

# **FresaProtect and BerryProtect: mixes of parasitoids to control all common aphid species on protected soft fruit crops**

## **Product development and case studies from three years of experience**

By N DASSONVILLE, T THIELLEMANS and V GOSSET

*Viridaxis S.A., Rue Louis Blériot 11, Gosselies 6041, Belgium, info@viridaxis.com*  
Corresponding Author Email: ndassonville@viridaxis.com

### **Summary**

FresaProtect, a mix of six aphid parasitoid species with complementary host spectrum, was developed between 2007 and 2010 by Viridaxis to control the most commonly found aphid species on strawberry crops. The release device followed an adaptation process to optimize ease of use. By working on the nutrition of the parasitoids and by mixing mummies of different ages, a product with a long lasting effect was obtained which allows the releases to be spaced in time (every 3 weeks) while guaranteeing the permanent presence of fresh adult parasitoids in the crop. Parasitoids show best efficacy in preventive conditions and must be introduced into the crop before the appearance of aphids. For berries (raspberries, blueberries...), the composition of parasitoids was adapted to the different aphid spectrum: BerryProtect contains a high proportion of *Praon volucre*, giving a good control of the large raspberry aphid, *Amphorophora idaei* and the blueberry aphid, *Ericaphis fimbriata*. Case studies of FresaProtect and BerryProtect in different European countries and in different production systems are shown. When used preventively, FresaProtect and BerryProtect offered good protection against the different aphid species attacks, decreasing the need for insecticide.

**Key words:** Natural aphid control, parasitoid cocktail, ready-to-use units

### **Introduction**

Aphids are a major pest in soft fruit production and chemical control is becoming difficult for at least three reasons: the demand of the retailers to have fruits without pesticide residues, reduction in availability of active substances, and occurrence of insecticide resistance in some aphid strains. This is, for instance, the case for some strains of the large raspberry aphid, *Amphorophora idaei* (McMenemy *et al.*, 2009) and the peach-potato aphid, *Myzus persicae* (Foster *et al.*, 1998). For those reasons, and to reduce pesticide exposure of farm workers and the environment, many growers opt for an integrated pest management (IPM) of aphids using parasitoids (*Braconidae* and *Aphelinidae*, Hymenoptera), anticipating the new European regulation on sustainable use of pesticides (2009/128/EC) and its implementation in national action plans.

Viridaxis is a Belgian company, spinoff from the Université Catholique de Louvain (UCL), specialized in the rearing of beneficial insects. In 2007, Viridaxis started the development of a product, called FresaProtect. This product was designed to be easy to deploy by growers and to control all aphid species on strawberry crop.

### Complementary host spectrum

As each parasitoid has its own host spectrum, the idea was to associate several parasitoid species so that each aphid species found on strawberries would be parasitized. A survey throughout Europe revealed that aphids commonly found on strawberry plants belong to 14 different species (Table 1). Trials in cages and semi-field conditions (Salin *et al.*, 2010) and in large scale commercial plots showed that a mix with six parasitoid species (*Aphidius colemani*, *A. ervi*, *A. matricariae*, *Aphelinus abdominalis*, *Praon volucre* and *Ephedrus cerasicola*) could control all these aphid species. Table 1 shows the host spectrum of those six parasitoid species included in the FresaProtect mix.

Table 1. List of the most important aphid species attacking strawberries

<b>Aphid/ Parasitoid</b>	<i>Aphidius ervi</i>	<i>Aphidius matricariae</i>	<i>Ephedrus cerasicola</i>	<i>Praon volucre</i>	<i>Aphidius colemani</i>	<i>Aphelinus abdominalis</i>
<b><i>Acyrtosiphon malvae</i></b>	++			+++		
<i>Aphis craccivora</i>		++		+	+++	X
<i>Aphis fabae</i>		+		+	X	X
<i>Aphis forbesi</i>		X			X	
<b><i>Aphis gossypii</i></b>		++	X	+	+++	X
<i>Aphis nasturtii</i>		++		+		
<i>Aphis ruborum</i>		++			++	
<b><i>Aulacorthum solani</i></b>	++	X	+++	++	X	++
<b><i>Chaetosiphon fragaefolii</i></b>			X	X		X
<b><i>Macrosiphum euphorbiae</i></b>	+++			+++	X	+++
<b><i>Macrosiphum rosae</i></b>	++			+++	X	++
<i>Myzus ascalonicus</i>		X	X	X		X
<b><i>Myzus persicae</i></b>	+	++	++	++	+++	++
<b><i>Rhodobium porosum</i></b>	++		X	X		+++

(Left column, in bold are the most common species or those which are economically important). The parasitoids present in FresaProtect are listed in the first line. Their efficacy in the control of the different aphids is indicated by '+' for proven control under field conditions (+++: very high efficacy, ++: high efficacy, +: good efficacy) or 'X' for control under laboratory and semi field conditions.

