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AphidInnov - Biological solutions for controlling aphid populations in protected crops

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Summary

Inundative biological control with natural enemies produced by specialist suppliers is the main alternative to insecticides in protected crops. However, major pests such as aphids are still poorly controlled, due to a lack of efficiency or availability of biological control agents that would give solutions for the disappearance of neonicotinoids. The AphidInnov project has shown, on seed cabbage, strawberry and cucumber crops, that effective parasitoid species and populations adapted to each agricultural context could be selected, taking into account the ecological specialisation of parasitoids and their genetic variation, and that effective solutions could be proposed by combining parasitoids, predators and cultural practices. The production of these biological control agents could create new business models, involving more directly professional organizations and growers.

Key words: Protected crops, aphids, parasitoids, specialization, population genetics, inundative biological control, effectiveness, release methods, mass rearing.

1 - Introduction

One of the major challenges facing agriculture today is the significant reduction of plant protection products use, while maintaining high levels of production in terms of both quantity and quality. The reduction in the plant protection products use, including the disappearance of certain families of products such as neonicotinoids, requires the development of alternative methods to control crop pest populations. One widely explored approach against pest insects is biological control, which involves using natural enemies of pest species to limit their populations. In open fields, numerous studies focus on conservation biological control, aimed at enhancing the action of natural enemies present in the crop environments by modifying the surroundings (hedgerow management, floral diversity in the landscape, etc). For protected crops, the primary strategy, developed over many years, is inundative biological control. This strategy involves releasing large numbers of natural pest enemies, such as parasitoid insects (parasitic organisms whose development leads to the death of the host), or predators (often more generalists), which are mass-produced by specialist companies.

However, for some major pests of protected crops, like aphids, biological control solutions are not always available. This may be due to the insufficient effectiveness of beneficial Biological Control Agents (BCAs), particularly parasitoids, or the absence of commercially viable solutions.



1.1 Background and objectives

The AphidInnov project was initiated in response to the observed inefficiency of biological control methods using macro-organisms, such as parasitoids and predatory insects. This inadequacy was expressed by various stakeholders in the field, including experimental stations and producer organisations. This perception aligned with the conclusion presented by ANSES (Saisine n°2016-SA-0057), which highlighted the lack of efficiency, operationality, and practicality of these organisms as an alternative solution to neonicotinoids, despite their good sustainability ranking. Consequently, the AphidInnov project aimed to enhance the efficiency, operationality, feasibility and practicality of these macro-organisms.

This issue particularly concerned aphids (Hemiptera: Aphididae) in protected crops, where inundative biological control has long been developed with a wide range of commercial BCAs, many of them being parasitoids. Aphids were also the primary targets of neonicotinoid insecticides. Improving the effectiveness of parasitoid insects in biological control of aphids was therefore a crucial objective to overcome the technical impasses faced by many protected crops.

The effectiveness of parasitoids highly depends on the parasitic match between the parasitoid individual and the host individual selected by the adult female. This host suitability is based on very close physiological interactions and observed failures could result from a mismatch between the target aphids and the introduced parasitoids. Aphid parasitoids, mainly belonging to Hymenoptera, Braconidae, Aphidiinae, exhibit a gradient of parasite specialisation, ranging from monophagous species or strict specialists to those capable of parasitising several dozen host species, known as generalists (Navasse, 2016). However, the host range may not be uniform within a parasitoid species, with populations possibly specialising on certain host species (Stireman *et al.*, 2006). Additionally, within a parasitoid's host range, certain species are preferred, providing advantages for parasitoid development. These variations in host preference can lead to the formation of specialised biotypes or even distinct species (Navasse *et al.*, 2017). Population genetics approaches can thus be employed to identify cases of parasitic specialisation (Stireman *et al.* 2006).

Parasitoids may also prove ineffective due to host resistance to parasitism. In aphids, this resistance is often associated with the presence of secondary symbiotic bacteria, notably *Hamiltonella defensa* (Schmidt *et al.*, 2012).

Based on these hypotheses, the AphidInnov project aimed to develop operational biological solutions. This involved selecting and using species or biotypes of BCAs that are effective against each targeted aphid in various protected cropping systems.

