5% [estimated in terms of their fresh mass and sugar content compared to the fully ripe stage]", explains Isabelle

Grechi. “Other levers would require some technical training and financial incentives to make it easer for growers to adopt them”. The development of a tool to improve support for the conception of approaches that use alternative methods is being continued by the ODACE project which is carrying on where ECOVERGER left off.

Introducing nematode-killing ground cover plants to combat the grapevine fanleaf virus vector

> *It is quite clear that there is not just one, but a number of levers to be implemented*

The grapevine fanleaf virus, which is transmitted by the *Xiphinema index* nematode, causes major damage in the wine-growing industry. The infected plants wilt and the disease can remain in the soil as long as the grapevine remains there: growers are therefore obliged to completely remove any affected vines. The BIOCOU project concerns the use of Fabaceae such as sweet clover, red clover, trefoil and sanfoin, for their antagonist effects on this nematode. “Sanfoin and trefoil extracts are more effective under laboratory conditions, their root extracts can achieve 80% effectiveness on this nematode”, explains Lise Negrel, researcher for Multifolia. “Whereas in the vineyards, the use of Fabaceae alone as an intercrop did not turn out to be effective. However, spreading Vitifolia granules [granulated sanfoin marketed by Multifolia] after replanting the vines has managed to reduce the return and spread of the disease”. The high potential of these granules is starting to be thought of as being a potential long term solution. Olivier Lemaire, INRAE researcher, explains: “Since 2018, we have been monitoring the physical recontamination of plots where *Xiphinema index* was known to be present. These fields were left fallow with alfalfa for two years. Then at the start of the third year, we spread 10 tonnes per hectare of sainfoin granules before planting new vines during the spring of that year. The results were promising”. There are still some aspects to be clarified, such as the maximum spreading dosages or the influence of soil-climate conditions on the effectiveness of the granules. “It is quite clear that there is not just one but a number levers that will need to be implemented to be able to deal with fanleaf virus without using phytosanitary products. Nematode killing granules and plants are proving to be a viable and easy to implement lever in this context.

Flax retting in Normandy. *Photo: © Jean Weber, INRAE*

▶ **The views of Thierry Goujon,** Director of the Terre de Lin Cooperative

"Combining different levers throughout the production cycle"

Thierry Goujon, director of the Terre de Lin Cooperative which includes 700 Normandy flax producers, tells us about some progress made in the sector: "We are working on adapting our growing methods: we prioritise mechanical weed control or false-sowing techniques to deal with weeds. These methods are not perfect, especially since they are a highly dependent on climate conditions, but they do allow us to reduce our reliance on phytosanitary products". Another lever: the use of resilient varieties developed through genetic innovations, has helped us to cope with fusarium which was rife around twenty years ago. The cooperative has also changed its seedling preparation methods. A steam disinfection technique has been developed to eliminate pathogens. "Its like a pasteurisation process for seedlings which complies perfectly with the applicable health standards. For 2021 we disinfected between 7,000 and 8,000 tonnes of seedlings in this way without having to use any phytosanitary products. The results are promising, which is helping us to strengthen the sector's reputation as the source of a quality natural product: a clear marketing advantage.

Colony of green aphids on a peach tree. *Photo: © Yvon Robert, INRAE*

Fruit fly damage on mangos. The ECOVERGER project.
Photo: © Isabelle Grechi, CIRAD

Webinar 9

Looking beyond the fields

The Cantal landscape near to Salers. *Photo: © Nicolas Bertrand, INRAE*

Session programme

▶ **RESCAPE**	RESistance of agricultural landSCAPEs to pesticide transfers in soils and living organisms (Pesticides 2014)	Céline Pelosi, Colette Bertrand (INRAE UMR EcoSys) Clémentine Fritsch (Chrono-Environnement CNRS/UBFC)
▶ **RESYST**	The REsistance of Tropical agricultural SYStems to the reduction of pesticide pollution pressure on the watershed scale (Pesticides 2014)	Charles Mottes (CIRAD)
▶ **TRAJECTOIRES**	Hereditary trajectories and the use of pesticides in major agricultural territories (Pesticides 2014)	Carole Barthélémy (University of Aix Marseille) Gilles Armani (University Lumière Lyon 2)
▶ **ALT'CIM**	Heading towards zero phyto in Normandy cemeteries (JEVI 2016)	Flore Diradourian (ASTREDHOR)
▶ **ALTHERCOL**	Weedkiller alternatives in urban allotment gardens. (JEVI 2016)	Francesca Di Pietro (University of Tours, UMR CITERES)

You can see the complete recording of this session on the symposium's YouTube channel

Looking beyond the fields

Reducing dependency on phytosanitary products will require the management of smaller crop volumes per field and some major changes to growing practices. However, the issues involved in the implementation of this dynamic in agricultural or non-agricultural regions are not going to be played out uniquely from an agricultural point of view. By "looking beyond the fields" we can see that they are an integral part of their surrounding landscape, with their own specific characteristics, restrictions and opportunities; and that the use of phytosanitary products also depends on the influences of the various parties involved (farmers, local government, citizens, etc.).

Planning for a regionally coordinated phytosanitary product policy

"The prairies and hedges will therefore retain a major importance in the landscape"

A certain portion of the phytosanitary products used on a given field will affect the neighbouring ones via the atmosphere and/or the ground. The RESCAPE project is concerned with classifying these transfers on a countryside scale by measuring the contamination of farmland soil and the exposure of non-target wild-life to pesticides. "We have worked on 60 landscape slots representing different aspects of the farmland patchwork, with varying degrees of hedging or proportions of untreated zones [prairies and organic farming land]", explains Céline Pelosi, INRAE researcher. Fungicide, pesticide and herbicide residues were not only looked for in soil samples but also in the tissues of earthworms, ground beetles and small mammals. "We have been able to demonstrate the almost systematic contamination of all of the samples we studied: at least one pesticide residue was detected in each one of the soil or small mammal samples, and very frequently we found a cocktail of fungicides, herbicides and insecticides, such as epoxiconazole, diflufenicanil and imidacloprid". 40% of the soil samples contained concentrations higher than the chronic toxicity threshold for earthworms listed for at least one pesticide product. "We also completed a risk assessment for pesticide mixtures, concluding that there is a high risk factor for almost half of the soil samples studied, including the majority of conventional farmland samples but also a good number of the untreated zones. Fortunately these untreated zones are significantly less contaminated, which highlights their potential as "refuge zones" protecting living organisms from pesticides. The prairies and hedges which have not been treated with phytosanitary products will therefore retain a major importance in the landscape. Especially since a computer model of the atmospheric dispersion of pesticides has shown that the hedges act as a "barrier" restricting such products from reaching neighbouring non-target zones. Consequently, researchers now recommend that agro-ecosystems are managed on a countryside scale and not just in terms of individual fields, thus increasing the number of untreated zones and agro-ecological infrastructures such as hedges and shrub plantations. This management approach will require the effective coordination of the various parties involved in managing the countryside.

The importance of such coordination was also highlighted by the RESYST project under the leadership of Charles Mottes, researcher at CIRAD. The main interest of the project is to encourage change in the agricultural practices used in and around the Galion watershed in Martinique in the hope of restricting the pollution of the river. "Based on the observation that the island's three main industries [sugar cane, bananas for export and diverse productions for the local market] operate in isolation to each other, we needed to coordinate everyone involved so that they could work together to construct fifteen new region-wide innovations, notably including new grassing over techniques, that we then tested in real-world situations". The stated ambition: "to show that the countryside can provide resources and levers that the individual industries cannot come up with in isolation", by constructing new ties between farmers, by promoting the land and its resources, whilst always taking the specific nature of each local environment into consideration.

The work of the Trajectoires project highlights the need to adapt to the local restrictions encountered by professionals

Successful transition by allowing for socio-cultural factors

The implementation of new agricultural practices will also be determined by socio-cultural factors. The Trajectoires project lead by Carole Barthélémy, professor-researcher in sociology, concerns the sociological causes that decide whether pesticide use reduction initiatives work or not. They have identified three trajectories that may or may not lead to change: the hereditary trajectory of changing phytopharmaceutical product usage from generation to generation, the material trajectory of the development of the phytosanitary products themselves and the trajectory of land-use transformations. “For the four agricultural regions that we studied, those who answered often spoke of the post-war period when phytosanitary products were being used systematically. Since then there has apparently been a rise in awareness, we were able to observe that individual or collective initiatives, both old and new, are seriously questioning this accepted norm”. Trajectoires also raised the question of the effectiveness of the CERTIPHYTO training programme, the mandatory passport for all operatives who use phytosanitary products. “We wanted to identify the limits of this system, that was conceived with a philosophy of uniformity although addressed to social groups with very different cultural and economic capitals: some consider this training to be pointless whilst others can’t afford to put its teachings into practice”, says Carole Barthélémy. These works highlight the need to adapt to the real local limitations that are encountered by professionals.

The ALTHERCOL project is also interested in the links between the socio-demographical origins of allotment gardeners and their motivations to implement alternative weed control strategies. “The survey looked both at the organisations that manage these gardens (associations or local councils) which promote pesticide-free gardening without wanting to leave too much room for wild or spontaneous flora, and on the gardeners themselves, who could be divided up into 4 classifications depending on their pesticide use”, explains Francesca Di Pietro, professor-researcher at the University of Tours. “Our surveys showed that the use of pesticides depended on the proximity between the gardeners’ rurality, where they learned to garden during their childhoods, and their current residence. Additionally we discovered that young gardeners coming from built up areas had a higher tolerance for wild or spontaneous flora”. And so, to help these gardeners on their way to reducing their use of phytosanitary products, the ALTHERCOL project proposes the implementation of training programmes, the diffusion of printed information and the organisation of debates between gardeners to encourage the exchange of knowledge and good practices.

Upstream from one of the RESYST project’s pilot zones: the Galion drainage basin
Photo: © Charles Mottes, CIRAD.

> *A large majority of users support the use of such vegetation since it encourages biodiversity and makes cemeterie*

Encouraging biodiversity in cemeteries

The ALT'CIM project aims to develop an alternative vegetation for cemeteries that will not require the use of phytosanitary products. A number of different plant species and introduction techniques were tested on eight cemeteries in Normandy. Grassing over the paths with trample-resistant Ray-Grass, herbaceous plants like camomile between and behind the tombs, wild flower prairies and pre-planted rolls are all examples of plantations that can be maintained easily without phytosanitary products. The extent to which these new planting schemes were accepted by cemetery users was surveyed, revealing that a large majority of users support the use of such vegetation since it encourages biodiversity and makes cemeteries more attractive, even if this did involve more work for the landscape managers.

Hommet d'Arthenay cemetery (50) – Hydroseeding. Alt'Cim. *project Photo: © David Philippart, FREDON Normandie*

Farmlands of the ZAPVS (Zone Atelier Plaine & Val de Sèvre), crop field and neighbouring hedges. Rescape project. *Photo: © Céline Pelosi, INRAE*

CONCLUSION

Three visions of the Ecophyto Research & Innovation Symposium

> *It was very important to take the time to share the results...and to debate the performances that might be achieved using the levers proposed by these projects*

The views of Marion Bardy,
deputy to the Food and Agriculture Ministry
vice-director of research, innovation
and international cooperation

This symposium was organised within the framework of the Ecophyto plan, which was launched in 2008 in response to high public expectations concerning the preservation of human health and the environment. The plan's ambitions have been regularly revised and we are now in the Ecophyto II+ plan. The projects presented during the symposium, which took the highly original form of a series of themed webinars, have been financed by the plan and are opening up new ways forward by proposing different phytosanitary product usage reduction strategies, either in an agricultural or JEVI (Jardins, Espaces Végétalisés et Infrastructures - Gardens, Parks and Infrastructures) context. "It was very important to take the time to share the results of these projects through this symposium and to debate the performances that might be achieved using the levers proposed by the projects as well as to identify the most suitable ways to implement them in the real world" explains Marion Bardy. "Additionally, the discussions that continued throughout these webinars will serve to feed reflections on the different orientations that the plan's Research & Innovation axis could take", she adds. These research projects contribute not only to knowledge but also to structuring research communities around these subjects. And what's more they are being expressed through projects from other programmes financed by the Ecophyto Plan and which are also supporting this agro-ecological transition such as the "Grow and protect differently" and "Maturation" calls for projects. And finally, it is important to spread knowledge of all that is produced, not just on a nationwide scale. "As for research, the INRAE has set up a European alliance around the question of constructing a pesticide-free agriculture. And as for the institutions, we are currently working with our European opposite numbers as part of the European Standing Committee on Agricultural Research SCAR. ".

The views of Xavier Reboud,
president of the "Research & Innovation"
Scientific Orientation Committee (CSO R&I)

The subjects covered by this series of webinars fits perfectly with the scientific policy of the CSO R&;, notably the promotion of the acquisition of the knowledge that has been identified as essential for the achievement of the Ecophyto plan objectives. The session formats were set out in advance such that they present the projects financed by the Ecophyto plan in an informative way, and such that

> *Some of the projects presented over the sessions managed to initiate partnerships to develop the marketed alternative solutions*

useful exchanges between those presenting and those listening could occur. "We decided to attach an external viewpoint for each session, by inviting one or a number of guest experts. This allowed us to gain some perspective on the subjects" says Xavier Reboud. Some of the projects presented over the sessions managed to initiate partnerships to develop the marketed or almost-marketed alternative solutions, and we clearly noted that the promising content of some of the projects could also have taken things further. "Market repercussions will require the conjunction of two factors: the first being the expression of high expectations, the second being the presence of project supporters who are prepared to take on the risk associated with these opportunities. So it remains crucial to link these partnerships together as early as possible in order to ensure that both the scientific and the practising communities can work together", he says. Another positive aspect that was highlighted was the informational format of these sessions, the diffusion of which could reach a broader public and thus encourage wider partnerships. The symposium resources are available to the general public: the videos on the symposium's YouTube channel and the webinar brochures on the symposium's EcophytoPIC page.

The views of Lucile Gauchet,
Ecophyto plan project leader for the Ecology Transition Ministry's General Planning, Housing and Nature Directorate

> *What really hit me was the diversity of the propositions, the new approaches for finding alternatives at different levels*

Amongst the projects presented during the symposium, the spotlight was placed on alternative techniques, such as biocontrol, to replace the use of synthetic phytosanitary products. "What really hit me was the diversity of the propositions, the new approaches for finding alternatives at different levels highlighting the various levers that will need to be incorporated into the technical routes and also promoting reflections beyond the management of individual fields" she says. Following this, it should be noted that the presented projects all come from the programmes launched a few years ago (2015 - 2016), which really demonstrates the anticipatory work of the Research & Innovation axis. "The work of the R&I CSO supported by the events hub, contributes to structuring the scientific community on the subject of reducing pesticide use". It should also be noted that the Ecophyto plan Research & Innovation axis also covers other crucial and current aspects such as health and environmental impact through other research proposition calls which were not presented at this symposium. Lucile Gauchet also points out that France has done pioneering work by setting the ambitious Ecophyto Plan objectives to reduce the use of phytopharmaceutical products, requiring further research efforts, and this has lead to an increase in the resources allocated to this axis via Ecophyto II+. The effects that are leading us to change in the here are now are becoming evident, as the European Union has also set ambitious goals via the Green Charter through the "from the Farm to the table" and "EU biodiversity" strategies." And finally there is the question of the scope of these research projects, especially in terms of passing on the necessary know-how to those concerned: farmers, consultants, public institutions etc. It would now seem essential to reconsider the resources necessary to make the best use of these leverage effects.

Appendix

Project description sheets

ACAROSOL
ALT'CIM
ALTHERCOL
AM&BAS
AttractMyFly
BIOBOT
BIOCCYD
BIOCOU
BIOLIM
BIOTI-VIGNE
DAS-REVI
DESHERBAL
DICABIO
ECOVERGER
ENFIN !
FLEUR
LIPOCONTROLE
MacroPlus
MDRisque
MilPomBio
NABUCO
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ACAROSOL

Biological control of spider mite on Solanaceae

Launch year: 2015
Completion year: 2018

Partners
Institut Agro-Montpellier SupAgro ; INRAE UMR CBGP ; CTIFL-Ballandran ; SONITO ; Koppert

Scientific manager
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Financing
Total cost of the project: € 527,989
Ecophyto grant: € 90,347

Key words:
Spider-mite; Biological control; Tomatoes; Biodiversity; Agro-ecological transitions; Management via macro-organisms

Context and main objectives

Spider mite can cause significant crop damage on tomatoes (as much as 65%), resulting in the need to use pesticides, which is highly unsatisfactory in environmental, financial and social terms. Predatory spider mites, Phytoseiidae, are the most effective natural enemy of the pest spider-mites. However, these auxiliaries have little effect on tomato crops because of the plant's physical and biochemical characteristics. This project is therefore concerned with identifying endemic European predators that are suited to Solanaceae and then testing their predatory characteristics in the laboratory and in experimental greenhouses. The effects of predators present in the non-cultivated areas of crop-fields were also studied. The main hypotheses tested were as follows:

1. As yet unidentified predators exist that are both endemic to Europe and suited to the tomato plant.
2. They have useful predatory characteristics.
3. They can be raised in large quantities for release within the crop fields.
4. They are naturally present on the plants in the non-cultivated areas bordering the fields.

Principal results and interest in relation to the Ecophyto plan

Generally speaking, the project results met these expectations regarding the biological control of pest spider mites on tomato crops; notably the most widespread species, *Aculops lycopersici*, which causes tomato russet. The work was organised over three major phases.

1. Identification and classification of "useful" and original biodiversity

A number of field missions were completed; more than 100 plants (33 species of Solanaceae) were collected in France and Morocco. Four main species of Phytoseiidae were found.

Laboratory assays identified the predatory differences between the species and populations studies. One predator species *(T. (A.) recki)* was selected to continue the tests on a broader scale. Additionally, molecular and morphological analysis demonstrated the relations between these characteristics, the host plants and the predatory capacities of the identified *(T. (A.) recki)* populations.

2. Classification of the predator's dispersion capacities on tomato plants.

Laboratory tests have shown that females of the predators move around freely on the stems, and that they are attracted by food (pollen or *Aculops lycopersici*). However, the results of preliminary tests on whole plants were deceptive, showing low predator dispersion along the stems of the tomato plants. Consequently the perspective of large scale breeding by an industrial partner has been abandoned and an alternative solution had to be found.

3. Survey of biological effectiveness in experimental conditions under glass.

With large-scale breeding now considered to be impossible, two other predator "introduction methods" were studied:

Companion planting: plants serving as a reservoir for natural enemies. During the predator diversity survey, we have shown that the selected species was present in very large numbers on plants of the mint family. Consequently, we have decided to study the impact of the presence of mint as a factor in the control of pests present on nearby tomato crops. Experiments showed that *T. (A.) recki* spreads from the mint to the infested tomato plants both on isolated leaves or whole plants. A planting density of 6 tomato plants to 3 mint plants seems sufficient to ensure this dispersion. *T. (A.) recki* seems to spread more effectively when the tomato plants are infested. This result opens up the perspective of pest control approaches involving relay plants.

Inoculation via predator introduction; considering the presence of large numbers of the predator on mint leaves, branches of mint were placed around the bases of the tomato plants under experimental greenhouse conditions. The branches of mint were introduced followed by the inoculated pests one week later (*A. lycopersici*). The greenhouse experiments demonstrated an unprecedented and satisfactory level of biological control over *A. lycopersici*.

It should be noted that the selected predator species has also been observed in the sample non-cultivated zones surrounding outdoor tomato crop fields which also opens up the perspective of an organic control approach via the preservation of biodiversity.

Prospects for transfer or research

Transfer:

This project has opened up a pathway to the development of two biological control approaches:

- *Use of companion planting.* Our results have opened up new market prospects for (i) nursery owners as the suppliers of companion plants and (ii) companies that supply auxiliaries and/or companion plants already colonised by populations of the desired auxiliary.
- *Biological control by increase.* Our results have provoked interest from biocontrol companies which would be able to develop additional testing programmes to prepare for marketing. The economical stakes are very high given the high global demand for biological control solutions for *A. lycopersici*.

Research:

The project results are very positive because (i) this is the first time that a predator species has turned out to be so effective in controlling *Aculops lycopersici* and (ii) the fact that new biological control prospects using companion plants can now be envisaged. This work is being continued in an Ecophyto Maturation project, BioLycTom, with the more operational objective of testing (i) the ideal conditions for the introduction of Phytoseiidae, (ii) the impact of the predator on other tomato pests, (iii) the positive and potentially negative effects of the presence of companion plants inside crop-fields in biological, technical and socio-economical terms.

Publications and scientific symposia:

Technical workshops and scientific symposia:

- Gard B., Douin M., Tixier M.-S. 2021. **A new hope for the biological control of *Aculops lycopersici* (Acari: Eriophyidae) with the predatory mite *Typhlodromus* (Anthoseius) *recki* Wainstein (Acari: Phytoseiidae)**. International Congress of Entomology, Helsinki. Planned for 2020 and postponed to 2021.

Scientific publications:

- Tixier M.-S., Douin M., Kreiter S. 2020. Phytoseiidae (Acari: Mesostigmata) on plants of the family Solanaceae: results of a survey in the **south of France and a review of world biodiversity.** Experimental and Applied Acarology 81 : 357-388
- Tixier M.-S., Perez Martinez S., Douin M. 2020. **Markers for life traits: the example of variations in morphology, molecular and amino acid sequences within the species *Typhlodromus* (Anthoseius) *recki* Wainstein (Acari: Mesostigmata: Phytoseiidae).** Journal of Linnean Society accepted. https://doi.org/10.1093/biolinnean/blaa103
- Tixier M.-S., Douin M., Oliva R., Gonzalez L., Pount B., Kreiter S. 2020. **Distribution and biological features of the species *Typhlodromus* (Anthoseius) *recki* (Acari: Phytoseiidae) on *Tetranychus urticae*, *T. evansi* (Acari: Tetranychidae) and *Aculops lycopersici (Acari: Eriophyidae)*.** Acarologia Acarologia 60 (4) : 684-697. DOI: 10.24349 / acarologia / 20204396

ALT'CIM

Heading towards zero phyto in Norman cemeteries

Launch year: 2017
Completion year: 2021

Partners
ASTREDHOR ; FREDON Normandie

Scientific manager
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Financing
Total cost of the project: € 193,845
Ecophyto grant: € 145,383

Key words:
JEVI; Zero-phyto; Integrated landscape management for cemeteries; Planting solutions; Restricted spaces; Landscaping memorial sites

Context and main objectives

The use of phytosanitary products in cemeteries will become illegal in 2022. Experience has shown that this kind of change can be very difficult for this type of site.
These environments involve a lot of hard surfaces, making it very difficult to tolerate even the smallest weed. This means that weedkillers are used massively to keep the sites "clean".
The project objectives are as follows:

1) Define and classify the different types of cemetery in Normandy and the specific issues that they face whilst also listing all of the currently available approaches and solutions.
2) Promote changes to cultural perceptions of cemeteries through specific communication and awareness initiatives.
3) Support local governments in their transition to a sustainable zero-phyto approach for their cemeteries by testing planting solutions that would reduce time spent on maintenance and encourage the acceptance of vegetation
4) Respect the purposes of the sites
5) Plantations must respect and improve both landscape and heritage.

Principal results and interest in relation to the Ecophyto plan

The plantation tests and trials were completed between autumn 2017 and spring 2018. These tests took a number of different forms: grassing over, herbaceous planting, pre-planted solutions and mixed wildflower seed.

The grassing over techniques were generally tested on the cemeteries' main or secondary footpaths. Hydro-seeding was used for the sites where grassing over was more generalised. Whilst complicated to implement, this method has the benefit of assisting the initial growth stages. The results were rapid and encouraging, although the costs were higher than for more traditional grassing over techniques.

The grass was mostly seeded when it was to be used on the main, secondary and transversal paths.

Outside of any specific restrictions (localised shade) and within the scope of our trials, the best ground cover results were achieved with Top Green Euronature TPV1 and Routemaster 3 grasses. These varieties/mixes caught up with the performances of the hydro-seeded areas after only a few months.

Where the areas between the tombs were too narrow for grass, other solutions such as herbaceous planting were tested. They were planted as single species or in modules of a number of species. Varieties were tried on a number of sites. The study analysed the ground cover percentage, invasive impact, aesthetics... The following table provides a summary of the results.

	Establishment	Competition with weeds	Invasive impact	Aesthetic
Centaurea simplicaulis	+++	++++	++	++++
Prunella vulgaris	++++	++++	+++	+++
Acaena microphylla	++++	++++	++++	+++
Frankenia laevis	+++	+++	++++	++
Muehlenbeckia complexa	++	+++	++	++
Erigeron karvinskianus	+++	++++	+	++++
Chamaemelum nobile	++++	+++	+	++++
Polygonum affine 'Dimity'	+++	+++	++++	++++
Sedum album 'Coral carpet'	++	+++	+	++++
Sedum spurium 'Woodoo'	++	+++	+	++++
Origanum vulgare 'Compactum'	+++	++	+	+++

Summary comparison of the most effective herbaceous varieties based on the criteria of adaptation and development, ALT'CIM test programme. *Credits: Agnès Langlois, ASTREDHOR*

Observations have identified some species that are interesting both for their adaptability and ground-cover capacity: *Ceantaurea simplicaulis, Prunella vulgaris* and *Chamaemelum nobile.*

Arnières / Iton (27), *Chamaemelum nobile. Photo: © David Philippart, FREDON Normandie*

Régneville / mer (50): *Prunella vulgaris. Photo: © David Philippart, FREDON Norrmandie*

Vaucelles, Caen (14): *Centaurea simplicaulis. Photo: © David Philippart, FREDON Normandie*

On the contrary, others have displayed a highly invasive nature: *Acaena microphylla, Frankenia laevis* and *Polygonum affine 'Dimitry'.*

A specific number of species were tested in association, planted between high tombs, which potentially invasive plants would not be able to cover. Independent to the plant combinations,

Vaucelles, Caen (14), *Polygonum affine 'Dimitry'. Photo: © David Philippart, FREDON Normandie*

Vaucelles, Caen (14), *Frankenia laevis. Photo: © David Philippart, FREDON Normandie*

Hernaria glabra, Polygonum affine, Thymus serpyllum, Saponaria ocymioides and Stachys byzantina provided the best results.

Other planting solutions such as pre-grown rolls were used on three sites. These were installed around the tomb stones. The pre-planted rolls or mats includes both the plants (sedum or herbaceous) and the planting media necessary for their growth with or without coco matting to hold it all together. These systems are very quick to install and provide immediate aesthetic results. These were compared to individually planted sedum.

The pre-grown rolls are a very effective planting solution, since they cover the ground directly thus avoiding the emergence of spontaneous plants and making establishment easier. The sedums have the advantage of being closer to the ground than the herbaceous plants which makes them more acceptable near to monuments. Whilst the planted rolls have shown quicker and more effective results, they will be kept for restricted and strategic areas because of their high cost.

Regnéville / mer (50): herbaceous mat. *Photo: © David Philippart, FREDON Normandie*

Regnéville / mer (50): sedum mat. *Photo: © David Philippart, FREDON Normandie*

Wildflower mixes were tested in different locations over three sites. Generally speaking, wildflower mixes are recommended for 2-3 years. Beyond this the mix loses it diversity, and a new sowing operation should be done.

Prospects for transfer or research

Transfer:

The results obtained by the ALT'CIM project will provide a source of guidance for local councils who want to go zero-phyto in their cemeteries. The diversity of the solutions tested means that one or more solutions can be proposed for each of the complex spaces of these memorial sites: main and secondary footpaths, inter-tombs, inter-rows, waiting areas, decoration of some monuments...

The project has shown that grassing over is one of the easiest solutions to implement. However, given that configurations are different for each cemetery, planting with herbaceous plants or sedums has also been shown to be of interest.

Publications and scientific symposia:

Technical workshops and scientific symposia:

- The "**Que vont devenir les cimetières en Normandie, et ailleurs ?** " symposium, 30-31/08 and 01/09/2017. CCIC Cerisy-la-Salle (FLORYSAGE Intervention), by the 'Fédération Normande pour la sauvegarde des cimetières et du patrimoine funéraire'.
- The FLORYSAGE 2017 "**Paysage et entretien des cimetières**" symposium, 29/11/17. Eure Departmental Council.
- "**Vers le zéro-phyto dans les cimetières de la Manche**" half-day conference. Manche Departmental Council, February 2018.
- "**Végétalisation des espaces contraints : massifs de voiries et cimetières**" technical workshop, 06/04/18. Jean Rostand Professional College (Offranville, 76).
- CAUE14/FLORYSAGE "**Quand le zéro-phyto nourrit le projet de paysage"** technical workshop, May 2018.
- "**Zéro-phyto dans l'espace public : pour aller plus loin que la réglementation"** Technical workshop, Caen la Mer and Plante & Cité, in partnership with FLORYSAGE, Hortis, Unep, September 2018.
- ASTREDHOR Open Days, Seine-Manche and Pays de la Loire, September 2018.
- Second "Champs d'Innovation" forum organised by the Normandy Chamber of Agriculture, Caen, 18/10/18.
- "**Le zéro-phyto au quotidien dans ma commune : je végétalise le cimetière**" Technical half-day workshop, partners: FREDON/Le Havre Seine Métropole / FLORYSAGE, April 2019.
- Intervention at the Salon Vert: "**Sensibiliser et accompagner les collectivités dans la végétalisation de leur cimetière, afin de faciliter la transition zéro-phyto"**, Saint-Chéron, 23/09/2020.

Prtactical / educational articles:

- Paris Normandie, October 2017: "**Le « zéro pesticides » arrive au cimetière de La Bonneville-sur-Iton**".
- Ouest France, October 2018: "**Quand on dit zéro phytos, c'est zéro**".
- Le Lien Horticole, January 2018: "**Cimetières normands : Accompagner les collectivités dans la transition zéro-phyto de leur cimetière"**.

Other practical works:

- "**(Ré)aménager et entretenir les cimetières de Normandie**". Brochure published in 2018.
- "**Diagnostic du paysage funéraire normand**". Brochure published in 2019.
- "**Passer au zéro-phyto dans mon cimetière : la solution de la végétalisation**". Unpublished brochure.
- "**Info'Charte" n°13 and n°15**. FREDON Haute-Normandie (April and October 2018).
- " **Comment (Ré)aménager et entretenir les cimetières en Normandie**?" . Unpublished handbook.
- "**Végétaliser son cimetière pour le gérer sans produit phytosanitaires**", FLORYSAGE Technical data-sheet, (For members, 2020).
- **ALT'CIM** "Information kit" (Poster, panels, flyers).

ALTHERCOL

Weedkiller alternatives in urban allotment gardens

Launch year: 2017
Completion year: 2020

Partners
University of Tours UMR CITERES; France Nature Environnement Centre Val de Loire (FNE-CVdL)

Scientific manager
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Financing
Total cost of the project: € 174,760
Ecophyto grant: € 119,760

Key words:
Allotment gardens; Horticultural practices; Pesticides; Socio-demography; Spontaneous flora; Gardeners; Motivations; Representations; Urban biodiversity; Biocontrol

Context and main objectives

Two thirds of pesticides on the non-agricultural market are aimed at private gardens; pesticides for amateur gardeners have an impact on a vast majority of the population. In allotment gardens, as with private gardens, people use the whole range of horticultural practices, from the consumption of vast quantities of pesticides to the most environmentally friendly techniques. But due to the nature of collective gardens, they can serve as an ideal place for gardeners to experiment and pick up or learn about biocontrol methods. We have postulated that **allotment gardens are an opportunity to encourage the appropriation of biocontrol methods and the abandon of pesticide use in amateur gardening.** It would seem like an important action to assist gardeners in making this transition towards more environmentally respectful practices and towards the adoption of biocontrol methods, especially given that pesticide stocks could still last for a few more years and that inappropriate alternative practices are still sometimes implemented.

Principal results and interest in relation to the Ecophyto plan

We have mobilised specific methodologies for:

1) Identifying all of the allotment gardens in two urban centres: Tours and Orléans (see photo and maps)
2) Interviewing allotment garden managers (17 interviews)
3) Interviewing gardeners (30 initial interviews and 150 questionnaires)
4) Learning about the structure and spontaneous flora of allotment gardens (150 allotment structure surveys, 150 botanic allotment surveys, 150 botanic footpath surveys)

Our survey showed that, according to the questionnaires completed by gardeners in allotment gardens, ecological gardening practices are increasingly wide spread, and most of the garden managers have committed to these new practices through regulations, training and discussions with

Allotments and footpaths, two very distinct spaces within family gardens. *Photo: © F. Di Pietro, University of Tours*

the gardeners. However, for many of them, this change can be reduced to simply replacing one set of products with other, less toxic, ones (example: 'bordeaux mixture). Additionally allotment gardens are considered to be vegetable production areas by their managers. Even if auxiliary life is accepted or even encouraged there, "weeds" are the subjects of hostility since they are considered to be completely contrary to the production function.

Family garden allotments can be intensively cultivated spaces.
Photo: © F. Di Pietro

Initial interviews with allotment gardeners allowed us to classify the diversity of the gardening practices in use as well as the motivations and attitudes of the gardeners in relation to this activity. More specifically we have also defined the spectrum of synthetic pesticide usage and the implementation of alternative methods. We have highlighted a large number of links between the positions of individual gardeners on this spectrum, their type and their socio-demographic profile. This classification allowed us to target the problems raised by each type of gardener and imagine ways to resolve them. The use of synthetic pesticides is not influenced by socio-demographic factors, but related to where the gardener grew up (countryside) and their identification with the countryside. However, age and social class, as well as where they grew up influence not only how they accept the wild flora and fauna, but also how they manage the soil, fertilisation and choice of vegetable varieties as well as their openness to changing habits. These elements were confirmed by a larger sample of 150 allotment gardeners that were interviewed via questionnaires.

Structural analysis of the gardens and the wild plants in the allotments and footpaths have shown that **the internal structure of the allotments** varies significantly between the different sites and gardens, and the associations have a significant influence on the number of trees planted by the gardeners. Women tend to cultivate less surface area than men. The wild plants found in the footpaths correlate strongly with gardening practices and the structure of the gardens: the share of perennial species increases significantly with the proportion of hedges and, rather surprisingly, diminishes with the proportion of non-cultivated areas and the proportion of vine planting. **The wild plants found in the allotments** vary in terms of the number of ornamental trees (related to perennial species). Additionally, the gardening practices, especially hand tilling of the soil and use of 'bordeaux mixture', have a major influence on the specific variety and proportion of perennial species present in the allotments and footpaths.

Prospects for transfer or research

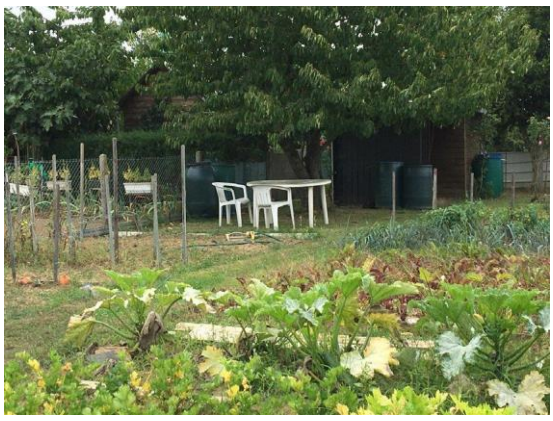
Transfer:

4 orientations were found to be useful for supporting the transition of gardeners towards more environmentally friendly practices:

1) Organise "debates" involving gardeners and a mediator: a priority for those gardeners who use the most synthetic pesticides.
2) Distribute printed information (even if this might exclude gardeners who are illiterate or do not speak much French) and on-line content (even if this is largely the domain of gardeners who do not use a lot of pesticides).
3) Train gardeners on the basis of the techniques they already use.
4) Train the garden managers on biological gardening and inform them about other aesthetic garden forms.