### Conception of the release point

One important requirement during the development of FresaProtect was to have an easy-to-use product. The research focused then on the development of a release point avoiding the fastidious traditional method of spreading mummies in the crop. The first release point tested was a plastic tube with holes in the lid (Fig. 1). Those tubes were rapidly discarded because the condensation inside was so high that the mummies rotted. The obvious solution was to work with a more breathable material, such as cardboard. Cardboard boxes of different shapes and sizes were tested.

All designs assured the parasitoids quality. The selection was thus based on logistic aspects. The selected model is a small cardboard tube (L: 8 cm, Ø: 3 cm, Fig. 1) closed with two plastic lids (one plain lid and one perforated) (Fig. 1). This model protects the mummies and is easy to ship and to carry in large greenhouses during the installation of the tubes. Feeding parasitoids with honey increases their life-span and fecundity (Bezemer *et al.*, 2005). Using a prototype, a drop of honey was put on top of the cardboard tube, a manipulation which was not user friendly and impossible to apply in a commercial crop. Hence, a plastic lid was designed, including a recipient for honey (organically produced), exit holes for the parasitoids and a support system for a stake (Fig. 1).

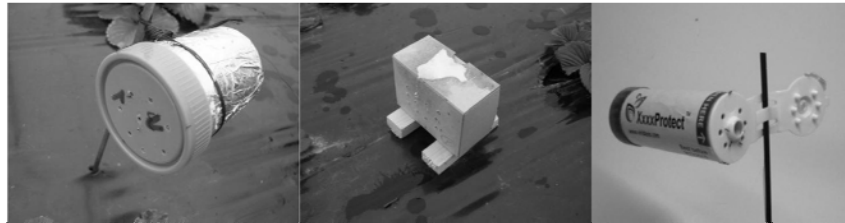


Fig. 1. Successive steps in the design of the aphid parasitoid release points. From left to right: plastic tubes with holes in the lid, cardboard box with wooden feet, Actual cardboard tubes with integrated feeding point (honey) in the plastic lid.

During the first year of commercial experience of FresaProtect, it was observed that sometimes, the tubes are visited by ants which carry away the mummies and completely empty the tubes reducing the number of emerging adults in the crop. It was found that hanging the tubes with a fine nylon string avoids that the ants access the tubes. A new device was thus developed. It includes a roof to protect the tubes from direct sunshine and water overhead and is hung by a fine nylon string to the structure of the greenhouse (Fig. 2) or to a metal hook planted in the soil. The results shown below have been obtained with this new device.



Fig. 2. Protection of the tubes against water, direct sunshine and ants: tubes are clipped on a plastic roof hung to the structure of the glasshouse by a nylon string to avoid access by ants.

#### *Prolonged emergence*

One objective was to obtain a product with a long lasting effect which allowed the parasitoid releases to be spaced in time. We showed that, for *A. abdominalis*, *Praon volucre* and *E. cerasicola*, mixing mummies with different development stages allowed the extension of the emergence period up to 17 days at an alternating temperature regime (7°C at night and 22°C during the day) (Fig. 3, from Rosemeyer *et al.*, 2012). In the crop, depending on the temperature, emergence will last 2 to 4 weeks. With this extended emergence period and regarding the life span of the different species ranging from 2 weeks for *Aphidus* spp. to 2 month for *Aphelinus abdominalis*, one release every 3 weeks is enough to guarantee a constant presence of fresh adult parasitoids in the crop.

#### *Preventive use*

Parasitoids show their best efficacy by using preventive applications to the crop. Using field observations and pest/parasitoid monitoring, de Menten (2011) showed that the first release of FresaProtect must be done before appearance of aphids in order to keep aphid populations below the economic damage threshold.