1.2 Target crops and methodology

Three crops grown under conventional or organic farming methods were the focus of this project, each facing a series of extremely damaging aphid pests, with some infesting multiple crops. Aphid management posed a significant technical barrier to reducing insecticide use, or even to production itself. These are:

- Brassica seed production for organic farming: recurrent aphid attacks posed a major concern in organic seed production, which must adhere to organic practices since the disappearance of rotenone. Producers have to use untreated conventional seed under a derogation. When the derogation was no longer allowed, seed production for organic farming could potentially be reduced to zero. Conventional seed production is also in a very fragile situation following the ban on neonicotinoids.
- Conventional protected strawberry production: among strawberry pests, aphids have been identified as a major obstacle to reducing pesticide use on this crop, susceptible to be attacked by a wide variety of aphid species (Turquet *et al.*, 2019). Among insecticides authorised for this crop, Thiacloprid (Calypso®, neonicotinoid) was the only molecule effective against some of them, given their resistance to other molecules.



- Protected production of cucumbers in organic farming which is developing strongly due to the conversion dynamic and the need for crop rotation in this context, particularly with tomatoes. But, growing cucumbers organically is still poorly controlled, as it is difficult to control attacks by aphids of different species during the production cycle. Uncontrolled attacks could result in the total loss of a crop in about ten days. Technical control of aphids became more complex in conventional cucumber production following the disappearance of neonicotinoids.

The main goal of the project was to identify and develop parasitoids truly adapted to specific targets, in terms of species, host plant and seasonality for these three protected crops, and that could serve as models for subsequent developments on other crops. To achieve this objective, the first step involved identifying for each of the three crops, the parasitoid species and populations best suited to the target aphid species and populations. Molecular analysis methods coupled with biological parasitism tests were employed. This included extensive sampling of parasitoids spontaneously colonising greenhouses, at regional or national levels, depending on the case, and including strains of commercial parasitoids. A second objective was to optimise the use of predatory BCAs, in association with parasitoids, in terms of conditioning systems, development stages released and application methods. The aim was to assess the conditions for optimum aphid control, based solely on parasitoids or a combination of parasitoids and predators.

1. 3 Partnership

The AphidInnov project involved five partners, forming a continuum between research (UMR IGEPP), experimentation (Terre d'essais, OBS), production of BCAs (Savéol Nature, If Tech) and the interface with growers of protected vegetables crops (Terre d'essais, OBS, Coopérative Maraîchère de l'Ouest - Savéol). The 'Fraises de France' association was closely involved in the project through a CIFRE thesis (Estelle Postic, employed by 'Fraises de France' and supervised by A. Le Ralec and Y. Outreman, UMR IGEPP), part of whose work was funded by AphidInnov. This partnership structure has proved to be effective: it has enabled both academic studies on the characterisation of species and populations and evaluations of the effectiveness of BCAs in conditions close to those in production greenhouses.

Experiments on brassica seed production were carried out by OBS, those on strawberry production were partly carried out by Savéol Nature and partly as part of E. Postic's CIFRE thesis, and those on cucumbers by the 'Terre d'essais' experimental station. IfTech contributed to the production of certain BCAs and the improvement of application systems. Finally, IGEPP, the project leader, was particularly involved in selecting the parasitoids for use in strawberry cultivation. It conducted parasitism tests in the laboratory, performed genetic analyses using molecular biology methods, identified all aphids and parasitoids collected in the framework of the project and supported the design of experimental protocols with the partners.

2. Main results and points for discussion

2.1 Identification and selection of parasitoids of interest

To identify potentially effective parasitoid species and populations in limiting aphid populations in the three target crops, aphid-parasitoid communities resulting from natural colonisation were studied under production conditions. The releases of BCAs in the monitored greenhouses were recorded to distinguish between the two sources of BCAs.

The aphid-parasitoid community of vegetable brassicas is very simple, comprising only two aphid species: a specialist, the cabbage aphid *Brevicoryne brassicae*, and a generalist, the green peach aphid *Myzus persicae*. These two aphid species are parasitized by a single species, the Aphidiinae *Diaeretiella rapae* (Hymenoptera, Braconidae) (Figure 1). Previous studies showed that this species, considered to be one of the most generalist Aphidiinae, exhibits local specializations and at least one cryptic species. However,



there are no genetic differences between individuals developing locally on these two aphid hosts (Le Ralec et al., 2011; Navasse et al., 2017).

