

## **FresaProtect: the use of a cocktail of parasitoids against aphids in strawberries - a case study**

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**Abstract:** A cocktail of six species of parasitoids has been used to control aphids on strawberry in biological and integrated pest management cultures. As the aphid populations are different from one year to another and from one place to another, a mix of different species of parasitoids covering all the aphid species possibly occurring on that plant is the easiest way to work and have good result. The design of the release points has been studied and optimized in order to simplify the manipulations necessary to deploy them. After three years of R&D in the laboratory and in the field and two years of large scale field trials in Belgium, FresaProtect has proven to be an efficient treatment for aphid control.

**Key words:** strawberry, aphids, parasitoids, IPM, FresaProtect

### **Introduction**

Starting in 2007, collaborative experiments and trials between the Biodiversity laboratory at the Catholic University of Louvain (UCL, Belgium) and the R&D team at Viridaxis showed that under laboratory conditions, in cages and in semi-field conditions, a cocktail of six species of aphid parasitoids (*Aphidius colemani*, *A. ervi*, *A. matricariae*, *Ephedrus cerasicola*, *Aphelinus abdominalis* and *Praon volucre*) should be enough to control all aphids species on strawberries (Salin et al. 2010). It has also been observed that the parasitoids could fly and disperse easily over 200m<sup>2</sup>. In 2009 and 2010, while the UCL team continued working on fundamental aspects of the cocktail (trade name: FresaProtect), large scale field trials were carried out in Belgium by the R&D team of Viridaxis. The field trials aimed to examine the efficacy of this cocktail and to optimize a good and practical way to release it in commercial crops.

Each year, more pesticides are withdrawn from the market and banned from use in different cultures. The solutions are either to use the remaining chemical products, fearing more and more resistances happening every time faster, or use alternative solutions as pest management techniques. Most of the growers realize that systematic chemical treatment presents risks for the environment, the consumers, and themselves, and a growing pressure is coming from European and national governments as well as from the consumers to get more environmentally friendly products. They are open to biological or integrated pest management methods but fear the difficulty, the time consuming way of application or the slowness of such methods

One of the possible solutions to reduce the use of aphicide on a crop is bio-control agents. Two choices exist: Predators or parasitoids.

Parasitoids are very selective insects, attacking only a small number of aphid species. Up to now, a correct identification of the aphid present, which is cumbersome and often needs the support of a specialist, was the only way to choose which parasitoid species to use.

The advantage of parasitoids over predators (ladybird, predatory bug, lacewing...) is their extended action over time (successive generations) and their very high detection capaci-

ty. The parasitoids are capable of finding very small aphid colonies, even isolated aphids entering the glasshouse or tunnel. The predators are most efficient when relatively big colonies of aphids are already installed. As soon as the aphid population drops under a certain level, the predators tend to move away and eventually leave the glasshouse or tunnel. The population of pest will then grow back and become a threat again.

The main objective of FresaProtect is to be as easy to use as any chemical treatment. Growers shouldn't have to worry about identifying or counting aphids. Releasing the product should be fast and easy with as few manipulations as possible. The product has to be reliable and cost effective for the grower.

Founded in 2004 as a spin-off from the UCL, Viridaxis is a company specialized in the mass-production of parasitoids against aphids. Several species of parasitoids already available on the market are produced (*Aphidius colemani* since 2005, *Aphidius ervi* since 2008 and *Aphelinus abdominalis* since 2010) and, more recently, other indigenous species never commercialized before have been reared.

## Material and methods

### *Field trials*

Both in 2009 and 2010, fifteen Belgian growers participated in the trials and used FresaProtect to control aphids in one or more of their tunnels or greenhouses. The treated protected area ranged from 150m<sup>2</sup> to 2400m<sup>2</sup>. One release point per 200m<sup>2</sup> was installed in every greenhouse treated. Experience from former years demonstrated that an early introduction is necessary and that three releases are sufficient. The first release has to be done 1 or 2 weeks after the start of the vegetating phase (end of February, beginning of March). The second release 3 to 4 weeks later and the third and last release 3 weeks after the second one. With 3 releases, the crop should be covered at least until mid-harvest by the emerging parasitoids from the release points. If aphids are present in the crop, a population of parasitoids will install itself and the control will last even longer.