Research:

Research perspectives point towards the conditions surrounding the place where food and ecological objectives meet (conservation of urban biodiversity). The results are currently being analysed in detail and published in scientific journals. Within the framework of the Ecophyto programme events and the ALTHERCOL project, exchanges have been able to form ties with the Orléans INRAE (Jérome Rousselet). These exchanges have resulted in a partnership for the OSTils project (direction: Jérôme Rousselet), one of the major chapters will cover private gardeners' reasons for their planting choices and tree species preferences. The financing for this project has been approved and it has now been launched.



Organise "debates" involving gardeners and a mediator: a priority for those gardeners who use the most synthetic pesticides.
Photo: © F. Di Pietro

Publications and scientific symposia:

TECHNICAL WORKSHOPS AND SCIENTIFIC SYMPOSIA:

- Di Pietro F. (2020), hosting of Workshop 3 (**Pourquoi jardinez-vous ?**), Biocontrol for amateur gardeners symposium, Paris - La Défense (France), 11 February 2020.
- Di Pietro F. & Poiré M. (2018), **Évolution des fonctions et des pratiques horticoles dans les jardins familiaux,** oral presentation, Biocontrol symposium. Les nouvelles pratiques pour un jardinage au naturel, Fondettes (France), 13 December 2018

SCIENTIFIC PUBLICATIONS:

Published

- **Faire nature en ville. Les jardins familiaux entre production alimentaire et loisirs : quelle place pour la biodiversité ? Attitude des gestionnaires vis-à-vis des pratiques de jardinage, de la faune et de la flore spontanées.** Di Pietro F. & Poiré M., Carrière JP. *et al.* (Eds.), 2021. Published by Harmattan.

Pending publishing

- Dynamiques Environnementales n°47: **Nature spontanée, nature maitrisée : quelle place pour la biodiversité dans les jardins familiaux ?** Poiré M. & Di Pietro F. (submission)
- Espaces et sociétés : **Motivations, pratiques de jardinage et rapport à la nature dans les jardins familiaux.** Di Pietro F., Gardair E. & Poiré M. (to be published in early 2023)
- Nouvelles Perspectives en Sciences Sociales n°17(2): **Rapport à la nature et contrôle de l'alimentation dans les jardins familiaux.** Di Pietro F., Gardair E., Poiré M. & Gosset S.

Two other science papers are currently being prepared, one discussing the questionnaire results, the other on the botanical analyses.

Practical / educational articles:

- Microscoop, Hors-série n° 18 (October 2018, pp14-15) : **Jardins collectifs en ville : nature spontanée, nature maîtrisée.** Di Pietro F. & Poiré M. Link to document: http://www.dr8.cnrs.fr/CNRS-Hebdo/Documents/542/Document.aspx

AM&BAS

Agro-messages and treatment of insect pests in seedling production and large-scale crops

Launch year: 2015
Completion year: 2017

Partners
INRAE IEES Paris; ARVALIS; FNAMS; M2i

Scientific manager
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Financing
Total cost of the project: € 277,432
Ecophyto grant: € 100,000

Key words:
Tychius aureolus; Ostrinia nubilalis; Alfalfa; Sweetcorn; Chemical landscape; Attraction; Biocontrol; Chemical mediators; Kairomone; Mass trapping; Improving varieties

Context and main objectives

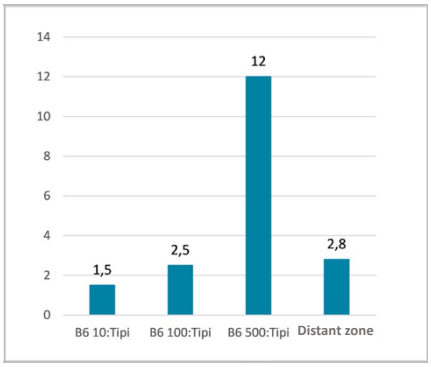
Following the ban on the majority of conventional insecticides due to their negative impact on public health and the environment, a number of industry sectors now find themselves in a technical no-man's land, especially seedling specialists and large-scale crop producers. These producers will now have to evolve in keeping with the Ecophyto plan, but with their priorities being to maintain the performance, quality and sustainability of their operations.
Chemical mediators are one of the biocontrol solutions which can be used as a viable alternative to conventional insecticides. The objective of the AM&BAS project is to use them to control European corn borer and alfalfa weevil populations. The project could be seen as a detailed investigation of the process of taking a proposal from the laboratory to the field; it aims to validate the potentials of kairomone-type chemical mediators for the control of two pest species. A priority was placed on developing biodegradable diffusers to add a further technical benefit. The project involves an industrial producer, an inter-professional, technical institutes and an INRAE research laboratory working together to explore the attractive potentials raised by previous research programs.


Trials with INRAE diffusers
Photo: © Brigitte Frérot, INRAE

Principal results and interest in relation to the Ecophyto plan

The AM&BAS project continues existing laboratory research work which has shown that insect host-plant location and recognition processes are very closely related to the olfactory detection of specific chemical signals emitted by the plant.
There is no doubt that host-plants attract fertilised female corn borers and that fruiting-stage alfalfa plants attract *Tychius.* The chemical signals emitted by these plants have been identified. The perception of of certain compounds by the olfactory organs of the two insects has been validated.
Behavioural laboratory tests on European corn borers have validated the attraction of an identified mixture and have provided evidence of an impact on egg-laying. This action has also been confirmed in the field. The attraction of the mixture has not yet been confirmed in the field, but none of the traps tested were suited to capturing the female of the European corn borer. Studies on the perception of the identified molecules by the insects' antennae has shown that there are key molecules that block the insect's localisation of the plant if they are not emitted. The attraction of fertilised females raises a new approach to corn crop protection but this does involves a complex system in which the insect's detection capacities have been demonstrated to be very precise.
A specific mixture appears to attract *Tychius aureolus*, it seems to be able to concentrate insects within the zone where the mixture was diffused. It could be used to establish trap areas. However, for the moment we have not identified a trap suited to the behaviour of this insect. Certain compounds essential to the relation have also been identified, especially for the seedpod-development stage.

The hypothesis of the attraction of pests outside of the field or along its edges has been validated for *Tychius aureolus.* The positive first year results for the European corn borer were not validated for the second year, perhaps due to the influence of excessive population levels.
The diffusion parameters of the INRAE and M2i diffusers have been calibrated. Experiments have allowed us to improve and advance the M2i diffusion matrices. These matrices are biodegradable and easy to use. The proposed attractor for the project was evaluated using a trap that has already proven its usefulness for capturing male moths attracted by a sexual pheromone. In the context of our study, the results were not able to validate the effectiveness of the attractor for capturing females. Further work would be merited.



Concentration of European corn borer eggs - Average number of eggs counted on 10 corn plants within areas close to the B6 attractor at different doses
Image: © Brigitte Frérot, INRAE

Prospects for transfer or research

Transfer:

The INRAE has submitted an Invention and Usable Results Declaration (DIRV) for the Corn plant - Corn borer pairing. Competition in the presence of the host plant must be reinforced but we have all the information necessary to improve the assemblies of synthetic molecules.
A scientific article is currently being drafted for the Alfalfa-*Tychius* pairing.
For both of the pairings, we have identified the key compounds of the process whereby the host-plant is colonised by the insect. This knowledge could be used to select resistant varieties. Knowledge built up on the olfactory relation between the insect and the host-plant will enable the selection of resistant plants that do not emit the key chemical signals.

Research:

Research work on the olfactory relations between pest insects and their host-plants must continue since it could produce new pest insect control solutions or new selection criteria for crop varieties resistant to pest insects. This latter point raised a new research path concerning key chemical signal genes. They could be selected to produce plants that specialist pest insects would be unable to identify. This selection could be advanced as a compatible clean and biological pest control solution. Since the gene maps of most cultivated plants are already known, it would be easy to remove a light alcohol and a terpene.

Publications and scientific symposia:

SCIENTIFIC SYMPOSIA:

- **Perspective de lutte contre les insectes monophages. Du paysage chimique à la protection des plantes.** *AFPP* – Sixth alternative protection methods for integrated production conference. Ene LEPPIK, Centina PINIER, Céline ROBERT, Véronique BIARNES, Pierre TAUPIN, Jean-Baptiste THIBORD, Brigitte FREROT - Lille, on 21, 22 and 23 March 2018.
- **L'attraction des femelles fécondées, une nouvelle voie de recherche pour la protection des plantes cultivées :** AFPP - New contributions to crop protection from pests. Ene LEPPIK, Centina PINIER, Magali GRANGER, Jean-Baptiste THIBORD and Brigitte FREROT – Montpellier on 24 October 2017.

PRACTICAL / EDUCATIONAL ARTICLES:

- **Peut-on leurrer la pyrale avec le parfum du maïs ?** Phytoma, n° 707, 26-29 (2017). Ene LEPPIK, Centina PINIER, Magali GRANGER, Jean-Baptiste THIBORD and Brigitte FREROT
- **L'attraction des femelles fécondées de lépidoptères ; une nouvelle voie de recherche pour la protection des plantes cultivées.** Phloem (2018). Brigitte FREROT.
- **Ravageurs du maïs - Le « parfum » du maïs synthétisé**. Perspectives-agricoles (2018). See with Paloma Cabeza-Orcel – p.cabeza@perspectives-agricoles.com
- **« Tromper les insectes ravageurs des cultures grâce à l'écologie chimique »** (21/06/2018) Article available here

OTHER PRACTICAL WORK:

- Confirmation of previous results of work on the identification of signals emitted by the host-plant: **DIRV** (Invention and Usable Results Declaration) for an attractor for European corn borer
- Optimisation of biodegradable distributors (M2I)
- **2018 and 2019 agricultural shows** with Arvalis and Passion céréales 2018 "using odours in Agriculture"
- **Un parfum pour protéger les plantes /** Passion céréales: Video available online here

ATTRACTMYFLY

Development of attraction and entomopathogenic fungus auto-dissemination techniques to control melon fly, *Zeugodacus cucurbitae*

Launch year: 2015
Completion year: 2018

Partners
CIRAD UMR PVBMT; ARMEFLHOR; AB7 Innovation Natural Plant Protection SA and Betel Réunion SAS (Arysta LifeScience Group)

Scientific manager
Laurent Costet, UMR PVBMT CIRAD
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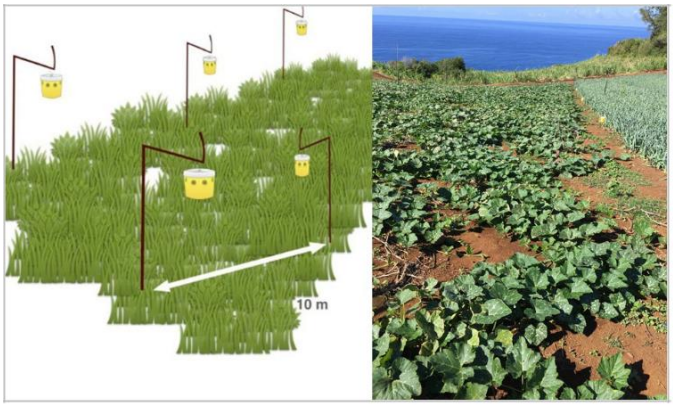
Financing
Total cost of the project: € 262,426
Ecophyto grant: € 100,000

Key words:
Olfactory pest control and traps; Elementary alternative practices; Vegetable crops; Specific attractors for fruit fly; Kairomones; Entomopathogens; *Beauveria spp*; Auto-dissemination; "Attract and disseminate"

Context and main objectives

Fruit fly (*Tephritidae*) are pests that damage fruit and vegetable crops in tropical and temperate zones, including mainland France and its overseas territories. Synthetic insecticides are used in vast quantities to control them. Alternative control methods, such as large-scale trapping, have produced encouraging results, but their development remains limited for certain species where no specific attractor for the female insects that cause the damage has yet been identified. *Zeugodacus curcurbitae*, the melon fly causes a lot of damage to cucurbitaceous crops. Kairomones, substances emitted by the host-plant that attract the female, have been identified. One of the objectives of the AttractMyFly project was to develop a mass female fly trapping system including a polymer kairomone diffuser. Another objective was to evaluate an auto-contamination-dissemination strategy involving entomopathogenic fungus.



Experimental in-field trap evaluation system: the traps are attached to an iron post 50cm above the ground. They are located along the edge of the field with a distance of 10 metres between each trap.

Image and photo: © Anne-Sophie Zoogones, CIRAD

The availability of a specific attractor for males raised the possibility of attracting them into the traps in order to use them as vectors for spreading spores to contaminate the females during mating.

Principal results and interest in relation to the Ecophyto plan

Starting from 10 compounds potentially involved in attracting females of the melon fly, *Zeugodacus cucurbitae*, a combination of two volatile synthetic compounds, C1 and C2, were identified in the laboratory and considered to be attractive in the same way as the odour of fresh fruit. One of the objectives was to incorporate kairomones into a polymer diffuser and develop a mass trapping system for the females.

These polymer diffusers were developed and then assessed in the laboratory using a one-way olfactometer. Polymers with C1 and C2 were found to be just as attractive as the odour of fresh fruit. The trapping capacity of the various systems was then assessed under semi-controlled conditions inside large outdoor cages. We tested the different traps using different combinations of the kairomones inside different polymers. Kairomones C1 and C2 were confirmed as attractive but were ten times less effective than they had been under laboratory conditions. A TephriTrap system filled with detergent and water was selected. This system's trapping capacity was then assessed in the field between June 2017 and January 2018 on thirteen different *cucurbitaceae* crop fields on Réunion Isle using different quantities of the C1 and C2 kairomones in the polymers. The systems with quantities higher than or equal to 27 mg of C1 and C2 trapped significantly larger quantities of females than the control. During the project we also developed an "Attract and Kill" system based on the kairomone attractors that target females.

It was also possible to attract the males into a system containing spores, using a specific attractor: cue-lure. These were then used as vectors for the spores in order to contaminate the females during mating. Another objective was to develop an auto-contamination-dissemination system for entomopathogenic fungus.

Beauveria hoplocheli strain.
Photo: © Isabelle Merle, CIRAD

The first step was to identify a biocontrol agent that could be approved for marketing. We evaluated the pathogenic capacity of three commercially available strains of *Beauveria* on *Z. cucurbitae*. The I-2961 strain was the most pathogenic for the melon fly and was selected for the rest of the study.
The auto-contamination-dissemination concept specifically targeting the females is based on the possibility of transferring spores of *Beauveria* from the male onto the female during mating. The second step was to test this transfer process on *Z. cucurbitae*.
The transmission of spores from the males onto the females during mating will significantly increase the mortality of the female if the males are able to mate less than an hour after contamination. However, 24 hours after contamination, there was no mortality amongst females.

Finally, we have designed an attraction contamination system. This is based on attracting males with a polymer emitting cue-lure, a formulation of spores of the I-2961 strain and a modified Tephitrap. This system works in the laboratory. We were therefore able to prove the effectiveness of this auto-contamination-dissemination method. However: on one hand, the window of opportunity during which the spores can be transferred successfully seems too short for this strategy to have an effect on populations in the field, and on the other hand, we have shown that a part of the inoculate transported by the males was deposited into the surroundings thus creating a potential for spreading this disease to other individuals.

From left to right. Schematic representation of a female of the melon fly. *Image: Toulassi Nurbel, CIRAD. Beauveria hoplocheli and auto-contamination system. Photo: Isabelle Merle, CIRAD*. Female melon fly affected by Beauveria hoplocheii. *Photo: © Brice Derepas, CIRAD*

Prospects for transfer or research

Transfer:

The mass trapping strategy for the melon fly, based on the use of kairomones, has a short-term transfer and usage potential. It could be possible to use the traps developed during the project after a validation phase. The pathogenicity tests on the commercially available strains of *Beauveria* have shown that the concept could be immediately extended for other pest species. The *Beauveria* spore auto-dissemination strategy targeting females shows promise but requires further research work.

Research:

The continuation of research work on the mass-trapping strategy using kairomones should focus on the optimisation of the traps by considering the diffusers, attractor quantities and numbers as well as the trapping system. The use of kairomone compounds would also require further studies on the specificity of the traps and toxicological issues. As for the *Beauveria* spore auto-dissemination strategy targeting females, further work should be done to optimise the auto-contamination system especially concerning the spore formulation and to evaluate its genuine field potential.

Publications and scientific symposia:

TECHNICAL WORKSHOPS AND SCIENTIFIC SYMPOSIA

- ***International Congress on Invertebrate Pathology and Microbial Control (24-28/07/2016): Abstract of the 49th Annual Meeting of the Society for Invertebrate Pathology***: *Characterization of the pathogenicity of commercial or precommercial Beauveria sp. strains against the melon fly Bactrocera cucurbitae.* Rohrlich C., Merle I., Payet-Hoarau M., Télismart H., Besse S., Nibouche S., & Costet L. (2016). Tours, France [Poster FU 5].

SCIENTIFIC PUBLICATIONS

- ***PLOS one,*** *13(7), e0199199: Variation in physiological host range in three strains of two species of the entomopathogenic fungus Beauveria*. Rohrlich C., Merle I., Hassani I. M., Verger M., Zuin M., Besse S., Robene I., Nibouche S. & Costet L. (2018). https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0199199.

PRACTICAL / EDUCATIONAL ARTICLES:

- ***Fertile: Bulletin de l'Association Réunionnaise pour la Modernisation de l'Economie Fruitière Légumière et HORticole (Armefhlor).*** *(n°41, p 14) : Lutte biologique contre la mouche du melon : des résultats prometteurs.* Graindorge R. et Costet L. (2018).

PRESENTATION TO PROFESSIONAL OR DECISION-MAKING BODIES:

- AttractMyFly steerage committee, Launch meeting, 10/04/2015
- AttractMyFly steerage committee, 22/09/2016

OTHER PRACTICAL WORK:

Educational

- ***PhD thesis in Population Biology and Ecology - Université de la Réunion****: Lutte biologique à base de champignons entomopathogènes du genre Beauveria en zone tropicale.* Presented on 3 October 2018. 149 p. Rohrlich C. (2018)
- ***Dissertation Master 2 Healthcare Sciences and Technology - Plant Biology and Technology, Plant Production and Technology (Protev), Université d'Angers*** *: Caractérisation de la capacité de transfert de spores de champignon entomopathogène Beauveria bassiana des mâles aux femelles de la mouche du melon Zeugodacus cucurbitae lors de l'accouplement. 33 p.* Verger D. (2017)
- ***Dissertation, Agronomics Engineer studies, ESA d'Angers*** *: Caractérisation de la capacité de transfert de spores de Beauveria bassiana des mâles aux femelles de la mouche du melon Zeugodacus cucurbitae lors de l'accouplement. 84 p.* Venard J. (2016)
- ***Dissertation Agronomics Engineer Studies, ENSAIA, specialisation: crop protection and Université de Lorraine*** *: Caractérisation du pouvoir pathogène de souches de Beauveria sp. sur la mouche du melon Bactrocera cucurbitae et de la capacité de transfert horizontal des spores du champignon lors de l'accouplement.* 39 p. Merle I. (2015)

BIOBOT

Optimisation, including the global and integrated management, of biocontrol solutions for the treatment of grey mould on grapevines

Launch year: 2015
Completion year: 2019

Partners
IFV; INRAE UMR SAVE; Gironde Chamber of Agriculture

Scientific manager
Nicolas Aveline,
IFV (French Vine and Wine Institute)
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Financing
Total cost of the project: € 141,394
Ecophyto grant: € 96,845

Key words:
Wine-making; Botrytis; Grey mould; Biocontrol; Micro-organisms; Biological control agents; Decision-making tools; Changes to technical approaches

Context and main objectives

Grey mould is a disease that affects grapes towards the end of the growing season (powdery greyish mould), once it affects one grape it rapidly spreads over the whole of the bunch. In addition to its effect on harvests (quantity), the fungus affects the taste of the wine produced if more than 5% of the harvest is affected. Generally speaking, the phytosanitary treatment for this disease can reach between 1 and 2 TFI, which regularly results in the presence of product residues in the wine.

Biocontrol alternatives exists, they were even amongst the first biocontrol products for wine-growing to be officially registered. Unfortunately the use of these products remains restricted and difficult to evaluate: especially the very diverse operational methods which raise a number of questions on their ideal role and application strategies. In addition, Botrytis is difficult to treat preventively and its sudden development at the end of the year can be explosive.

Grapes affected by Botrytis.
Photo: © Nicolas Aveline, IFV

Principal results and interest in relation to the Ecophyto plan

A lot of work has been done to assess the biocontrol products available for treating Botrytis on vines; over 3 years of assays on two different locations in the Gironde region: 14 products or substances were tested as part of a series of specially designed comparative experiments: 6 applications during the season and, for some of the products, application of the “Ciliberti index” decision-making tool to organise the post-ripening applications as efficiently as possible. The results generally showed a low level of protection effectiveness. One of the tested products, Armicarb® (potassium bicarbonate) seems to be the only solution that makes a consistent difference when compared to an untreated control. Some of the products are micro-organisms from the *Bacillus* family: Amylo-X®, S38 (INRA model bacteria) and Rhapsody® have regularly shown themselves to be effective. The other products produce results that are too variable from year to year for any conclusions to be reached on their usefulness.

The validity and above all consistency of the results obtained by the project remain complex: the 3 harvests were contrastingly different in terms of climate (heavy frosts in 2017) and the botrytis effect was mostly middling and light. Additional measurements (vigour, tightness of bunches, grape worm effect, etc.) were taken and still have not shown any clear intra-vineyard correlations. *B. cinerea* contamination rate tests on flower caps have turned out to be interesting in spite of inconsistancies from one year to the next and identification limitations. It is useful for the knowledge gained about the disease and as an indicator of parasite impact at the start of the season. It is a tool with potential for the evaluation of the early effects of biocontrol products on the pathogen, but it still requires some methodological improvements.

The post ripening “Ciliberti Index” decision making tool developed by INRAE can be used to simulate a daily botrytis development climate index (temperature and hygrometry) covering the 4 major risk categories. It allows for effective and judicious end-of-season biocontrol applications in relation to pre-identified risk periods.

It was tested on a number of candidate products on a large vineyard in 2017; one year later it was transferred to the partner experiment specialists. Interpretation and the decision making rules have changed during the project to finish with the 2019 on-line calculation version.

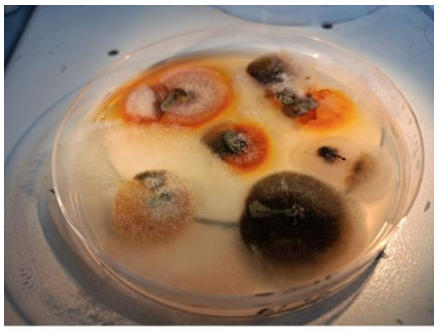
Links with the Ecophyto plan:

- **Biocontrol development:** the strategies proposed and studied by the project are 100% biocontrol, no traditional products used in alternation or association were assessed. With the strict theme of lowering intrants, this project aims to define the biocontrol strategies that would cope with the residue issues generally raised by more traditional anti-botrytis treatments.
- **Optimising the use of biocontrol products:** the use and organisation of biocontrol products has been studied, especially at the end of the season and right up to just a few days before harvesting, the crucial period when the use of chemical products is restricted. We now have new intervention possibilities for dealing with botrytis.
- **Development of a decision-making tool:** the Ciliberti index was used and improved (definition of decision making rules and thresholds) to judiciously program the use of certain biocontrol products either after ripening or during maturity.

Prospects for transfer or research

Transfer:

The candidate biocontrol solutions which produced interesting performance results are currently in use (2019, 2020, etc.) in other regional applied research programs (Alt’Fongi Biocontrol project, BEE project). The aim is to include them in a global protection programme that uses as many biocontrol products and preventive approaches as possible along with an absolute minimum of conventional pesticides.



Petri dish culture of flower caps.
Photo: © Nicolas Aveline, IFV

The use of the Ciliberti index opens up research potential on the position of anti-Botrytis products and the crucial phases of the disease’s development. Its transfer through global tools distributed by the IFV (French Wine and Vine Institute), such as the on-line solution DECITRAIT®, should provide the means to integrate it into global protection management and provide optimised biocontrol application recommendations for grey mould.

The experimental “flower cap contamination rate” early warning indicator is very useful for evaluating the botrytis pressure on the inflorescence as well as measuring the effect of some of the biocontrol products applied during this period. Improving identification techniques will require more work (the molecular tool?) to identify and evaluate botrytis more effectively. This could then be integrated into the Botrytis Receptivity Potential (PRB) indicator published annually by the INRAE and allow for the publication of an early-use effectiveness rating for the tested solutions.

Finally, our trials have shown that the *B. ginsengihumi* S38 bacteria, isolated by the INRAE, has a very interesting anti-Botrytis potential for grape vines.

Research:

The work completed during the project has shown the importance of advance knowledge, both concerning Botrytis and its development and on the operation of biocontrol products. Questions remain on the pathogen’s epidemiology and the prediction of its development, but some clear progress has been achieved with certain risk indicators used by BIOBOT. Research programmes and projects have been launched on this subject by the UMT SEVEN, including a project for the identification and evaluation of aerial spore dissemination on grapevines, or dynamic studies of the agro-climate factors that influence the spread of the disease. All of this research work will help any control indicators that can be developed to improve protection management and potential biocontrol applications.

It is worth noting that there is a need for a biocontrol micro-organism traceability tool (for use with all strains) to know if the strain remains present on the plants and fruit, to have a better knowledge of its installation and survival conditions and organise suitable scheduling decisions.

The work by INRAE UMR SAVE on the *B. ginsengihum* S38 bacteria (Calvo et al., 2019) confirms how the micro-organisms naturally present in vineyards can be enormously useful. Research work could be continued to improve the use of this type of bacteria; especially in terms of mass production and formulation.

Finally, and more generally speaking, research on biocontrol and biostimulant products considered potentially effective in the treatment of grey mould should be continued: action methods such as defence stimulation, physical barrier strengthening etc., are not that common in the biocontrol list. The European “basic substances” status could also turn out to be a good source of alternatives to chemical fungicides which could have a regular and significant effect on grey mould.

Publications and scientific symposia:

TECHNICAL WORKSHOPS AND SCIENTIFIC SYMPOSIA:

- ***Rencontres Viticoles d'Aquitaine, Lycée Viticole de Blanquefort 2018:*** *Le biocontrôle pour lutter contre la Pourriture Grise : the BIOBOT and ALB's projects (RVA 2018) [PPT]***.** Aveline N.

SCIENTIFIC PUBLICATIONS:

- ***Frontiers in Plant Science (n° 10, 2019, p 105):*** *Microbial Antagonism Toward Botrytis Bunch Rot of Grapes in Multiple Field Tests Using One Bacillus ginsengihumi Strain and Formulated Biological Control Products.* Calvo Garrido C., Dupin S., Aveline N., Roudet J., Davidou L., Fermaud M.

PRACTICAL / EDUCATIONAL ARTICLES:

- ***Guide ACTA: Guide ACTA Biocontrôle 2018:*** *Lutte contre la Pourriture Grise sur Vigne.* Aveline N., Fermaud M., Chantelot E.
- ***Guide ACTA: Guide ACTA Biocontrôle 2019:*** *Lutte contre la Pourriture Grise sur Vigne.* Aveline N, Fermaud M, Chantelot E.

PRESENTATION TO PROFESSIONAL OR DECISION-MAKING BODIES:

- ***Union Girondine des Vins de Bordeaux (2017, pp 53-54)*****:** Utiliser les produits de biocontrôle pour protéger la vigne contre la pourriture grise en viticulture biologique : résultats des essais menés dans le RESAQ Vitibio. Aveline N., Davidou L.

OTHER PRACTICAL WORK:

- ***Bordeaux ISVV: Master 2 course: Grape vine sciences, oenology, EU wine-growing pathology (2017):*** *Epidémiologie et lutte contre Botrytis cinerea au vignoble.* Aveline N., Fermaud M.
- ***Poster presentation, Perpignan (2018):*** *BIOBOT: Evaluation de produits de biocontrôle contre la pourriture grise (B. cinerea) au vignoble.* Aveline N., Dupin S., Davidou L., Calvo Garrido C., Roudet J., Fermaud M.

BIOCCYD

Biological control of the codling moth, *Cydia pomonella* (BIOControl of *CYDia pomonella*)

Launch year: 2015
Completion year: 2018

Partners
Institut Sophia Agrobiotech; BIOLINE Agrosciences

Scientific manager
Nicolas Ris, INRAE ISA
nicolas.ris@inrae.fr r

Financing
Total cost of the project: € 303,142
Ecophyto grant: € 103,918

Key words:
Acclimatisation; Augmentation; *Cydia pomonella*; Biological control; Parasitoid

Context and main objectives

Codling moth, *Cydia Pomonella* (Lepidoptra: Torticidae) is one of the major macro-organism pests affecting apple orchards in France, and across the globe; due to (i) its extensive geographical distribution, (ii) the economic impact of the damage it causes, (iii) the excessive use of pesticides it provokes and (iv) the limits of the currently available control solutions. Amongst the available biocontrol solutions, a lot has been invested in a sexual confusion approach and the use of carpovirus; but both of these methods have major limitations. Elimination methods based on releasing sterile insects have also been tried locally but have yet to prove effective. Finally, the possibility of effectively controlling codling moth by encouraging the local biodiversity (organic control through conservation) also seems to have its limits. It would therefore appear that biocontrol methods need to be diversified to complement the already existing approaches and, potentially, limit the possibility of this pest developing resistance to them. In this context, the BIOCCYID project aims to evaluate two biocontrol methods based on two types of entomophagic macro-organisms: egg-eating parasitoids of the *Trichogramma* genus (*Hymenoptera*: Trichogrammatideae) and a pre-pupal parasitoid *Mastrus ridens* (*Hymenoptera:* Ichneumonidae).

Principal results and interest in relation to the Ecophyto plan

Regarding the “Organic pest control by increase using trichogramma” part of the project: trichogramma sample collections were taken between 2014 and 2016 over a large part of the South of France in a coordinated program involving the “BIOCCYD”, “INDREGARB” PSPE1 2013-2015 and “TRIPTIC” ANR 2014-2019 projects. For the potential host plants for *Cydia Pomonella*, the trichogramma collection rate was established as mid to low. The diversity of the species collected was estimated using molecular approaches (COI region “barcoding”) and compared to those observed on other plant categories: (i) Rosaceae not host to *C; Pomonella* and sympatric with *C. pomonella* hosts; (ii) other non-host plants for *C. Pomonella* and sympatric to *C. pomonella* hosts; (iii) Rosaceae not host to *C. pomonella* and allopatric to *C. Pomonella* hosts. Except for the molecular taxon known as “misG” (only to be found in cultivated apple trees), the main species found on *C. pomonella* hosts turned out to be common and not very specialised. Regarding “misG”, studies are currently under way to define its precise taxonomical status. Considering both these trichogramma diversity results and the strategy of BIOLINE Agrosciences, investigations on these subjects was ceased by mutual agreement.

Regarding the “Organic pest control by acclimatisation using *Mastrus ridens*” a number of *M. ridens* strains have been successfully introduced into our facilities (from New Zealand: LOA n°FR15PA00001 dated 22/05/2015; from Chilli: LOA n°FR16PA00001 dated 19/01/2016 and LOA n0FR16PA00002 dated 01/03/2016) after authorisation had been received (19/05/2015). *M. ridens* is relatively easy to reproduce for population level maintenance purposes, but much more difficult to reproduce in large numbers. Based on (i) the introduction applications made in other countries, (ii) articles on the subject and (iii) experiments we have completed on non-target hosts, an application for its introduction into the environment was submitted to the DGAL and the ANSES on 04/03/2016. This 37 page report summarises knowledge acquired on the biology of *M. ridens*, describing the communities of natural enemies to *C. Pomonella* present in France and across the world, and sets out the potential advantages and risks that could be associated with such an introduction into the environment. On this basis and with the recommendations of independent experts, the ANSES issued its approval on 04/01/2017 and a decree cosigned by the Ministry of Agriculture and the Ministry of the Environment was officially issued on 09/05/2017. As with all first-time introductions of exotic auxiliaries, the aim is now to use these rare opportunities to set up multi-site post-release monitoring programmes over a number of years, in order to evaluate the performance of *M. ridens* as precisely as possible and answer population biology issues.

Considering the available logistical and financial resources, only one site in the Vaucluse was used for releasing *M. ridens* during the second half of 2018. Attempts to raise further funding to complete a more precise evaluation of the establishment capacities of *M. ridens* were unsuccessful in 2017 and 2018. However in 2019, financial support was obtained from FranceAgriMer ("BIOCCYD-Mastrus" project; Manager: Nicolas BOROWIEC, INRAE).

Link with the Ecophyto plan:

The initial objective of the BIOCCYD project was evidently to reduce the use of phytosanitary products to control the codling moth, *C. pomonella*, in combination with other biocontrol methods (sexual confusion, carpovirus, biological pest control through conservation). Considering the results achieved, one particularly interesting perspective raised by our work is the possibility of introducing *Mastrus ridens* into France and its potential establishment. If this is successful a new eco-systemic service will be created that regulates coddling moth populations at no cost to the agriculturalists involved.

Prospects for transfer or research

Transfer:

Organic pest control by acclimatisation is generally seen as a public service mission: (i) "anticipatory" evaluation of candidate auxiliaries, (ii) introduction into all or part of the territory and (iii) post release evaluations are all generally completed by research institutes and technical-agronomic bodies (Technical institutes, Experimental stations, Farming networks, etc.). The involvement of the private sector (bio-factories) may be possible (see the "chestnut gall wasp" case) but is generally limited over time.

Research:

The most evident research perspective is clearly the **multi-year and multi-site evaluation of *Mastrus ridens*** in terms of its local dynamic (local establishment and populations), geographical dispersion and the ecological niches generated (effective use against *C. pomonella* on the various host-plants; any impact on non-target species). In many ways, this post-release evaluation will respond to scientific expectations (biology of introduced populations) and farming expectations (evaluation of the service provided by *M. ridens* and its impact on other codling moth control methods).

Through the introduction of *Mastrus ridens* in the Vaucluse (2018) and perhaps some other similar operations, **the influence of environmental heterogeneity** around the release site (< 1km) on local dynamics and dispersions is currently being evaluated (Thesis of David MURU; Institut Sophia Agrobiotech).

Another particularly interesting question is **the evaluation of the gender determination method (sI-CSD) of *M. ridens* on its population dynamics.** The lack of genetic variability at the sI-CSD locus leads to the production of diploid males that are reproductively non-functional which then compete with the normal (haploid) males to mate with females. Monitoring the frequency of diploid males in the wild and restoring genetic diversity at the sI-CSD locus, are both issues that deserve further specific investigation.

Publications and scientific symposia:

TECHNICAL DOCUMENTS:

- Borowiec N., Malausa T., Ris N. 2015. **Document technique relatif à l'introduction en milieu confiné de l'hyménoptère parasitoïde exotique, *Mastrus ridens*.** 18pp.
- Borowiec N., Muru D., Malausa T., Ris N. 2016. **Document technique relatif à l'introduction dans l'environnement de l'hyménoptère parasitoïde exotique, *Mastrus ridens.*** 37pp.
- Marchand A., Sellier N., Warot S., Ion-Scotta M., Ris N., Groussier-Bout G. 2017. **Formalisation d'un Centre de ressources biologiques dédié aux parastoïdes oophages : CRB EP-Coll.** Cahier des Techniques de l'INRA

PRACTICAL / EDUCATIONAL ARTICLES:

- Muru D., Auguste A., Fauvergue X., Malausa T., Ris N., Thaon M., Vercken E., Borowiec N. 2018. **Un parasitoïde exotique pour lutter contre le carpocapse.** Phytoma, la Défense des Végétaux. 710: 37-41.

SCIENTIFIC PUBLICATIONS:

- Muru D., Marchand A., Calcagno V., Cruaud A., Rasplus J-Y., Ris N., Vercken E., Warot S., Groussier G. in prep. **Survey of the diversity of *Trichogramma* species in France and neighbouring areas with information related to their host plants and habitats**
- Warot S., Cruaud A., Groussier G., Malausa T., Martinez-Rodriguez P., Pintureau B., Seguret J., Ris N. **Insights into the molecular diversity and species delineation in the genus *Trichogramma* with a focus on West Palearctic**

BIOCOU

Biological control of the grapevine fanleaf virus: the impact of integrating growing techniques involving intermediate crops and the use of a resistant root-stock on nematode populations

Unfortunately this summary data-sheet is not available.