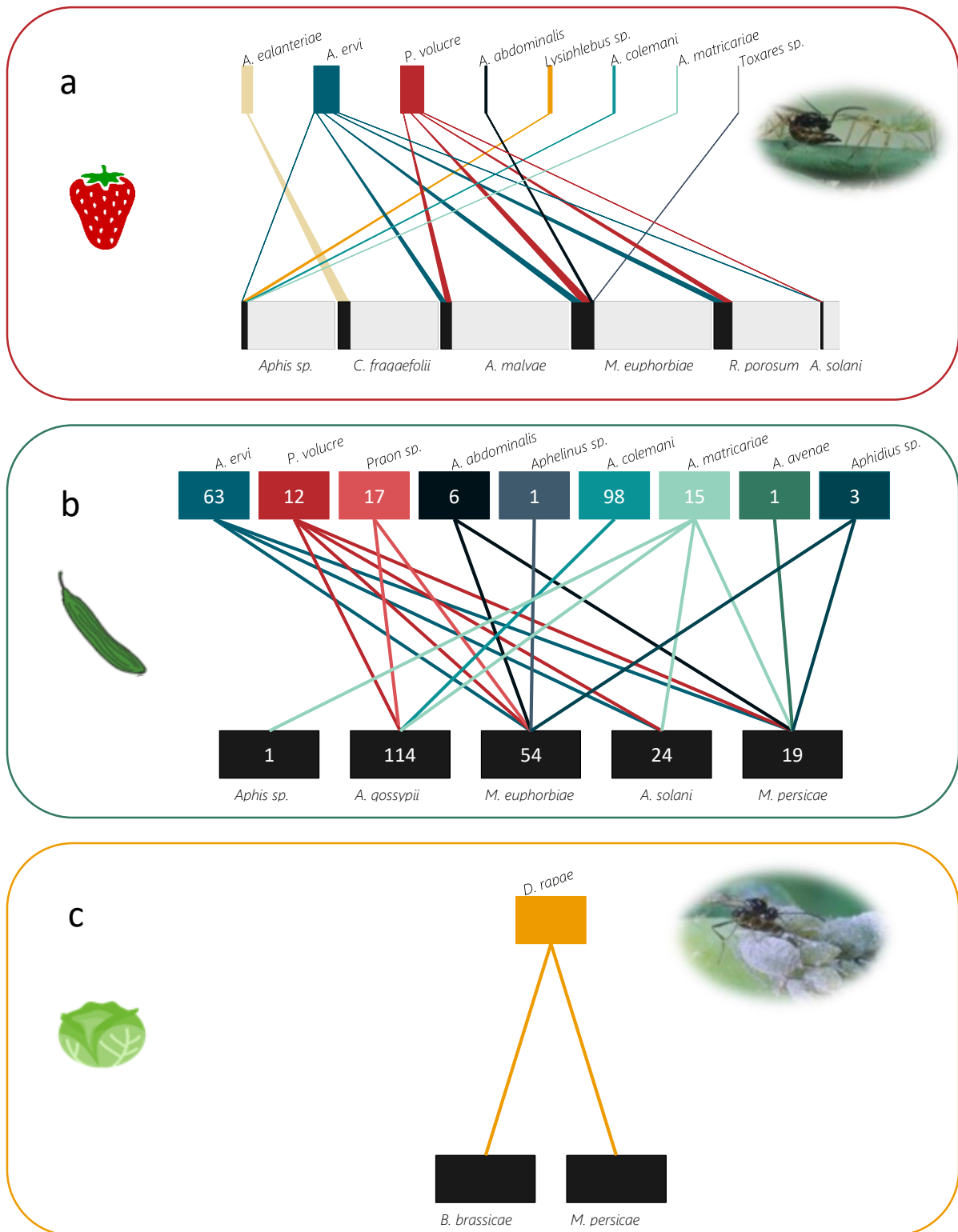


Figure 1: Ecological networks between aphids (bottom) and parasitoids (top) sampled a) in strawberry crops (quantitative network: the width of the rectangles is proportional to the number of individuals of each species (inset, *Aphidius ervi* parasitizing the aphid *Acyrtosiphon pisum*); for aphids, the black part represents the proportion of aphids parasitized, from Postic et al., 2020 a); b) in cucumber crops (semi-quantitative network: the numbers in the rectangles indicate the number of individuals of each species, according to Saliou, 2019); c) in seed cabbage crops



(qualitative network) (inset, *Diaeretiella rapae* parasitizing the aphid *Brevicoryne brassicae*). Photos B. Chaubet, INRAE.