The apparition and the evolution of the aphids colony is observed the same way a grower follows it: Presence of aphids (Yes or no), Necessity to spray (Yes or no). In addition, aphid species are identified, the presence of mummies in the colonies observed and the parasitoids species determined. The fluctuation in the parasitoids population is monitored and correlated with the different chemical products used in the crop (fungicides, acaricides, and herbicides) to verify the compatibility.

### *Release points*

To develop a practical, efficient and easy to use release point is one of the key elements of the pre-commercial phase. Different prototypes are made in order to find out the best materials and construction to use for the grower's ease and to guarantee the quality of the insects. Plastic tubes of different sizes and shapes are used, combined with differently sized mesh grid for ventilation. Cardboard boxes and tubes of different sizes and shapes are also tested. Different types of feeding points are made and included on or by the tubes.

All the different prototypes are tried out under field conditions in commercial crop. A prototype enclosed in a mesh grid bag is put in every site to count the emerging adults.

Every new prototype version is given out for use to a grower and practical issues are discussed. The prototype is then finely tuned to fit all requirements.

## Results and discussion

### Field trials

Both years, the total treated area was just over 20000m<sup>2</sup> in 40 different greenhouses in Belgium. The top 10 commercially used varieties of strawberry were treated (Darselect, El Santa, Lambada) and most of the cultural techniques were represented (beds, soilless, plastic tunnels, plastic and glass greenhouses, with or without floating row cover.).

In 2010, On top of the Belgian trials, 12ha were treated with FresaProtect around Europe (in France, Spain, UK, Germany, etc.) with different partners (research centres and companies).

Due to the different species present and the fluctuating temperature in the field, the emergence is continuous for a long time. Different batches of each species are mixed to expand that long lasting emergence and ensure that some individuals will emerge everyday for up to 4 weeks (depending on the average temperature).

In the control release points, adults are still alive and wandering around from 10 to up to 40 days (depending on the temperature and the species) when provided with a food source. Without it they would die in 3 to 4 days. A honey point provides them with a good kick-start.

Table 1. Aphids-parasitoids relationships.

<b>Aphid/parasitoid</b>	<i>A. ervi</i>	<i>A. matricariae</i>	<i>E. cerasicola</i>	<i>P. volucre</i>	<i>A. colemani</i>	<i>A. abdominalis</i>
<i>Acyrtosiphon malvae</i>	xx			xxx		
<i>Aphis craccivora</i>		xx		x	xxx	
<i>Aphis fabae</i>		x		x	(x)	
<i>Aphis forbesi</i>		(x)			(x)	
<i>Aphis gossypii</i>		xxx	(x)	x	xxx	
<i>Aphis nasturtii</i>		xx		x		
<i>Aphis ruborum</i>		xx			xx	
<i>Aulacorthum solani</i>	xx	(x)	xxx	xx		xx
<i>Chaetosiphon fragaefolii</i>			(x)	(x)		(x)
<i>Macrosiphum euphorbiae</i>	xxx			xxx		xxx
<i>Macrosiphum rosae</i>	xx			xxx		xx
<i>Myzus ascalonicus</i>		(x)	(x)	(x)		
<i>Myzus persicae</i>	x	xx	xx	xx	xxx	xx
<i>Rhodobium porosum</i>	xx		(x)	(x)		xxx

xxx: very high efficacy. xx: high efficacy. x: good efficacy. Under all conditions. (x): observed efficacy in lab and semi field trials, not yet observed in field trials. **Bold:** most frequent / dangerous species.

In all the cases where aphids are observed, mummies appeared and parasitoids populations installed themselves. Depending not only on the aphids present in the greenhouses but also on the conditions (temperature, humidity, and precocity of the attack) one or another species would install itself and build up a population (Table 1). In many cases, traces of cleaned hotspots (honeydew and exuviae) are observed but no aphids can be found (Figures 1-5).