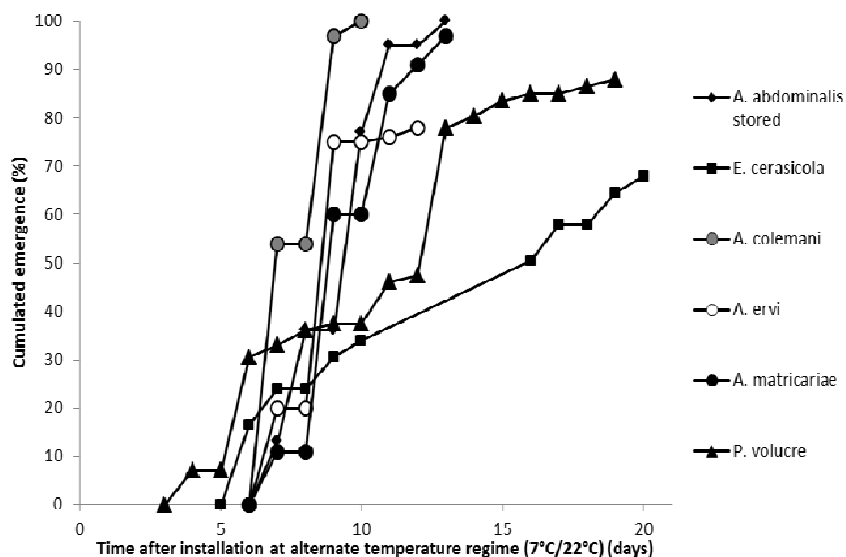


Fig. 3. Optimised emergence of the parasitoids under alternate hatching temperature regime (7/22°C). The emergence spreading is enlarged for *E. cerasicola* and *P. volucre* by using two different batches. The emergence of *Aphelinus abdominalis* is accelerated by a storage at temperatures above 7°C during several weeks. Reproduced from Rosemeyer *et al.* (2011).

### BerryProtect

A similar product to FresaProtect was developed for berries (raspberries, blueberries...). Its composition was adapted to the different aphid spectrum. BerryProtect contains five species of parasitoids with a high proportion of *Praon volucre*, giving a good control of the large raspberry aphid, *Amphorophora idaei*, and the blueberry aphid, *Ericaphis fimbriata* (Table 2).

Table 2. List of the most important aphid species attacking berry crops

Aphid/ parasitoid species	<i>Aphidius ervi</i>	<i>Aphidius matricariae</i>	<i>Praon volucre</i>	<i>Aphidius colemani</i>	<i>Aphelinus abdominalis</i>
<i>Acyrtosiphon malvae</i>	++		+++		
<b><i>Amphorophora idaei</i></b>	++		+++		
<i>Aphis gossypii</i>		++	+	+++	X
<b><i>Aphis idaei</i></b>		++		++	
<i>Aphis pomi</i>		X		X	
<b><i>Aphis schneideri</i></b>		+		++	
<i>Aphis spiraecola</i>		++	X	++	X
<i>Cryptomyzus ribis</i>		X		X	
<b><i>Ericaphis fimbriata</i></b>	++		+++		++
<i>Hyperomyzus lactucae</i>	++		+++		++
<b><i>Illinoia pepperi</i></b>	X		X		
<i>Macrosiphum euphorbiae</i>	+++		+++		+++
<i>Myzus persicae</i>	+	++	++	+++	++
<b><i>Rhodobium porosum</i></b>	++		X	X	

(Left column, in bold are the most common species or those which are economically important). The parasitoids present in FresaProtect are listed in the first line. Their efficacy in the control of the different aphids is indicated by '+' for proven control under field conditions (+++: very high efficacy, ++: high efficacy, +: good efficacy) or 'X' for control under laboratory and semi field conditions.

Here, we show case studies of FresaProtect and BerryProtect between 2011 and 2013 in different European countries in strawberry and raspberry crops in different production systems.