BIOLIM

Innovative biocontrol techniques for the management of crop damaging land molluscs: Looking for an evaluation methodology suited to their ecophysiology

Launch year: 2015
Completion year: 2018

Partners
ACTA; Université de Rennes; ARVALIS Institut du végétal; Phyteurop; Bayer-CropScience; De Sangosse

Scientific manager
André Chabert, ACTA
andre.chabert@acta.asso.fr

Financing
Total cost of the project: € 194,696
Ecophyto grant: € 100,000

Key words:
Slugs; Biocontrol; Large-scale crops; Ecophysiology; Elementary alternative practices; Changing techniques; Redesigning crop systems; Management via macro-organisms

Context and main objectives

For most annual plants that are subject to slug damage, chemical treatment becomes practically systemic during high rainfall years. This project aims to contribute to the development of new biocontrol approaches and therefore contribute to reducing the use of non-biocontrol phytosanitary products. The main contributions are as follows:

- Evaluate the performance of new biocontrol approaches and products for dealing with slugs;
- Use chemical ecology to explore new evaluation approaches to study the semiochemical relations between slugs and plants as well as ground beetles;
- Evaluate the effectiveness of biological control methods in terms of the agricultural levers that can be mobilised to deal with this pest;
- Define the conditions that apply to their use in reducing populations of this pest within crop systems through the combination of identified and evaluated levers to re-design integrated protection strategies;
- Create an innovation research dynamic by assembling a public/private sector partnership.

The methodologies were first communicated to the partner experimentation networks of the BIOLIM project which has facilitated the exploration of new research approaches by private companies; they were then communicated to the principal activity sectors concerned by slug damage.

Principal results and interest in relation to the Ecophyto plan

This BIOLIM PSPE2 project includes a public partner (University of Rennes 1) for its expertise in mollusc eco-physiology along with technical institute and private business partners for their contribution to experimentation procedures and their knowledge of crop systems. Consequently this slug biocontrol product dedicated project has facilitated the compilation of a panorama of all current knowledge on the subject and the launch of new experimental programmes. It has also established an inventory of preventive agricultural levers and their effectiveness on slug populations.

The first phase was dedicated to completing a meta-analysis type approach based on:

i) Data from the CasDAR RESOLIM project

ii) Textual sources covering slug control techniques

iii) The various available biocontrol approaches.

The specific quality of this approach was to allow for interactions between slug biology, crop systems and all of the currently available biocontrol techniques. These resources are diverse and reflect the relations existing between slugs and their biotic and abiotic environments (see Fig 1 on the next page).

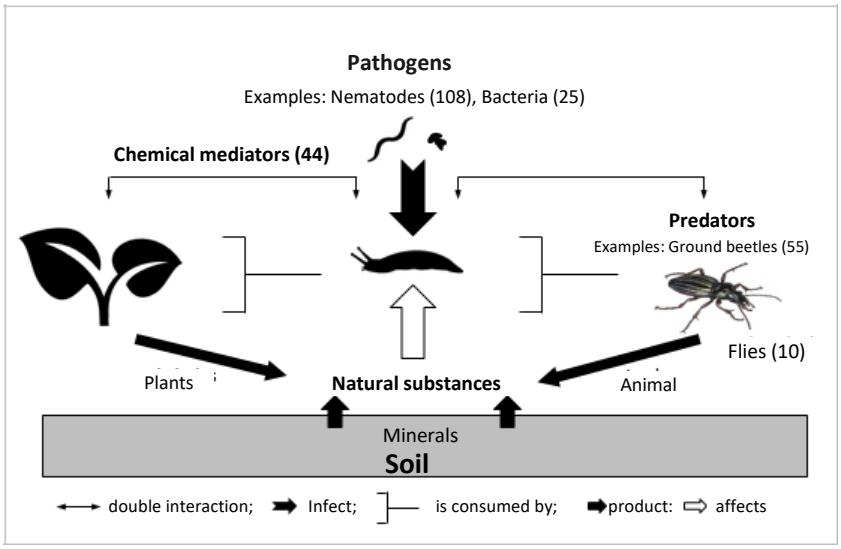


Figure 1: Biotic and abiotic relations of slugs with their environment. The number of listed publications (between 1992 and 2018) for each biocontrol method is shown here in brackets. *Image: © André Chabert, ACTA*

Following this, these organic pest control methods have been studied using specially designed experimental methodologies to evaluate their performance, allowing for the specific aspects of slug life-cycles, and their potential for integration into crop systems. These studies could be resumed as follows:

1) The use of service plants as an intermediary crop

In order to clarify the usage potential for service plants, the University of Rennes studies were designed to evaluate the biochemical interactions between the slugs and the plants when the former are eating the latter. A protocol was set up to establish the feeding reaction of the slugs to the rapeseed metabolites emitted at the seed leaf stage. It has been shown that the phenylalanine present in the seedlings produces a phagostimulant effect, but this property is lost when glucosinolate emitted when the plant is attacked is added to the mix. Slugs are therefore always capable of eating rapeseed seedlings even if their natural defences are active, but their consumption is reduced.

The ARVALIS laboratory studies concentrated on the use of repellent mustard varieties as intermediate crops. They were able to evaluate the feeding preferences of slugs in the presence of 4 different mustard varieties. These methodologies clearly showed the differences between the plant species that need to be known in order to anticipate their real effects on slug populations and thus provide suitable recommendations.

2) Natural (mineral or plant) substances with toxic or repellent properties

ACTA lab-tested a caffeine based formulation on grey slugs, this provoked mortalities on contact but did not function as bait. The companies that own these formulations decided not to continue with the development of this method. Other studies have shown that decoctions of garlic for plant protection did not have a sufficiently repellent effect to restrict attacks from slugs.

3) Macro-organisms: predators such as ground and rove beetles, spiders or parasites such as nematodes

Monitoring campaigns using traps were completed in Boigneville to try to find out if ground beetle populations could regulate those of slugs. These trapping campaigns showed that slug populations were much higher in fields where the preceding crop was barley compared to those where the preceding crop had been rapeseed. However the total number of ground beetles (*Poecilus cupreus* and *Pterostichus melanarius*) trapped was almost exactly the same for both fields. In this case, no relation has been detected between the two populations. For this study it would appear that variations in slug population levels are more closely related to the preceding crop.

▶ Results summary

The performance results for the biocontrol techniques tested were summarised collectively by all of the partners. We have calculated ratios to compare the different works taken from the bibliography with those completed within the context of the project. Table 2 in the appendix, shows ratios that evaluate the impacts of different techniques on slugs, and the damage they incur calculated from the analysis of the bibliography and the works completed by BIOLIM.

Prospects for transfer or research

Transfer:

Regarding slugs present in large-scale crops, and probably any other crop-fields, it is difficult to isolate the agricultural practices from the implementation of biocontrol techniques. This might seem obvious, however the knowledge required, the practical issues raised by the implementation of biocontrol phytosanitary products are numerous, complex and therefore often avoided when there is no available expertise on the subject. This then leads to over-simplified recommendations that are possibly unsuited to the myriad of different localised and climate-related situations. This is where working as part of a network is important. However, a large number of scientific and technical questions concerning the evaluation of biocontrol projects could only be resolved by analytical tests which require significant resources if they are to be done independently.

Research:

One research approach could be to collate the large sets of data obtained at different levels (laboratory, fields, systems, farms, etc.), which would require the collective work done here to continue, doubtless along with further collaborations around other projects. The continuation of this work will need to concentrate on these two points:

1) Maintain an experimental network concerning slugs in order to evaluate biocontrol phytosanitary products independently and maintain a network of expertise in place. Current chemical ecology knowledge would need to be shared and applied to find new biocontrol approaches for slugs.

2) This network should also contribute to the enlargement of the current meta-analysis approach as well as the development of slug risk evaluation tools. These two approaches could be extended further to cover other pests affecting large-scale or other crops.

Publications and scientific symposia:

TECHNICAL WORKSHOPS AND SCIENTIFIC SYMPOSIA:

- **REOLIM project results presentation symposium. 18/03/2016, Paris:** Presentation of the BIOLIM project and its current works.
- **Terres Inovia Workshop 26/01/17, Évreux:** presentation of the RESOLIM and BIOLIM project results.

SCIENTIFIC PUBLICATIONS:

- ***Phytoma (n°694, pp 34-37)****: État des connaissances sur le biocontrôle vis-à-vis des limaces.* Mottin E., Tamine M., Chabert A. and Charrier M. (2016)
- *Discrimination of oilseed rape seedlings by the field slug Deroceras reticulatum and storage of salicylic acid in its digestive gland.* Tamine M., Jonard C., Van Der Linde M. & Charrier M. Article in preparation

PRESENTATION TO PROFESSIONAL OR DECISION-MAKING BODIES:

Contacts established with various Ecophyto plan participants during the results presentations:

- At the PSPE seminar, 2 December 2017
- At a workshop with farming professionals in Bourges, January 2017
- To people involved in one of the Ecophyto projects (action 30,000) on the reduction of the use of anti-slug products, 10 September 2018

PRACTICAL / EDUCATIONAL ARTICLES:

- **Creation of an instruction sheet for a biocontrol anti-slug product as part of a solution contract**
- **Creation of part of the ACTA biocontrol index**
- **Master 2 Natural heritage and Biodiversity, University of Rennes (2015, 21 p):** *Quels moyens de biocontrôle pour les ravageurs de culture ? Le cas des limaces.* Tamine M.
- **Master 2 Natural heritage and Biodiversity, University of Rennes (2016, 25 p):** *Étude expérimentale de l'interaction trophique entre le colza et son ravageur, la limace grise Deroceras reticulatum : facteurs stimulant la nutrition et réponses métaboliques interspécifiques.* Tamine M.
- **Master 1 Natural heritage and Biodiversity, University of Rennes (2017, 15 p):** *Sélection gustative de métabolites du colza par la limace grise Deroceras reticulatum et devenir des phytohormones dans les tissus de la limace.* Van Der Linde M.

MATERIALS AVAILABLE ON DEMAND:

- Mendeley data-base deliverables including the 310 articles
- Excel spreadsheets with the analysis of around one hundred articles
- Various seminar presentations
- Final project report
- Project web-site link: https://ecophytopic.fr/recherche-innovation/proteger/projet-biolim

BIOTI-VIGNE

Biotisation of young nursery-grown grapevines to prevent trunk diseases

Launch year: 2015
Completion year: 2017

Partners
UMR SAVE INRAE ; Mercier Frères SARL and Biovitis

Scientific manager
Dr Marc Fermaud, UMR SAVE INRAE
marc.fermaud@inrae.fr

Financing
Total cost of the project: € 253,938
Ecophyto grant: € 88,848

Key words:
Wine growing; elementary alternative practised; Biotisation, Biological pest and disease management; Biocontrol

Context and main objectives

The French grape and wine industry is a major chemical pesticide user: around 20% of all pesticides applied to only 3% of French agricultural land. The industry is also suffering from a state of vegetative decline, largely due to grapevine trunk diseases (GTD). Biocontrol for GTD is rapidly becoming a major subject considering the dire need to avoid any increase in the use of chemical fungicides to deal with these diseases. The biotisation of plants that have been grafted in nurseries, which is at the heart of this project, involves inoculating the plants with beneficial micro-organisms which will improve their tolerance to biotic stress (GTD pathogen fungus infections).

Vine wood with brown bands under the bark, a common sign of Esca.
Photo: © Pascal Lecomte, INRAE

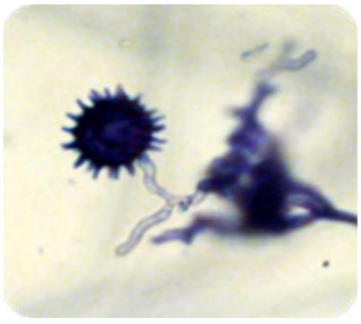
The project's originality is based on:

1) Various biotisation agents, micro-organism strains, all taken from French vineyards
2) Isolation and/or combination tests
3) Application to plants at a very early age

The 2 major objectives are:

1) Improving and limiting the possibility of varying protection levels using the candidate biotisation agents identified by our research work, such as bacterial strains and *Pythium oligandrum*
2) Evaluating the effect of root-stock choice on the persistence and effectiveness of biotisation agents in nurseries.

Principal results and interest in relation to the Ecophyto plan

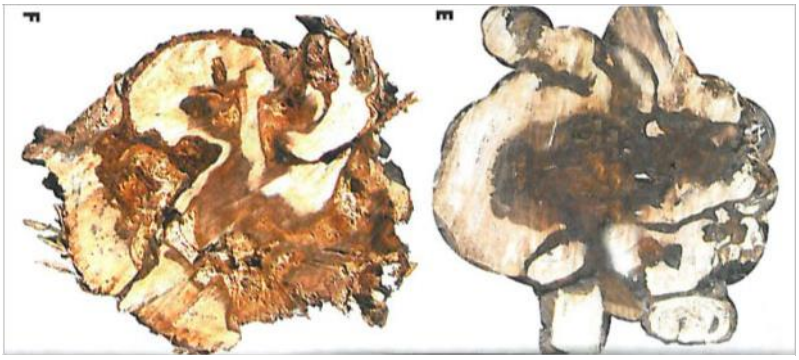


Microscope image of *Pythium oligandrum*, successful biotisation agent for controlling the fungi known to be the causes of grapevine trunk diseases.
Photo: © Jonathan Gerbore, BIOVITIS

Two extensive experimental campaigns were completed in 2015 and 2016. The main objectives, methodology and results of these were presented in a scientific article (L. Daraignes et al., 2018 Biological Control 119:59–67, doi: 10.1016/j.biocontrol.2018.01.008 https://hal.inrae.fr/hal-02626154) and on a dedicated Web-page(http://www.maladie-du-bois-vigne.fr/Programmes-de-recherche/Les-projets-en-cours/Les-projets-de-biocontrole/BIOTIVIGNE/Les-resultats-du-projet).

▶ The first conclusion confirmed that the selected strain of *Pythium oligandrum* "Po" (oomycete) is a biocontrol or biotisation agent with a very high potential for the protection of grapevines from GTD. Biotisation assays have demonstrated effectiveness, as early as during nursery production, of the tested biotisation agents against 2 major GTD pathogen fungi: Phaeomoniella chlamydospora (Pch) and Neofusicoccum parvum (Np).

For both assay campaigns, the extent of dieback was significantly reduced by as much as 39-40% for Pch and 52-64% for Np in plants that had been biotised with *P. oligandrum* (Po). The absence of contact between the pathogen, present in the graft, and the Po biocontrol agent which colonises the root stock, indicates that the action occurs remotely through the stimulation of the vine's defence system. The mechanism is therefore an induction by Po in the roots with a vegetative elicitation which limits the infection and colonisation of the trunk by the two studied pathogenic fungi. In addition to this the Biotisation agent bacteria, Pantoea agglomerans (Pa) also significantly reduced dieback associated with the Np pathogen by 22-38%.



Cross-section view of mother plant root-stock trunks showing the extent of, central and/or sector type, Esca associated dieback.
Photo: © Jean-Michel Liminana, INRAE

- The second major conclusion came from the combined implementation of Po in association with bacteria strains pre-identified as having a high biocontrol potential (UMR SAVE, INRAE results). These biotisation agent bacteria are confirmed as effective but less so than Po. In addition to this, the accumulation of different biotisation agents in the same plant does not generate a synergic effect (greater effectiveness compared to Po alone). This may not be general to other disease systems and/or other microbe combinations. Consequently the combination of two Biotisation agents (Po and Pa) has reduced the extent of Np associated dieback by 32-52% depending on the year.

- The third major conclusion shows that the presence of Po in the rhizosphere and/or roots of the grafted vine cuttings can be maintained for long periods of time, 2 to 3 months. Po's high capacity for colonising the rhizosphere of grafted vine cuttings has therefore been shown for the first time and in an innovative and applicable way for the wine growing industry (previous studies had only shown this for cuttings that had not been grafted).

- The fourth conclusion, of major interest for the development of future innovations, concerns the very promising levels of grafted plant protection using the two tested root stocks (101-14 and SO4), which are widely used in France and other wine-growing countries. The extrapolation of our results to cover wine-growing techniques has therefore been significantly increased, even if this project was based on assays carried out in nursery greenhouses (MERCIER), further experiments will be necessary under different wine-growing conditions. These have been launched and an essential applied perspective, as a direct follow-up to the BiotiVigne project, will be the process set up to achieve authorisation for the *Pythium oligandrum*-strain biocontrol agent that was used for these tests.

Prospects for transfer or research

Transfer:

A certain degree of consistency can be noted in this study, the tested micro-organisms also show antagonistic capacities under other conditions, as shown in other biocontrol studies on pathogen fungi affecting grapevines (such as the research works by UMR SAVE, INRAE Bordeaux). For the partner company Biovitos, this project has confirmed the interest and effectiveness of the ground surface application of the *P. oligandrum* (Po) micro-organism. The company is therefore planning to follow the Biotivigne project with field trials in vineyards, in order to get authorisation to market this biocontrol agent. Thanks to this project, Mercier has confirmed its decision to explore the development of one or a number of commercial products ranges involving biotised graft cuttings.

Research:

In addition to opening up new potential biocontrol approaches, this project has raised a number of essential research issues. The biotisation procedure needs to be taken further in order to evaluate the duration of its beneficial effects, which are notable on young plants; this research would need to cover the whole productive life of a long-lived plant like the grapevine. One other question which is raising debate is that of the consistency of our major results: the association of a number of antagonist biocontrol micro-organisms is not always more effective than introducing a single one of the targeted candidate micro-organisms (this will almost certainly depend on the patho-system and/or the association of micro-organisms in question).

Publications and scientific symposia:

- ***Biological Control (n°119, pp 59–67, 2018):*** *Efficacy of P. oligandrum affected by its association with bacterial BCAs and rootstock effect in controlling grapevine trunk diseases*. Leslie Daraignes, Jonathan Gerbore, Amira Yacoub, Laure Dubois, C. Romand, O. Zekri, Jean Roudet, P. Chambon, and Marc Fermaud. Doi: 10.1016/j.biocontrol.2018.01.008. URL: https://hal.inrae.fr/hal-02626154

PRACTICAL / EDUCATIONAL ARTICLES:

- INRA biocontrol brochure "**les conquêtes de l'INRA pour le biocontrôle"** to which Marc Fermaud and Jonathan Gerbore have made contributions (including the BIOTIVIGNE project), INRA or INRAE, July 2018 (https://www.inrae.fr/sites/default/files/pdf/180529_presse_BIOCONTROLE__BD_.pdf)

OTHER PRACTICAL WORK:

- **The project's dedicated web-site:** https://www.maladie-du-bois-vigne.fr/Programmes-de-recherche/Les-projets-en-cours/Les-projets-de-biocontrole/BIOTIVIGNE
- **BiotiVigne RESULTS WEB-page:** http://www.maladie-du-bois-vigne.fr/Programmes-de-recherche/Les-projets-en-cours/Les-projets-de-biocontrole/BIOTIVIGNE/Les-resultats-du-projet

DAS-REVI

Socio-technical development and appropriation of plant variety resistance in sustainable wine-growing

Launch year: 2015
Completion year: 2019

Scientific manager
François Hochereau, INRAE UMR SADAPT francois.hochereau@inrae.fr
Laurent Delière, INRAE UMR SAVE laurent.deliere@inrae.fr

Partners
INRAE UMR SADAPT ; INRAE UMR SAV; IFV (French Grapevine Institute); Chambers of agriculture (Gironde, Hérault, Aude)

Financing
Total cost of the project: € 306,197
Ecophyto grant: € 134,868

Key words:
Wine growing; Improvement through variety selection; Disease resistant varieties; Environmental innovation; Observatory; Socio-economic evaluation

Context and main objectives

The aim of the project was to study pioneering disease resistant grapevine variety introductions in France; in order to identify the main regulatory, technical and socio-economical levers and obstacles that come into play when deploying this kind of plant variety innovation (task 4).

German and Swiss context studies, where such varieties have been marketed since the early 2000s provide a rich source of feedback and a better understanding of the definitive methods which can be applied to a participatory experimental framework associating the different “involved parties” (task 2).

At the same time, we considered how the regulatory and professional bodies of the wine growing industry, notably the INAO, have influenced the development of the regulatory framework covering plant variety experimentation (task 1).

Finally, the project's objective was also to federate these pioneering disease resistant variety adoption practices by constituting a plant variety-related resistance observation network. This network is structured around the shared application of a protocol for the collection and sharing of data concerning the behaviour of disease resistant varieties (task 3).

Principal results and interest in relation to the Ecophyto plan

In 2019, the disease resistant grape variety observation network covered 87 individual vineyards distributed over 43 different sites (wine growers, cooperatives, chambers of agriculture), most of which are in the Aquitaine and Occitanie regions. The observations were made between 2017 and 2019 and concerned 23 varieties of French, German and Swiss origin. It has shown a reduction of 96% in the TFI for fungicides compared to the agreste 2016 control reference with 80% of the treatment applications around the flowering season.

The study of restraints on plant variety innovations in France has shown:

- Plant variety innovation cycles are too long and above all obscure. French VATE (Environmental and Technical Agricultural Value) experimentation does not sufficiently integrate the wine-growers themselves in testing the wines produced or involve the nurseries in how they should prepare the plants for distribution.
- The very high degree of rigidity surrounding ‘authentic regional origins’ in the wine trade imposes endless experimentation constraints and ‘origin’ typicity criteria that are too strict.

German and Swiss wine makers are quite the opposite, they have a very flexible and much more participatory regulatory and experimental approach.

France has had to soften its experimental framework in the face of what is demanded in the field, even if this still remains too bureaucratic and tightly structured around the INRAE and IVF professional associations. In the same way the INAO has completely changed its own framework such that experimentation is now possible even if it is still restricted by a deep-seated hierarchical structure concerning the various 'regional origins'. A number of regional variety selection programs should lead to a major renewal of the grape variety landscape. To this end, the dynamic is now much more powerful in France than elsewhere, where the use of grape varieties remains secret even after 20 years of history.

As for those winegrowers who are prepared to adopt new varieties, the priority is placed on the quality of the wines produced rather than the variety's resistance to disease. Evidently all of this is central to the choices of new varieties, or there would be no reason to change the grape varieties. Professionals prefer a variety that produces greater quality even if it is less disease resistant (provided it isn't completely decimated by pests). They feel that they can better manage the risk of moderate levels of disease resistance with *ad hoc* control practices rather than face the commercial risk that the wine will not please consumers. The practice of wine blending in the South of France should favour the integration of a percentage of disease resistant varieties in the IGP regions, less rigid than the AOP 'regional origins' when it comes to homogeneous wine-typicity, and more interested in the marketability generated by the low environmental impact of their wines. Amongst the IGP of the North of France, where single grape variety wines are dominant, these varieties could find their place in the production of sparkling wines, since the wine-making qualities of the grape variety are less decisive. This is also the case (possibly even more so) for spirits such as Cognac and Armagnac.

More globally speaking the profession is waiting for the arrival, in about a dozen years, of varieties from regional retro-breeding programmes, where they hope to be able to combine wine typicity with variety related disease resistance. In the meantime, initiatives are concentrating on the zones of friction between wine-growers and residents of the new urban fringes, as a backdoor route to introducing varieties requiring little or no pesticide treatment. It would be probably best to support and federate experimental approaches for the IGP and cooperatives in order to build on the agricultural, pathological and quality capital of these collective organisations, since the observatory itself remains a simple research tool and sanitary watchdog before anything else.

Prospects for transfer or research

Transfer:

Originally the project was very optimistic considering that the distribution of disease resistant grape varieties remained quite confidential. Recent regulatory changes meant that planting did not really start until 2019. This is why regional exchange workshops have been planned in continuation of the project. Due to Covid, they will not be organised before the end of 2021.

The disease resistant grape variety monitoring observatory will ensure better education of wine-growers on the subject of how grape varieties behave in the face of disease. A doctorate on wine-making using disease resistant grape varieties is planned which should do a better job of analysing the relation between variety-related disease resistance and wine quality, and therefore provide a better response to the expectations of growers.

Research:

The project has facilitated the launch of the follow-on project OSCAR, which aims to consolidate and extend the disease resistant grape variety observation structure. It will now extend into the PPR Curare on zero pesticides on grapevines, with a planned doctorate that aims to put together an experimental variety evaluation table, combining pathological, agricultural and oenological aspects.

Publications and scientific symposia:

SCIENTIFIC PUBLICATIONS:

- S. Tabouret, 2019, **Setting up a "good" experimentation: the case of vine varieties testing in Languedoc,** Open Agriculture Journal, Elsevier.
- S. Guimier, F. Delmotte, A.S. Miclot, F. Fabre, I. Mazet, C. Couture, C. Schneider, L. Delière, 2019. **OSCAR, a national observatory to support the durable deployment of disease-resistant grapevine varieties.** Acta Horticulturae
- S. Tabouret, 2020, **Résister ! Une aptitude commune aux vignes, aux agents pathogènes, aux professionnels et aux scientifiques,** L'immunité des plantes : Pour des cultures résistantes aux maladies (C. Lannou, D. Roby, V. Ravigné, M. Hannachi, B. Moury,- eds.), Paris, Quae, p.235-46
- F. Hochereau, 2020, **La résistance variétale, un objet frontière à construire,** L'immunité des plantes : Pour des cultures résistantes aux maladies (C. Lannou, D. Roby, V. Ravigné, M. Hannachi, B. Moury, eds.), Paris, Quae, p. 257-58

TECHNICAL WORKSHOPS AND SCIENTIFIC SYMPOSIA:

- F. Hochereau, 2019, **Classement, déclassement et reclassement de l'innovation variétale : Une comparaison franco-suisse,** RT 29 Sciences et Techniques en Société, AFS congress, Aix en Provence
- F. Hochereau, 2019, **L'évolution des catégorisations du risque par l'adoption de cépages résistants en viticulture,** RT 38 sociologie de l'environnement et des risques, AFS congress, Aix-en Provence
- F. Delmotte 2018. **OSCAR — a national observatory for a sustainable deployment of disease-resistant grape varieties.** XII International Conference on Grapevine Breeding and Genetics
- L. Delière. 2017. **Déploiement des variétés résistantes en viticulture : observatoire Oscar. Rencontres régionales de la recherche, du développement et de la formation.** Montagne, 13 December 2017
- L. Delière. 2017. **Un Observatoire du déploiement des cépages résistants.** SITEVI conference, 29 November 2017
- F. Hochereau, 2017, **Sélectionneurs et pépiniéristes: Métiers ou Expertises au carrefour des mutations du monde de la vigne,** Métiers de la vigne et du vin, 20-21 November, Paris Saclay

PRESENTATION TO PROFESSIONAL OR DECISION-MAKING BODIES:

- L. Delière 2018. **Table ronde Cépages résistants : quelles avancées ?** Cognac 2025
- L. Delière. 2018. **Comment accompagner le déploiement des nouvelles variétés de vigne résistantes au mildiou et à l'oïdium ?** Presented to: Séances de l'Académie d'Agriculture « Révolution variétale en viticulture ! De nouveaux cépages résistant aux maladies : création, déploiement et impact sur la filière viticole », Paris, France (2018-05-30 - 2018-05-30)

PRACTICAL / EDUCATIONAL ARTICLES:

- **Un observatoire des cépages résistants pour lutter contre des maladies de la vigne.** Dépêche AFP, Bordeaux, 9 January 2017
- **Les cépages résistants en observation.** PHYTOMA n°718, November 2018

DESHERBAL

Development and study of the effectiveness of allelopathic substances to improve weed control for particularly resistant species

Launch year: 2017
Completion year: 2020

Scientific manager
Claire Richard, Clermont-Ferrand Institute of Chemistry / CNRS claire.richard@uca.fr

Partners
ICCF; Laboratoire de Physique et Physiologie Intégratives de l'Arbre en environnement Fluctuant (PIAF); INRAE; Mairie d'Aubière

Financing
Total cost of the project: € 287,550
Ecophyto grant: € 87,346

Key words:
JEVI; Weed control; Allelopathy; Phytotoxicity of secondary metabolites; Rock Rose; Hyssop; Mulch; Macerate; Cypress Leylandii

Context and main objectives

The ban on chemical weedkillers has raised a number of practical difficulties for JEVI (Gardens, Parks and Infrastructures) maintenance managers. Manual weed control is time-consuming and expensive whilst weed control by heat is not very effective since the weeds re-grow very rapidly. Using plants to control weeds has become an attractive alternative. This involves making the best use of the plants' ability to produce and diffuse phytotoxic compounds.

We have selected plants that, on the basis of material published to date, are known to contain large quantities of terpenes and have set up experiments to evaluate their ability to control weeds when the terpenes are:
(i) Emitted by the plants;
(ii) Diffused via mulch or
(iii) Applied as a macerate.

These experiments should be able to reveal the allelopathic properties of terpene-rich plants and thus show how best they can be used. This project has a twofold objective: to propose new potential weed control solutions and increase specific knowledge in this field through fundamental research.

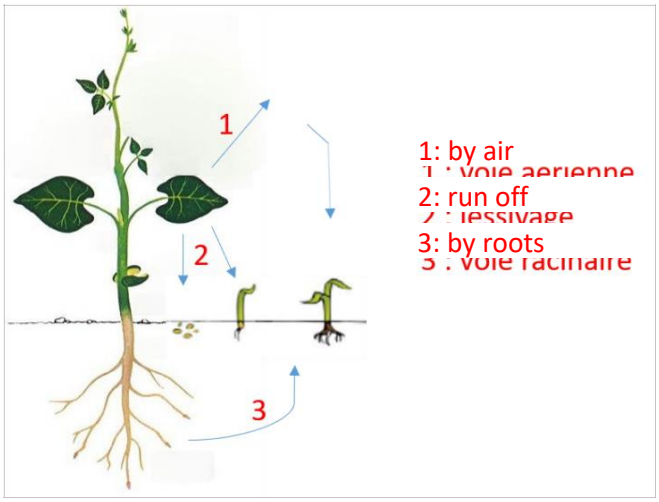


Figure 1: The phytotoxic compound emission paths of allelopathic plants *Image: © Claire Richard, ICCF/CNRS*

Principal results and interest in relation to the Ecophyto plan

Experiments on whole plants:

Lomelosia cretica; Centaurea bella; Hyssopus officinalis; Cistus creticus; Veronica polifolia were initially selected because they are strong-scented, hardy (-15°C), aesthetically pleasing, non-invasive, medium-sized (< 60 cm), evergreen and considered to be allelopathic by certain growers. Chemical analyses have shown that, apart from *Veronica polifolia*, all of these plants emit terpenes.

An initial series of tests in the Aubière municipal conservatory gardens have shown that rock rose and hyssop are the most promising of these plants (Fig. 2, following page); they were selected for a second series of tests under real conditions, where they were planted around the bases of trees in a school playground. This second series of experiments lasted barely more than a year, which did not provide the plants with enough time to develop and show their full capacities. However it was concluded that the results did confirm those obtained in the conservatory

gardens, rock-rose does appear to have interesting weed-control properties when planted alone or in combination with hyssop. And further tests in pots have also shown that rock-rose clearly reduces the growth of clover by reducing its access to water (fig. 3). This property could contribute to the effect it has previously been observed to have on weeds.

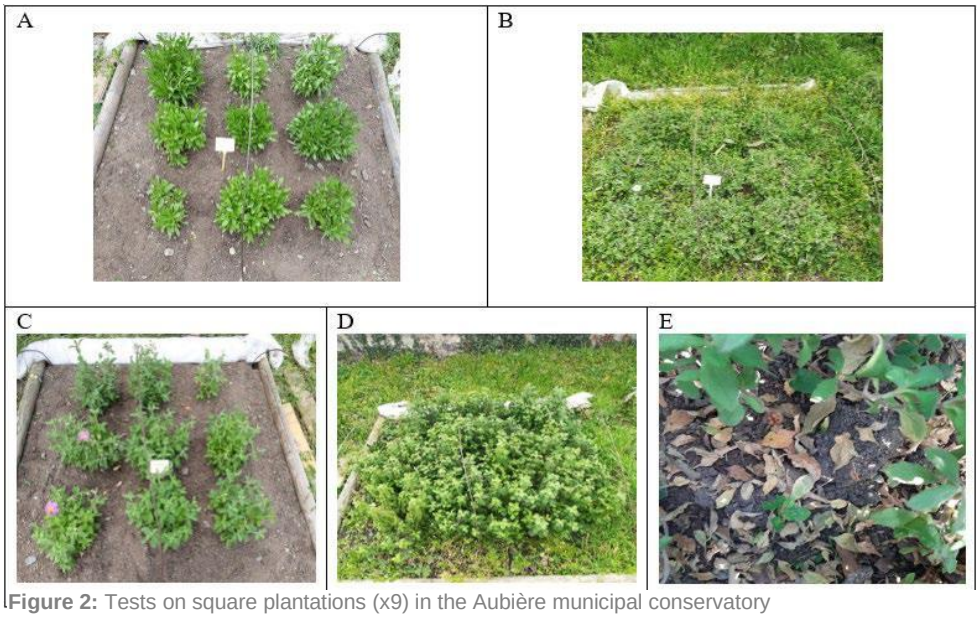


Figure 2: Tests on square plantations (x9) in the Aubière municipal conservatory gardens. A: *Lomelosia* May 2019; B: *Lomelosia* March 2020; C: Rock rose May 2019; D: Rock rose March 2020; E: Rock rose March 2020; showing ground underneath. *Photo: © Claire Richard, ICCF/CNRS*

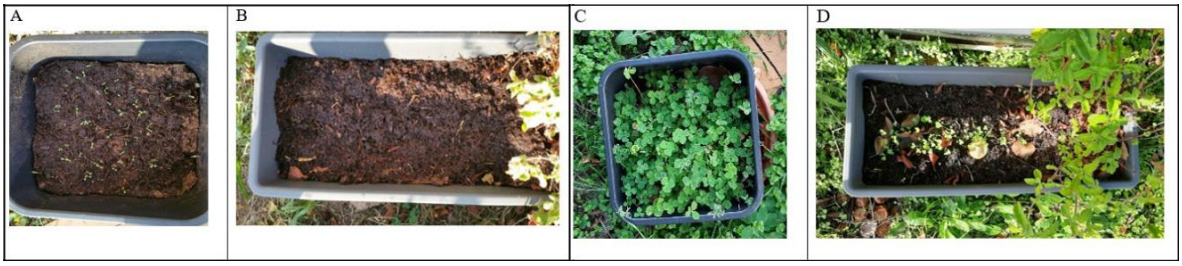


Figure 3: Effects of rock rose on the germination and growth of clover. A: Control, 9 days after seeding; B: Pot with rock-rose planted on right, 9 days after seeding; C: Control, 5 month after seeding; D: Pot with rock-rose planted on right, 5 months after seeding. *Photo: © Claire Richard, ICF/CNRS*

Experiments with Leylandii cypress extract:

Leylandii cypress, also terpene-rich, was selected for use as a mulch and a macerate.
This choice was based on information from published articles which presented encouraging results.

Germination tests under controlled laboratory conditions have shown that cypress needles, when applied as a concentrate or as a mulch, can very significantly reduce the germination or growth rates of clover and cress. The inhibition rate was > 90 % using an 8 day macerate. When applied as a 5cm layer of mulch, cypress needles reduce seedling growth rate by almost 50%. Experiments were also completed under real conditions around the bases of trees in a school playground. After a few months, the mulch turned out to be much more effective for weed control than a layer of the same thickness of chipped plant material (fig. 4, following page).