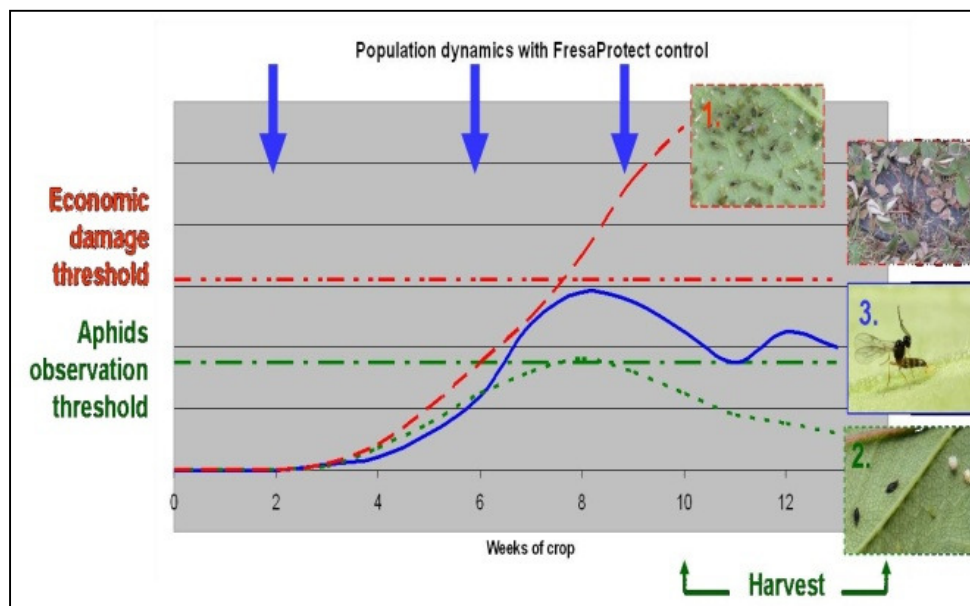


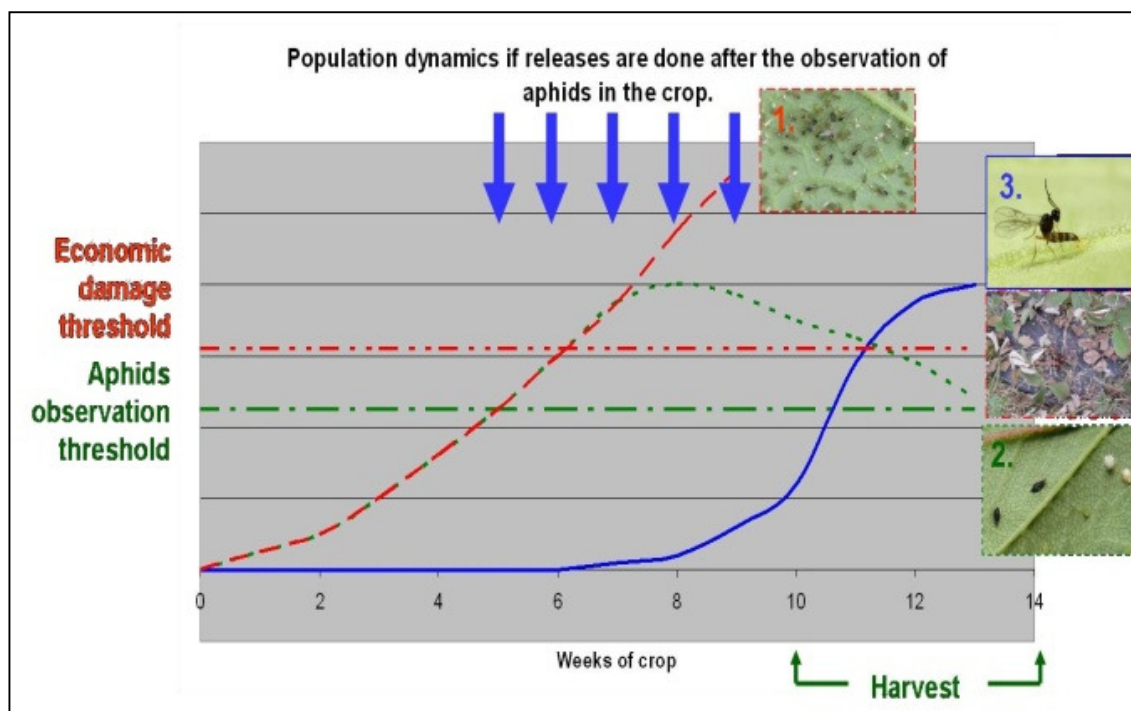
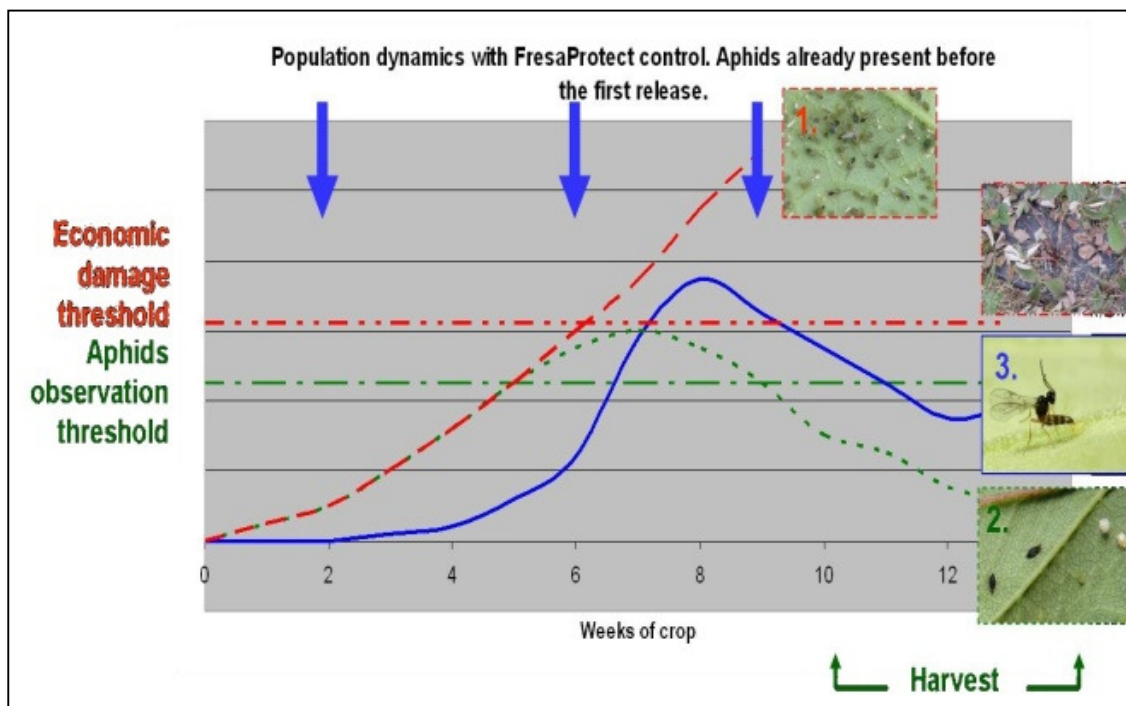
Figures 1-5. Mummies of the different species found on strawberry plants during the trials. 1. *Ephedrus cerasicola*. 2-3. *Aphidius* sp. 4: *Aphelinus abdominalis*, *Praon volucre* and *Aphidius ervi*. 5. *A. ervi*.

Two very common strawberry aphids in Belgium (*Macrosiphum euphorbiae* and *Acyrtosiphon malvae rogersii*) were found in almost every greenhouse and served as excellent reservoir of population for three species in the cocktail, ensuring at least a second generation of those parasitoids and overlapping their cycles with the ones from the releases.