## Materials & Methods

FresaProtect and BerryProtect have been used commercially since 2011 in many European countries, including Belgium, France, Germany and the United Kingdom, in different cropping systems (heated glasshouses or unheated tunnels, full ground or soilless) and varieties of strawberries, raspberries and other berry crops (currants, blueberries, blackberries). Releases start on a clean crop (in the absence of aphids) just after planting or covering of the tunnels (or after an insecticide ‘clean up’ if necessary). Releases are done every 3 weeks at the recommended rate of one tube for 200 m<sup>2</sup>. In 2012 and 2013, aphids and mummies in some commercial and several research stations, crops were regularly monitored (at least monthly). Aphids and mummies were counted on a fixed number of randomly chosen plants depending on the surface of the plot (<800 m<sup>2</sup>: 25 plants, 800 to 2000 m<sup>2</sup>: 40 plants) using the counting key shown in Table 3. For FresaProtect, we provide here quantitative data from three research stations with different characteristics (cropping systems, varieties...), representative for what can be seen in the majority of commercial crops. To give a complete picture of the efficacy of the product in commercial environment, we also provide non quantitative results obtained with commercial use of FresaProtect and BerryProtect at English and Belgian farms. This type of non-quantitative assessment, more adapted to large plots, is not based on counting on a limited number of plants but made by walking slowly in the crop and looking for signs of aphid presence (honeydew, sooty mold, skins or aphid colonies) and mummies throughout the field.

Table 3. Key for aphid and mummies counting

Key for aphid “intensity”		Key for mummy ”intensity”	
0	Absence of aphids	0	absence of mummies
1	1–4 aphids per plant present	1	1–2 mummies per plant present
2	5–10 aphids per plant present	2	> 2 mummies per plant present
3	spot with colonies	3	> 10 mummies per plant present

## Results

### *FresaProtect*

#### *Start with clean crop (Fig. 4)*

The first parasitoid release was done in week 15 on a clean Elsanta crop. Aphid pressure was low and all arriving aphids (*Acyrtosiphon malvae*) were rapidly parasitized. The presence of winged *Aphidius mummies* (Fig. 5) highlights the efficacy of parasitoids to find isolated aphids.

#### *Start after a cleaning treatment (Fig. 6)*

A cleaning treatment with pirimicarb (0.4 kg ha<sup>-1</sup>) was done in week 17 before the first release. After that, the first aphids were well controlled until week 25. As no release was done during 5 weeks, a new cleaning treatment was applied in week 26 and releases restarted in week 27. After that, aphids were under control until the end of the harvest.

#### *Start after organic cleaning treatment (Fig. 7)*

Just after covering the tunnels, individuals of *Chaetosiphon fragaefolii* were detected. They were treated with a foliar application of pyrethrum. After which, *Acyrtosiphon malvae* and *Macrosiphum*