Chemical analyses of the macerate and cypress leaf ethanol extract were completed. In both cases a number of compounds were detected including terpenes that had oxidised to varying degrees. However, no clear relation has been established between the presence of any one particular compound and the observed property. The soil to which the mulch was applied was also tested after a year. This presented no unusual degree of acidification.
With these results, the Desherbal project has opened up prospects on the types of research to be undertaken in order to explore and understand the role played by terpenes in allelopathic phenomena. It also helped to implement operational solutions. Consequently, rock-rose, with or without the association of hyssop, and Leylandii cypress needle mulch can be used to help control weeds.

Figure 4: Test involving Leyland cypress mulch applied tot he base of a tree. A: Chipped plant material, September 2019; B: Chipped plant material, June 2020; C: Cypress mulch, September 2019; D: Cypress mulch, June 2020. *Photo Credit: © Claire Richard, ICCF/CNRS*

Prospects for transfer or research

Transfer:

This project has shown that rock-rose, either alone or in association with hyssop, can be used to control weeds in flower beds or around the bases of trees. Once in place, they require very little watering or maintenance. *Cistus creticus* produced better results than *cistus purpurus* but this could be a problem related to their growth-rates since the former was tested over two years whilst the latter was only tested over a year.

Additionally, Leylandii cypress clippings can be used as a mulch around the base of trees or in flower beds. Soil acidification remained very minimal.

Research:

For the part of the project that concerned allelopathic plants, the project has been able to highlight the interesting properties shown by terpene-rich plants, but room for more precise or detailed research on the subject remains. Other terpene-rich plants could also be tested.

For the Leylandii cypress mulch part of the project, further soil analyses should be completed to establish whether compounds in Cypress leaves can be detected in the soil and to ensure that they are innocuous.

Prospects for transfer or research (cont.)

Publications and scientific symposia:

TECHNICAL WORKSHOPS AND SCIENTIFIC SYMPOSIA

- **IUPAC on Crop plant protection. 19-24/05/2019. Gand, Belgium:** *Characterization of myrigalone photoproducts and evaluation of their anti-germinative properties.* Poster. A. Khaled, M. Sleiman, Y. Arbid, C. Sac, A. Corson, C. Bertrand, P. Goupil, C. Richard
- **European meeting on Environmental Chemistry (EMEC19). 03-07/12/2018. Clermont-Ferrand, France**: *Photodegradation of myrigalone, a natural herbicide, in water and on leaves.* Poster (Best poster prize). A. Khaled, M. Sleiman, C. Bertrand, C. Richard

Planned conference presentations

- **Congrès Biocontrole Perpignan (planned for September 2020, postponed due to Covid)**: *Can cypress leaves be used as mulch to control weeds?* Amina Khaled, Etienne Darras, Guillaume Barrès, Mohamad Sleiman, Claire Richard
- **Congrès Biocontrole Perpignan (planned for September 2020, postponed due to Covid)**: *Potential use of Cistus to control weed growth.* Amina Khaled, Etienne Darras, Guillaume Barrès, Mohamad Sleiman, Claire Richard

SCIENTIFIC PUBLICATIONS

- **Journal of Agricultural and Food Chemistry** *(n° 67, pp 7258-7265) : Photodegradation of myrigalone A, an allelochemical from Myrica gale: photoproducts and effect of terpenes.* Amina Khaled, Mohamad Sleiman, Etienne Darras, Aurélien Trivella, Cédric Bertrand, Nicolas Inguimbert, Pascale Goupil, Claire Richard*. 2019. https://pubs.acs.org/doi/10.1021/acs.jafc.9b01722
- **Forests** *(n°11-11, 1177, pp 1-12) : Phytotoxic Effect of Macerates and Mulches from Cupressus leylandii Leaves on Clover and Cress: Role of Chemical Composition.* Amina Khaled, Mohamad Sleiman, Pascale Goupil and Claire Richard*. 2020. Open access https://link.springer.com/article/10.1007/s10311-020-01137-z; https://www.mdpi.com/1999-4907/11/11/1177

Other practical works:

Article published in “La Montagne” http://ville-aubiere.fr/2018/07/26/espaces-verts-plantes-allelopathiques/-article published in “Métropole”, December 2019 - January 2020

DICABIO

Evaluation of dicaffeoylquinic and dicaffeoyltartric acids as natural Biocontrol substances

Launch year: 2016
Completion year: 2020

Scientific manager
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Partners
INRAE PACA Avignon; SBM Développement

Financing
Total cost of the project: € 431,035
Ecophyto grant: € 110,658

Key words:
Natural control methods; Plant extracts; Bio-aggressor resistance methods; Aphids; Fungal diseases; Bio-pesticides; Natural substances; Alternative elementary practices; Large-scale crops; Horticulture

Context and main objectives

The aim of DicaBio is to develop natural substances for use as bio-pesticides. Whilst a large number of publications have shown that a number of botanical substances can have pesticide properties, this has only very rarely resulted in their use as biocontrol solutions due to the large number of obstacles to their development.

DicaBio aims to develop plant-based phenolic molecules, dicaffeoylquinic (diCQ) and dicaffeoyltartric (diCT) acids, as organic aphidicides and fungicides for use on a variety of crops. The lack of diversity in the range of aphid control methods currently on offer is in itself a factor for the increasing resistance of these pests and the resulting crop epidemics. For example, in 2020, viruses affecting beetroot transmitted by aphids led to major production losses and resulted in the return of neo-nicotinoid insecticides onto the market. The alternative to chemical products that we are proposing here aims to provide a very specific control solution for the target insects which can also be respectful of the environment and public health.

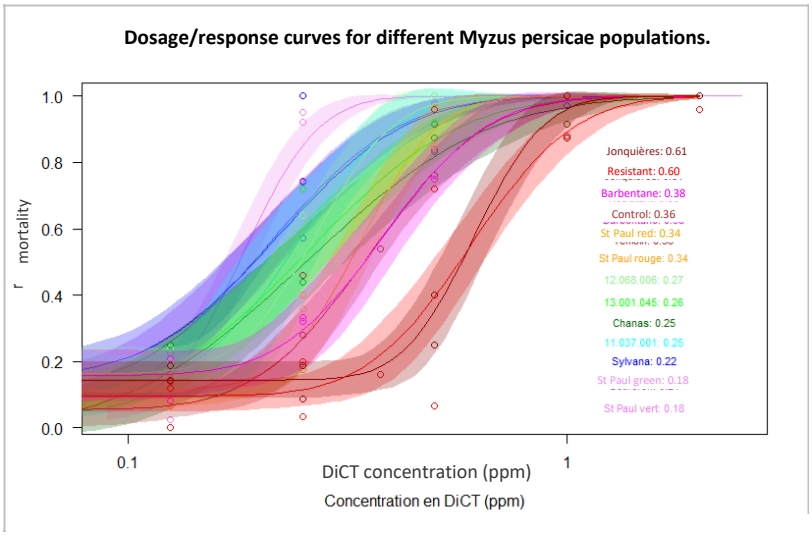
Principal results and interest in relation to the Ecophyto plan

The results obtained by DicaBio cover every step of the bio-pesticide product development process.

- We have identified new natural production sources of diCT which could guarantee supplies, using dandelion or peanut for example.
- We have studied the chemical properties of diCT and diCQ. These two molecules are highly anti-oxidising. They breakdown rapidly through oxidation to neutral pH; diCT is slower than diCA, but both remain stable at low pH levels. These results made it possible for the partner company, SBM, to produce adapted formulations.
- Amongst the 5 geneartions of formulation developed, the most recent presents an environment in which the molecule remains stable. After developing a laboratory testing protocol for the aphidicide properties, the various formulation types (liquid, wettable powder, suspension, liquid concentrate) were studied for their aphidicide properties on the various species of aphid, with and without the addition of agents to facilitate the penetration of the active ingredient. In spite of the gradual adaptation of formulations to resolve issues as they arose, the laboratory tests did not show a clear aphidicide action. Our work on this action of the formulation has shown that the problem lies in the failure of the preparation to penetrate the foliar tissues in spite of the addition of wetting agents. Once these formulations actually penetrate into the leaves they show that they are truly effective. Our results have also indicated that the substance has a low persistence on leaves and in foliar tissue, indicating that it is probably metabolised very rapidly. This property demonstrates its very low environmental impact.

▶ We have also shown that diCT, like diCQ, has a potential fungicide effect under laboratory conditions against cereal seedling pathogens and septoria leaf blotch in wheat.

▶ Works on the risk of aphids adapting to treatment with diCT or diCQ have shown that, to the extent of existing knowledge, both of these active substances have a long action duration as an aphidicide on Myzus persicae, a species which is known to have developed resistance to a number of chemical insecticides. There is no reason for the observed resistance to chemical insecticides passes on to these two active substances. However we have observed that one Aphis craccivora clone was not affected at all by the two substances. Better knowledge of this insensibility phenomenon could help us to both understand how these substances function and better manage the risk of adaptation by aphid populations.



DiCT dosage/response curves for different Myzus persicae populations. *Image: © Myriam Siegwart, INRAE*

▶ The risks of unwanted side-effects were evaluated on domestic bees. Acute exposure to diCQ and diCT, either by contact or ingestion, or over a number of days does not cause mortality. However a reduction in feeding was noted after chronic exposure for diCT but not for diCQ. These results have raised potential unwanted effects which could be suffered by beneficial insects if these substances are not used correctly. However, the impact of these natural substances on bees appears to be much less serious than that of the chemical insecticides currently used in farming.

Prospects for transfer or research

Transfer:

We have clearly identified that the absence of product penetration is a serious obstacle to the expression of the full potential of these substances as aphidicides. Once the problems is resolved, new biocontrol products that respect both the environment and public health could be available for use in a number of agricultural domains such as large-scale crops, beetroot, rapeseed, or in fruit orchards, all of which are suffering from a serious lack of new solutions. The results obtained here are a major step along the road to using diCT and diCQ as bio-pesticides and, in a more general way, a significant contribution to the innovative development of other botanical biocontrol substances.

Research:

It now seems necessary that innovative bio-pesticide product formulation approaches must be developed, such as nanoformulations; these are the subject of significant global research at the moment and could provide new solutions to resolve the low levels of active product penetration into foliar tissue, which has become the main product effectiveness obstacle raised by the DicaBio project. Additional research based on these innovative formulations would also be necessary in order to evaluate the possibility of developing a diCT-based fungicide to control wheat septoria leaf blotch and other fungal diseases.

Publications and scientific symposia:

Scientific symposia:

▶ **Séminaire de restitution intermédiaire des appels à projets « PSPE 2 » et « Pesticides 2014 » (14-15/12/2017)**, Paris, France
▶ **Natural Products and Biocontrol Conference (25-28/09/2018)**, Perpignan, France
▶ **Resistance'19. (16-19/09/2019**), Rothamsted, UK

Technical workshop:

▶ **Journée Métabolites Secondaires (16/09/2018)**, Avignon, France

Scientific publications:

▶ ***Mode of action and sustainability of the use of caffeic acid derivatives in the control of aphids in agriculture*** (in preparation). Siegwart M., Sauge MH., Lecerf E., Mascle O., and Poëssel JL.

ÉCOVERGER

Designing low pesticide-use technical approaches for orchards that respect the current farming restrictions and objectives. An applied modelling approach for peach and mango trees

Launch year: 2015
Completion year: 2019

Partners
CIRAD UPR HortSys; INRAE UR PSH; University of Montpelier

Scientific manager
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Financing
Total cost of the project: € 348,935
Ecophyto grant: € 88,921

Key words:
Fruit crops; Socio-economic evaluation; Technical route modification; Alternative treatment methods; Modelling; Growing practices; Production; Fruit quality; Farming strategy classification; Co-design

Context and main objectives

Fruit tree farming is subject to strict social and governmental requirements in terms of reducing its impact on the environment and public health whilst, at the same time, increasing the production quality. Even if producers are increasingly turning to alternative methods, orchard pest and disease management still relies heavily on chemical products. Agricultural practices will need to be totally redefined to invent economically viable systems capable of producing quality fruit whilst using fewer pesticides.

The objective of the project was to develop specific technical route (or solution) design support tools and approaches for orchard productions; evaluated on the basis of their capacity to restrict the development and the damages caused by pests and diseases whilst also meeting agricultural performance and economic viability obligations. The project attempts to identify and develop viable alternatives to the use of pesticides in the form of integrated control and hands-on methods that will increase the resistance of orchards to pests and disease. The approach has been tested and proven on two different fruit crops: mangos in La Réunion and peach-nectarines in mainland France.

Peach-tree orchard
Photo: © Michel Génard, INRAE

Principal results and interest in relation to the Ecophyto plan

The Ecoverger project has used three case studies to evaluate the performance of a number of pest and disease control levers, which were selected for their action and their standards of innovation as pesticide alternatives for brown rot - peach, fruit fly - mango and gall midge - mango blossom.

Amongst the evaluated levers, those that aim to restrict crop disease and contamination are useful for limiting the development of pests and diseases along with the damage they cause, but they can have a potential negative impact on harvests. Moisture stress and moderate fruit loads will reduce the incidence of brown rot, increase fruit quality but reduce calibre and harvest potential. Early harvests of mango, when they are green-ripe and just turning yellow, will minimise the risk of fruit fly infestation on the fruit and will only have a minor effect on fruit quality and quantity.

The other evaluated levers to restrict pest and disease development are less consistent in their results but have no impact on harvests. Covering the ground with woven textiles or high weeds will reduce gall midge populations and the damage they cause to mango blossom. Blossom synchronisation using suitable practices would also reduce gall midge populations, but

only if the orchard is not exposed to a high degree of outside pest pressure. Winter sanitation harvests, involving the removal of any dessicated twigs or fruit from previous seasons, will only control brown rot incidence if the primary inoculum is almost entirely eliminated from the orchard in the first place. Removing infected peaches appears to be more effective when it is done during the growing season. Due to such partial effects, these levers should be preferred only in combination with other levers.

The substitution of phytosanitary treatments with certain levers may generate additional expenditure due to investment in equipment or increased labour resulting from the introduction of new technical operations (covering the ground with woven textile) or increase the technicality of a practice already in use (harvesting fruit at specifically targeted ripeness stages). Other levers are quite easy to implement since they are simply based on adapting practices already in place in the technical pathway (changes to weed-cutting dates, less irrigation and cleaning out).

The value, productivity and visual aspect of the fruit are the most important performance criteria for most producers, whereas indicators concerning the environmental pollution related to phytosanitary treatments are perhaps of lesser importance (at least in the mango trade). This implies that there is little motivation to move over to eco-responsible practices if there is no return on the investment, for example through a higher market value, or carefully adjusted subsidies to compensate for any additional expenditure or a diminished harvest.

To find out if these levers can be substituted, either totally or partially, for pesticides, it would be necessary to achieve a greater degree of objectivity on their performance; and establish the conditions for success and adoption when implemented in the field. The diversity of production profiles promotes the idea of seeking out solutions adapted for each specific production context allowing for the specific constraints and objectives of the users.

Prospects for transfer or research

Transfer:

The Ecoverger project has studied alternative control levers to pesticides for dealing with pests which attack the reproductive organs of fruit trees in temperate and sub-tropical climates. General and specific rules concerning the effectiveness and deployment methods for these levers have been established in terms of their action and the nature of the pests. Additionally, considering that certain potential obstacles to their adoption are essentially economic, measures to incite the adoption of eco-responsible practices along with better promotion and marketability for products with environmental and public health benefits could help to remove such obstacles.

Research:

The Ecophyto II+ Plan ODACE[1] project continues the work begun by the Ecoverger project with the operational and functional implementation of an orchard management approach which is less dependent on pesticides. To do this, it will use a larger range of tools and expertise as well as trying to do away with some of the Ecoverger project limitations. More specifically, it will develop an interactive evaluation tool to facilitate dialogue between researchers and growers, in which the combination of a number of alternative levers, multiple pest and disease vectors, and sustainability dimensions will be taken into account.

Publications and scientific symposia:

TECHNICAL WORKSHOPS AND SCIENTIFIC SYMPOSIA:

- HortiModel 2016: Vth International Symposium on Models for Plant Growth, Environment Control and Farming
- Management in Protected Cultivation. 19-22 September 2016, Avignon, France: **A compartmental epidemiological model for brown rot spreading in fruit orchards.** Bevacqua D., Génard M., Turion-Quilot B., Oliveira Lino L., Mercier V., Lescourret F. (2016a).
- Plant Biology Europe EPSO/FESPB 2016 Congress. 26-30 June 2016, Prague, Czech Republic: **A compartmental epidemiological model for brown rot spreading in stone fruit orchards** [poster]. Bevacqua D., Génard M., Turion-Quilot B., Oliveira Lino L., Mercier V., Lescourret F., Bolzoni L. (2016b).
- XXVII Congresso Nazionale della Società Italiana di Ecologia, 12-15 September 2017, Napoli, Italy: **A model for temporal dynamics of brown rot spreading in fruit orchards.** Bevacqua D., Quilot-Turion B., Bolzoni L. (2017).
- XII International Mango Symposium, 10-16 July 2017, Baise, China: **How different pruning intensities and severities affect vegetative growth processes in "Cogshall" mango trees.** Persello S., Grechi I., Boudon F., Normand F. (2017).

[1] ODACE: Outil d'évaluation et de Dialogue entre acteurs et chercheurs, pour Accompagner la conCEption de solutions de protection des plantes dans le contexte arboricole (Evaluation and Dialogue Tool for professionals and researchers, to Support the design of pant protection solutions in an orchard context) (ECOPHYTO II+ OFB 2021-2023)

- XII International Mango Symposium, 10-16 July 2017, Baise, China: **Effects of some cultural practices on**
- **mango inflorescence and fruit pest infestation and damage in Reunion Island: recent progress, on-going studies and future steps** [poster]. Ratnadass A., Grechi I., Caillat A., Préterre A.-L., Normand F., Graindorge R. (2017).
- International conference: Models in Population Dynamics, Ecology and Evolution. 9-13 April, Leicester, UK: **A model for temporal dynamics of brown rot spreading in fruit orchards**. Bevacqua D., Quilot-Turion B., Bolzoni L. (2018a).
- XXVIII Congresso Nazionale della Società Italiana di Ecologia, 12-14 September, Cagliari, Italy: **Climate effects**
- **on the spread of brown rot disease: insights from an epidemiological model.** Bevacqua D., Vanalli C., Casagrandi R., Gatto M. (2018b).
- 30th International Horticultural Congress (IHC2018). 12-16 August 2018, Istanbul, Turkey: **Assessment of**
- **mango tree - blossom gall midge management solutions from in-silico experiments: overview of an on-going modeling approach** [poster]. Grechi I., Saint Criq L., Soria C., Ratnadass A., Normand F., Amouroux P, Boudon F. (2018).
- UMT Si-Bio seminar, 21 June 2019, Avignon, France: **Évaluation d'itinéraires techniques pour la gestion de la moniliose en verger de pêcher-nectarine**. Borg J., Kerdraon M., Plénet D. (2019).
- The International Society for the Ecological Modelling - Global Conference 2019. 1-5 October 2019, Salzburg, Austria: **The mango tree – blossom gall midge system: *in-silico* assessment of its functioning and management.** Grechi I., Reyné B., Saint-Criq L., Memah M.M., Ratnadass A., Normand F., Boudon F. (2019).
- 2nd annual ESA International Branch Virtual Symposium. 8-10 April 2019, USA: **Plant organ hardness as a factor of crop resistance to insect pests** [poster]. Ratnadass A., Caillat A., Chantereau J., Chillet M., Fliedel G., Grechi I. (2019).
- EEF: Ecology across borders. Embedding ecology in sustainable development goals. 29 July-2 August 2019,
- Lisbon, Portugal: **Climate change and the spread of brown-rot disease in peach orchards: insights from an epidemiological model**. Vanalli C., Bevacqua B., Casagrandi R., Gatto M. (2019).
- 4th TEAM Meeting, 5-9 October 2020, La Grande Motte, France: ***Potential of some cultural levers for fruit fly management on mango in Réunion*** [poster]. Ratnadass A., Caillat A., Préterre A.L., Brunet-Lecomte C., Lardenois M., Grechi I. (2020)

SCIENTIFIC ARTICLES:

- Frontiers in Ecology and Evolution (n°5, p 170): **The crop load affects brown rot progression in fruit**
- **orchards: high fruit densities facilitate fruit exposure to spores but reduce the infection rate by decreasing fruit growth and cuticle cracking.** Bellingeri M., Quilot-Turion B., Oliveira Lino L., Bevacqua D. (2018). https://doi.org/10.3389/fevo.2017.00170
- Phytopathology (n° 108, pp 595-601): **A model for temporal dynamics of brown rot spreading in fruit orchards**. Bevacqua D., Quilo-Turion B., Bolzoni L. (2018). https://doi.org/10.1094/PHYTO-07-17-0250-R
- Scientific Reports (n°9, p 8519): **Coupling epidemiological and tree growth models to control fungal diseases spread in fruit orchards.** Bevacqua D., Génard M., Lescourret F., Martinetti D., Vercambre G., Valsesia P., Mirás-Avalos J.M. (2019). https://doi.org/10.1038/s41598-019-44898-6
- European Journal of Agronomy (n° 104, pp 85-96): **Nature abhors a vacuum: Deciphering the vegetative reaction of the mango tree to pruning.** Persello S., Grechi I., Boudon F., Normand F. (2019). https://doi.org/10.1016/j.eja.2019.01.007
- Acta Horticulturae (n° 1244, pp 159-166): **Effects of some cultural practices on mango inflorescence and**
- **fruit pest infestation and damage in Reunion Island: recent progress, on-going studies and future steps.** Ratnadass A., Grechi I., Graindorge R., Caillat A., Préterre A.L., Normand F. (2019). https://doi.org/10.17660/ActaHortic.2019.1244.24
- Annals of Botany (n° 126, pp 745-763): V-Mango: **A functional-structural model of mango tree growth, development and fruit production.** Boudon F., Persello S., Jestin A., Briand A-S., Grechi I., Fernique P., Guédon Y., Léchaudel M., Lauri P-E., Normand F. (2020). https://doi.org/10.1093/aob/mcaa089
- Acta Horticulturae (n° 1281, pp 633-641): **The mango tree - blossom gall midge system: toward in-silico assessment of management practices.** Grechi I., Saint Criq L., Ratnadass A., Normand F., Soria C., Brustel L., Amouroux P, Boudon F. (2020). https://doi.org/10.17660/ActaHortic.2020.1281.83

Other promotional works:

- Educational documents for schools and researchers: *« L'agroécologie à la croisée des disciplines scientifiques »* organised by the Cirad and Montpellier SupAgro (Saint-Pierre, Ile de la Réunion, 28 November – 2 December 2016) for which the Ecoverger project served as study material: Data-sheet: **« Présentation du terrain mangues »** - video: **« La mangue, la culture de compromis »**
- Tp dispensed in BTS GPN1 (Gestion & Protection de la Nature) at l'EPLEFPA in St-Paul, Ile de la Réunion

ACTIVITY REPORTS FOR PRODUCERS:

- **Analyse systémique des exploitations productrices de mangues à La Réunion : identification des déterminants influençant les choix techniques et les changements de pratiques des producteurs** pour la co-conception d'itinéraires techniques innovants apportant une alternative aux pesticides – Summary presentation of orchard types. 8p. Marchetti M. (2016).

- Compte rendu **d'étude de l'effet de différentes modalités de couverture du sol sur la cécidomyie des fleurs du manguier, la phénologie de l'arbre, et sur l'infestation par les mouches des fruits des mangues précocement tombées au sol.** 9p. Brustel L., Soria C., Wilt M., Ratnadass A. (2017).
- **Evaluation des dynamiques des mouches des fruits, d'abondance/maturation des mangues et des niveaux d'infestation des fruits en vergers de manguiers.** Préterre A.L., Grechi I., Ratnadass A. (2017). 2 x 8p.
- Compte rendu de l'**analyse des résultats d'enquête et de de classification : identification des profils de production.** Kerdraon M. (2019). 4p+Appendix.
- **Communication and educational posters/data-sheets:**
- **Quels leviers pour maîtriser les mouches des fruits et leurs dégâts sur mangue ?** Grechi I., Caillat A., Préterre A-L., Brunet-Lecomte C., Ratnadass A. (2019).
- **Un dispositif de recherche et de formation sur les options agroécologiques de régulation de la cécidomyie des fleurs du manguier.** Ratnadass A., Amouroux P., Billot T., Briandy A., Brustel L., Grechi I., Normand F., Payet R-M., Reyné B., Saint Criq L., Soria C., Auré A., Wilt M. (2019a).
- Quels leviers pour maîtriser les cécidomyies des fleurs et leurs dégâts sur manguier ? Ratnadass A., Brustel L., Briandy A., Billot T., Grechi I. (2019b).

SURVEY / PLACEMENT REPORTS:

- Ecoverger project – **Déterminants des pratiques & indicateurs d'évaluation des systèmes techniques en vergers** de manguiers. Cirad. 22p. Parrot L., Michels T., Brulard N. (2019).
- **Analyse systémique des exploitations productrices de mangues à La Réunion : Identification des déterminants influençant les choix techniques et les changements de pratiques des producteurs pour la co-conception d'itinéraires techniques innovants apportant une alternative aux pesticides.** Dissertation, ISTOM, 79p + Appendices Marchetti M. (2016).
- **Comprendre les déterminants de la décision technique pour mieux accompagner l'innovation : le cas des producteurs de mangues réunionnais.** Dissertation, UniLaSalle, 71p + Appendices Girard G. (2017).
- **Evaluation des dynamiques des mouches des fruits, de l'abondance/ maturation des mangues et des niveaux d'infestation des fruits en vergers de manguiers et évaluation de la relation entre l´infestation des mangues et leur état de maturité.** Placement year dissertation, AgroParisTech, FacForPro affiliation, 38p + Appendices Préterre A.-L. (2017).
- **Effet de la taille sur la croissance végétative et la floraison du manguier Cogshall.** Placement year dissertaion, Montpellier SupAgro, 29p. Stahl A. (2017).
- **Evaluation de l'effet de pratiques culturales (paillage/ enherbement du sol/ récolte prophylactique précoce) en vergers de manguiers (*Mangifera indica L.*) sur la régulation de bio agresseurs de la floraison et de la fructification : les cas de la cécidomyie des fleurs (*Procontarinia mangiferae*) et des mouches des fruits (*Diptera : Tephitidae*).** Dissertation, Ecole d'ingénieurs de Purpan, 95p + Appendices. Brustel L. (2018)
- **Développement d'un outil pour co-concevoir des itinéraires techniques économes en pesticides en vergers**. Master 2 dissertation, University of Grenoble Alpes, 29p + Appendices. Girard J. (2018).
- **Modélisation du système manguier-cécidomyie des fleurs pour une évaluation de modes de gestion du ravageur et de ses dégâts.** Master 2 dissertation, Université Paul Sabatier, Toulouse III, 60p + Appendices. Saint Criq L. (2018).
- **Climate change and the spread of brown rot disease in peach orchards: insights from an epidemiological model.** M2 dissertation, Politecnico di Milano. 109p. Vanalli C. (2018).
- **Développement d'une démarche d'analyses statistiques de données d'un réseau pluriannuel d'expérimentations systèmes de culture en vergers de pêchers.** Dissertation , ISPED - University of Bordeaux, 40p + Appendices. Bostal C. (2019).
- **Evaluation du potentiel de leviers de gestion culturaux dans la lutte contre la mouche des fruits Bactrocera dorsalis (Hendel) sur manguier.** Dissertation, ISTOM 80p + Appendices. Brunet-Lecomte C. (2019).
- **Modélisation du système manguier-cécidomyie des fleurs pour une évaluation de modes de gestion du ravageur et de ses dégâts.** Master 2 dissertation, Montpellier Science Faculty, 44p + Appendices. Reyné B. (2019).
- **Growth and nutrient partitioning in deciduous trees: a modelling framework linking seasonal and inter-annual dynamics.** M2 dissertation, Politecnico di Milano. 63p. Salvagno P. (2019).
- *Evaluation de l'effet de pratiques culturales (type de couverture du sol / temps passé au sol par les fruits) et d'états physiologiques (couleur des fruits / état d'abscission / stade de maturité) sur l'infestation des mangues par les mouches des fruits (Diptera : Tephritidae) à la Réunion*. Placement year dissertation, AgroParisTech, FacForPro affiliation, 36p + Appendices. Lardenois M. (2020).
  Project web-site link : https://cosaq.cirad.fr/projets/ecoverger.

ENFIN !

Development of a new plant protection concept for apple scab disease

Launch year: 2022

Completion year: 2024

Partners
INRAE UMR IRHS, CTIFL, IFPC, UEH d'Angers, UMR
SADAPT de AgroParisTech

Scientific manager
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Financing
Total cost of the project: € 450,000
Ecophyto grant: 100 %

Key words:
Technical approach modification; Plant stimulation; Arboriculture; Socio-economic evaluation, Management via macro-organisms; pesticide reduction

Summary

Apple scab disease is caused by the *Venturia inaequalis* fungus and is the principal disease affecting apple-trees in temperate climates. Apple scab disease takes the form of blotches on the fruit making them unfit for consumption. Most of the leading commercial apple varieties are sensitive to this disease and phytosanitary products are used in orchards to cope with it. This represents an enormous financial and environmental cost (up to 30 fungicide applications per year to treat the disease effectively).

Apple scab disease

Photo: © INRAE

The ENFIN! project aims to test out a breakthrough technical approach which seeks to provoke a drastic fall in the population of this pathogen in orchards and thus considerably reduce the need to use fungicides. This approach uses a totally new biocontrol strategy which combines two INRAE patented inventions (FR 1915276 patent). The first invention renders the fungus non-virulent. It targets the reproductive phase of *V. inaequalis* and forces it to reproduce with non-pathogenic strains from the same species, thus generating non-virulent descendants for the following spring. The second innovation involves applying the same non-pathogenic strains during the spring time to provoke immunity in the apple trees, which will protect them from later apple scab disease infections. The project also aims to evaluate the durability of this approach in orchards (4 French experimental sites) and to evaluate the apple growing industry's acceptance and appropriation conditions for new inventions.

Ecophyto call for projects - Maturation (2021 edition) - Levers that can be implemented for a transition towards changing systems

In order to encourage research work that will contribute to reaching the Ecophyto II+ Plan objectives, the Ministries for Ecological transition and solidarity, Higher Education, Research and Innovation, Agriculture and Food, Solidarity and Health (Ministères de la Transition Ecologique et Solidaire, de l'Enseignement Supérieur de la Recherche et de l'Innovation, de l'Agriculture et de l'Alimentation, des Solidarités et de la Santé), have decided to launch a second edition of the "Ecophyto Maturation" call for projects.

This call for projects will promote the maturation of scientific works that have already succeeded in the context of a previously existing research program. The objective will be to take a breakthrough solution up to the stage where it can be applied using products, technologies or services. More precisely, the objective of this call for projects is to incite consortia of academic researchers and socio-economic entities (businesses, technical centres and institutes, professional bodies, consular chambers, NGOs, etc.) to work together to develop the research work necessary to be able to propose a solution which has been proven and rendered operational in the field whilst also responding fully to end-user requirements. On the TRL maturity scale, this would be the equivalent to level 5 or more.

This call for projects was operated by the Agence Nationale de la Recherche (ANR) which also co-financed it with the Office Français pour la Biodiversité (OFB).

FLEUR

Manipulating the floral biodiversity of cereal crop fields

Launch year: 2015
Completion year: 2018

Partners
University of Rennes-CNRS; INRAE / Agrocampus Ouest; Institut Sophia Agrobiotech; INRAE-AgroParisTech; SA PINAULT; Ter-Qualitechs; Dervenn; LTER Armorique

Scientific manager
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Financing
Total cost of the project: € 721,009
Ecophyto grant: € 141,748

Key words:
Floral plantings; Floral traits; Biological regulation; Trophic resources; Parasitoids; Predators; Winter season; Biological control by conservation; Global warming; Annual and perennial flower beds

Context and main objectives

In conventional large-scale cereal crop farming phytopharmaceutical products, amongst others, are used to coat seedlings. This is to limit aphid populations which spread virus during the winter. However, such seedling treatments have been banned since September 2018. For rapeseed crops, very rare in organic farming, pest beetle species are resistant to insecticides. The FLEUR project aims to set up a biocontrol by conservation approach to increase the population and diversity of the natural enemies of these pest insects by increasing the plant biodiversity surrounding the fields through perennial and annual flowerbeds. The objectives of the FLEUR project were to (1) identify the plant species best suited to increasing the effectiveness of natural enemies, (2) define the most efficient spatial arrangements for these flowerbeds in relation to the crops to be protected, (3) define the risks to neighbouring crops associated with these flowerbeds.

Aphidius avenae laying eggs inside an English grain aphid *Sitobion avenae*.

Photo: © Sonia Dourlot, University of Rennes 1

Principal results and interest in relation to the Ecophyto plan

The main product of this project is a wild flower mix of 4 species of inexpensive and clean plants that do not attract other pests and are used by alternative hosts allowing parasitoid populations to develop; they flower in winter when there is no frost (therefore producing nectar that can be used by the parasitoids) and are attractive to parasitoids due to their dominant yellow colour (mustard); when located near to the crops they serve as a refuge for predator arthropods (ground and rove beetles, spiders) thanks to their attractive micro-climate. This mixture includes a number of varieties of buckwheat likely to flower right through the autumn, mustard which is very frost resistant and attractive, various varieties of broadbean which flower between autumn and winter, as well as cornflower.

In conventional agriculture in Brittany, a region where cereal crops (wheat and corn in rotation) are common, this annual mix can be sown amongst the winter cover crops that the farmers grow between September and March in areas where they plant corn in the spring. In organic farming, since farmers do not use winter cover crops, a part of the field surface area must be given over to the flowers in place of cereal (lost surface area). The results achieved are very similar for both conventional and organic farming, highlighting the lack of risk involved in growing these flowers near to crops.

The winter season has now become a favourable period for the implementation of biological pest control methods that use conservation, thus opening up the possibility of year-round biological pest control through plant diversity. With the effects of global warming, natural enemies are present in fields throughout the year and are dangerous in winter, the period when the aphids transmit virus such as the Barley Yellow Dwarf Virus.

Our results have highlighted the positive effects of these flowerbeds on parasitism rates and the predatory regulation of crop pests by their natural enemies and show how important these flower beds can be to encourage auxiliary populations throughout the year.

Demonstrating the benefits of planting annual flowerbeds near to crops during winter is a first for biological control by conservation which until then had been mostly concentrated on the growing season. Additionally, this project has also been able to highlight the benefits of perennial flowerbeds to maintain the population levels of natural enemies.

Winter manipulation (looking for aphid) in a wheat field.

Photo: © Ouest-France 05 April 2018

Prospects for transfer or research

Transfer:

Our results show the benefits of planting wild flower mixes near to cereal crops during winter. In conventional agriculture in Brittany, which involves the rotation of wheat and corn crops, the transfer is relatively simple since farmers have to use winter cover crops and most of the farmers we contacted were not especially resistant to the idea of sowing a wildflower mix over the whole of the surface of their winter cover crops. If this method was adopted on a broad scale, it would probably have a synergistic effect over the whole of the landscape. Mass production of the wildflower mix is not in place yet.

Research:

The benefits of perennial or annual cultivated plant diversity on the scale of farming landscapes would need to be evaluated. For the rapeseed and pulse crops that are attacked by pest beetles, these insects and their parasitoids only complete one reproductive cycle per year and disperse very widely in the surrounding landscape. This means that the effects of the perennial planting which facilitates natural regulation shifts over time and is diluted over the land and thus farmers find little reason to invest in this approach. Annual flowering winter cover crops that are widely distributed over the farming landscape would not only be beneficial to neighbouring fields but also to the whole of the landscape.