An interesting observation made by the growers themselves was that the number of insects species spotted in their greenhouses was definitely higher than usual. As they did not spray any insecticide for 1 or 2 years, honeybees, *Orius* sp., *Chrysopa* sp., hoverflies, were observed in their tunnels and contributed in their equilibrium.

Mummies were spotted in the adjacent greenhouses after the second release. In the middle of the aphid season, the growers prepared to treat in their “traditionally” run greenhouses and spotted mummies in the colonies. Enough mummies in some tunnels to actually control the population. So still less use of chemicals.





Figures 6-8. Aphids and parasitoids population dynamics in the crop with use of FresaProtect. Different situations exposed. Models based on observations. - - - Uncontrolled aphids, . . . Aphids controlled by Fresa Protect, — Parasitoids individuals, ↓ Releases of FresaProtect

After two years of observations in the field, the action dynamic of the cocktail was modeled to better understand what is happening in the crop and optimize the way to use it. It appears that when the parasitoids are introduced and there are no aphids in the crop yet, the

aphids might not be seen through the season (green line · · ·), but some mummies might be spotted or an adult parasitoid might be seen flying around (blue line —). Following the same dynamic, an uncontrolled population of aphids would explode and damage the crop (red line - - -) (Figure 6). If the parasitoids are introduced early but some aphids are already on the plants, the aphids might be seen and even raise close to the damage threshold, but the parasitoids will control the situation. Mummies will be spotted in the colonies (Figure 7). Finally, if the parasitoids are introduced after spotting the first aphids, the introduction rate will be higher and might not even be sufficient to control the situation, the grower will register some losses or have to spray with an insecticide (Figure 8).

### ***Release points***

The first release points tested were plastic tubes (Figure 9) wrapped in aluminium foil with a bottom of mesh grid for aeration. Those tubes were eliminated, after only two weeks of use because the condensation was so high that the mummies, empty or not, rotted.

The obvious solution was to work with a more breathable material, such as cardboard boxes on feet to avoid soil humidity (Figure 10). That design assured the parasitoids quality and protection but was not practical. Every now and then an intervention in the crop would damage the box.

The next model were cardboard tubes (L: 11cm, Ø: 5.5cm, Fig. 11) on a stake with two plastic lids, one drilled with 3mm holes, the other one plain. Those tubes were easy to put in place, could withstand the other interventions done in the field, protected the mummies from condensation and humidity and included a honey point (in the plain lid). Many trials were done with them. They worked fine for small tunnels, but when shipment was involved or if they were used in large greenhouses their size became an issue.



Figures 9-13. The evolution of the release points from the beginning of the trials in 2009 to the commercial tubes sold at the end of 2010.

New smaller cardboard tubes were developed (L: 8cm, Ø: 3cm, Fig. 12) and tested. They met the main expectations, protected the mummies perfectly and were easy to ship and to carry along in a large greenhouse. The remaining difficulty was the inclusion of a honey point; a drop had to be put on top of the cardboard, a manipulation which is not user friendly and impossible to apply in a commercial crop. A plastic lid was designed for them, including a recipient for honey, exit holes for the parasitoids and a support system for the stake. To put one of those tube (Fig. 13) in a crop takes between 3 and 5 seconds.

## Conclusions

FresaProtect is a new tool to fight aphids in strawberries. It has been proven efficient in large scale field trials in Belgium and in other parts of Europe. The release points developed for it are perfectly adapted to the product and provide a long lasting protection for the mummies until their emergence. The integrated honey point ensures a better fitness to the freshly emerged individuals and at the same time provides the growers with an easy way to set them up in their crop. The application is at least as fast and easy as any traditional chemical treatment. The fact that the six species present in the cocktail have different life cycles allows a continuous emergence for up to 3-4 weeks and a permanent presence of freshly emerged individuals.

It fills a gap in nowadays IPM strategies in strawberries where aphids from a wide range of species are still a recurring problem.

It is a preventive tool and has to be used before the appearance of the aphids. No immediate effect will be noticed; mummies generally start to appear in the colonies at the earliest two weeks after the first release.

The product is commercialized since September 2010 and is a good example of a fruitful collaboration between university, a private company and growers.

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