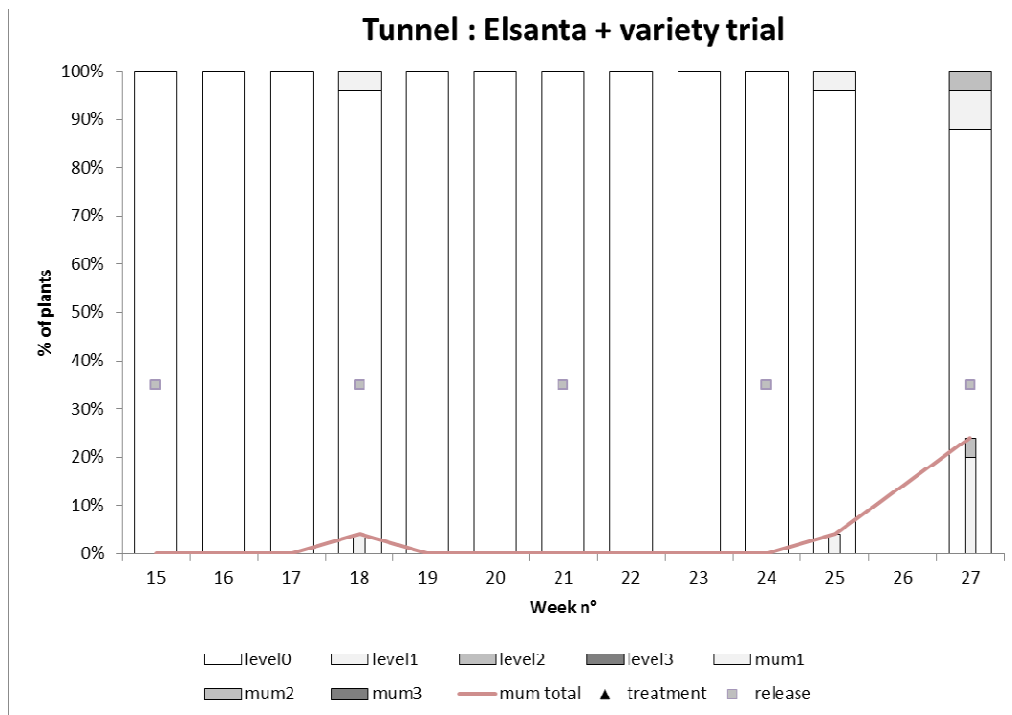


Fig. 4. Percentage of plants (N=25) with aphids (wide bars: level 0: no aphid, level 1: 1–4 aphids plant<sup>-1</sup>, level 2: 5–10 aphids plant<sup>-1</sup>, level 3: Spot with colonies) and mummies (narrow bars: mum 1: 1–2 mummies plant<sup>-1</sup>, mum 2: 2–10 mummies plant<sup>-1</sup>, mum 3: >10 mummies plant<sup>-1</sup>) in a 800 m<sup>2</sup> polythene protected tunnel with a table top crop of strawberries (Elsanta + other June bearer varieties). Proefcentrum Hoogstraten, April–July 2013.



Fig. 5. Mummy of Aphidius on a winged *Acyrtosiphon malvae*.

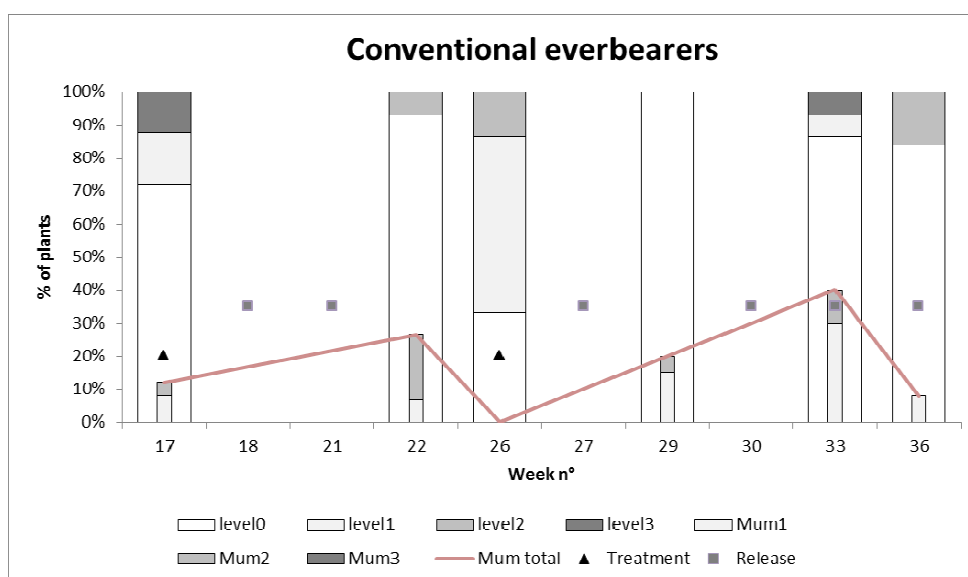


Fig. 6. Percentage of plants (N=40) with aphids (wide bars: level 0: no aphid, level 1: 1–4 aphids plant<sup>-1</sup>, level 2: 5–10 aphids plant<sup>-1</sup>, level 3: Spot with colonies) and mummies (narrow bars: Mum1: 1–2 mummies /plant, Mum 2: 2–10 mummies plant<sup>-1</sup>, Mum 3: >10 mummies plant<sup>-1</sup>) in a 1600 m<sup>2</sup> plastic tunnel with a table top crop of strawberries (everbearers, different varieties). Landwirtschaftskammer Nordrhein-Westfalen, Auweiler, April–September 2012.

*euphorbiae* were observed on a relatively high number of plants (more than 90% of the plants in week 20, see Fig. 7). However, the parasitism rate was very high (Mummies on 66% of the plants, Fig. 7) (mummies of *Aphelinus abdominalis*, *Praon volucre* and *Aphidius* sp. were observed) and the latter two aphid species never reached the economic damage threshold.