Publications and scientific symposia:

SCIENTIFIC PUBLICATIONS:

- *Agriculture Ecosystems and Environment (n°247: pp 418-425):* ***Change in plant phenology during winter increases pest control but not trophic link diversity****.* Damien M, Le Lann C, Desneux N, Alford L, Al-Hassan D, Georges R, Van Baaren J. 2017.
- *Frontiers in Ecology and Evolution-Population and Evolutionary Dynamics :* ***Changes in host-parasitoid communities over the years in cereal crops of Western France: Does climate warming matters?*** Tougeron K, Damien M, Le Lann C, Brodeur J & van Baaren J. 2018.
- *Behavioral Ecology and Sociobiology (73:156):* ***Food or host : do physiological state and flower type affect foraging decisions of parasitoids ?*** Damien M, Barascou L, Ridel A, Van Baaren J, Le Lann C 2019. https://doi.org/10.1007/s00265-019-2758-9
- *Entomologia generalis (40(2): 147 – 156):* ***How does floral nectar quality affect life history strategies in parasitic wasps?*** Damien M, Llopis S, Desneux N, Van Baaren J and Le Lann C. 2020. Art No. ESP146004002003 DOI: 10.1127/entomologia/2020/0906S

OPERATIONAL / EDUCATIONAL ARTICLES:

- *Science ouest (n° 341, Avril 2016):* ***Des fleurs contre les pesticides.*** http://www.espace-sciences.org/sciences-ouest/341/actualite/des-fleurs-contre-les-pesticides
- *Horizon (n°130, April/May 2016):* ***Les parasitoïdes, des alliés actifs en hiver à favoriser.***

LIPOCONTROLE

The search for new lipopeptides for use as biopesticides, by screening a collection of Pseudomonas

Launch year: 2015
Completion year: 2020

Scientific manager
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Partners
University of Lille; INRAE; JUNIA; ULCO; Lipofabrik

Financing
Total cost of the project: € 254,261
Ecophyto grant: € 100,000

Key words:
Biocontrol product; Large scale crops; Natural substances; Lipopeptides; Pseudomonas syringae; High flow-rate screening; Cryptogamic diseases

Context and main objectives

Phytopathogenic fungi are one of the main pests and diseases affecting large-scale crops. Plant protection is currently largely based on the use of resistant varieties and chemical pesticides. There is a strong desire for the development of biocontrol plant protection products so that agriculture can become more sustainable and respectful of the environment and public health. Amongst these products, natural substances, mostly sulphur or plant based, occupy 63% of the market (in turnover, IBMA France 2019). Natural substances of microbial origin, such as lipopeptides produced by Bacillus and Pseudomonas bacteria, are little represented on the market but have been shown to have biocontrol potential against fungal diseases. The objective of the LIPOCONTROLE project is to identify new lipopeptides for use as biocontrol agents either in complement to or substitution of chemical pesticides in the treatment of the fungi that cause the cryptogamic diseases that cause the most economic damage.

Principal results and interest in relation to the Ecophyto plan

The LIPOCONTROLE project has identified a number of new lipopeptides, extracted from cultures of *Pseudomonas* bacteria, which are effective against a wide range of fungal pathogens:

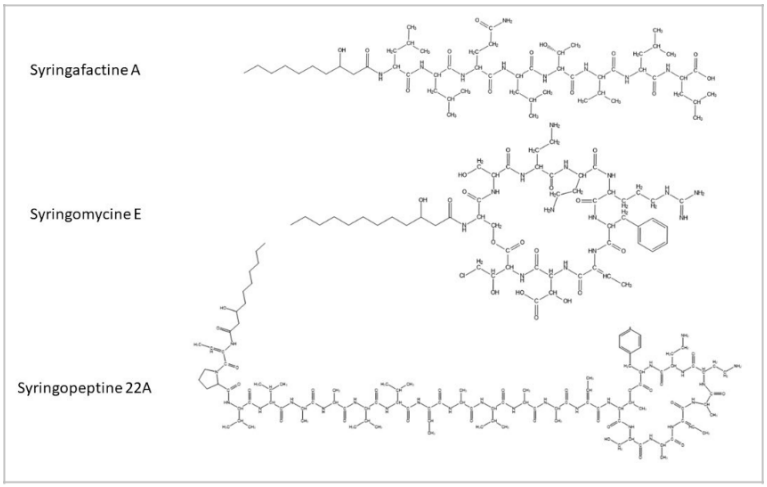

Figure 1: Lipopeptide structures produced by strains of the *P. syringae* complex. Linear or cyclic lipopeptides all have a hydrophobic fatty acid and a hydrophilous peptide chain.
Image: © Alice Rochex, University of Lille

- *Botrytis cinerea* (responsible for grey mould on a number of plant species including grape vines and tomatoes)
- *Sclerotinia sclerotiorum* (responsible for white mould on a number of plant species including rapeseed and sunflower)
- *Zymoseptoria tritici* (responsible for wheat septoria leaf blotch)
- *Phytophthora infestans* (responsible for potato blight)
- *Blumeria graminis f.sp. tritici* (responsible powdery mildew on cereals)
- *Oidium neolycopersici* (responsible for oidium on tomatoes)

These new lipopeptides could become the active ingredients of future biocontrol products that would be effective in protecting large-scale crops, such as cereals, oil-seed and grapevines, from the principle fungal diseases. These biofungicide products would be classed as natural biocontrol substances and could be used in complement to or substitution for chemical fungicides. By proposing new natural biofungicide substances the results of the LIPOCONTROLE project will contribute to growth in biocontrol products and the reduction of the use of conventional phytosanitary products.

Using one molecule presents a number of advantages over the use of living bacteria. They avoid the storage and viability constraints during packing, formulation and field application as well as ensuring constant treatment effectiveness. These molecules are formulated as a liquid and can therefore be used and stored in the same way as a conventional phytosanitary product with no further constraints in comparison to current common practice.

The first limitation in the results is that the effectiveness of biomolecules has only been proven *in vitro* for *B. cinerea* and *S. sclerotiorum*, and *in vitro* and under glass for *Z. tritici, P. infestans, B. graminis f. sp. tritici* and *O. neolycopersci.* For *B. cinerea* and *S. sclerotiorim*, a protocol will have to be developed for *in planta* tests. For all of the pathogen-systems studied, the effectiveness of identified lipopeptides will have to be confirmed in the field.

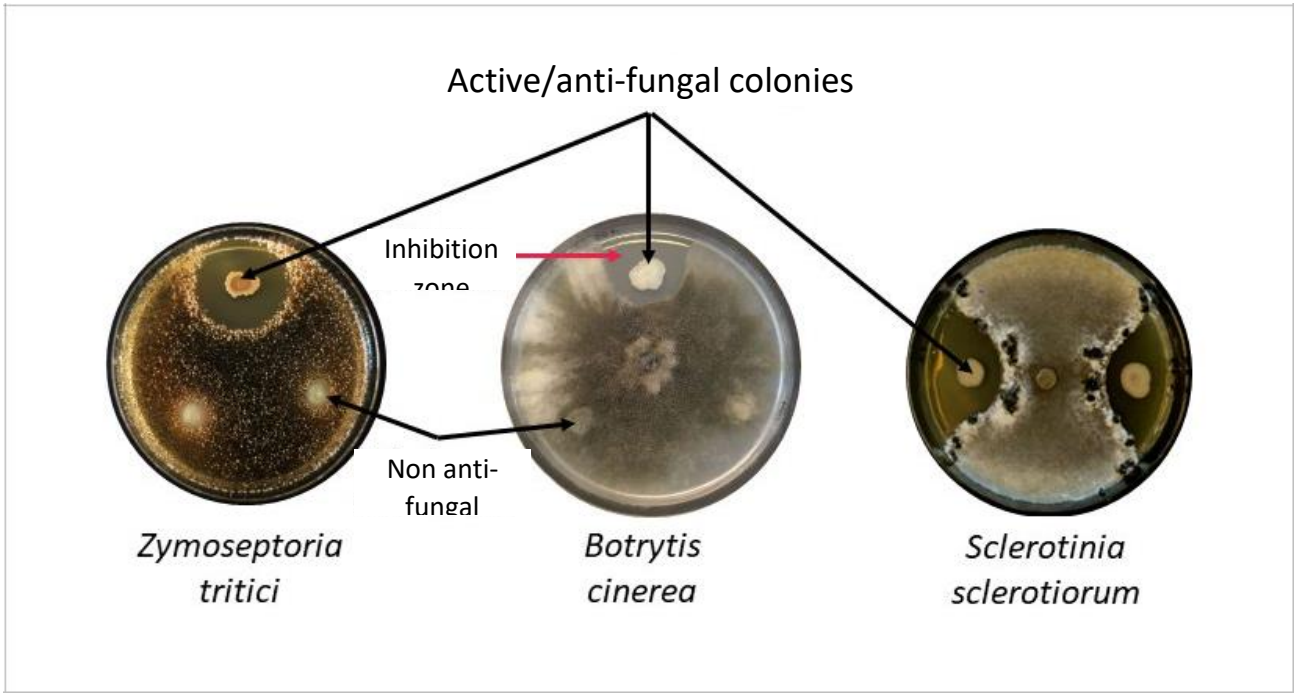

Figure 2: Laboratory tests on the anti-fungal activity of *P. syringae* bacteria. The anti-fungal bacteria inhibit the growth of fungi (inihibition zone). *Photo Credit: © Alexandre Bricout, University of Lille*

à The second limitation of these results is that the lipopeptides have only been produced on a laboratory scale (100 mL to 2L). A larger scale production and extraction process for these molecules will need to be designed and developed, this will need to produce enough of the molecule to fully evaluate their effectiveness in the field and show the feasibility of industrial scale production.

Finally, for the development of a new biocontrol product based on lipopeptides, there will need to be an evaluation of its toxicity, ecotoxicity and sustainability; and a formulation will have to be developed that will guarantee conservation and effective plant protection.

Prospects for transfer or research

Transfer:

Psudomonas lipopeptides are biocontrol products that show a high potential in spite of one major technological setback concerning its potential for mass industrial scale production: they are produced in very small quantities by the bacteria. Once the production and purification processes are available, the project results will be transferred to Lipofabrik a company specialised in the production of lipopeptides.

Research:

The works of LIPOCONTROLE will be continued by the ANR-19-ECOM-0007 CERES project (17 April 2020 - 27 October 2023), led by ULille and financed by APR ANR Ecophyto Maturation 2019. The CERES project aims to show that products based on Pseudomonas bacteria culture extracts containing lipopeptides are an operational biocontrol solution for managing wheat septoria leaf blotch.

Publications and scientific symposia:

SCIENTIFIC PUBLICATIONS:

- ***The diversity of lipopeptides in the P. syringae complex parallels phylogeny and sheds light on structural diversification during evolutionary history.*** Bricout A., Morris C.E., Chandeysson C., Boistel C., Chataigné G., Lecouturier D., Jacques P., Leclère V., Rochex A. Publication soumise.

PHD THESIS:

- ***Mise en évidence d'une forte diversité structurale de lipopeptides chez P. syringae, un complexe bactérien aux activités antifongiques prometteuses*. Bricout A. (2020). Ademe - University of Lille**

SCIENTIFIC SYMPOSIA (ORAL PRESENTATIONS):

- **Online conference, 16/06/2020, Akureyri, Iceland.** *Deciphering the structural diversity of lipopeptides produced by strains of the P. syringae complex. P. syringae.* Bricout A., Morris C.E., Bardin M., Nicot P., Chataigné G., Lecouturier D., Jacques P., Leclère V., Rochex A.
- **14th IUPAC International Congress of Crop Protection Chemistry. 19-24/05/2019, Gand, Belgium:** *Screening of Pseudomonas sp. strains for the biocontrol of septoria tritici blotch of wheat*. Bricout A., Morris C.E., Bardin M., Nicot P., Chataigné G., Siah A., Lecouturier D., Jacques P., Leclère V., Rochex A.
- **Biocontrol conferences. 04/04/2019, Reims, France:** *Stratégies de criblage pour la découverte et la caractérisation de lipopeptides antifongiques utilisables comme agents de biocontrôle*. Bricout A., Morris C.E., Bardin M., Nicot P., Chataigné G., Siah A., Lecouturier D., Jacques P., Leclère V., Rochex A.
- **Young Researchers Workshop Condorcet. 18-19/01/2018, Amiens, France:** *Développement de stratégies de criblage pour la recherche de lipopeptides antifongiques utilisables comme biopesticides.* Bricout A., Morris C.E., Bardin M., Nicot P., Chataigné G., Jacques P., Leclère V., Rochex A.

MACROPLUS

Facilitating the installation of the whitefly, spider mite and aphid predator *Macrolophus pygmaeus*

Launch year: 2015
Completion year: 2018

Partners
GRAB; SERAIL; APREL; Chambre d'Agriculture des Bouches du Rhône; INRAE UE Alénya Roussillon

Scientific manager
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Financing
Total cost of the project: € 198,550
Ecophyto grant: € 99,233

Key words:
Management via macro-organisms; Redesigning crop systems; Functional biodiversity; Vegetable crops; Host-plant; *Macrolophus*; Service plant; Co-design work with farmers

Context and main objectives

Macrolophus pygmaeus is a predatory plant bug which has been used to control white fly on tomatoes for the last twenty years. This plant bug is native to the South of France and can be used to regulate red spider mite, aphid, moths and tomato leaf miner populations provided that the crops are not intensively treated. Unfortunately this essential auxiliary species is costly and difficult to introduce sustainably.

The MacroPlus project aims to work on these last two points by developing techniques to obtain larger numbers of *M. pygmaeus* at a lower cost and earlier in the season. The MacroPlus project has developed strong ties with motivated producers that are curious about the planned experiments. For the experiments, the techniques were based on the use of *M. pygmaeus* host-plants such as the common marigold. These plants were studied as winter refuge zones or relay plants.

Macrolophus larvae and adults on marigolds.
Photo: © SERAIL

The central issue for the MacroPlus project is that, beyond supplying technical references, it is the involvement of producers which will need to be strengthened such that they participate in the development of a biological control approach in which they play an active role rather than just being the clients of a supplier.

Principal results and interest in relation to the Ecophyto plan

Amongst the twenty species tested by the GRAB since 2007, it is the common marigold (*Calendula officinalus*) that has turned out to be the most interesting for encouraging the presence of *Macrolophus*. This hardy plant allows *Macrolophus* to feed, since they can feed off the plant juices via the stems and the pollen in the flowers. *Macrolophus* also finds prey on them. Observations during mid-winter have shown that *Macrolophus* were present and active on marigolds both inside and outside shelter areas and that they were able to reproduce during this, usually unfavourable, period.

The strategy throughout the project was to work on beds of marigolds as winter refuges for these predator plant bugs. Tests established in the fields of growers turned out to be conclusive. Marigolds were planted or sown under plastic sheets, inside unheated plastic greenhouses before the crop plants were ripped up in autumn. These marigolds were then able to serve as a refuge for *Macrolophus* during the winter where they could also reproduce; the marigold were then able to serve as a source of *Macrolophus* the following spring. Crops grown in tunnels bordered with marigold plantations had higher populations of *Macrolophus* and lower populations of pests than those without marigolds. Further assays have proven that ripping up or cutting the marigold plants served as a way to force the *Macrolophus* to transfer over to the crops at the best possible time and thus harmonise *Macrolophus* populations on the crops and strengthen their effectiveness as a biocontrol vector.

Cut Marigold stems bearing *Macrolophus* placed near to crops. *Photo: © GRAB*

Transfer from the *Macrolophus* bearing marigold stems is very simple and can be just as easily done in the same tunnel as in another one. It would be useful to adjust the quantity of cut marigold stems in terms of the actual *Macrolophus* populations on the marigolds. The issue here is that there is no sudden interruption in the provision of a habitat and food to the auxiliaries, either on the marigolds or the crop plants.

When the marigolds cannot be planted or seeded in the ground, they could also be used as relay plants. They could then be sown or planted in pots or planters. These pots can be placed inside the tunnels with the tomato plants when they are ripped up at the end of the season. The *Macrolophus* then find refuge in the marigolds since the tomatoes have dried up and are no longer attractive. These pots are then placed in a tunnel (which does not need to be heated) for the winter where they should be watered sufficiently. The installation of insect-proof nets (or P17 in colder regions) over these pots will confine the *Macrolophus* populations and/or protect the plants and insects from the cold.

In springtime, after checking for the presence of *Macrolophus* and absence of pests (such as *Nesidiocoris*, white fly) on the marigolds, the pots can then be placed as early as possible near to the crops to be protected. The use of pots is also better suited where insecticide treatments are used on winter cultures or solarisation in summer. They can easily be moved from one tunnel to another to allow for crop rotation.

The results obtained in different production contexts (organic or conventional farming, different soil types) are consistent allowing for reliable conclusions. The success of strategies based on those worked on in the Macrolophus project in other regions of France further confirms these results. These references were acquired within a cold-frame growing context in the ground.

Prospects for transfer or research

Transfer:

Transfer has already begun through the growers' involvement in the project. This involvement was assisted by the close relations maintained by the experimentation stations with the professionals. The implementation of simplified monitoring on the sites of certain growers and the relay provided by the Dephy Fermes networks have amplified the application of the project's conclusions taking them beyond the experimental sites. Right from the start of the project, the care taken in listening to the expectations and constraints of growers ensured that the strategies would be realistic and that the transfer into the field would be rapid.

Research:

In spite of the declared ambitions, budget limitations imposed certain choices. Winter cultures were not monitored for example, even if *Macrolophus*, still active in the flower beds during this period, could be used to regulate certain other pests (such as aphids on lettuce). The references were acquired within the project in a cold-frame, ground planting context. Hydroponic production constraints will be different. *Macrolophus* is an essential but costly auxiliary for these systems. It would therefore be very interesting to transpose the knowledge acquired for *Macrolophus* into a hydroponic production context. In the same way, working with other host-plant/auxiliary combinations such as *Geranium/Dicyphus* could also strengthen agro-ecosystem stability. The CASDAR ACOR project has taken on these objectives since it launched in 2020.

Publications and scientific symposia:

There have been a very large number of communication initiatives. These have been largely aimed at growers and technicians (professional journals, visits, conferences and participation in technical meetings). Some application operations have concentrated on scientific partners (Ecophyto, GTN functional biodiversity, GTN PBI symposia). The project results have also been integrated in to functional biodiversity and Organic and Integrated P&D control training programs.

EDUCATIONAL/FUNCTIONAL ARTICLES:

- Culture Légumière (n°158 March April 2017): **Favoriser *Macrolophus pygmaeus*, un auxiliaire indigène**
- Biofil (n°111 May June 2017): **Lutte biologique sous abri : optimiser *Macrolophus pygmaeus***
- Serre et plein champ (n°378 June 2017): **Utilisation des soucis comme refuge hivernal pour les *Macrolophus***
- Phytoma n°724 May 2019: ***M. pygmaeus,* une punaise pleine de ressources**

TECHNICAL DATA-SHEETS:

- Technical resources data-sheet September 2018: **Le souci, plante-hôte de *Macrolophus*** 4p.

MDRisque

Evaluation of the potential for multi-drug resistance in the wheat septoria leaf blotch agent, *Zymoseptoria tritici*

Launch year: 2016
Completion year: 2019

Partners
INRAE UMR BIOGER; Anses; Arvalis

Scientific manager
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Sabine Fillinger, INRAE UMR BIOGER
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Financing
Total cost of the project: € 358,494
Ecophyto grant: € 103,120

Key words:
Efflux; Adaptation; Fungicides; Resistance mechanisms; Genetics; Hybridisation; Field results; Pest and disease resistance management; Large-scale crops

Context and main objectives

Wheat septoria leaf blotch caused by Zymoseptoria Tritici is the most common disease affecting wheat in Europe. This disease is largely controlled with fungicides which have the (unintentional) effect of provoking the natural selection of the more resistant individuals within populations. A new mechanism associated with a MultiDrug Resistance (MDR) phenotype was detected in 2007 although its complete genetic architecture remains to be fully elucidated. MDR is provoked by the intense efflux of fungicides with various action methods and which results in a non-specific resistance to a number of active ingredients. As a general mechanism, intense efflux can even have a potential impact on synthetic or natural active ingredients that will be marketed in the near future. The issue at the heart of this project is to promote wide ranging anti-fungal usage practices that would limit the selection of the MDR phenotype in order to maintain their sustainability and avoid unnecessary or useless applications.
In this context the objectives of MDRisques were:

1. Formally demonstrate the involvement of the previously identified MDR mutations in the MDR phenotype
2. Detect and evaluate MDR mutations in populations
3. Evaluate the practical risk of MDR mechanism related resistance in *Z. tritici*.

Principal results and interest in relation to the Ecophyto plan

Work completed within the MDRisque project provided a better description of the origin and characteristics of MultiDrug Resistance (MDR) in the major wheat pathogen *Z. tritici.* We have used a functional genetic approach to confirm that three types of *MFS1* promoter inserts are responsible for the intense fungicide efflux mechanism in *Z. trttici* and, therefore, the MDR phenotype. We have developed a PCR tool that can detect these alleles on isolated strains. A quantification tool based on the amplicon sequencing technique using Nanopore® technology is being used routinely but still requires further work.
Through a Mendelian genetic approach and the phenotyping of descendants from two hybridisations, we were also able to show that the combination of efflux and target modification mechanisms, already common amongst populations, will significantly increase the risk of practical resistance to SDHI and IDM fungicides. This risk of association between specialist and generalist resistance mechanisms is high and realistic *in natura* due to the frequent and intense sexual reproduction of *Z. tritici.* Paradoxically, recombination would largely add to the risk associated with the alleles presenting low levels of resistance to SDHI (such as the $sdhC_{T79N}$ allele, which is constantly increasing its presence in French populations). In fact, if the risk of real-world resistance is low for strains with just this allele or the MFS1 alleles, their combination would lead to resistance levels compatible with a loss of effectiveness in the field. Additionally, no especially high costs associated with each of the five alleles studied were detected in the project, regardless of whether they were carried alone or in combination. This reduces the possibility of a natural decrease in the frequency of these alleles in the absence of natural selection.

Finally, we have shown that MDR was selected preferentially within a network of field assays by SDHI based programs. We have also demonstrated that the introduction of a multi-site such as chlorothalonil, not affected by these resistance mechanisms, into the programmes, especially at T1, tended to reduce the natural selection of MDR.

Prospects for transfer or research

Transfer:

This project has allowed professionals to develop their own awareness of the indirect risks associated with generalised resistance mechanisms such as intense efflux. This risk concerns active ingredients currently in use as well as those likely to be marketed in the near future including biocontrol fungicides The identification of practices which favour MDR has strengthened reflections on optimising treatment strategies, especially by limiting the use of SDHI in programmes, and will assist the transition towards other control methods, such as variety selections. MDR is now monitored annually in French populations using the diagnostic tools developed by this project.

Research:

The MDRisque project has been associated with a 3 year training programme for an apprentice engineer.

This project has demonstrated the use of studying resistance mechanisms that are not related to the target in phytopathology, intense efflux being mainly studied by the medical community. The data obtained will also be used to explore the spatio-temporal adaptation dynamics observed in *Z. tritici.*

Finally, this project has led to the emergence of a thesis project (under way) to classify the distribution of MFS1 alleles in European populations and to elucidate the genetic mechanisms that regulate intense efflux in *Z. tritici.*

Publications and scientific symposia:

TECHNICAL WORKSHOPS AND SCIENTIFIC SYMPOSIA:

- Walker A-S. (2019). **Research, monitoring and resistance management in France**. *In:* Annual meeting of the Phytopathological Society of Japan, Tokyo, Japan.
- Walker A-S. (2019). **Assessing the risk of resistance towards SDHIs in France.** *In:* Plant Health 2019, American Society of Phytopathology, Cleveland, USA.
- Couleaud G., Walker A-S., Maumené C. (2015). **Etude au champ de l'efficacité de la pression sélective exercée par des associations fongicides sur la sensibilité des populations de Zymoseptoria tritici ces dernières années en France.** In: *Proceedings of the "11ème Conférence Internationale sur les Maladies des Plantes"* (ed. AFPP), Tours (France).
- Omrane S., Audéon C., Ignace A., Duplaix C., Sghyer H., Aouini L., Kema G., Fillinger S., Walker A-S. (2015).
- **La résistance de type MDR chez l'agent de la septoriose du blé: dernières avancées sur un mode d'action**
- **original.** In: "11*ème* Conférence Internationale sur les Maladies des Plantes", Tours (France).
- Couleaud G., Maumené C., Maufras J-Y., Walker A-S. (2018). **Prévenir la sélection de la résistance MDR chez *Zymoseptoria tritici.*** In: *Proceedings of the « 12ème conférence internationale sur les maladies des plantes »* (ed. Végéphyl). Tours, 11 and 12 December 2018.
- Gazeau G. and Walker A-S. (2018). **Mécanisme de la résistance multidrogues (MDR) et prévention du risque de résistance aux anti-septoriose chez *Zymoseptoria tritici.*** In: *Proceedings of the « 12ème conférence internationale sur les maladies des plantes »* (ed. Végéphyl). Tours, 11 and 12 December 2018.
- Omrane S., Audéon C., Ignace A., Duplaix C., Sghyer H., Aouini L., Kema G., Walker A-S., Fillinger S. (2016).
- **Several mutations of *Zymoseptoria tritici* field strains lead to MFS1 overexpression and multi-drug resistance (MDR)** (poster). In: 18th International Reinhardsbrunn Symposium on Modern Fungicides and Antifungal Coumpounds, Friedrichroda (Germany).
- Walker A-S., Gazeau G. (2019). **Mechanism of multidrug resistance and risk assessment towards fungicides in *Zymoseptoria tritici.*** In: 19*th* International Reinhardsbrunn Symposium on Modern Fungicides and Antifungal Compounds, edited by H. B. Deising, B. Fraaije, A. Mehl, E. C. Oerke, H. Sierotzki and G. Stammler. Frierichroda, Germany: Deusche Phytomedizinische Gesellchaft, Braunschweig.
- Omrane S., Audéon C., Ignace A., Duplaix C., Sghyer H., Aouini L., Kema G., Walker A-S., Fillinger S. (2015). **Genetic analysis of multi-drug resistance (MDR) in *Mycosphaerella graminicola (Zymoseptoria tritici)*** In: 28th Fungal Genetics Conference, Asilomar, USA.
- Omrane S., Audéon C., Ignace A., Duplaix C., Sghyer H., Aouini L., Kema G., Walker A-S., Fillinger S. (2015). **Multi-drug resistance (MDR) in septoria leaf blotch** (oral pres.). In: *XVIII International Plant Protection Congress, Berlin (Germany).*
- Walker A-S., Gazeau G. (2019). **Mechanism of multidrug resistance and risk assessment towards fungicides in *Zymoseptoria tritici*.** In: *International Symposium on Cereal Leaf Blights,* 22-24 May 2019, Dublin, Ireland.

- Gazeau G., Auclair C., Delestre G., Suffert F., Walker A-S. (2016). **Assessing the risk of recombination between MDR and specific resistance towards SDHIs in *Zymoseptoria tritici.*** In: *18th International Reinhardsbrunn symposium on modern fungicides and antifungal compounds,* Friedrichroda, Germany.

SCIENTIFIC PUBLICATIONS:

- Omrane S., Audéon C., Ignace A., Duplaix C., Aouini L., Kema G., Walker A-S., Fillinger S. (2017). **Plasticity of the MFS1 promoter leads to multidrug resistance in the wheat Pathogen *Zymoseptoria tritici.*** *mSphere* 2 (5).
- Garnault M., Duplaix C., Leroux P., Couleaud G., Carpentier F., David O., Walker A-S. (2019). **Spatiotemporal dynamics of fungicide resistance contrast quantitatively in the pathogenic fungus *Zymoseptoria tritici.*** Pest Management Science 75 (7), 1794-1807.
- Gazeau G., Duplaix C., Walker A-S. *(in prep)* **Assessing the risk of recombination between MDR and specific resistance towards SDHIs in *Zymoseptoria tritici*.**

OTHER OPERATIONAL WORK:

- Shared notes « Gestion de la résistance des maladies des céréales à paille ». https://www.r4p-inra.fr/fr/notes-communes/

MILPOMBIO

Potato blight: identification and development of biocontrol products for integrated crop protection

Launch year: 2015
Completion year: 2018

Partners
VEGENOV; INRAE UMR IGEPP; ARVALIS Institut du végétal; Bretagne Plants Innovation

Scientific manager
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turner@vegenov.com

Financing
Total cost of the project: € 235,561
Ecophyto grant: € 104,950

Key words:
Biocontrol; Blight; Potatoes; Combination of levers; Integrated protection; Variety resistance

Context and main objectives

Potato blight (caused by *phytophthora infestans*) is the main disease affecting French potato crops. Conventional chemical treatments are widely used to cope with this pathogen resulting in a very high TFI for this crop (fungicide TFI > 10). At the time that this project was launched no biocontrol solution for this disease was available.

The objective of the MilPomBio project was to identify biocontrol products that could be effective against potato blight and then propose them to growers. These products have been integrated into technical approaches that associate them with the use of other phytosanitary products at lower doses, more resistant varieties and the Mileos DA tool to organise effective treatment campaigns. Under field production conditions, this has reached symptom levels compatible with satisfactory harvests as well as reducing TFI.

Assays under controlled conditions.
Image: © MMedia

One of the difficulties faced was the transfer of results from controlled conditions to the field, where a significant loss in protection was noted.

Principal results and interest in relation to the Ecophyto plan

The first phase of the MilPomBio project involved evaluating the effectiveness of around 40 biocontrol products (seaweed extracts, plant extracts, micro-organisms, minerals, organic compounds, phosphites) for the management of potato blight under controlled conditions after artificial inoculation. Under these conditions about 15 of the products were shown to be effective in reducing symptoms.

The most interesting of these products were then selected to:

1) Better understand how they work (natural plant defence stimulation and/or direct effects on the pathogen)

2) Study the effectiveness of these products in the field when they interact with the level of genetic resistance and the plant's structure

3) Evaluate the field protection effectiveness in combination with reduced fungicide doses for one variety.

Field screening was applied with the products whose effectiveness had been proven in the growing chambers. One plant extract based product also contained copper and did provide effective protection. This could not be included in a list of biocontrol products and was excluded from the study. A number of products containing phosphites were also shown to be effective in the field.

Some work was done on the importance of foliar structure in the disease's development as well as the performance of the biocontrol products. The conclusion was that a more open structure did not favour the disease. Over the 4 genotypes studied, the same phosphite-based product provided the same results. The effect of structure was visible but limited; no direct relation between biocontrol product performance and foliar structure was demonstrated.

The final stage of the project involved evaluating, under production conditions, a number of integrated protection strategies combining varieties with variable degrees of resistance, the Mileos DA, a biocontrol product and fungicides likely to maintain the disease at an acceptable level. This work was completed over two different sites with different soil types. A product was also evaluated on about a dozen different varieties to further investigate interactions with the plant's genetics.

In conclusion for the project, the use of a group of levers will reduce the need to use conventional phytosanitary products to treat potato blight. Notably, the results obtained with phosphite-based biocontrol products are encouraging and have lead to a reduction in the use of phytosanitary products of between 40% to more than 90% depending on the variety. These products are effective in protecting against potato blight for all of the varieties tested by the project, especially those with partial or high levels of resistance.

Prospects for transfer or research

Transfer:

Promising results will be communicated within the biocontrol consortium, notably the XP-BC potato blight/mildew network, such that assays can be completed on other crops.

Partnerships with businesses developing effective phosphites have been engaged which should lead to the registration of this solution for use on potato crops, or even for other crops.

Work on the evaluation of the active ingredient actually applied has been done. This has been broadened to cover other crops and protocols have been set up to assist producers to get a better understanding of the issue with the logic of encouraging them to reduce and optimise the quantity of products applied.

Research:

These prospects concern different aspects of the project. A better understanding of how phosphites work would help to optimise their use in the field. Notably, research into the interaction between the plant's genetics and biocontrol products should be continued.

Work to identify other biocontrol products would appear essential such that a wider range can be made available to growers. A better understanding of why the results obtained under controlled conditions do not transfer into the field will also be crucial to the development of biocontrol solutions. Research into the combinations of integrated protection levers should be continued.

Publications and scientific symposia:

- ***Phytoma (n° 721 February 2019):*** *« Lutte contre le mildiou : allier le biocontrôle à d'autres méthodes (Projet MilPomBio) ».* A. MENIL, J-E. CHAUVIN, R. PELLE, M. BOUSSEAU, D. GAUCHER, G. BEAUVALLET, C. MAUMENE, D. ANDRIVON, C. PASCO, F. VAL, A. BARBARY et M. TURNER
- ***Potato Planet (n°39, July 2016, p. 82-83):*** *« Traitements alternatifs. Vegenov teste de nouveaux produits de biocontrôle »* - M. TURNER, A. MENIL

TECHNICAL WORKSHOPS AND SCIENTIFIC SYMPOSIA:

- **AFPP Congress « Conférence internationale sur les maladies des plantes ». 10-12/12/2018, Tours:** *« Mildiou de la pomme de terre : recherche et maîtrise des produits de biocontrôle dans un schéma de protection intégrée des cultures (projet MilPomBio) »*. Oral presentation.
- **« Natural Product and Biocontrol » Congress. 25-28/09/2018, Perpignan:** *« Mildiou de la pomme de terre : recherche et maîtrise des produits de biocontrôle dans un schéma de protection intégrée des cultures (projet MilPomBio) ».* Oral presentation.

NABUCO

New Bio-Marine Agents for Biological Control Use

Launch year: 2015
Completion year: 2018

Scientific manager
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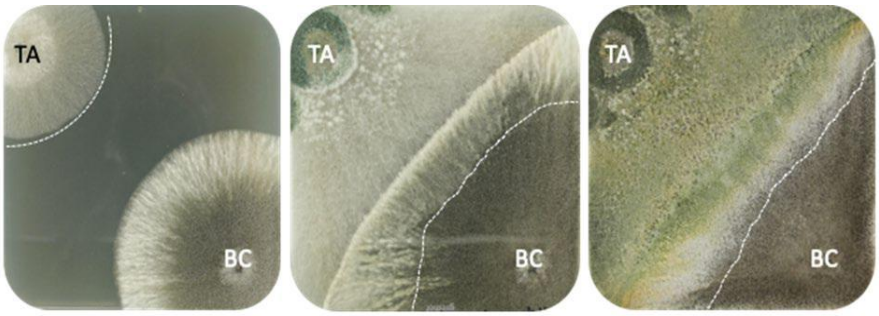
Partners
UMR 1345 IRHS University of Angers; EA 2160 MMS University of Nantes; Société AGRAUXINE by Lesaffre

Financing
Total cost of the project: € 534,412
Ecophyto grant: € 99,521

Key words:
Biocontrol; Cryptogamic diseases; Marine fungi; *Trichoderma*; Secondary metabolites; Screening; Antagonism

Context and main objectives

At the moment, biocontrol agents represent only a very small part of the global phytosanitary market and even that is dominated almost entirely by a narrow range of biological agents and substances. In this context, we are proposing to identify new biological control agents based on marine fungal micro-organisms. Marine environments are filled with an incredible microbial biodiversity which is able to synthesise molecules with diverse biological properties, in terms of plant protection agents we have barely scratched the surface of this biodiversity.



A marine isolate of *Trichoderma atroviride* MMS1295 (TA) confronts the phytopathogenic agent *Botrytis cinerea* (BC) in a petri dish. The dotted white lines show the front lines of the MMS1295 colony. *Photo: © Franck Bastide, IRHS*

The identified biocontrol agents are intended for use in protecting against fungal pathogenic agents for which few effective biocontrol agents are currently available and which are responsible for high volumes of phytosanitary product consumptions. The objective is also to combine cultural and molecular tools and implement a method which could later be applied to other bio-resources in order to optimise and accelerate the sorting of strains and molecules for potential use in biological control agents.