In strawberry protected crops, due to diversity of aphids likely to cause damage and the lack of knowledge about the BCAs that can control them, an analysis of aphid-parasitoid communities on strawberry plants was carried out. Extensive sampling was performed in five strawberry production basins across France in the spring and summer of 2017 and 2018, to identify the aphid species colonising strawberry production greenhouses in the different regions and their associated parasitoids (Postic *et al.*, 2020a).

A total of 13 aphid species were identified. On average, each greenhouse hosted three different aphid species, with a maximum of seven species observed in a single one. Common species included *Macrosiphum euphorbiae*, *Acyrtosiphon malvae*, *Rhodobium porosum*, *Chaetosiphon fragaefolii*, *Aphis gossypii* and *Aulacorthum solani*. Aphid communities exhibited high variability, depending on the region, year and season, but this variability was primarily explained by local factors. The vast majority of greenhouses were colonised by parasitoid insects, with eight different species identified, showing local variability (Postic *et al.*, 2020, a). Networks of aphid-parasitoid interactions (Figure 1) were used to identify potentially interesting species for biological control. *Aphidius eglanteriae*, a parasitoid specialist of the aphid *C. fragaefolii*, was identified - a significant finding as there are currently no commercial parasitoids targeting this damaging strawberry aphid. Two generalist parasitoids, *Aphidius ervi* and *Praon volucre*, were also frequently found and parasitize three of the most common aphid species: *M. euphorbiae*, *A. malvae* and *R. porosum*. While these parasitoids are included in various commercial products used by strawberry growers, their low effectiveness was confirmed by the absence of released specimens in our samples. Conversely, presence of parasitoids in greenhouses without releases suggests existence of wild populations capable of parasitizing strawberry aphids, in the vicinity of the greenhouses they spontaneously colonise.

In organic cucumber crops, local sampling in northern Brittany at five growers in 2019 and 2020 (Saliou, 2019; Allard, 2020) confirmed the colonization of cucumber crops by three main aphid species, at different times. Early contamination of greenhouses by plants is possible for the two earliest species (*A. solani*, *M. euphorbiae*), detected as early as March in 2019. The third species, *A. gossypii*, arrives later, around mid-May. Monitoring in 2020 confirmed the dominance of these three species and their different time patterns. Nine parasitoid taxa were identified on the different aphid species (Figure 1). Similar to strawberry cultivation, the aphid *M. euphorbiae* was frequently parasitized by the generalist parasitoids *A. ervi* and *P. volucre*, which also attacked *A. solani*. The aphid *A. gossypii* is mainly parasitized by *Aphidius colemani* and occasionally by *P. volucre* and *Aphidius matricariae*. The latter species has also been found on *A. solani*. Notably, in greenhouses where commercial mixtures of parasitoid species were used, some were rarely found during sampling¹, such as *Ephedrus cerasicola* and *Aphelinus abdominalis*.

The analysis of aphid and parasitoid communities in protected crops has therefore revealed a high level of specific diversity and frequent spontaneous colonisation of these structures by wild parasitoids. These findings facilitated the selection of parasitoids with potential interest for biological control of the various aphid species:

- *Diaeretiella rapae*, the only parasitoid shared by the two brassica aphids,
- *Aphidius eglanteriae*, a specialised parasitoid of the strawberry aphid *C. fragaefolii*,
- *Aphidius ervi* and *Praon volucre*, two generalist parasitoids frequently found on several strawberry and cucumber aphids, in particular *A. malvae* and *R. porosum* on strawberries, *M. persicae* (*A. ervi*) and *A. gossypii* (*P. volucre*) on cucumbers, and *M. euphorbiae* and *A. solani* on both crops.

The first two species are not available as commercial products while the last two are. However, their effectiveness is low, and they were rarely recovered on strawberries after releases in our study. They were found more regularly in cucumber crops.

¹ This is also the case for predators released by cucumber growers, such as ladybirds.