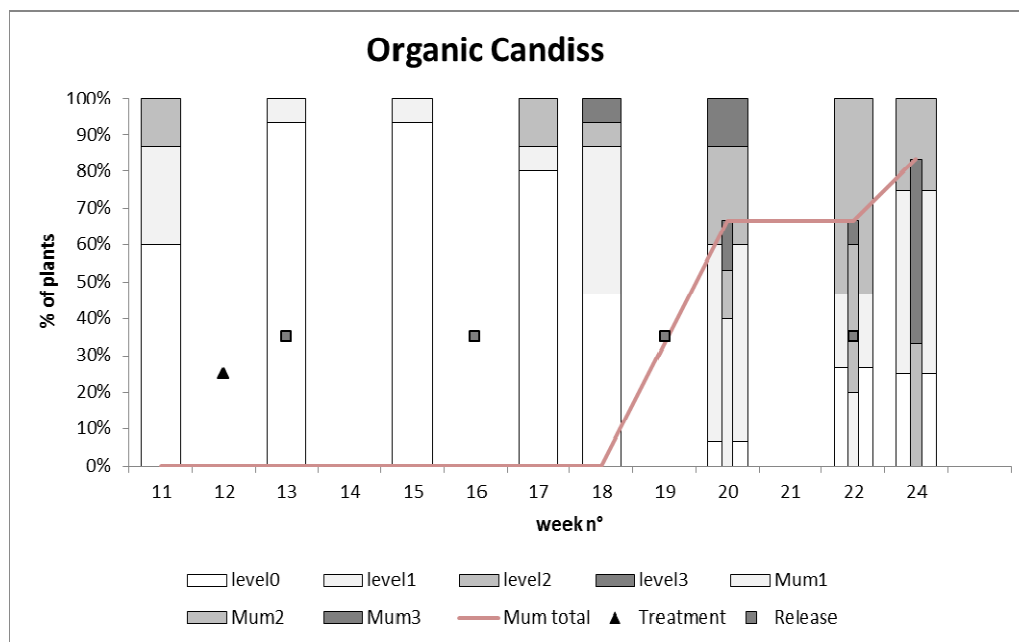


Fig. 7. Percentage of plants (N=25) with aphids (wide bars: level 0: no aphid, level 1: 1–4 aphids plant<sup>-1</sup>, level 2: 5–10 aphids plant<sup>-1</sup>, level 3: Spot with colonies) and mummies (narrow bars: mum 1: 1–2 mummies plant<sup>-1</sup>, mum 2: 2–10 mummies plant<sup>-1</sup>, mum 3: >10 mummies plant<sup>-1</sup>) in a 200 m<sup>2</sup> plastic tunnel with an organic full ground strawberry crop (Var. Candiss). Provinciaal Proefcentrum voor Klein fruit, Pamel (PPK), March–June 2013.

In the UK, in 2013, a non-quantitative follow-up (see definition in Materials & Methods section) was realized at some growers using FresaProtect. 3.4 ha from five fields of strawberry crops where FresaProtect was used were monitored. In all situations aphids stayed under control. The level of aphid presence was variable but in all situations, no aphid damage was recorded and no chemical correction was needed. Here, we show two examples.

In Sonata strawberries (soil, unheated tunnel) (releases in week 14, 17 and 20), the red form of *Macrosiphum euphorbiae* appeared in May and was immediately parasitized by *Praon volucre* and *Aphidius* sp. The parasitoid population established and control of the aphid was maintained until the end of the harvest (week 26–27) without additional parasitoid releases.

In unheated tunnels with table top Elsanta strawberries (releases from week 14 until week 25), the same species of aphid appeared in week 21 in one of the 17 tunnels. In week 23, aphids were present in all tunnels and the number of mummies was still limited. However, the examination of the aphids showed that most of them were already parasitized and that they would soon be mummified. No additional treatment was applied and in week 25, very few living aphids remained and mummies of *P. volucre* could be found on nearly all plants. No damage by aphids was found.

In Wallonia (southern part of Belgium), strawberry growers mainly grow June bearer strawberries (Darselect, Lambada, Elsanta) in full ground, in unheated tunnels. Between 2010 and 2013, 17 growers used FresaProtect during four seasons on a cumulated surface of 16.1 ha. On 95% of this area, aphids (belonging to 11 different species) were efficiently controlled, no aphid damage was recorded and no chemical correction was needed. On 5% of the surface, chemical correction has been applied for the following reasons:

- First release done too late (not preventively): If parasitoids are released when aphids are already present, the control is difficult to achieve due to the faster population

dynamics of the aphids compared to parasitoids. Before parasitoids will gain control over the aphids, there is a risk to exceed the economic damage threshold. In order to avoid this risk, the growers tend to make a treatment before aphids are effectively controlled. This situation has been observed by one grower in 2011 in five of his 30 tunnels (4000 m<sup>2</sup>).