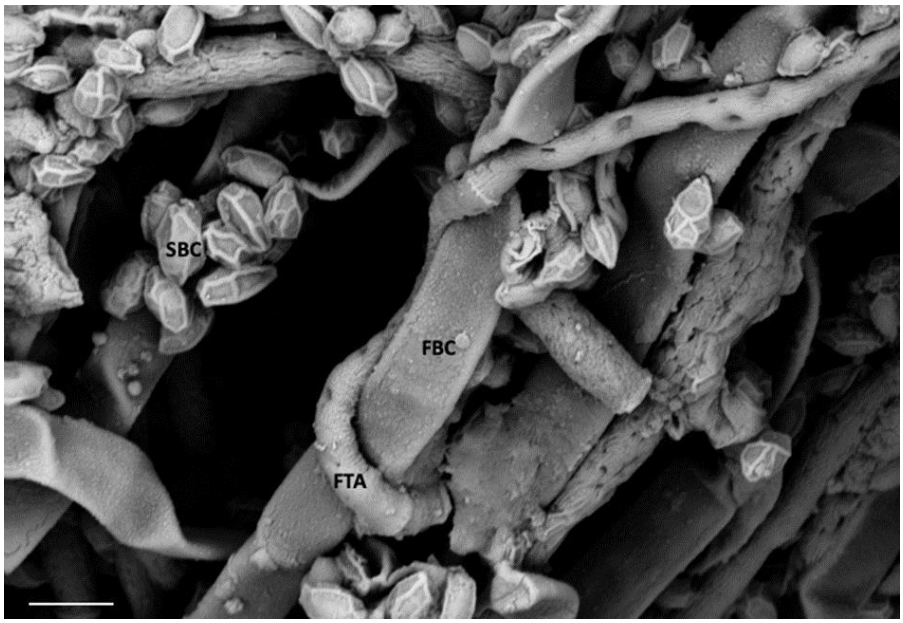
Principal results and interest in relation to the Ecophyto plan

This project has led to a number of advances in the search for new biological protection solutions to deal with fungal diseases affecting plants. Fungi of the genus *Trichoderma* are useful biological control agents since they have an action on phytopathogenic agents either through direct antagonism (parasitism, antibiosis) or indirect antagonism by stimulating the plant's natural defences.

First of all, a strain screening method was developed and applied to more than 50 marine *Trichoderma* and 11 secondary metabolites in order to evaluate the best candidates for use as biological control agents This covered both cultural characteristics (growth rate, spore production), environmental stress tolerance, mycotoxinogenic properties, compatibility with various pesticides, antagonist potential (antibiosis and parasitism) as well as the capacity to stimulate the natural defences of plants.

Various fungal growth monitoring tools (laser nephelometry micro-culture, time-lapse) or plant defence activation monitoring tools (qPFD) were used to optimise the screening process. qPFD remains difficult to apply to a large number of samples due to its relatively high cost. This screening process was useful for its capacity to highlight the major differences between the tested strains and then select the best ones. According to the analysed criteria, 4 marine strains of *Trichoderma* showed cultural potential and antagonistic activity superior to 4 other commercially available strains. This result validates the initial hypothesis that suggested that marine *Trichoderma* could be good candidates for use in plant protection.

At the same time, different secondary metabolites were extracted and purified from a variety of marine *Trichoderma* strains. This work was an opportunity to isolate and purify a series of secondary metabolites which had never before been described for this genus of fungi: peptaibols made up of 15 residues. *In vitro*, these secondary metabolites have demonstrated powerful inhibiting effects on pathogenic agents. These anti-fungal activities depend on the target pathogen and the nature of the secondary metabolites tested. The main difficulty with this action resides in the capacity to extract sufficient quantities of secondary metabolites compatible with the plant application thresholds.



Trichoderma atroviride filament wrapping and parasiting filaments of the phytopathogenic agent *Botrytis cinerea*. Scale: 10 µm. FBC: filament of *B. cinerea*; FTA: filaments of *T. atroviride*; SBC: spores of *Botrytis cinerea.*
Photo: © Franck Bastide, IRHS

Finally, certain candidates were tested for their capacity to reduce apple scab disease and wheat septoria leaf blotch. When used as preventive treatment on the above ground parts of the plants without any wetting agent or additive, a secondary metabolite and the strain that produced it will achieve 24% level protection against wheat septoria leaf blotch. Under the same conditions, similar protection levels were achieved against apple scab disease after application of the same secondary metabolite with a slightly higher result (34%) after application of the strain itself.

The project's private sector partner is currently leading work to define the exact ecological/toxicological risks associated with the selected candidates (secondary metabolites and/or the *Trichoderma* that produce them), continue the research and development assays, evaluate the costs of applying for the necessary commercial registration and then finally to assess industrial and financial feasibility.

Prospects for transfer or research

Transfer:

This project must lead to the identification of new biological protection solutions to combat pathogenic agents that attack crops and lead to the intensive use of pesticides for which no alternative non-chemical solutions are currently available. Agrauxine by Lesaffre is continuing the work to define the exact ecological/toxicological risks associated with the selected candidates (secondary metabolites and/or the *Trichoderma* that produce them), continue the research and development assays, evaluate the costs of applying for the necessary commercial registration and then finally to assess industrial and financial feasibility.

Research:

Our results demonstrate that within the same species of *Trichoderma*, individual strains are far from presenting the same antagonistic performance and their parasitic capacities can vary significantly. In order to increase knowledge of these infectious processes and optimise the effectiveness of these biocontrol agents, it would appear essential to take a closer look at the fungal mechanisms that would explain variations in intra-species myco-parasitism. This type of approach should allow us to identify the molecular myco-parasitism aptitude markers that could be deployed for a more rapid screening of *Trichoderma* strains.

Publications and scientific symposia:

TECHNICAL WORKSHOPS AND SCIENTIFIC SYMPOSIA

- **3rd International AFERP STOLON Symposium. 18-20/07/2018, Rennes, France:** *The NABUCO project: a search for new marine biocontrol agents against plant diseases.* Ruiz *et al.*
- **12èmes Rencontres de Phytopathologie-Mycologie, Société Française de Phytopathologie. 15-19/01/2018, Aussois, France:** *Study of new fungal biocontrol isolates and their secondary metabolites against plant diseases.* Bastide *et al.*
- **1st International Symposium on plant bioprotection sciences and technologies. Reims, France 27-30/06/2017**: *Screening strategy to select new fungal biocontrol products against plant diseases.* Bastide *et al.*

SCIENTIFIC PUBLICATIONS

Pentadecaibins I–V: *15- Residue Peptaibols Produced by a Marine-Derived Trichoderma sp. of the Harzianum Clade.* Van Bohemen AI, Ruiz N, Zalouk-Vergnoux A, Michaud A, Robiou du Pont T, Druzhinina I, Atanasova L, Prado S, Bodo B, Meslet-Cladière L, Cochereau B, Bastide F, Maslard C, Marchi M, Guillemette T, Pouchus YF. 2021. In press.

OPTIM'PHERO

Optimising Pheromones and Transposing results obtained with the pine processionary to other insects, pest lepidoptera in Non-Agricultural Zones
Innovative biocontrol solutions for other lepidoptera.

Launch year: 2015
Completion year: 2018

Partners
INRAE UEFM; FREDON PACA; M2I Life Sciences

Scientific manager

Jean-Claude MARTIN, INRAE UEFM

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Financing

Total cost of the project: € 271,199
Ecophyto grant: € 98,393

Key words:
Biocontrol; Ecophyto; JEVI; Sexual confusion; PBI

Context and main objectives

The OPTIM'PHERO project has its origins in the realisation that something was lacking in the alternative control strategies for pest lepidoptera in JEVI (Gardens, Parks and Infrastructures or non-agricultural zones). Four teams have combined forces and their multi-discipline skill base, covering fine chemicals, innovative patents and applied entomology. The objective of this project was to propose innovative biocontrol solutions for 3 pest insects: the pine and oak tree processionaries and box tree moth which all represent major threats. OPTIM'PHERO aimed to extend the range of available biocontrol products through new technology developed with their French partner M2i Life Sciences which proposes the controlled release of pheromone substances and innovative sexual confusion application methods suited to specific plant configurations. A biodegradable support for the pheromone (no diffuser, no system dismantling) would reduce costs whilst also optimising efficiency thanks to better suited application methods designed specifically for each insect and situation. A number of original application methods were tested over large surface areas including the development of paintball ammunition filled with micro-encapsulated pheromones or the application of a pheromonal gel directly onto the plants. These solutions represent interesting alternatives to the ground-level (aerial treatment being forbidden) treatment methods currently used to control pine processionary, such as pick-up mounted turbines or lances. These latter are used to spray to great heights and are not at all ecologically friendly due to the vast quantities of product that falls back to the ground.

Photo: © M2i LIFE SCIENCES
PHEROBALL PIN - Ball filled with micro-encapsulated pheromone for application by Paintball gun

Targeted application of micro-encapsulated pheromone responds to a high demand for solutions to deal with pests that depend on trees (Pheroball) as well as direct manual methods applied to the shrub layer (micro-encapsulated pheromone gel).

Principal results and interest in relation to the Ecophyto plan

Pine Pheroball

Three years of experimental and optimising work on the Pine Pheroball, a product that results directly from the project, has demonstrated an average reduction, through sexual confusion, of processionary populations of between 29% (300 balls/ha, over a 4 ha field) and 58% (30 balls per pine tree, isolated trees in urban areas), depending on the tested dose. The study was completed in wooded and urban zones.

This study raised a number of limits associated with this control strategy:

1) **The effectiveness** of the doses tested in 2017, between 300 and 400 balls per hectare over large wooded areas (4 ha), corresponds to a population reduction between 29 and 36% whilst the tests were conducted on low population levels of pine processionary, a situation which is more favourable for a sexual confusion control strategy.

2) In terms of the **ball application time** (6 to 8 hectares per day for 2 operatives) and frequent gun cleaning. In forests, the precise ball application route following a 10m x 10m grid to apply is very time consuming which increases the overall cost of this control strategy. However the cost and ease of use remains attractive when compared to traps or BT.

3) In terms of **cost**: Pine Pheroball requires further experimental work to evaluate the effectiveness of higher doses.

Firing PINE PHEROBALL In urban zones (Avignon 2017)

Photo: © INRAE ; M2i LIFE SCIENCES

Pheromone gel

Tests of targeted applications of biodegradable pheromone gel were completed on pine processionary and box tree moth as a control strategy using sexual confusion. Results were encouraging for pine processionary with a more than 87% descent reduction; the pheromone gel was applied onto the crowns of the trees. This was done with a single testing dose; the experiment needs to be repeated to validate the result. For box tree moth, a population reduction of 25%, compared to the control, may well be significant but does not reduce foliar damage. Further work is required to establish the correct dose.

On oak processionary, the complexity involved in synthesising the pheromone was a genuine technical obstacle throughout the 3 test seasons.

Prospects for transfer or research

Transfer:

By the end of the project, M2i obtained a Biocide AMM for pine tree confusion solutions in France, by derogation for 2017 and then permanent as of 2018 followed by the same for Spain, Portugal and Algeria. This sexual confusion pheromone application method has been available in agriculture since a paintball product for walnut trees has just been authorised for the French market. OPTIM'PHERO has also contributed to the development of a sexual confusion product for box tree moth, this is now authorised for sale in France with a biocontrol AMM since 2019 and the same for Benelux, Italy, Spain, Portugal and the United Kingdom.

UEFM will continue to develop the AGIIR (Alerter et Gérer les Insectes Invasifs et/ou Ravageurs - Alert and Manage Invasive Insects and/or Pests) smartphone application as a control support tool for the project's 3 target pests. This application can be downloaded free for Android and IOS. The publication of the technical guide and real-time updates will continue.

Research:

Pine Pheroball in forests: the effectiveness of Pine Pheroball has been demonstrated by the programme but its use in forests (large densely wooded areas) remains to be proven. M2i will continue forest application protocol optimisation experiments after the end of the project until authorisation for sale is awarded.

Pheromone gel and effectiveness: M2i has developed a new application protocol for box tree moth based on the project work which increases dosage and the number of treatments and includes the combination of a larvicide to develop a fully effective solution.

The solution's conclusive results on pine processionary open up prospects to extend application to other pest lepidoptera that affect JEVI or even agricultural zones thanks to its innovative pheromone application method.

Sexual confusion with oak processionary

M2i will be continuing its research since sexual confusion assays will be launched in cooperation with Cardiff Univeristy, Great Britain for 2022. The INRAE has continued its research work on this pest through other projects and has yet to identify a pheromone that is suitably effective for monitoring, or other methods involving sexual pheromones (low or non-existent capture rates for pheromone comparisons).

Publications and scientific symposia:

TECHNICAL DOCUMENTS:

- Brinquin A.S. and Martin J-C., 2017: **Les clés pour lutter contre la processionnaire du pin.** https://www6.paca.inra.fr/entomologie_foret_med/Insectes-ravageurs-et-protection-durable/Aide-a-la-decision

TECHNICAL WORKSHOPS AND SCIENTIFIC SYMPOSIA:

- Technical workshops organised by the UEFM and its institutional partners to train managers on new alternative pest control techniques.
- Box tree bioaggressor symposium Tours 16 & 17 October 2018 organised by Végéphyl https://www.academie-agriculture.fr/sites/default/files/agenda/programmebuisenfrancaisv17-09.pdf

PRACTICAL / EDUCATIONAL ARTICLES:

- E. Pal, M. Corréard, F. Rei, J. Thévenet, S. Lantus, E. Pezzini, M. Buradino, A-S Brinquin and J-C. MARTIN (2018). **Processionnaire du pin : un traitement bille en tête.** Phytoma 715, 28-32.
- E. Pal, M. Correard, M. Buradino, E.Morel, S. Touzeau, J.Thévenet, D. Vauthier, O.Gilg and J-C. Martin (2017). **Processionnaire du pin : le piégeage se perfectionne.** Phytoma 709, 27-29.
- J-C. Martin, M. Buradino, A-S. Brinquin, M. Correard, J. Thévenet, D. Vauthier, E. Morel, A. Gilli, M. Vénard et E. Tabone (2018). **Réguler la pyrale du buis Cydalima perspectalis : limites d'utilisation de la phéromone sexuelle de synthèse.**

PRO-BIO-TAUPIN

Evaluation of biocontrol solutions for protecting crops from the damage caused by wireworms

Launch year: 2016
Completion year: 2019

Partners
ARVALIS; UMR DGIMI INRAE - University of Montpellier; ACTA; CTIFL; Invenio; ACPEL; APREL; SudExpé; Sonito

Scientific manager
Jean-Baptiste Thibord, ARVALIS
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Financing
Total cost of the project: € 210,807
Ecophyto grant: € 113,891

Key words:
Wireworm; Biocontrol; Entomopathogen nematodes; Entomopathogen fungi; natural substances

Context and main objectives

Protection against wireworms is often essential to maintaining large-scale and vegetable crop harvests and/or quality standards. For a number of years growers have seen the number of phytopharmaceutical solutions become increasingly rare, with those that remain on the market often limited or insufficient in terms of effectiveness. It has therefore become necessary, even urgent, to develop protection strategies integrating biocontrol solutions either as a substitution to phytopharmaceutical solutions for low wireworm population situations, or in addition to conventional solutions to limit the damage caused by the pest in high population situations.

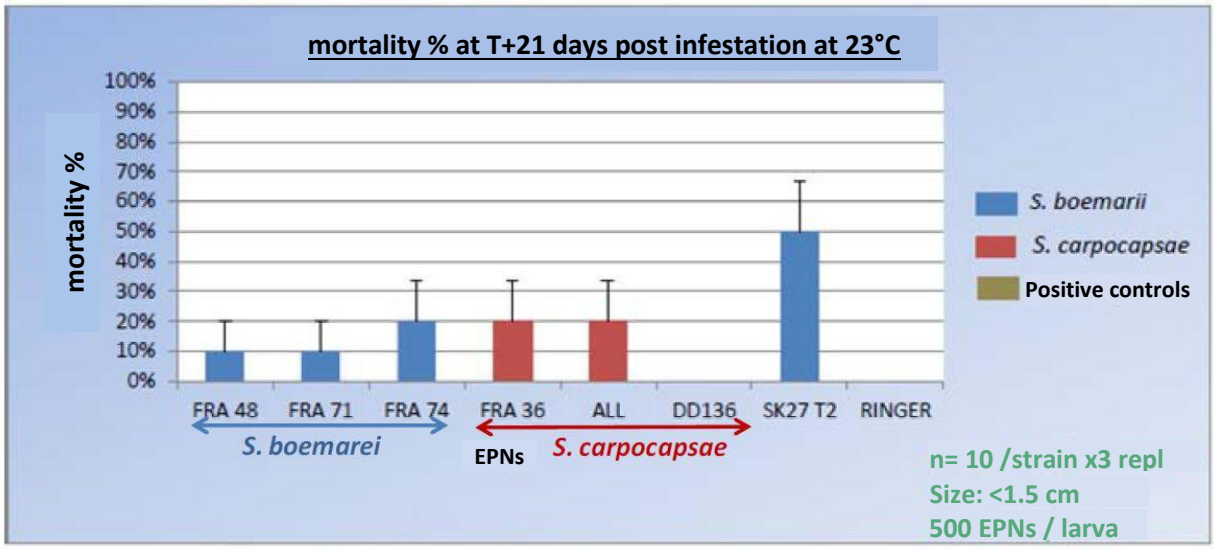
The objective of the PRO-BIO-TAUPIN project was to explore the technical interest of various potential biocontrol solutions;

- Entomopathogen nematodes;
- An entomopathogen fungus, more specifically a product based on *Metarhizium anisopliae*.
- Natural substances, essentially glucosinolate based products.

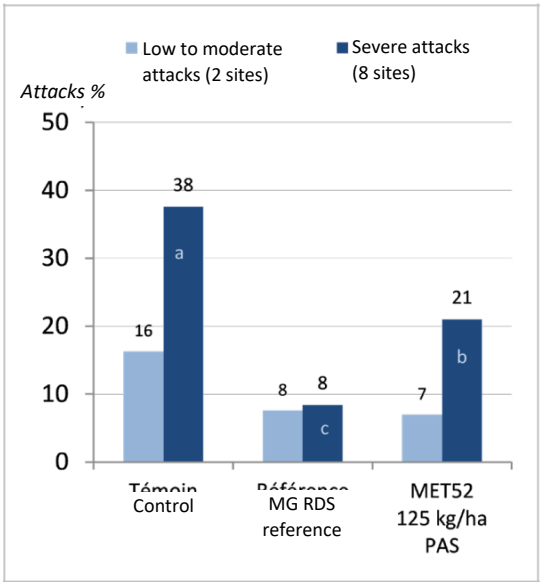
The assays were completed under controlled conditions (entomopathogen nematodes) and field conditions (entomopathogen fungus, natural substances) to protect various crops: potato, carrot, melon, asparagus, tomato and corn.

Principal results and interest in relation to the Ecophyto plan

Implementation work on entomopathogen nematodes (EPNs) has highlighted the fact that the insect's physical barriers (particularly its highly resistant cuticle) clearly restrict the entrance of nematodes into the larvae thus explaining the weak performance of these biocontrol agents on wireworm (see image on next page). This key stage of the nematode's parasitic cycle is the main obstacle to be dealt with before considering any field application of EPNs to control wireworm. The direct injection of EPN symbiotic partner bacteria into the insects' haemolymphe has been able to show that it could be interesting to use a high performance bacteria strain to kill wireworm larvae: *X. kozodoii* FR48. To our knowledge, this is the first time that an EPN symbiotic bacterium is pathogenic in isolation to wireworm larvae. Bio-assays involving injections have also shown that other species of the *X. kozodoii* species were conversely quite ineffective on wireworm larvae. This result means that it would be interesting to launch genome studies comparing the virulent strain, *X. kozodoii* FR48 and the other non-virulent strains of *X. kozodoii* to identify any genes or genome regions involved in the pathogenic property of *X. kozodoii* FR48 on wireworms. Complete genomes of the pathogenic *X. kozodoii* FR48 strain and the non-virulent FR71 and FR74 strains have been sequenced.



Entomopathogen nematode mortality tests associating different bacteria on wireworm larvae. The histograms show the average percentage of wireworm larvae mortality (3 replicas) 21 days after infection with strains of *S. boemarii* and *S. carpocapsae.* N = 30 wireworm larvae; infestation dose = 500 EPNs per larva; negative control = Ringer liquid; positive control = SK27 T2. *Image: © INRAE*

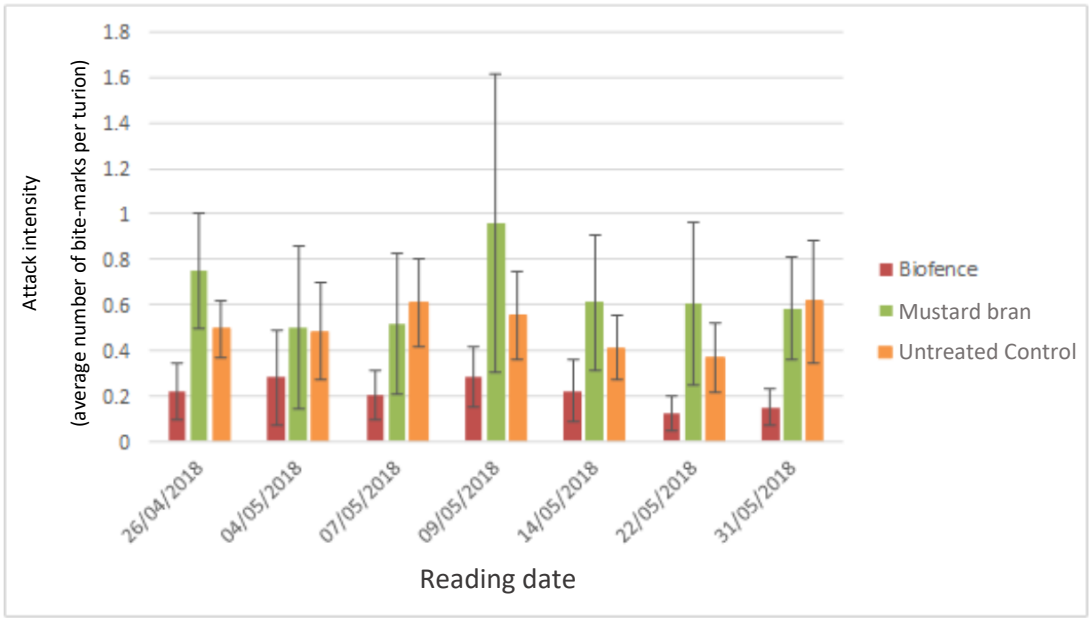


The study of entomopathogen fungi (axis 2) was concentrated on the MET 52 granule commercial speciality, based on Metarizhium anisopliae F52. Plant protection assays involving wireworm larvae produced very encouraging results with, in some situations, a degree of effectiveness comparable to those achieved with conventional products (50% effectiveness).

However protection level variations, the high cost of the currently available commercial product and supply chain problems mean that this solution cannot be used as a year round crop protection solution. Initial works were implemented as part of a strategy to lower wireworm populations through repeated introductions into the fields. Whilst the initial results were encouraging, further work needs to be done to confirm the interest of this control method.

Evaluation of MET 52 GR applied and incorporated into the ground before sowing corn. 10 assay summary (2012-2017) - *Image: © ARVALIS*

A number of liquid formulations involving glucosinolates are available to growers. Having been unable to test them all, those that were the subject of experiments organised by the project's partners (axis 3) did not produce particularly interesting results in spite of the diversity of application methods tested. However a formulation of Abyssinia mustard seed, *Brassica carinata*, press cakes as sold by Biofence, could be a solution to reduce wireworm attacks on corn and potato crops. This seems to be less effective on other crops but might be useful against other under-soil pests - such as symphyman (on asparagus) and cyst nematodes (on potatoes). However the required doses could imply a lack of selectivity under certain circumstances. With the Biofence product being very expensive, doses were reduced and locally targeted. The results on corn crops were interesting but such experiments would need to be taken further to prove the effectiveness of the techniques in the event of severe attacks. At the same time, a number of assays were completed to evaluate the effectiveness of mustard bran which is authorised as a base substance and can be used as a substitution product. Whilst the results were not very encouraging, improvements could be made by improving the chemical composition of the bran used (see image on next page).



Wireworm attack intensity on asparagus leading to rejected harvests.
Image: © INVENIO

Prospects for transfer or research

Transfer:

Even if none of the solutions evaluated by the PRO-BIO-TAUPIN project turned out to be satisfactory for crop protection under production conditions (especially for financial reasons), the results have been presented to growers in the various crop industries. Additionally, these results have enabled the launch of further research work programmes. The work on wireworm pathogen bacteria will be continued by UMR DGIMI. New work on entomopthogen fungi has been launched as part of a collaboration with the University of 'Pau et des Pays de l'Adour'. Finally, further works are planned on the production of glucosinolates in the field via service plants.

Research:

The results obtained via the first axis of the PRO-BIO-TAUPIN project are very promising thanks to the identification of a pathogen bacterium that is effective against wireworm larvae. However it will be difficult to implement this strategy in the field for the moment considering that the *Xenorhabdus* entomopathogen bacteria cannot survive without its host nematode. One possibility would be to identify the pertinent toxins present in these bacteria.

The technical interest of *Metarizhizium anisopliae* and certain press cakes containing glucosinolates has been confirmed. However, it will be necessary to analyse the active ingredients that affect wireworm larvae in order to develop knowledge on the most effective strains and substances and define the conditions which would favour their presence in the field. This would allow for an optimisation of doses and render these solutions compatible with the budgetary constraints involved in producing these crops.

Publications and scientific symposia:

SCIENTIFIC SYMPOSIA:

- **International Congress Natural Products and Biocontrol (Perpignan, France. 25-28/09/2018):** *Évaluation des nématodes entomopathogènes et leurs bactéries symbiotiques pour lutter contre les taupins (poster présenté).* Frayssinet Marie, Pagès Sylvie, Lawac Floriane, Duvic Bernard, Givaudan Alain and Ogier Jean-Claude
- **11ème Conférence Internationale sur les Ravageurs et Auxiliaires en Agriculture, Montpellier 25-26/10/2017:** *Intérêt de l'utilisation de Metarhizium brunneum pour lutter contre les taupins (Agriotes sp.) en grandes cultures.* Larroudé Ph. and Thibord J.B. – 2017

SCIENTIFIC PUBLICATIONS:

- **M1 placement report (IMHE master, University of Montpellier):** *Étude des facteurs de virulence de la bactérie entomopathogène Xenorhabdus kozodoii vis-à-vis du ravageur taupin (Agriotes sordidus).* Océane Bueno, 2018.
M1 placement report (Master in Agronomics and Food industry, SEPMET, SupAgro Montpellier): *Évaluation de la virulence des complexes némato-bactériens entomopathogènes contre les taupins.* Floriane Lawac, 2017.

REGPUC

Designing irrigation and fertilisation strategies for controlling green aphid populations in peach tree orchards?

Launch year: 2015
Completion year: 2019

Scientific manager
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Financing
Total cost of the project: € 248,235
Ecophyto grant: € 90,000

Key words:
Alternative elementary practices; Technical approach changes; Irrigation; Fertilisation; Orchards; *Prunus persica*; *Myzus percicae*; Branch composition; Branch development

Context and main objectives

"Bottom-up" methods, involving controlling pests and disease by modifying the host-plant's vulnerability, are not very well known. This vulnerability is related to the dynamic equilibrium between plant development and structure; it can be optimised through various techniques, such as irrigation and fertilisation. However the effects of these techniques will vary depending on the plant's initial condition (size, shape and structure). The implementation of these techniques will therefore have to involve an assessment of the variability of their effects in terms of the trophic and hydric states of the plants. This is the main subject of this project which concentrates on the possibility of adjusting irrigation and nitrate fertilisation practices to control green aphid infestations on peach tree orchards.

This objective involves:

1) Identifying the specific operational characteristics of the plant to which aphids are attracted
2) Identify the effects of irrigation and fertilisation practices on these key plant-pest/disease interactions and on fruit production
3) Select the most adequate seeming practices from these combinations

This is the approach we have adopted, and which we will combine with experimental and modelling techniques.

Damage to fruit in production orchards.
Photo: © Alice Diot, INRAE

Principal results and interest in relation to the Ecophyto plan

The project was divided into three phases:

1) Experiments under controlled conditions to identify the main determining factors for plant vulnerability
2) Multi-criteria and multi-year (2 year) validation of results in commercial orchards that are subject to severe natural infestations.
3) Modelling work: development and simulation of the long-term effects of the tested practices.

For container planted trees, infestation severity is positively related to branch development (leaves, secondary structures) and the non-structural amino acid and carbon content in the apical buds, and negatively related to polyphenol concentrations. These relations depend on the variety's hydric state.

In orchards, the trees that have been subjected to a Nitrate limitation were able to maintain their development and production potential during the first year by drawing on their own reserves. However they were infested later on, but less severely. During the second year, infestations were less severe on the least vigorous branches and those trees which had been limited in their Nitrate uptake. Again, these practices had no effect on production.

Reducing Nitrate fertilisation therefore reduces the prevalence of infestations, but the frequency of periods where the tree is fertilised correctly such that it can reconstitute its reserves has yet to be defined. The impact of irrigation depends on the environment surrounding the orchard: the apparition of hydric stress will be later in relation to winter precipitation quantities. However this is only effective if it is applied (even temporarily) at the start of the infestation and not around the peak.

Simulations using "simple models" show that growth is more sensitive to fertilisation than to the presence of aphids. Production is only affected during the second year: the decrease in the growth of shoots related to severe infestation will reduce the number of fruit for year (n+1) since these are produced on year old wood. This drop in production stabilised after 4 years.

RegPuc has facilitated the integration of QualiTree (a function structure model), a module which formalises the absorption of nitrates from the soil, its distribution amongst organs and the effects on development and growth. This was a preparative for the inclusion of the "aphid model" which is currently being developed.

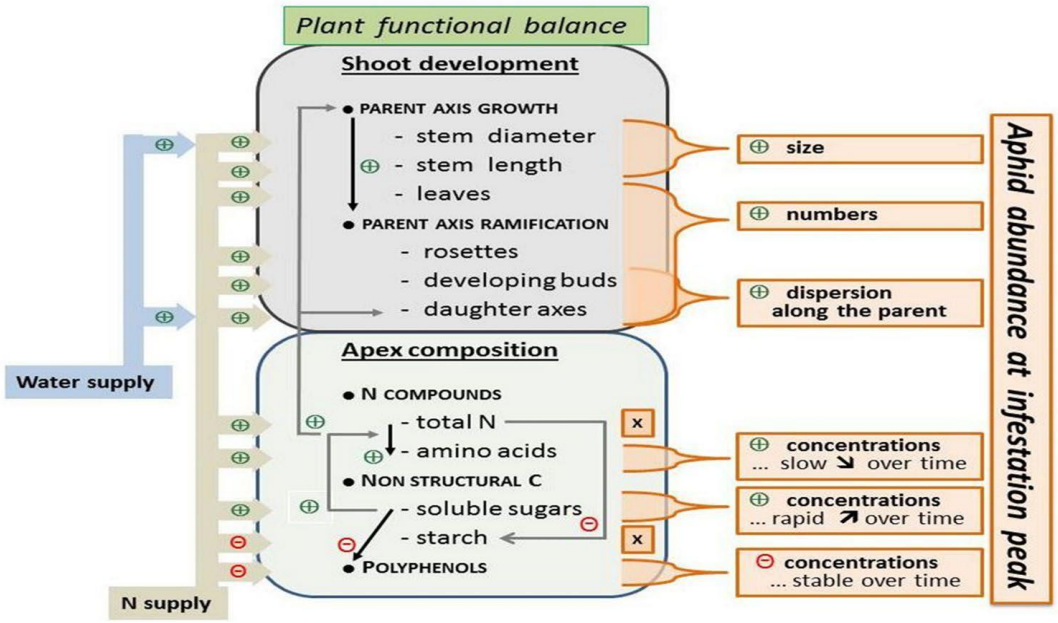

Figure 1: Tree-aphid interaction structure. Impact of development variables (in pale grey) and those relating to structure (in pale green) on aphid populations, shown in orange. Positive if marked with + and negative is marked with ⊖. The interdependence of "plant ○ variables" is indicated by arrows, black where the result comes from the work presented here and pale grey if it is drawn from previous work (*Jordan et al. 2011*). Details of the effects of irrigation and fertilisation on plant variables are shown on the left side of the graphic. *Image: © Marie-Odile Jordan, INRAE*

Our results confirm that fertilisation and irrigation practices can be adapted to meet a twofold objective: maximised production and aphid control. However further studies will have to establish any long-term consequences (> 2 years) on the durability and productivity of orchards. Additionally, other practices such as hedge planting, ground cover plant selection could also be applied at the same time to increase performance, notably by encouraging predators. Consequently growers will have to have access to a set of methods to be applied depending on the specifics of each orchard. These can be more or less vulnerable to aphids depending on their surroundings which will determine the number of fundatrices, and the condition of the tree on which the future of the first colonies will depend. However, no existing alternative control approach targets all of these aspects. They should therefore be combined to reach the same degree of effectiveness as pesticides. The effects of these method combinations have not been studied to any great extent so far.

Prospects for transfer or research

Transfer:

Since one of the partners is an association of tree farmers, the transfer has occurred in "real-time": project progress presentation to growers from the network during themed workshops, (assay) site visits and publications in the network's news-sheet. Finally, once sanitary conditions allow, the presentation of the final results will be organised in the form of a summary presentation to growers in the network and the technical coordination units of the PACA and Languedoc-Roussilon Regions. Finally a shorter presentation will be published in *"La France Agricole"* as part of a collaboration between the magazine and the GRCETA.

Research:

The results of RegPuc have enabled the INRAE partner (PSH unit) to participate in APITree, a European C-IPM project investigating the aphid's choices in terms of host-tree when returning in autumn. This project was an opportunity to initiate collaboration with the G. Alins (IRTA Lleida ESP) and L. Sigsgaard (University of Copenhagen, Sweden) teams.

Additionally, INRAE PSH is currently coordinating a PPR project: CAP Zéro Phyto, for which it is implementing a long-term experimentation programme (4 years) in peach-tree orchards concerning the combined use of different levers (fertilisation, irrigation, ground-cover and service plants, mechanical stress, etc.) on aphid-plant interactions.

Publications and scientific symposia:

Scientific publications:

- Ecological Modelling (n° 338, pp 11-16): **The consequences of aphid infestation on fruit production become evident in a multi-year perspective: Insights from a virtual experiment.** Bevacqua D. Grechi I. Génard M. Lescourret F. (2016)
- Agronomy for Sustainable Development (n° 38, pp 1-17): **Harnessing the aphid lifecyle to reduce insecticide reliance in apple and peach orchards: A review.** Rousselin A. Bevacqua D. Sauge M-H. Lescourret F. Moody K. Jordan M-O. (2017)
- Pest Management Science (n° 76, pp 797-806): **Chemical and growth traits of the peach tree may induce higher infestation rates of the green peach aphid, Myzus persicae (Sultzer).** Jordan M-O., Sauge M-H., Vercambre G. (2020)
- Journal of the royal society Interface (n°17: 20200356): **An eco-physiological model of plant-pest interactions: the role of nutrient and water availability.** Zaffaroni M., Cunniffe N. J., Bevacqua D (2020)

Scientific symposia:

- International Crop Modelling Symposium: Crop Modelling for Agriculture and Food Security under Global Change (15-17/03/2016. Berlin, Germany): **Consequences of aphid infestation over fruit tree production: the peach tree-green aphid case.** Bevacqua D., Grechi I., Genard M., Lescourret F.
- 9th international conference on integrated protection of fruit crops, 4-8/09/2016, Tessaloniki (Greece). IOBC WPRS Bulletin, (n° 123, pp 79-85): **Combining irrigation, fertilization and pruning techniques helps control aphid populations in apple and peach orchards.** Jordan M-O. Rousselin A. Vercambre G. Sauge M-H.
- IIIrd International Symposium on Horticulture in Europe – SHE2016 (17-20/10/2016, Chaina, Greece): **Modelling fruit plant-pest interactions, their control by cultivation practices and their link to fruit quality.** Lescourret F. Gibert C. Grechi I. Génard M. Bevacqua D.
- BAPOA (Biologie Adaptative des Pucerons et Organismes Associés) network workshops, (19-20/10/2017, Colmar, France): **Variables écophysiologiques (croissance, architecture, composition biochimique) affectant l'attractivité du pêcher pour le puceron vert.** Jordan M-O. Rousselin A. Sauge M-H.
- 10th International Conference on Dynamical Systems Applied to Biology and Natural Sciences (DSABNS) (03-06/02/2019, Napoli, Italy): **A general eco-physiological framework to model the interactions between phloem feeder pests and plants.** Zaffaroni M., Bevacqua D.
- XXIX Congresso SItE. (10-12/09/2019, Ferrara Italy): **An eco-physiological model coupling plant growth and aphid population dynamics.** Bevacqua D., Cunniffe N., Zaffaroni M.
- 14th International Symposium « Ecology of Aphidophaga » (16-20/09/2019, Montreal, Canada): **Limiting irrigation and fertilisation enables to limit the aphid populations in commercial apple *(Malus domestica)* and peach (*Prunus persica*) orchards.** Jordan M-O., Drevet Giraud A., Vercambre G., Hucbourg B.
- The International Society for Ecological Modelling Global Conference (ISEM) (01-05/10/2019, Salzburg, Austria): **An agro-ecological model coupling plant growth and pest population: highlights on the role of fertilization and irrigation.** Zaffaroni M., Bevacqua D.