2.2 Wild and commercial populations of parasitoids of interest

The spontaneous colonisation of greenhouses by wild populations prompted us to characterise them from a genetic and biological perspective. This was done to understand the lack of efficiency of commercial products and to assess their potential to better regulating aphid populations.

Firstly, molecular analyses were carried out on several parasitoid species, *A. ervi*, *A. matricariae* and *P. volucre*, using a barcoding approach with a fragment of the CO1 mitochondrial gene to detect high levels of genetic variability. The results revealed genetic differentiation within the *A. ervi* species, with no association between wild or commercial status, geographical origin or the original host of the individuals (Postic et al., 2021). This result confirms the generalist nature of *A. ervi* (Derocles et al., 2015, 2020). For *P. volucre*, no clear differentiation was demonstrated. However, a marked structuring was observed between individuals collected in greenhouses and those from commercial products in *A. matricariae* (Saliou, 2019), reinforcing the hypothesis of a commercial biotype that may be poorly or not adapted to some of the targets advertised by suppliers. Further studies are required to clarify the taxonomic status of this entity and link the genetic groups to exploited host ranges.

Secondly, genetic variability associated with the host plant, host aphid, geographical location and origin, whether wild or commercial, was examined in *A. ervi* using microsatellites. While no structuring linked to the host plant or aphid species was observed, there was a clear genetic differentiation between wild and commercial individuals (Postic et al., 2021). These results could partly explain the observed lack of efficiency of *A. ervi* releases as the low genetic variation in commercial populations may impact the adaptation of parasitoids to target hosts and, consequently, their performance.

To assess the relationship between low genetic diversity and parasitism rates on strawberry aphids, commercial parasitoids were tested on the most damaging potential host species (as indicated by suppliers): *A. malvae*, *M. euphorbiae* and *R. porosum*. The parasitism rates obtained were higher on the first two species, but generally low, at 38%, 28% and 7% respectively, on different aphid lines not carrying secondary symbiotic bacteria. A large-scale study of the prevalence of these symbiotic bacteria, which can confer resistance to parasitoids in aphid hosts, was conducted based on a national sample (Postic et al., 2020, b). Parasitism tests comparing lines carrying and not carrying the *Hamiltonella defensa* bacterium, alone or in co-infection with the *Regiella insecticola* bacterium, with commercial *A. ervi* were carried out. They showed a sharp reduction in the rate of parasitism in the *A. malvae* aphid, for lines carrying *H. defensa* (Postic et al., 2020 b). These parasitism rates were compared with those obtained with parasitoids from wild populations. The results, which are still preliminary, show that wild parasitoids are more effective than commercial parasitoids, particularly on aphid lines for which low parasitism rates had been observed with commercial parasitoids (Figure 2).