- Stress of the grower: This was the case for one grower on 1200 m<sup>2</sup> of tunnels in 2012. It was the first year he used parasitoids and he was not aware of the lag time between oviposition and mummification. Two weeks before the start of the harvest, *Myzus persicae* was present and the number of mummies at that time was considered too low by the grower. He, thus, applied a pirimicarb treatment (0.4 kg ha<sup>-1</sup>). This treatment was not completely efficient (70% mortality) and the examination of the surviving aphids showed that most of them (75%) were parasitized. One week after, a lot of mummies were observed in the crop. The chemical treatment was thus not needed.
- Ants: Ants have been shown to empty tubes with sometimes negative impact on aphid control. This has been observed by five growers in 2010 and 2011 with repercussion on aphid control in some tunnels which justified local aphicide treatments on aphid hotspots. These observations motivated the development of the release device shown in Fig. 2. No ant problem has been observed where this new device has been used.
- Too long interval between two releases: the advised interval between two releases is 3 weeks. For various reasons, this interval is not always respected by growers. In 2012, one grower was not able to make his second release in mid-April in due time. With one week of delay, there was a gap in parasitoid presence in the crop and a population of *Acyrtosiphon malvae* succeeded to settle on approximately 1600 m<sup>2</sup> of crops (two tunnels out of 20) leading to an unbalanced situation needing a chemical correction in those tunnels.

#### *BerryProtect*

For BerryProtect, a non-quantitative follow-up was done in two commercial raspberry crops in Belgium in 2012 (soil, unheated tunnels) and at two commercial plantations in the UK (pot Maravilla plants in double cropping system in unheated tunnels as well as in heated glasshouse) in 2013. In Belgium, in 2012, releases started in March. At one Belgium site, no aphid was detected during the whole season, and two mummies were observed in mid-May, indicating that the preventive strategy was effective. In this crop, no aphicidal product was applied. At the other plantation, *Amphorophora idaei* appeared towards the end of April and was rapidly controlled by *P. volucre* and *Aphidius* sp. No treatment against aphids was needed. In the UK, in 2013, releases of parasitoids were begun at the end of March in the heated glasshouse and in mid-May after a 'clean-up' treatments of thiacloprid against raspberry beetle and cane midge in the unheated tunnels. In the glasshouse, apart from 2 weeks after a thiacloprid treatment against capsids in week 17, *Amphorophora idaei* was observed throughout the first crop cycle (until end of first harvest beginning of July), but no significant aphid population increase was recorded and mummies from *Praon volucre* and *Aphidius* sp. were regularly observed. In the tunnels, individual aphids (*Amphorophora idaei* and *Macrosiphum euphorbiae*) were regularly seen until end of first harvest (mid-July) but no colonies were formed and *Aphidius* sp. and *P. volucre* mummies were observed.

### **Discussion**

From 2010 to 2013, in trials or in commercial crops, FresaProtect and BerryProtect offered a good protection against the different aphid species attacking. The use of a mix of five or six parasitoid species is thus an efficient alternative to chemical aphicides. However, FresaProtect and BerryProtect are part of an integrated aphid management strategy and chemical treatments can be necessary in some situations. As parasitoids are mainly efficient when used preventively, a clean-



up treatment can be needed before the first release if aphids are already presents at the beginning of the season. Apart from that, most aphicides used were applied after an inappropriate use of the product. The main reasons for chemical corrections were the non-respect of preventive use or of the 3 week interval between two releases. It was sometimes needed if the tubes were emptied by ants. This problem has however been solved with the new release device (Fig. 2).

According to growers, the parasitoids decreased their use of insecticides in the treated crops, saving, on average, between one and three treatments compared to their standard strategy. The ease of use of the parasitoid release system was appreciated by the growers for whom the mix of parasitoids is a good tool to reduce pesticide residues in fruit and to manage insecticide resistance.

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