Operational / educational articles:

- Info CETA (n°5, May 2017): Pêcher : **quelles stratégies d'irrigation et de fertilisation pour réguler les populations de *Myzus persicae***. Drevet Giraud A.

Technical workshops - GR-CETA Growers network:

- Participation in 11 technical workshops (2015-2019): presentation of assay and results.

Communication during technical coordination operations:

- 16-18/10/2019: **"Rencontre CETA des techniciens"** (involving CETA South-East region Technicians association members): results presentation

RESCAPE

RESistance of agricultural landSCAPEs to pesticide transfers into soil and living organisms

Launch year: 2015
Completion year: 2019

Scientific manager

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Partners

UMR EMMAH INRAE - Avignon University; UMR Chrono-environnement CNRS - University of Bourgogne Franche-Comté - Usc INRAE ; UMR EcoSys INRAE - AgroParisTech; Luxembourg Institute of Health - Dpt of Population Health; INSA Centre Val de Loire; UMR 7372 CEBC - CNRS – University of La Rochelle; Laboratoire d'Analyse des Sols d'Arras; UMR 5280 Institut des Sciences analytiques INRAE - CNRS - University of Lyon

Financing

Total cost of the project: € 721,208
Ecophyto grant: € 111,540

Key words:

Pesticides; Transfers; Contamination of agricultural environments; Exposure; Functional biodiversity; Earthworms; Ground beetles; Micro-mammals; Undesired effects; Landscape approach

Context and main objectives

Agricultural landscapes are made up of a patchwork of cultivated fields and semi-natural features (hedges, woods, prairies, etc.), which can be reservoirs of beneficial organism and pests but alsos sources and wells of pesticides (used on the crops and potentially transferred outside of the fields). The proportion and arrangement of these various agricultural and non-agricultural features can influence how landscapes resist pesticide transfer into the soil and living organisms.

The RESCAPE project fits into the landscape ecotoxicology framework, and aims to increase knowledge of the relations between phytosanitary practices, environmental contamination and biodiversity. The objective of the project was to identify the effects of land use (landscape composition and configuration) and agricultural management (cultural practices) on the spatial distribution of pesticides, the exposure of non-target organisms and impacts on them.

Principal results and interest in relation to the Ecophyto plan

The RESCAPE project has enabled the development of multi-residue analysis methods to measure soil and non-target organism exposure to pesticides, these revealed three major results:

1) The exposure of soils and living organisms is ubiquitous. We have found pesticide residues in all types of habitats (conventional and organic [AB] agriculture cereal fields, prairies and hedges). Pesticide concentrations measured in 180 soil samples demonstrated a high risk of negative effects on earthworm reproduction in 46% of cases, this applied equally to both cereal crop treated with insecticides and the untreated zones which are very important to biodiversity.

2) Exposure to cocktails of molecules involving at least one insecticide (imidaclopride), one fungicide (epoxiconazole) and one herbicide (most frequently diflufenican).

3) For both soils and non-target organisms, the pesticide treated zones were the most contaminated. AB fields and semi-natural habitats (such as prairies and hedges) often present lower concentrations and numbers of molecules, and could be used as refuges for organisms within the agricultural landscapes.

Additionally, a modelling approach has revealed that the predominant factors controlling the atmospheric diffusion of pesticides and their later entry into non-target ecosystems were (i) the positions of hedges in relation to the treated field, (ii) the width of the hedges, (iii) the physical-chemical properties of the pesticides and the application season. In this way, the presence of certain habitats (hedges, prairies) as well as an increase in the surface areas of untreated habitats (AB fields, wooded features) play a positive role in limiting soil and non-target living organism exposure to pesticides.

This project raised a number of questions on the following points:

- The pertinence and effectiveness of current assessment methods, currently in use for the environment and biodiversity, concerning the risks associated with pesticides (PEC and toxic risk measurements) and the innocuousness of authorised pesticides.
- The need to proceed with pesticide residue analyses for soils and non-target living organisms within the framework of post molecule registration monitoring.

Regarding the role of landscape context in limiting the unwanted effects of pesticides, the project results indicate:

- That local and agricultural landscape scale pesticide use should be limited (increase untreated zones *via* the installation of agro-ecological infrastructures).
- Consider the surface area and size of features (such as hedges) and their position within the landscape to ensure the effectiveness of agro-ecological infrastructures.

However, in order to be able to propose pertinent landscape management recommendations that will result in landscape feature arrangement optimisation propositions able to limit the unwanted negative effects of pesticides and favour the kind of agricultural biodiversity that would be a benefit to agro-ecosystems, it would seem necessary to consolidate these initial results by broadening the current data set (e.g.: over more years, more geographical zones and agro-soil-climate contexts). Additionally, it will be essential to work on a better evaluation of the eco-toxicological effects caused by the exposure of living organisms to the pesticides cocktails found *in natura.*

Prospects for transfer or research

Transfer:

The produced data could be useful to organisations in charge of pharmacovigilence (ANSES) as well as public decision-makers; it would contribute to national expertise on the molecules currently “in play”. The project results could also be used to contribute to establishing recommendations for limiting the unwanted effects of pesticides and encourage a beneficial and useful biodiversity within agro-ecosystems (e.g.: Increase the presence of agro-ecological infrastructures in the landscape; reduce the field and landscape scale use of pesticides by encouraging the presence of untreated zones within the landscape patchwork).

Research:

New research programmes will be necessary in order to propose management policies that would be pertinent and applicable for the whole of the country:

- Evaluate the temporal and geographical variability of pesticide contamination.
- Study other production systems (polyculture/livestock, vineyards) to evaluate the possibility of transposing recommendations.
- Consider other types of landscape configurations that could restrict unwanted side-effects.
- Improve the understanding of ecological and eco-toxicological mechanisms at work (e.g.: impacts on trophic chains and public health).

Publications and scientific symposia:

Technical workshops:

- Scientific workshops for the Sèvre Valley and plain workshop zone, 17/06/2016. RESCAPE. C. Pelosi & C. Fritsch.
- MP ECOSERV BIOSERV, MP SMACH ACE, MP SMACH ESPACE & MP SMACH PING (PSPE RESCAPE) projects presentation day 2018:
  - Approches spatiales en écotoxicologie du paysage sur la distribution spatiale de polluants dans les sols et les transferts à la faune. C. Fritsch.
  - C. Bertrand. Réponse des carabes aux facteurs locaux et paysagers.
- INRAE SMaCH / ACTA ITA day, 20 December 2018. PING and RESCAPE projects. C. Fritsch. & C. Bertrand.

Scientific symposia:

- French Pesticide Research Group Congress, Nancy, 2017. Gaëlle D., Lafay F., Pelosi C., Fritsch C., Bretagnolle V., Vulliet E. **Développement d’une méthode multi-résidus par LC-MS/MS pour la quantification de traces de pesticides dans les vers de terre.**

- SFECOLOGIE symposium, Rennes, 2018 :
  - Colette Bertrand, Pierre Zagatti, Sébastien Bonthoux, Gaëlle Daniele, Florent Lafay, Emmanuelle Vulliet, Vincent Bretagnolle, Clémentine Fritsch, Céline Pelosi. **Assessing the impact of farming practices and landscape heterogeneity on ground beetles' exposure to pesticides.**
  - S. Bonthoux, C. Fritsch, C. Bertrand, P. Zagatti, V. Bretagnolle, M. Coeurdassier, G. Danielef F. Lafay, F. Raoul, R. Scheifler, E. Vulliet & C. Pelosi. **Disentangling the effects of pesticides, soil characteristics and landscape features on earthworms, carabids and small mammals.**
- French Pesticide Research Group Congress, Montpelier, 2019:
  - Bertrand C., Daniele G., Lafay F., Vulliet E., Bretagnolle V., Zagatti P., Fritsch C., Pelosi **C. Exposition des sols agricoles et d'organismes non-cibles aux pesticides : quelle influence de la mosaïque paysagère ?** (Poster)
  - Fritsch C., Coeurdassier M., Raoul F., Scheifler R., Burkart L., Hardy E., Palazzi P., Schaeffer C., Bretagnolle V., Bertrand C., Appenzeller B., Pelosi C. **Exposition des micromammifères aux pesticides actuellement utilisés : différences entre espèces, rôle de l'habitat et du paysage.** (Poster)
  - Djouhri M., Loubet B., Benoit P., Mamy L., Bedos C. **Modélisation de l'effet de la configuration paysagère et des pratiques culturales sur la dispersion et le dépôt des pesticides utilisés en agriculture.** (Oral presentation).
- XVIth Symposium in Pesticide Chemistry Advances in risk assessment and management, Piacenza, 3-5 September 2019. Djouhri M., Loubet B., Benoit P., Mamy L., Bedos C. **Modélisation de l'effet de la configuration paysagère et des pratiques culturales sur la dispersion et le dépôt des pesticides utilisés en agriculture.**
- SETAC Europe 14th Special Science Symposium, Brussels, 19-29 November 2019:
  - Bertrand C., Zagatti P., Bonthoux S., Daniele G., Lafay F., Vulliet E., Bretagnolle V., Fritsch C., Pelosi C. **Ground-dwelling beetles' exposure to pesticides at large scales.**
  - Pelosi C., Bertrand C., Bonthoux S., Daniele G., Lafay F., Vulliet E., Bretagnolle V., Fritsch C. **Earthworm exposure to pesticides in agricultural landscapes.**

Scientific publications:

- Pelosi C., Bertrand C., Daniele G., Coeurdassier M., Benoit P., Nélieu S., Lafay F., Bretagnolle V., Gaba S., Vulliet E. Fritsch C., 2021. **Residues of currently used pesticides in soils and earthworms: a silent threat?** Agriculture, Ecosystems & Environment. 305, 107167, DOI: 10.1016/j.agee.2020.107167
- Daniele G., Lafay F., Pelosi C., Fritsch C., Vulliet E. 2018. **Development of a method for the simultaneous determination of multi-class pesticides in earthworms by liquid chromatography coupled to tandem electrospray mass spectrometry.** Analytical and Bioanalytical Chemistry 410(20):5009–5018. DOI: 10.1007/s00216-018-1151-2

Other practical work:

- La France agricole. **Produit phytosanitaire, Des résidus de pesticides dans les sols et dans les vers**. February 2021,
- INRAE website. **Des résidus de pesticides dans les sols et les vers de terre : une réalité omniprésente et insidieuse**. 14 January 2021. https://www.inrae.fr/actualites/residus-pesticides-sols-terre-realite-omnipresente-insidieuse
- Charlie Hebdo. **Lettre des animaux aux humains reconfinés : le lombric**. Allain Bougrain-Dubourg published on-line, 1 December 2020. https://charliehebdo.fr/2020/12/ecologie/lettre-des-animaux-aux-humains-reconfines-le-lombric/
- Journal La Libre Belgique. **Les pesticides intoxiquent aussi les vers de terre... et donc leurs prédateurs**. S. Devillers, 25 November 2020. https://www.lalibre.be/planete/environnement/les-pesticides-intoxiquent-aussi-les-vers-de-terre-et-donc-leurs-predateurs-5fbd5ad3d8ad586f5119c890
- Solagro, Osaé. **Des niveaux alarmants de pesticides mesurés dans les sols et les vers de terre**. 17 November 2020. https://osez-agroecologie.org/des-niveaux-alarmants-de-pesticides-mesures-dans-les-sols-et-les-vers-de-terre-168-actu-169
- Forskare (Swedish agricultural publication). **Omfattande spridning av växtskyddsmedel**, 12 November 2020. https://www.atl.nu/lantbruk/forskare-omfattande-spridning-av-vaxtskyddsmedel/
- Le Monde. **Des niveaux alarmants de pesticides dans les sols - Une étude montre une large contamination de l'environnement, où plusieurs produits s'accumulent**, 30 October 2020.
- Sciences et Avenir. **Des pesticides retrouvés partout dans l'environnement, même dans les vers de terre**, 2 November 2020. https://www.sciencesetavenir.fr/nature-environnement/pollution/les-pesticides-migrent-partout-dans-l-environnement_148889
- Leblob.fr. **Vers de terre : une étude alerte sur l'impact des pesticides**, 6 November 2020. https://leblob.fr/videos/vers-de-terre-une-etude-alerte-sur-impact-des-pesticides
- Life Sciences UPSaclay. **La menace silencieuse de la contamination des sols et des vers de terre par les pesticides**, 5 November 2020. http://sco.lt/5slHLk

Project web-site link: https://rescape.univ-fcomte.fr

RESYST

The REsistance of Tropical agricultural SYStems to the reduction of pesticide pollution pressure at the watershed scale

Launch year: 2015
Completion year: 2019

Partners
UPR HortSys CIRAD; UAG Ceregmia-INRAE; UMR Tetis CIRAD; UMR SCBPA CIRAD; UMR SADAPT AgroParisTech

Scientific manager
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Financing
Total cost of the project: € 246,623
Ecophyto grant: € 106,276

Key words:
Environment contamination; Territorial approach; Tropical crops; Co-design work with farmers and other local professionals

Context and main objectives

The French Caribbean (Antilles) is facing generalised pollution by chlordecone. And despite this situation, large quantities of pesticides continue to be applied across the archipelago. The Caribbean environment is extremely vulnerable to pesticide pressure. For the moment there is relatively little awareness of the factors which maintain such pesticide usage and the processes that lead to the pollution of waterways. It should also be noted that no tools are in place to support changes or agro-ecological transition over to agricultural practices better suited to managing pollution situations under tropical conditions.

In this context, the objective of the project is to construct a support structure that will accompany changes in socio-ecosystem practices in the watersheds affected by agricultural pollution. This will involve the participative construction of a shared representation of the risks related to pollution from pesticide use, including the agronomic, economic and social representations. The practical objective will be to limit the pollution of rivers by changing practices across whole regions.

Upstream from one of the RESYST project's pilot zones: the Galion watershed. *Photo: © Charles Mottes, Cirad*

Principal results and interest in relation to the Ecophyto plan

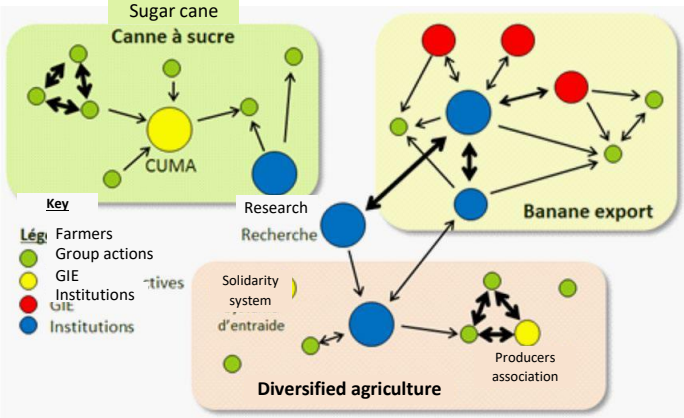
The first results from the correlated analysis of pollution pressures and the pollution observed in the waterways of the watersheds have shown a **high herbicide usage pressure** on the watersheds since such herbicide use is common to the various agricultural systems in place. This intense pressure (applied quantities and application frequency considering the scale of the watersheds) is **generating chronic pollution in waterways.**

Pesticide use is a two-edged sword for the community. The concept of utility and danger co-exist without necessarily generating a shared representation by the different members of the community In order to clear up this ambivalence, debates on perceptions of pesticide usage in the areas which influence local waterways have generated the need to create a space where points of view and knowledge can be exchanged on the subject. The second result concerns this space, which has taken the form of **action schools**.

Our analysis and participation in debates have revealed a **severe disconnect between the different professionals of the regions.** In this way changes in agricultural practices as well as the different agricultural sector support strategies for these changes are only mobilising the networks within the sectors without any interaction between them. Each sector favours its own production interests, highlighting its own constraint frameworks to the detriment of any regional development which would mobilise the diverse services and resources of the region. Our results have also shown that there is no "miracle" solution in terms of technical innovations that can be applied directly to the fields. Alternative techniques to the use of herbicides generate significant extra labour, operational and training costs.

However these costs could be offset by creating value on a region-wide scale through the available proximity resources (such as processing units, livestock, tourism, etc.) and by recognising the quality and diversity of local productions. In this way agriculture could have a leverage effect on tourism and promote the region through recognised "green" practices, such as excluding herbicide use, ending in a form of quality label for the region and its products. Agriculture would then be including in the strengthened economic development dynamic with the creation of an eco-tourism circuit in the watershed area combined with short supply chain sales outlets frequented by tourists that would also allow farmers to diversify their revenues sources. Products exported from the area (such as bananas) could also carry this image outside of the local region. This type of scenario shows the ambitions and powerful interconnections that are necessary for regional innovations when their objective is to create a virtuous circle of economic development associated with a reduction in pesticide use.

These innovations and how they will be presented have been co-constructed and debated with the local professionals thanks to the project's tools and models (such as DPSIR, region level C-K methodology, role playing).



Schematic representation of the professional network and influences concerning over-grassing management practices in the Galion watershed. *From Deffontaines et al. (2020)*

Prospects for transfer or research

Transfer:

Regional innovation seems to be a promising way to support change in agricultural practices. It would appear essential that such exchange and co-construction dynamics concerning the available options on a regional scale must be maintained between all professionals and all sectors. Environmental contracts must play a very specific role. The available tools (action schools, serious games) must facilitate these organisation and co-design systems. However, special attention must be paid to the long-term participation and commitment of the professionals involved. Sufficient and permanent resources must therefore be dedicated to this regional organisation.

Research:

The RESYST project has shown that it will be necessary:

1) to construct and maintain perennial meeting places where the future of agriculture can be debated in full, to propose localised objectives for new public policies and lead reflections and to negotiate and allocate the resources that will be necessary for the transition.

2) to consider the regional scaling in order to elaborate phytosanitary product usage reduction innovations and use more localised public policies to support their implementation and/or regional transitions. The "Sustainable Territories" project should integrate the analysis of government transition support policies.

Publications and scientific symposia:

TECHNICAL WORKSHOPS AND SCIENTIFIC SYMPOSIA:

- 14th ESA Congress. 2016, Edinburgh, Écosse: **Assessment of the pesticide pressure at the watershed scale: the challenge of the Water Framework Directive** (INRAE, 2 p). M. Lesueur Jannoyer, C. Mottes, M. Le Bail, P. Cattan.
- 14th ESA Congress. 2016, Edinburgh, Écosse: **Locating the contribution of cropping systems within a watershed to water pollution by pesticides** (INRAE, 2p). Mottes C., Lesueur Jannoyer M., Della Rossa P., Le Bail M., Cattan P.

- Tropentag 2017. 20-22/09/2017, Bonn, Allemagne: **Future Agriculture: Social-ecological transitions and bio-cultural shifts. Sociotechnical System Analysis of Weeding, A Key Step for Designing Agro-ecological Systems at the Watershed Scale.** University of Bonn, 1 poster. Della Rossa P., Mottes C., Cattan P., Le Bail M., Jannoyer M.
- Doctorate registered as a case study for the Idiss platform (INDISS spotlight project: INnovation and Design In Sociotechnical Systems) by Labex BASC (Biodiversité, Agroécosystèmes, Sociétés, Climat), seminar, 2017.
- OPDE symposium **"Concevoir, adapter, évaluer des dispositifs pour faciliter et étendre la participation".** Montpellier, France, OPDE network: Ecole-acteurs aux Antilles, un espace de représentation partagée de la question des pollutions agricoles. 13 p. Tonneau, J.-P., *et al.* (2016).

PRESENTATION TO PROFESSIONAL OR DECISION-MAKING BODIES:

- **Agriculture et qualité de l'eau, comprendre leurs relations pour mieux les gérer.** Presented to the "Assises de l'agricultures de Martinique", Agriculture, environnement et santé, Schœlcher, 5 December 2017.
- **Action schools** including professionals, institution representatives and researchers:

- Action school 1: November 2016, action school dedicated to the presentation of the action school concept
- Action school 2: March 2017, action school dedicated to innovations and innovation systems
- Action school 3: June 2017, action school dedicated to the theme: "awareness without guilt".
- Action school 4: November 2017, action school dedicated to the theme: "how to get by without glyphosate"
- Action school 5: February 2019, action school dedicated to population expectations regarding agriculture
- Action school 6: September 2019, action school dedicated to the theme: "What future for agriculture in Guadeloupe".
- Action school 7: Finally, an action school in Martinique, April 2019. The objective was to work on constructing a diffused pollution model for Martinique's drainage basins.
- Action school 8: November 2019, on the food industry.
- K Workshop: March 2018, knowledge exchange workshop on innovations between Martinique's agricultural professionals.

SCIENTIFIC PUBLICATIONS:

- Agricultural Systems (n° 179, 102769): **How farmers learn to change their weed management practices: Simple changes lead to system redesign in the French West Indies**. Deffontaines, L., Mottes, C., Della Rossa, P., Lesueur-Jannoyer, M., Cattan, P., Le Bail, M., 2020. https://doi.org/10.1016/j.agsy.2019.102769

- Agronomical Sustainable Development (n° 40, 10): **Innovations developed within supply chains hinder territorial ecological transition: the case of a watershed in Martinique.** Della Rossa, P., Le Bail, M., Mottes, C., Jannoyer, M., Cattan, P., 2020. https://doi.org/10.1007/s13593-020-0613-z
- Conception collective d'organisations territoriales innovantes pour une évolution coordonnée de systèmes de production agricoles (p328): **Cas d'une réduction de la pollution herbicide d'une rivière en Martinique**. Université Paris-Saclay, Saint Aubin, France. Della Rossa, P., 2020.

Other practical works:

- Presentation and use of WATPPASS-Game tools with a LEGTA class from Robert (December 2018).
- Presentation and training of technical staff from the Martinique Chamber of Agriculture on the use of the WATPPASS-Game solution (Novelber 2018).
- Réseau Canope – Gestion des pollutions à l'échelle des bassins versants (2017).
- France Antilles Martinique – Chlordécone : où en est la dépollution des sols ? - 9/10 June 2018.
- Bernard Crutzen, interview for the documentary: "Pour quelques bananes de plus" (June 2019).

RhizoDia

Regulating scale insect populations on black-current crops by releasing the scale-eating ladybird *Rhizobius lophantae* - Transposition to other fruit crops

Launch year: 2016
Completion year: 2019

Scientific manager
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Partners
CTIFL; Station d'Expérimentation La Morinière; Chambre Départementale d'Agriculture de Côte d'Or; INRAE PACA; KOPPERT

Financing
Total cost of the project: € 149,576
Ecophyto grant: € 87,128

Key words:
Scale insect; Biological pest control by augmentation; Scale-eating lady-bird; *Rhizobius lophantae; Pseudaulacaspis pentagona*; Black-current; Auxiliary releases; Perennial culture; Fruit crops; Multi-sites experimentation

Context and main objectives

Even if its is considered to be only a minor crop, black-current remains a major economic sector on a high value market where French production is much sought after (liquors, perfumes). For the last fifteen years, this crop has been severely affected by the white black-berry scale insect (*Pseudaulacaspis pentagona*), which was accidentally introduced into Italy in the 19th century via the silk road, since then it has spread further North to affect French black-current crops. This pest regularly causes crop losses or even the entire destruction of plantations; it is not very well controlled by chemical solutions and has become a major problem for the black-current industry. The RhizoDia project proposes a multi-site experiment programme, involving releases of the specific lady bird predator for scale insect *Rhizobius lophantae*, to demonstrate the effectiveness of a biological pest control by augmentation approach which could be applied by black-current growers. Beyond the model studied, it also aims to transfer this same biocontrol solution and its experimental methodology to other fruit crops that are affected by scale insects.

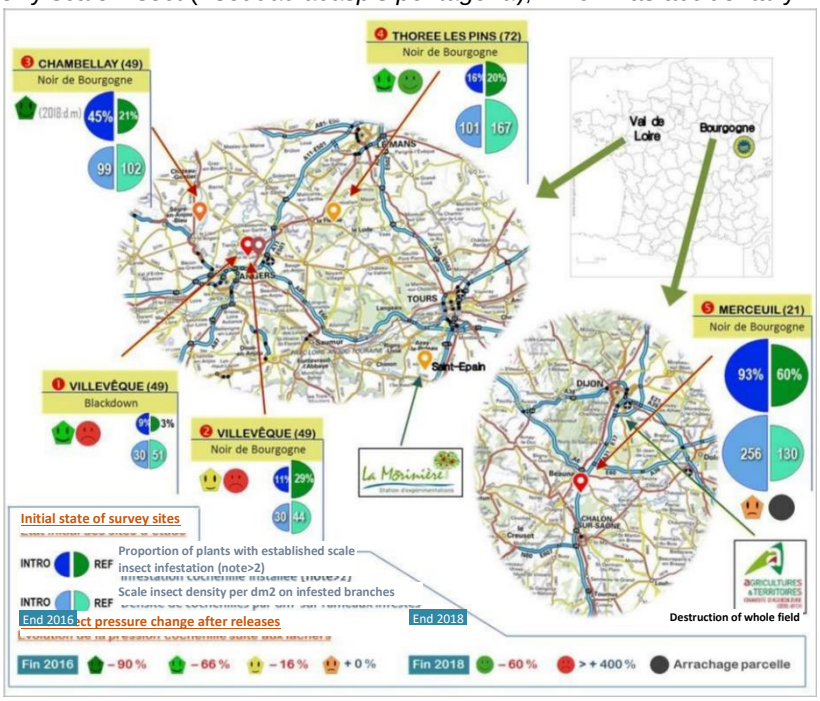

Figure 1: Initial condition of test sites and evolutions in the pest pressure for the release zones.
Image: © CTIFL/IGN (for map details)

Principal results and interest in relation to the Ecophyto plan

Experiments were completed in production orchards using conventional agriculture; it involved 5 sites in the Val-du-Loire and one in the Côte d'Or presenting a variety of different pest pressure situations. The experimental method used was proven during the project and can now be proposed for this type of study: 2 plantations of the same variety and of the same age were chosen, located in the same geographical zone but at least 500m apart, one was used for the introduction of auxiliaries (0.5 ha) and the other as a control site.

Figure 2: Winter shelters used by the project.
Left: cardboard strips. Right: bamboo bundles
Photo: © Station La Morinière

The control site was managed in keeping with a full phytosanitary treatment schedule including at least one specific scale insect treatment during the second swarming (REF(+)), except for a block of about 50 ares which was left without scale treatment, used as an "untreated" (REF(-)) control. The *Rhizobius* releases were annual, during each swarming period following a protocol which could also be used elsewhere for its ease of use: the auxiliaries were received ready for release, in bags with a ring which could be fitted to the base of the plant; each bag contained 200 adults, these were distributed across 15 release sites per 0.5 ha, so an installation time of 15 mins.

3 indicators were used to evaluate the effectiveness of this protection by release approach:

- The density of live scale insects per surface area unit, counted on samples of infested branches taken from the affected plants;
- The degree of plant infestation measured using a visual scale that covers the presence and severity of the symptoms;
- The number of *Rhizobius* captures, obtained by tapping the plants on a Japanese parasol.

The aptitude of the introduced auxiliaries to install themselves permanently was evaluated by analysing their ability to overwinter, measured by a hatchery experiment in which the viability of winter hatching was observed and by installing winter shelters where the occupation rates were observed at the end of winter to verify adult survival rates. A COI gene sequencing genetic classification protocol was applied to over 135 *Rhizobius* ladybirds captured before and after the releases as well as a sample taken directly from the producers, to attempt to confirm the contributions of the introduced auxiliaries over those of any established populations.

Figure 3: Bags of 200 *Rhizobius* at the base of infested black-current plants
Photo: © Station La Morinière

Under moderate pressure, the releases were effective during the first year of the project, with a scale insect regression rate of 48% in the introduction zones as opposed to 14% in the reference zones, a clear peak in *Rhizobius* re-captures after the releases and plants were visually in better health. But this was not confirmed later, since at the end of year 3 the situation degraded by 54% compared to the initial state of the introduction sites, whilst it remained stagnant for the reference sites. This failure could not be explained by phytosanitary pressure or global climate change, even if we can conclude that while the releases are effective, all of the factors for the method's success were not under control by the end of the project. Methodological improvements were also proposed, notably a more detailed classification of the fields, their surroundings and events occurring around the release operations in order to broaden the statistical analysis of the multi-sites experimentation in order to find explanatory factors.

Link with the Ecophyto plan:

The completion of the project did not result in the desired deployment of this biocontrol method. However it did confirm the highly variable effectiveness of phytosanitary treatments for the targeted pest, which further implies that work needs to continue to find alternative methods.

Prospects for transfer or research

Transfer:

The objectives laid down at the start of the project were not achieved; no transfer to application under production conditions can be planned at this time. Some methodological aspects, especially a number of points raised by the project report could be used for researchers working on similar projects.

Research:

In order to terminate exploration of the use of Rhizobius lophante to control scale insect on black-current, a new study program will test the method on young plants, targeting the zones where the first colonies appear and limiting release operations to the second summer swarming to allow for climate restrictions; the objective being to break up the pest's installation cycle as early as possible as well as lower the costs of this protection approach, under the project conditions costs were more than 40% of net product. Two research subjects will also be proposed: 1) the use of other gene portions or other genetic classification techniques on the auxiliary, the method used was not sufficiently discriminatory, and (2) research for non-destructive active scale insect detection tools.

Publications and scientific symposia:

PLACEMENT REPORTS:

- DAUFFOUIS, S. 2018. **Projet RhizoDia : essais en protection du cassis par *Rhizobius lophantae* contre la cochenille blanche du mûrier.** GIS-Fruits placement report 2018. https://www.gis-fruits.org/content/download/3876/38621/version/1/file/Simon_Dauffouis_RhizoDia2018.pdf
- GAY, A. 2017. **Etude de la régulation de populations de cochenilles diaspines en culture de cassis par des lâchers de coccinelles coccidiphages**. End of studies dissertation (unpublished)
- GOUVRION, J. 2016. **La coccinelle *Rhyzobius lophanthae,* agent de bio-contrôle pertinent dans la lutte contre la cochenille *Pseudaulacaspis pentagona* ?** GIS-Fruits placement report 2016. https://www.gis-fruits.org/content/download/3697/36570/file/RhizoDia-Gouvrion.pdf

PRACTICAL / EDUCATIONAL ARTICLES:

- FERNANDEZ, MM. 2021. **Cassis - Des coccinelles contre la cochenille.** Réussir Fruits et Légumes, n° 416: 36-37.
- FERNANDEZ, MM. 2020. **Une piste biocontrôle en culture de cassis ? Les lâchers de coccinelles contre la cochenille blanche du mûrier**. Infos CTIFL, n° 365: 18-32.

SCIENTIFIC SYMPOSIA:

- GUIGNEBAULT, P. 2018. **Biological control of the White Peach Scale on blackcurrant: the RhizoDia project.** Comments from the 6th World blackcurrant congress, Angers 6-8 June 2018. https://www.blackcurrant-iba.com/event/wp-content/uploads/2018/07/5-Rhizodia-program.pdf

SEMIOTRAP

Development of a biocontrol trap to monitor and control the palm borer moth, *Paysandisia archon*

Launch year: 2018
Completion year: 2021

Partners
INRAE – iEES Paris; Sauvons nos palmiers (SNP); M2i Group

Scientific manager
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Financing
Total cost of the project: € 226,902
Ecophyto grant: €120,000

Key words:
Attraction; Pheromone; VOC; Trap; Biocontrol; Chemical mediators; Palm trees; *Paysandisia archon*

Context and main objectives

Paysandisia archon is a moth of Uruguayan origin. It is not considered as a pest in its native habitat. It was unintentionally introduced into Europe at the end of the 1990s, where its larvae now cause significant damage to palm tree species. A pheromone has been identified which is emitted by the male to attract females. The insect is large with a wing span of 11cm. Conventional or biological control solutions are ineffective since it develops inside the trunk. The use of pheromones can help to adapt treatments or direct control techniques such as the mass trapping of fertilised females.

The project seeks to develop an understanding of how the pheromone produced by the male works and should conclude with its use for mass trapping or pest monitoring programmes. In order to achieve this, the project includes behavioural and physical-chemical studies to optimise the composition and dosage of the pheromone. The project is committed to designing a trap suitable for this moth; commercially available traps are not suited to the size and behaviour of this pest. After this the project concentrated on the Volatile Organic Compounds (VOC) emitted by the palm tree crowns where the eggs are laid.

Paysandisia archon
Photo: © Brigitte Frérot – INRAE iEES Paris

This project combines the work of three partners: The Versailles INRAE UMR iEES 1392 chemical ecology laboratory, M2i and th.e Sauvons Nos Palmiers (SNP - Save our Palm-trees) association, which is a collective of palm-tree growers. This collaboration serves to combine scientific expertise on the identification and formulation of chemical mediators with an industrial company specialised in synthesising Lepidoptera pheromones and formulating them on innovative biodegradable matrices. The SNP partner is deeply involved in communication and information concerning palm-tree pests. This partner played an essential role in the project by providing host-sites for the necessary field installations.

The hoped for result was a commercial biocontrol product including a trap and one or two types of lure designed to control the moth. This product will improve the epidemic-surveillance of the pest and its rapid management as well as its final control through mass-trapping operations. This type of solution will improve data collection and limit the need to use phytosanitary products. As part of an integrated control program in association with the currently available biocontrol solutions, the arrival of this solution should also help to achieve the “zero pesticide” objective for the control of this pest.

Principal results and interest in relation to the Ecophyto plan

I) Identification, formulation and observation of the effect of the pheromones

The SEMIOTRAP project continued work on:

1- the identification of the pheromone produced by the males and the demonstration that it attracts females independently to their visual senses.

2- the demonstration of the attraction of fertilised females by the odour of the palm-tree trunk.

During the synthesised pheromone attraction study; we demonstrated that the identified compound is never as attractive as the natural pheromone, and that the attraction rate remains much lower than that expected for a trapping system. The pheromone has therefore been re-investigated and two new minor compounds have been detected, only one of which has been identified: Z9-18: AC.