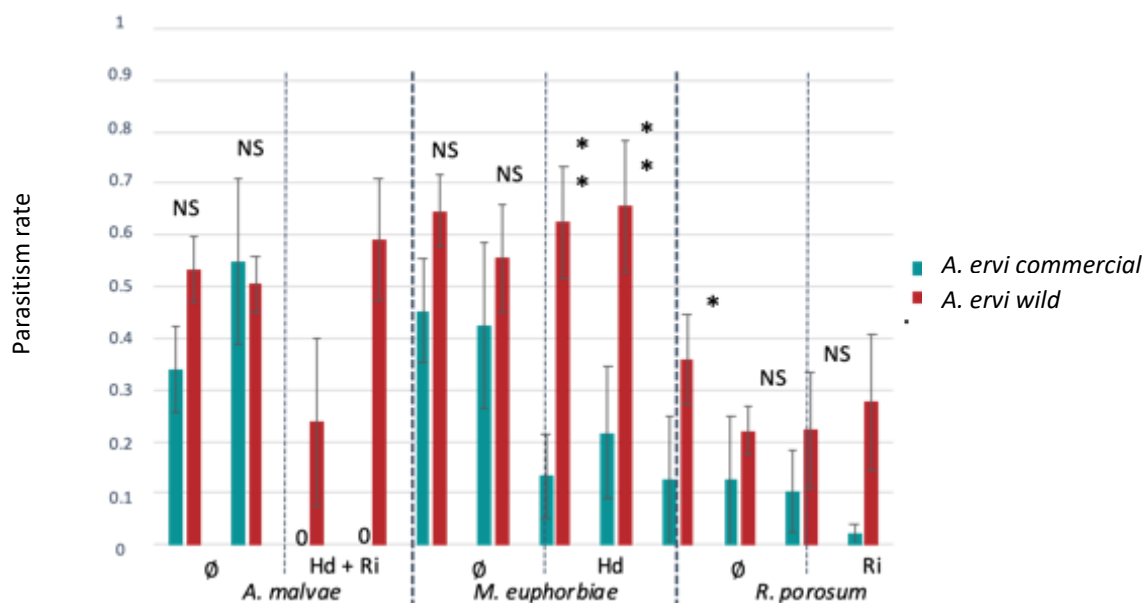


Figure 2: Parasitism rates obtained by females from 18 iso-female lines (6 commercial lines and 12 wild lines). Ø A: aposymbiotic, Hd: *Hamiltonella defensa*, Ri: *Regiella insecticola*. Error bars represent standard errors (Postic, 2020); * and ** indicate significant differences.

This difference in performance is particularly pronounced for *A. malvae* aphids co-infected with *H. defensa* and *R. insecticola*: while the rate of parasitism with commercial parasitoids is zero, it can be as high as 60% with wild parasitoids. The levels of parasitism obtained on the aphid *R. porosum*, although still lower than those obtained on the other two species, are nevertheless likely to contribute to the control of this pest, with parasitism rates that can exceed 30% (Figure 2).

These results are very promising for the biological control of three species of aphid pests of strawberries, two of which are also pests of cucumbers, by exploring the potential of wild populations of the generalist parasitoid *A. ervi*.

2. 3 Protection strategies combining different tools

For two of the crop systems for which we had potentially effective parasitoids, small-scale aphid protection strategies for organic protected crops were tested.

- Production of cabbage seed: early releases and use of a combination of BCAs.

Four experiments conducted between 2018 and 2021 showed that the early introduction of the parasitoid *D. rapae* in autumn was crucial for controlling populations of the aphids *B. brassicae* and *M. persicae* in spring. The presence of faba bean plants in the tunnels appears to promote the establishment of parasitism, likely through the continuous supply of extra-flower nectar. The release of the predator *Chrysoperla lucasina*, at the egg stage using a modified sprayer (Pulzor®), combined with repeated supplies of parasitoids (releases of the two beneficials every 15 days), was the only strategy that enabled a correct level of regulation of aphid populations. The photographs in Figure 3 illustrate this result: only this method resulted in the survival of male plants (centre), pollination of female plants and a harvest of seeds. However, less than a third of the female plants produced seeds, and none were viable. It seems necessary to introduce higher quantities of parasitoids for a better result.



Figure 3: Photos of the four treatments of an experimentation carried out at the OBS showing aphid damage to cabbage plants. a) Control treatment: no biological control agents introduced; b) *Chrysoperla lucasina* alone; c) *Diaeretiella rapae* parasitoid alone; d) *Chrysoperla lucasina* + *D. rapae*. Note the mortality of male plants (middle row), except in the last treatment (d). (Photographs taken on 15 April 2021, S. Perennec)

- Cucumber production: changes in crop management and the sustainable installation of biological control

The control of aphid populations on cucumbers is largely based on plant management, and in particular on a new trellising method, inspired by tomato practice, known as 'high-wire' trellising, which replaces the 'umbrella' technique traditionally used in low greenhouses (see Floury, 2019 for details). This method involves regular leaf removal from the vegetation. Observations made in 'Terre d'essais' station showed that this practice could significantly improve the crop health, by reducing aphid populations density. However, it may have a very unfavourable effect on the establishment of biological control by eliminating parasitized aphids and therefore limiting the production of a new generation. A trial involving regular releases of the parasitoids *A. ervi* and *A. colemani* was carried out to measure the effect of leaf removal and leaf export on aphid populations and on biological control by parasitoids. Using an augmentorium-type device (Deguine *et al.*, 2011), it was shown that the number of aphids removed from the greenhouse by leaf removal was high when populations were high, but that there was also a significant loss of parasitoids. However, the aphid-parasitoid ratio evacuated is largely in favour of the aphids for the first weeks. Then, when biological control is established in the greenhouse, the rate of parasitism increases, the aphid population density decreases and the proportion of parasitoids evacuated increases. These results indicate that the removed leaves must be first cleared out to reduce the development of aphid populations, then leave in the greenhouses to allow the emergence of parasitoids and thus achieve more sustainable biological control. We also confirm that the commercial *A. ervi* parasitoid used seems more effective on cucumbers than on strawberries against the *M. euphorbiae* aphid.



3 - Conclusion - Outlook

The work conducted by the various partners in the AphidInnov project has confirmed most of the hypotheses proposed to explain the poor efficiency of commercial BCAs against aphids in protected crops (unsuitable species, low genetic diversity, aphid resistance, etc.). It demonstrated the existence of natural parasitism in greenhouses, despite their isolation from the outside environment, and the existence of species or populations likely to be better adapted and more effective against aphids on the studied crops. The production of parasitoids that meet the specific needs of each crop appears to be possible. However, our results also underline the importance of maintaining the genetic variability of mass-produced populations and to the risk of selection by breeding hosts. Furthermore, in some cases, specialist species must be produced for a niche market, and therefore with low profitability for the large suppliers of BCAs. This challenge could be partly addressed by developing specialised production structures for a particular sector or groups of local producers. Such structures already exist, like Savéol Nature, which has producing BCAs for the tomato growers of the 'Coopérative Maraîchère de l'Ouest' (Savéol) in the Brest region (29) for many years.

At the end of the AphidInnov project, 'Fraises de France' and the 'Coopérative Maraîchère de l'Ouest' decided to use the project's results to found Frais'Nat, a dedicated company in 2021. This initiative, based in Guipavas (29), aims to provide growers with rapid solutions to the problem of aphids on strawberry plants, ultimately reducing or eliminating the use of insecticides. Initially, the company's focus is on producing the parasitoids *A. eglanteriae* and *A. ervi* as a service to the members of the two organisations. Supported by the French 'Ecophyto 2+ plan', this effort is part of a research project led by the Frais'Nat company and involving the UMR IGEPP and two producer organisations, under the 'ANR Ecophyto-Maturation 2021' call for projects. Entitled "Aphidius 2.0 - Optimised parasitoids and improved releases to open up new prospects for biological control", the project aims to develop a method for the biological control of aphids on strawberry plants using the generalist parasitoid *A. ervi* and the specialist parasitoid *A. eglanteriae*. Special attention is given to the issue of the genetic variability of breeding populations. The project also seeks to assess the possibility of using banker plants to facilitate the early and sustainable installation of the generalist parasitoid in greenhouses. The production of vegetable brassica seeds in organic farming could also be the subject of such a solution. Local production of the *D. rapae* parasitoid for seed producers by OBS could satisfy this niche market, which may have limited economic interest to major suppliers.

In addition to developing suitable, high-performance BCAs for inundative biological control, the AphidInnov project opens up new perspectives for the development of conservation biological control strategies, i.e. based on the management of the crop environment. Based on the results obtained regarding the spontaneous colonization of the greenhouses by insect pests and their natural enemies, a study assessing the relative contributions of cultivation practices, the surroundings of greenhouses, and the landscape on insect communities began in October 2022 (CIFRE thesis 'Fraises de France' - UMR IGEPP). Its aim is to propose prophylactic and biological management methods for these insects with a multi-pest approach. The knowledge acquired regarding the various aspects of the specialisation of aphid parasitoids will also be used as part of the EFFICACE project (CASDAR Connaissances) led by the CTIFL (2023-2026). The goal of this project is to propose conservation biological control strategies based on the use of service plants against aphids in vegetable crops.

Biological control remains an interesting method for managing aphid populations in protected crops, capable of offsetting the gradual disappearance of insecticides. This is achieved through the use of parasitoid insects selected and bred to adapt to different production contexts, as well as by exploiting natural parasitism and adapting cultivation practices (Figure 4).