Various combinations were tested in flight tunnels, but none of them were as effective as the natural extract, except for **E2Z13ODOL+ Z9-ODAC**

Retention time (min)	Retention index (RI)	Identified compound	Washingtonia	Trachycarpus	Phoenix
2.36	854.3	Hexanal	7.84%	6.69%	2.14%
2.95	890.5	Ethylbenzene	2.50%	2.39%	1.55%
3.05	896.4	p-Xylene	4.55%	3.63%	2.43%
3.2	905.5	3-Heptanone	2.20%	1.56%	2.50%
3.39	916.8	heptanal	5.40%	5.59%	2.86%
3.84	944.1	α-Pinene	1.04%	0.90%	3.79%
4.09	959.4	2-Ethylhexanal	3.89%	2.28%	3.03%
4.24	968.6	Benzaldehyde	5.56%	7.19%	9.79%
4.83	1004.1	Octanal	3.89%	3.10%	2.74%
5.26	1030.1	Limonene	20.34%	32.19%	22.47%
5.34	1035.6	Benzyl alcohol	14.50%	13.82%	11.92%
6.5	1105.5	Nonanal	7.10%	4.99%	5.48%
7.22	1147.2	3-Methylheptyl acetate	0.45%	0.48%	0.84%
7.4	1156.3	Isomenthone	0.37%	0.19%	0.00%
7.63	1171	2-Decenol	0.93%	0.69%	0.91%
7.85	1183.3	p-Ethylbenzaldehyde	0.00%	0.33%	0.67%
8.03	1194.6	Methyl salicylate	0.00%	0.08%	0.00%
8.25	1207	Decanal	5.43%	5.19%	4.66%
8.49	1220.8	2-phenoxyethanol	11.37%	8.38%	21.59%
9.15	1259.3	p-Anisaldehyde	0.89%	0.34%	0.00%
9.58	1285.5	Anethole	0.96%	0.00%	0.00%
12.24	1448.9	cis-Geranylacetate	0.78%	0.00%	0.64%

Identifications of the compounds emitted by various palm-tree species. (tested compounds in blue) *Image: INRAE*

(10µg+0,1µg), which provokes 20% of landings, but was only tested on a small number of individuals.

The second major result concerns the demonstration of the attraction of males to the pheromone that they or their peers emit. There can be no doubt that the males return to the spots marked by their peers of the same sex or those they marked themselves. We have also demonstrated that this was not the result of any significant pheromonal polymorphism in the males.

II) Trap assays:

None of the traps tested were effective. Electric harp or dome traps compatible with safety regulations do not produce a strong enough discharge to kill the insects. Net and cage traps are not compatible with the behaviour of this insect.

III) Palm-tree VOC classification:

In 2019 we broadened the range of molecules and tested most of the molecules produced by palm-tree crowns, along with some other molecules often emitted by the plants such as DMNT, caryophyllene, etc. Some of these products were very well perceived by the antennae of the unfertilised females with reactions close to 2 mV. This was the case with Methyl salicylate, 1-phenoxy-2-propanol, phenol, butyrolactone, anisaldehyde and, above all, caryophyllene. Alpha-pinene, present in the odours emitted by palm-tree crowns, described as an attraction inhibitor for RPW (Soroker et al.), is not very well perceived by *Paysandisia archon*.

Prospects for transfer or research

Transfer:

The *Paysandisia* is a complex insect in terms of its biology and dimension. The host plant is disseminated across the urban landscape.

Studies of the attraction of males and females by the synthetic pheromone and to identify the third compound need to be continued. This will be a long process but it could lead to reducing populations by trapping both sexes.

Current results have not lead to a solution but have contributed to furthering knowledge of the chemical mediators which affect the reproduction and egg-laying of this insect. We know that the plant attracts fertilised females and the VOC have been identified. Potential egg-laying site location inhibitors have been proposed and will need to be tested.

Research:

The marketing of a new technique based on chemical mediators will not be easy and will have to involve a long-term behavioural approach in cases where insects react outside of the known model.

An optimal pheromone mix will need to be identified; the roles of the VOC produced by the plants will need to be investigated in order to produce more than one lure: a sexual lure and a pre-egg-laying lure.

M2i will continue its research work, notably on optimising the design of a net trap for capturing attracted insects.

Publications and scientific symposia:

Operational / educational articles:

- RACHID HAMIDI AND BRIGITTE FRÉROT, INRAE, UMR 1392, IEES, Route de St-Cyr, Versailles F-78000. **Les médiateurs chimiques, clés de voûte du cycle de *Paysandisia archon.*** Study of chemical mediators that are involved in the palm-borer moth's development cycle must lead to new control solutions. Phytoma 740 May 2021

Technical workshops and scientific symposia:

- The presence of SNP members amongst the consortium members guarantees, *via* their annual "Les rencontres ravageurs de palmiers de Monaco" symposium and regular public interventions, the awareness of those involved regarding biological control and highlighted progress made by the programme with the local PACA region councils and the general public: https://www.sauvonsnospalmiers.fr/les-quatriemes-rencontres-ravageurs-de-palmiers-de-monaco.html?id_document=3402.

SERUM

Disinfecting soils in market gardens

Launch year: 2015
Completion year: 2019

Partners
CETU Innophyt, University of Tours; CIRAD; CIRAD/IRD/ SupAgro / UM2 UMR LSTM; INRAE; IRBI UMR CNRS 7261 University of Tours; EBI UMR CNRS 7267 Université Poitiers; GRAB-Avignon; CERTIS Europe; Delbon SARL

Scientific manager
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Financing
Total cost of the project: € 349,070
Ecophyto grant: € 140,930

Key words:
Tomato; *Allium; Crotalaria*; Nematodes; Bacterial wilt; DMDS; Mulch; Preceding crop; Ground-sheeting; Unwanted side-effects; Biostimulation; Allelopathy.

Context and main objectives

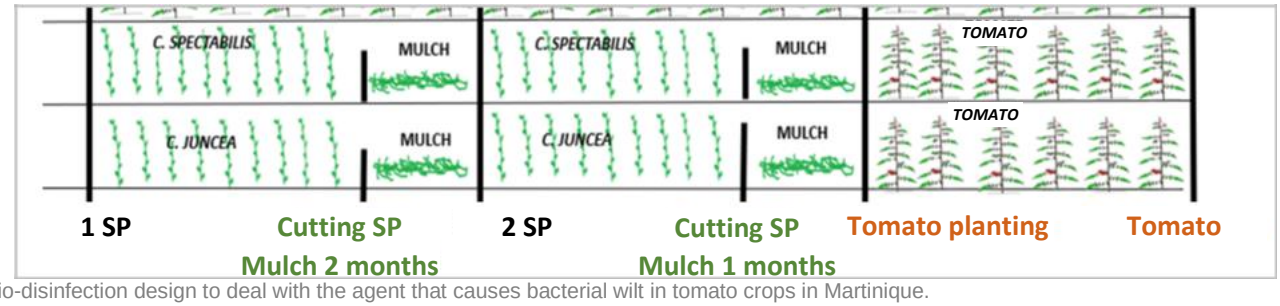
In Martinique, *Ralstonia solanacearum,* the bacteria behind bacterial wilt (quarantine organism), is the leading pest/disease affecting solanaceae and cucurbitaceae, resulting in losses of up to 100%. No authorised product currently exists to treat this. The root-knot nematode, *Meloïdogyne*, causes 40% losses in green-house grown tomatoes in the South of France. Only dichloropropene is currently authorised for use. There is now a very significant need for agro-ecological solutions to protect harvests for market gardeners. The biocontrol strategies studied by SERUM are based on the bio-disinfection of soil using service plants such as rattlepods and Alliaceae as precedent crops or mulch (figs 1 and 2) and/or incorporated dry or fresh chippings, or as companion planting. Dimethyl disulfide (DMDS), a volatile molecule emitted by Alliaceae, is a viable soil disinfection solution (nematodes, fungi, weeds), it has already received authorisation for sale as a soil fumigation agent for specific crops in a number of countries. Its antibacterial properties against bacterial wilt have been explored for use in an integrated agriculture context.

Figure 1: *C. Spectabilis* mulch.
Photo: © Ingrid ARNAULT, CETU Innophy

Principal results and interest in relation to the Ecophyto plan

The project has demonstrated the interest of service plants in soil disinfection for tomato crops from three different production and issue angles.

1) Against the **agent that causes bacterial wilt** in tropical agriculture, ***C. juncea*** as a **two cycle precedent crop followed by mulch** (see diagram) has been found to seriously reduce incidences of the disease.



Bio-disinfection design to deal with the agent that causes bacterial wilt in tomato crops in Martinique.
Image: © Ingrid ARNAULT, CETU Innophyt

However this usage does present some technical disadvantages for professionals. **The use of *C.juncea* as a powder or chipping mixed into the soil would be a more realistic solution.** A production industry for dry forms of *C. juncea* should be set up for better integration into crop systems.

As for the local *allium fistulosum* which has interesting anti-bacterial properties, its use as a mulch will not be possible since it is highly sought after for cooking.

2) Against **root-knot nematode**, *Meloidogyne*, in (French) mainland **market garden green-house productions**, *C. spectabilis* **as a preceding crop/mulch followed by incorporation into the soil** seems to reduce root-knot on lettuce. The incorporation of powdered rattlepod into the soil has also provided interesting results in the reduction of root-knot on tomatoes.

3) Against **root-knot nematode**, *Meloidogyne*, in (French) mainland **tunnel productions**, assays have demonstrated a slight interest in using **ground up chives and onions incorporated into the soil**. However, experimental design research needs to continue: the optimal dose of dry or fresh ground material incorporated into the soil combined with suitable ground-sheeting, use as a precedent crop/mulch, association with rattlepods and *Allium sp*., etc.

When parasite pressures are very high, as was the case for the nematode and *R. solanacearum* assays, service plants as crop precedents/mulch or as ground material incorporated into the soil did not always fully protect productions. A strategy involving pesticides during the growing phase could be envisaged to definitively reduce pathogens.

The project has highlighted the anti-bacterial properties of DMDS. This product is a promising alternative for use against this bacteria which is a pathogen in tropical region and potentially invasive in temperate climates. The prospects for the use of DMDS, currently unauthorised for use in this context, depend on the currently pending application for European commercial registration.

As for unwanted side-effects, observations have been mostly positive for the Service Plants. These Service Plants stimulate microbial populations in the soil and growth in tomatoes. The only toxic effect of *C. juncea* extracts and DMDS occur in very high doses, much higher than those used in the fields during the project, but should be considered for any potential effect on woodlouse.

Figure 2: Left: cross-section of *C. Spectabilis;*
Photo : © Ingrid ARNAULT, CETU Innophyt

Prospects for transfer or research

Transfer:

The question of the generic nature of the method is very important for biocontrol. As regards the project results, any transposition to other market garden crops would be possible but delicate, provided that the exact same production conditions apply: tunnel, green-house or field. For example, we have been able to demonstrate that *C. Spectabilis* has an anti-nematode effect as a precedent crop followed by application as a mulch for lettuce crops.

Research:

Strategies combining preventive approaches using Service Plants as precedent crops, and curative approaches using DMDS during the growth season for tomatoes, will permanently reduce pathogens.
Considering the prospects, and where possible, it would be useful to test this strategy with a crop rotation to reduce the infectious potential of the soils. Actions will be different and probably complementary.
Bio-stimulation of tomato plant roots and soil microbe populations via Service Plants suggests furthering studies of the rhizosphere and symbiotic interactions: mycorrhiza, antagonist bacteria, irregular bacteria, etc.

Publications and scientific symposia:

- Poster: Biocontrol and Natural Products, 25-28 September 2018, Perpignan. I. Arnault, P. Deberdt, G. Dubreuil, Y.Prin, P.Fernandes, H. Védie, P. Sunder, T. Fouillet, G. Dufretay, C. Souty-Grosset, N. Pourtau, M. Zimmermann, D. Giron. **Biodésinfection des sols en culture de tomate.**
- Poster: Future IPM 3.0: towards a sustainable agriculture. 16-20 October 2017, Italy. **Allelopathic effects of *Crotalaria juncea* and dimethyldisulfide (DMDS) on tomato plants in the future development of a biocontrol method against root-knot nematodes.** G. Dubreuil, N. Pourtau, N. Moreau, C. Leboissetier, M. Piot, D. Giron, and I. Arnault.
- Poster: 13e Rencontres Plantes-Bactéries. 29 January-2 February 2019, Aussois. **Beneficial effect of the use of the tropical legume Crotalaria spp for organic greenhouse vegetable production in nematodes-infested soil of South of France.** Y. Prin, F. Cussonneau, C. Le Roux, E. Tournier, B. Vincent, R. Duponnois, F. Deleuze, A. Galiana.
- Manuscript submitted to the European Journal of Agronomy (6 March 2019). **Beneficial effect of the use of the tropical legume *Crotalaria* spp for organic greenhouse vegetable production in nematodes-infested soil of South of France.** Y. Prin, F. Cussonneau, C. Le Roux, E. Tournier, B. Vincent, R. Duponnois, F. Deleuze, A. Galiana.
- Proceedings of IX International Symposium on Soil and Substrate Disinfestation (SD 2018) 9-13 September 2018, Heraklion, Crète. **Soil disinfestation with dimethyl disulfide (DMDS) to manage the bacterial wilt of tomato in the tropics.** P. Deberdt, R. Coranson-Beaudu, C. Thibaut, N. Le Roch, T. Fouillet, P. Sunder and I. Arnault.

SYSTEMYC

Conception of crop-growing SYSTEms based on the use of MYCorrhization for the biocontrol of earth-bound pests on tomato crops

Launch year: 2015
Completion year: 2018

Partners
UR 1321 ASTRO INRAE; University of the Antilles; INRA UR Ecodéveloppement; INRA UE Alénya-Roussillon; Institut Sophia Agrobiotech

Scientific manager
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Financing
Total cost of the project: € 528,074
Ecophyto grant: € 99,297

Key words:
Soil biodiversity; Mycorrhiza; Biocontrol; Redesigning crop systems; Vegetable crops; Co-design work with agriculturalists; Socio-technical blocking; Learning; Educational tool

Context and main objectives

French mainland and Caribbean market garden crops are currently affected by severe soil parasites resulting in serious damage to their harvests. With the recent withdrawals of authorisations to use certain phytopharmaceutical products (nematicides, methyl bromide, etc.), the development of biocontrol strategies has become a necessity. Interactions between plants and micro-organisms in the soil, such as mycorrhizal symbioses, could provide new solutions for the agro-ecological management of crop health. In this context; the SYSTEMYC project objectives were:
i. Co-design crop-growing systems based on the use of mycorrhiza for the biocontrol of under soil pests
ii. Construct systems able to identify the obstacles and levers involved in the use of mycorrhiza by farmers and the training that will be necessary to use them for biocontrol.
iii. Evaluate innovative strategies for using mycorrhiza in temperate and tropical zone market garden crop systems.

Principal results and interest in relation to the Ecophyto plan

If the “one pest/disease, one solution” approach remains valid for some biocontrol products, it does not apply to mycorrhiza. In fact, mycorrhiza 1) have a multi-pest/disease effect, 2) have different direct and indirect actions, and 3) are impacted by most agricultural practices.
Using mycorrhiza for biocontrol will therefore require an overhaul of crop protection approaches. The main results of the SYSTEMYC project are:

1. A collective MYMYX1 training system [1] (“Mimic Mycorrhizal networks”), which alternates participative workshops (figures 1 and 2) and farm surveys and has enabled the achievement of an understanding of the complexities and uncertainties associated with mycorrhiza processes and the identification of the obstacles and levers that will be involved in their use. From the 50 agriculturalists involved, we were able to distinguish two groups with different characteristics in terms of their proposition dynamics and the obstacles they identify regarding the implementation of mycorrhization: a “proactive group” and a “reserved group”.

Figure 1: The MYMYX game board.
Photo: © Marie Chave, INRAE

[1] MYMYX: (“Mimic Mycorrhizal networks”) is a support which can be used to get an understanding of under-soil interactions by creating and maintaining a network through the development of mycorrhizal filament segments (in white). For the players this involves constructing a strategy, a set of practices (crop/inter-crop, tilling, fertilisation, phyto-protection, introduction of commercial or other mycorrhiza, etc.) to design a crop system that will allow the roots (shown in the four corners of the board) to access the nutritive resources they need as rapidly as possible (e.g.: phosphates in yellow, water in blue, etc.) whilst maintaining protection from pestattacks (in red).

The “proactive group” agriculturalists display a capacity to adopt a re-designed approach whilst those of the “reserved group” show a lesser propensity to integrate different or new concepts. We have observed some diversity in regional responses to the propositions raised.

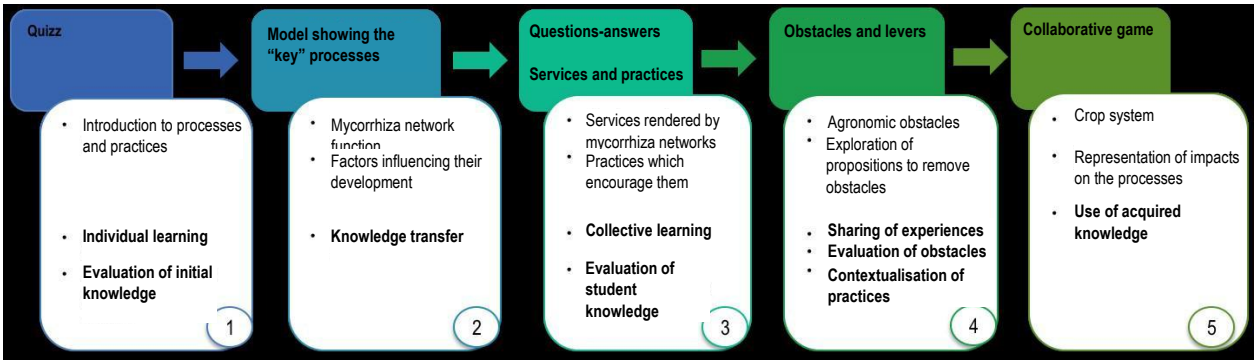

Figure 2: Organisation of 5 sequences of the participative “Sharing knowledge” workshop implemented in the 4 studied regions (Martinique, Guyana, Guadeloupe and Provence).

2. An innovative design approach based on the MYMYX system was able to open up mycorrhiza usage prospects for biocontrol purposes. When applied in a diverse range of contexts, it leads to the production of a hierarchical tree of the farmers’ propositions organised in three levels: concepts, sub-concepts and practices. The explored concepts are (C1) the introduction of standard commercial or native (natural) strain propagules, (C2) the connection of mycorrhiza fungi with plants through associations and crop rotations, (C3) the intensification of the mycorrhiza networks by reducing tilling operations and additives (fertilisers, phytopharmaceutical products) (figure 3). This large, yet still incomplete, range of solutions which can be adapted to each individual context as part of a systemic logic, will allow farmers to understand that they are all potential mycorrhiza producers.

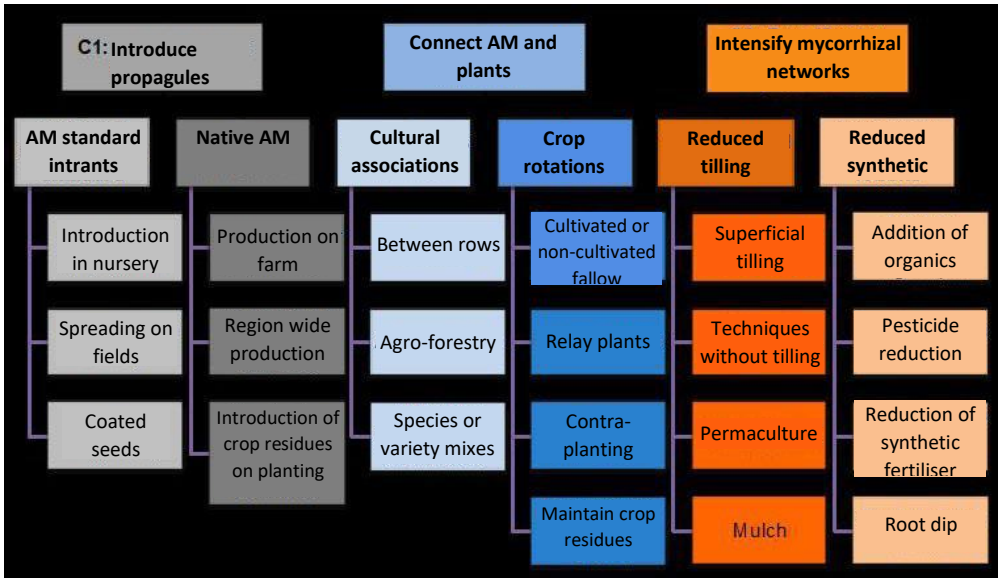

Figure 3: Extract from the mycorrhiza mobilisation levers propositions library (from the 4 study regions: Martinique, Guyana, Provence and Guadeloupe), CMA: Arbuscule Mycorrhizal fungi. *Image: © Marie Chave, INRAE*

3. The development of innovative strategies and systems has enabled the application of native mycorrhiza networks for the biocontrol of under-soil pests/diseases in market garden crops. The experimental evaluation of this strategy has been undertaken by local professionals in Martinique and Guyana. The evaluation of an innovative mycorrhiza strategy, completed at the same time by the experimental units (INRAE Alénya and PEYI), based on multiplying native mycorrhiza to ‘pre-mycorrhize’ tomato plants has shown: 1. in nurseries: the feasibility and effectiveness of this strategy in mycorrhization container prototypes, 2. in the field: the interest of early mycorrhization for crop health, 3. under controlled conditions: the effectiveness of the mycorrhization of a tomato plant using a mycorrhiza network in comparison to mycorrhization by propagule inoculation and the bio-protector effect of mycrorhization regarding root-knot nematodes.

Prospects for transfer or research

Transfer:

The results and tools developed by the SYSTEMYC project have been used by the CasDAR projects (Mycoagra and Mycado). The MYMYX educational tool has been redesigned with agricultural training and advice. This type of usage redesign approach is necessary to help professionals through the transition process, as they change their positions in order to produce/educate/advise/research in new ways.

Whilst the 'approach of applying biodiversity in the interests of crop health' developed by SYSTEMYC was concentrated on mycorrhiza, it remains generic and applicable to other natural regulation processes (mobilising auxiliaries for examples).

Research:

Continuing the analysis work on plant interactions via mycorrhiza networks will open up numerous crop health management possibilities combining the bioprotector and disinfection effects of mycorrhizotrophic plants (see the MultiServ, INRAE, SuMCrop projects).

On the crop systems scale, it will be necessary to continue field evaluations of the impacts of numerous mycorrhiza usage strategies for biocontrol (see the Dephy Expé Cabiosol project supported by FREDON Martinique).

On the territorial scale, analysis of the obstacles and levers involved in the implementation of biocontrol should be continued (see the Ecophyto Interlude project).

Publications and scientific symposia:

TECHNICAL WORKSHOPS AND SCIENTIFIC SYMPOSIA:

- **International Conference On Mycorrhizae, Mexico (30/06/2019 - 05/07/2019) :** *A participatory approach to harness native mycorrhizae***.** Chave M, Angeon V, Harter AC, Goasduff M, Paul G, Quinquenel S, Ster H.
- **International Conference on Plant Protection, Boston, USA (29/07/2018 - 03/08/2018) :** *Agroecological engineering for biocontrol of soil pests- examples from the French Caribbean.* Chave M, Angeon V.
- **Scientific Advisory Board of the SMaCH meta-programme, Versailles, FRA (29/01/2018) :** *Harnessing mycorrhiza for crop health: an agroecological approach*. Chave M, Angeon V.
- **54th Caribbean Food Crops Society symposium. Belize (07-15/07/2018):** *Agroecological production and consumption models in the context of transition: the approach of the economies of worth.* Angeon V, Crevoisier O.
- **Salon Tech & Bio. 20-21/09/ 2017. Valence***: Poster and workshop: "Cultivez vos mycorhizes !" .* Chave M., Paut R., Angeon V., Lefevre A., Dufils A., Tchamitchian M.
- **TANDEM network presentation seminar, Avignon, FRA (16/10/2017)** *: Les systèmes de culture maraîchers : des laboratoires pour la co-conception d'innovations agroécologiques ? Focus sur les réseaux mycorhiziens.* Chave M, Angeon V, Paut R, Tchamitchian M.
- **54th ASRDLF symposium, ERSA-GR conference, Athens, GRC (05-07/07/2017)** *: Mettre en œuvre la transition agréocologique : un défi d'apprentissage et de partage de connaissances par et pour l'action.* Angeon V, Chia E, Chave M, Auricoste C.
- **52nd Caribbean Food Crops Society symposium. Gosier, Guadeloupe (07-15/07/2016)** *: How to foster mycorrhiza ? From brakes to levers.* Chave M, Paut R, Angeon V, Dufils A, Lefèvre A, Tchamitchian M 2016.

PRACTICAL / EDUCATIONAL ARTICLES:

- ***INRA technical notebooks****: Des tomates mycorhizées dès la pépinière pour favoriser la nutrition et la protection des plantes : développement d'un dispositif-pilote.* Julianus P, Perrin B, Chave M. 2019.
- ***Data-sheet available online via geco.ecophytopic.fr:*** *« Les mycorhizes : des réseaux vivants au service de la protection des cultures ».* Chave M., Paut R., Perrin B., Dufils A. 2017. http://geco.ecophytopic.fr/documents/20182/21720/pdf_Cultiver_des_esp_ces___mycorhizes_2.pdf
- ***Data-sheet available online via geco.ecophytopic.fr:*** *« Multiplier des champignons mycorhiziens sur son exploitation ».* Chave M., Paut R., Perrin B., Dufils A. 2017 http://geco.ecophytopic.fr/documents/20182/21720/pdf_Multiplier_des_champignons_mycorhiziens_sur_son_exploitation_1.pdf
- ***INRA EA-SAD key-note 2016*** *: MYMYX, un dispositif participatif de conception d'innovations agroéco-logiques pour valoriser les réseaux mycorhiziens.* Chave M., Angeon V. 2016.

PRESENTATION TO PROFESSIONAL OR DECISION-MAKING BODIES:

- ***Annual APCA-RESOLIA training*** *(2019) : Développer les symbioses mycorhiziennes au profit des cultures. Formation à destination des conseillers agricoles.* Hirissou F, Chave M, Auricoste C, Lendure A.
- ***Presentation to the DEPHY Ferme Bouches du Rhône network*** *(28/01/2016) : Comment valoriser les mycorhizes en agriculture ?* Paut R., Chave M., Angeon V.
- ***INRA EA-SAD key-note 2016*** *: MYMYX, un dispositif participatif de conception d'innovations agroécologiques pour valoriser les réseaux mycorhiziens.* Chave M., Angeon V. 2016.
- ***Présentation dans le cadre du Forum territorial Culture Science PACA*** *(22/092016) : Accompagner la transition écologique en agriculture par le jeu.* Paut R, Chave M, Angeon V., Tchamitchian M.

SCIENTIFIC PUBLICATIONS:

- ***Agronomy for Sustainable Development*** *(n°39, p 48) : Codesigning biodiversity-based agrosystems promotes alternatives to mycorrhizal inoculants.* Chave M, Angeon V, Paut R, Collombet R, Tchamitchian M. 2019.
- ***Phytopathologia Mediterranea*** *(n°59[2], pp 377-384) : Protective effects of mycorrhizal association in tomato and pepper against Meloidogyne incognita infection, and mycorrhizal networks for early mycorrhization of low mycotrophic plants.* Rodriguez-Heredia M, Djian-Caporalino C, Ponchet M, Lapeyre L, Canaguier R, Fazari A, Marteau N, Industri B, Chave M.
- ***Agroecological transitions : from theory to practice in local participatory design*** *(pp 99-120) : A plurality of viewpoints regarding the uncertainties of the agroecological transition.* Magda D., Girard N., Angeon V., Cholez C., Raulet-Croset N., Sabbadin R., Salliou N., Barnaud C., Monteil C., Dubois Peyrard N. 2019. In: E. Audoin, JE. Bergez, O. Therond, dir.
- ***Innovations Agronomiques*** *(n°64, pp 97-111) : Du partage de connaissances à la co-conception d'innovations agroécologiques : Exemple de la mobilisation des mycorhizes en Guyane.* Chave M, Angeon V. 2018.
- ***Mycorrhiza*** *(n° 27, pp 719-723) : Rhizophagus irregularis MUCL 41833 transitorily reduces tomato bacterial wilt severity caused by Ralstonia solanacearum under in vitro conditions.* Chave M, Crozilhac P, Deberdt P, Plouznikoff K, Declerck S. 2017.

OTHER PRACTICAL WORKS:

- ***MYMYX,*** **board-game that aims to encourage knowledge sharing on mycorrhiza and the design of crop systems which will facilitate their success.** *(DI-RV-15-15-0020. Enveloppes Soleau n°582327 et n°582992. Marque n°174341832 déposée au nom de l'INRA en classes 9, 28 et 41.)* Chave M, Angeon V, Giraud N. 2017.
- **Redesign of the MYMYX tool and production of mobile elements.** N Giraud. 2016.
- **Development of a limited series of 4 MYMYX board games,** UE INRA Alénya-Roussillon. Girard G. 2016.
- **Les Mycorhizes. Kamannyok radio show.** Guadeloupe Première. Chave M., Angeon V. 2016. 22 October 2016

Trajectoires

Hereditary trajectories and the use of pesticides in major agricultural lands

Launch year: 2015
Completion year: 2019

Partners
LPED Aix-Marseille University; INRAE Lyon et Montpellier; Bureaux d'études Ressources

Scientific manager
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Financing
Total cost of the project: € 177,916
Ecophyto grant: € 89,991

Key words:
Sociological analysis; Hereditary trajectories; Pesticide use; Interdisciplinary approach; Wine growing; Rice growing; Oilseed culture; Tree culture

Context and main objectives

Within the framework of the national Ecophyto (2018, 2 and 2+) policy, and if we refer to the indicator used to evaluate the effects of this policy - the Number of Unit Doses (Nombre de Doses Unité - NODU), the overall results are disappointing in terms of the stated objective of reducing pesticide use by 50% over a ten year period[1] . Guichard et al.[2] have highlighted two major obstacles behind this result. The first is related to the lack of a systemic vision of the Ecophyto plan, which is exclusively concentrated on farmers and their consultants, thus failing to pay sufficient attention to "socio-technical blocks"[3], this aligns neatly with criticism laid out in the Potier report which mentioned a "vision that was too narrowly technical" and "not systemic enough"[4]. The second obstacle concerns the choice and use of monitoring indicators which remain essentially quantitative, thus blocking any chance of noting more intimate changes, or emerging changes.

It is in this context that the "Trajectoires" project seeks to contribute to reflections on changes in phytosanitary usage practices. As regards the limitations of an approach based on an evaluation using only quantitative indicators and figures, this research is mostly built on qualitative data from sociological interviews, taking into account the viewpoints and experiences of farmers and agricultural professionals.

Principal results and interest in relation to the Ecophyto plan

The sociological research was established on the basis of a comparison between four agricultural regions with different crops and crop systems and looked at how they could reduce their use of phytosanitary products: rice farming in the Camargue, oilseed culture in Alpilles-Sud; wine-growing in the Beaujolais and in the heart of the Hérault; and tree-farming in the Comtat (Nord-Alpilles). 146 semi-guided interviews were completed with 120 agriculturalists and 26 agriculture professionals, all of these were subjected to a comparative content analysis.

In order to encourage inter-disciplinary dialogue, four seminars were organised in each of the survey regions. Each seminar was organised around a visit to the region involving the project team and one or more agriculturalists and agriculture professionals. Group interviews were also organised, which encouraged the environmental science researchers to take interest in what the farmers had to say. An analysis exercise of certain statements was completed by these researchers. Finally, further inter-disciplinary dialogue was opened up between the ergotoxicology and sociology fields in order to confront these two disciplinary visions on the subject of the Certiphyto system.

The first element involved what was a largely stable point across all of the interviews, concerning the farmers' opinions of a systematic application period without any major protection from phytosanitary products. The structuring element was built on the fact that, for each of the regions, we have become aware of the individual and collective initiatives of various age-groups which suggest a desire to take a step back from the previously

[1] 2017 supervisory note, Ministère de la Transition écologique et solidaire (French Ministry for Ecological transition and solidarity), Ministère de l'Agriculture et de l'Alimentation (French Ministry of Agriculture and Food), July 2018.
[2] Laurence Guichard *et al.*, « Le plan Ecophyto de réduction d'usage des pesticides en France : décryptage d'un échec et raisons d'espérer », *Cahiers Agricultures* 26, no 1, 2017.
[3] Guichard *et al.*, p.1.
[4] Report by Dominique Potier, Pesticides et agro-écologie, les champs du possible, November 2014, p. 61.

described systemic referential and generate changes in practices regarding the use of phytosanitary products. This desire for perspective can be explained by a number of factors, the most evident are as follows: the weight of the bureaucratic norms and standards from private or government bodies/social pressure/solidarity and loyalty to families/attachment to the region.

The inter-disciplinary exchanges held within the Trajectoires project underline the limits in place regarding the knowledge trajectories that can be distributed within the framework of reducing phytosanitary product use. This has become too normalised within the framework of the Certiphyto system and unsuited to the public concerned; they remain too far from the technical preoccupations of farmers. Our reflections led to a questioning of this cognitive sectioning and call for the imagining of co-construction spaces which could be the subject of a public action.

Prospects for transfer or research

Transfer:

The mains issue of the Trajectories project was to continue existing comprehensive sociology work to provide elements to contribute to better efficiency in public actions and to share this approach with environmental science researchers. Four prospects were raised by our approach:

- Find a place within the register of ideas and culture.
- Support “transition” rather than categorise “change”.
- Encourage new knowledge and know-how.
- Co-construct research protocols that have a public action objective

Our results converged towards a certain number of already known elements as well as some that have been less commented in the literature, but which insist on the necessity of reconsidering how to support and evaluate public action in terms of reducing the use of phytosanitary products. In other words, it will also be up to public action to question its own function and representative system.

Research:

The Trajectoires project has contributed to feeding qualitative social sciences research that proposes another way to monitor, evaluate and support phytosanitary product use reduction policies. It also calls out for a dialogue between environmental sciences and social sciences in order to contextualise pesticide monitoring measures within a region.

Publications and scientific symposia:

Scientific publications:

- Carole Barthélémy, Aurélien Allouche, Gilles Armani, Gaïa Bonnet, Christelle Gramaglia, Laurence Nicolas, **Écologisation des pratiques agricoles et ancrages familiaux au territoire. Une comparaison de l’utilisation des produits phytosanitaires entre Camargue et Beaujolais,** Développement Durable et Territoires, Vol. 11, n°1, April 2020, Écologisation des pratiques et territorialisation des activités. https://journals.openedition.org/developpementdurable/16822
- Gaïa Bonnet, Christelle Gramaglia, **Les agriculteurs face aux risques induits par les pesticides : pratiques culturales, attachements familiaux et territoriaux chez les viticulteurs de l’Hérault,** *in* Lupton S., Chauveau-Aussourd V., Randrianasolo-Rakatobe H., Faire face aux risques en agriculture, Perspectives croisées de chercheurs et professionnels, L’Harmattan, 2019, pp.77-97.
- Armani G. **Analyse des stratégies d’utilisation des pesticides, des freins et leviers socio-culturels aux changements de pratiques viticoles dans le Beaujolais,** *in* Budzinski H. *et al.*, (éditeurs), Enjeux environnementaux et sanitaires associés aux pesticides, de leur usage à leur dispersion dans l’environnement en lien avec leur impact, Editions du GPF, 2017, pp. 188-191.
- Gilles Armani G., **Étude sociologique des pratiques viticoles dans le Beaujolais face aux enjeux environnementaux et sanitaires,** *in* La Tassée N° 191, Mai 2018, pp. 22-24.

Other practical works:

- Exchange seminars: organised in the Coeur d’Hérault and Beaujolais regions.
- Participation in Science events.
- Production of educational films: Videos produced for the Echo’s Phyto PACA social media network:
  - Allouche A, Nicolas, L : « Cultiver le riz autrement » https://www.youtube.com/watch?v=u8SqJBOagmg&t=1s
  - « Riz hybride et culture bio » https://www.youtube.com/watch?v=R8TH5GoRhqU&t=3s
- Production of 6 films by Gilles Armani:
  - Short film on the PolDiff laboratory, IRSTEA Lyon Villeurbanne, October 2017.
  - Short film on the LAMA and EMHA, IRSTEA Lyon Villeurbanne, October 2017.
  - Short film of an interview with two wine growers from the Beaujolais, October 2017.
  - Short film of an interview with an organic wine grower from the Beaujolais, October 2017.
  - Short film of comments by participants in the SAAM Seminar, 19 October 2017 in Lancié, October 2017.

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