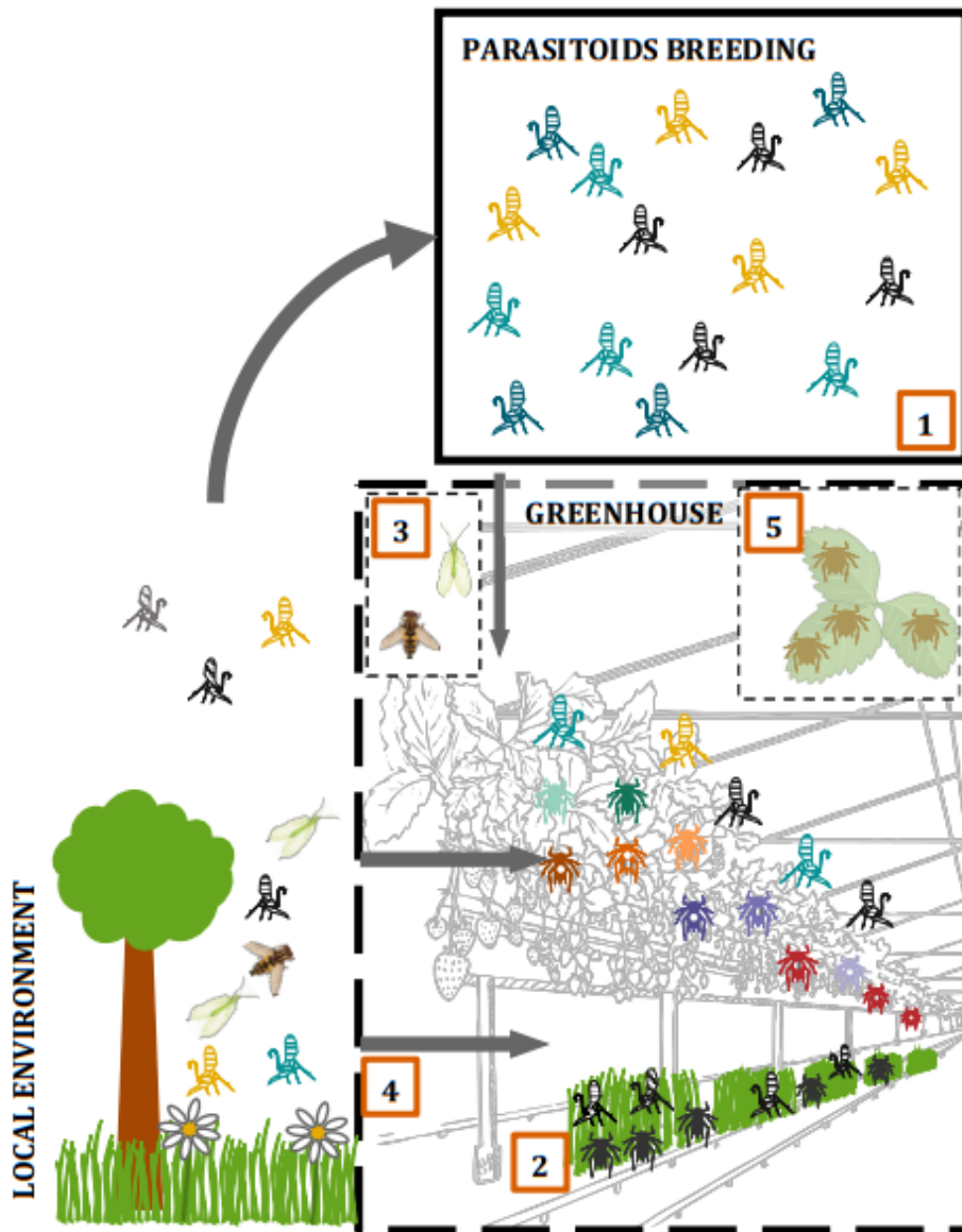


Figure 4: Diagram showing the levers for improving biological control under cover. (1) Breed specialist and generalist parasitoids adapted to the pests to be controlled. (2) Bring these beneficials in early and provide them with resources in the greenhouse using service plants. (3) Combine parasitoids with predators. (4) Encourage the spontaneous colonisation of greenhouses by beneficials by managing the surrounding area. (5) Manage leaf removal to enable biological control to take hold over the long term. Adapted from Postic, 2020. Background drawing: M. Charrois.



Ethics

The authors declare that the experiments were carried out in compliance with the applicable national regulations.

Declaration on Generative Artificial Intelligence and Artificial Intelligence Assisted Technologies in the Drafting Process.

The authors have used artificial intelligence-assisted technologies to translate from French to English.

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Declaration of interest

The authors declare that they do not work for, advise, own shares in, or receive funds from any organisation that could benefit from this article, and declare no affiliation other than those listed at the beginning of the article.

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