VIRIDAXIS

A technologically advanced biocontrol company with a future

MARILYN STEINER and STEPHEN GOODWIN report on an up and coming Belgian company with sights firmly fixed on the industrial production of aphid parasitoids.

Aphidius *species with mummified aphid. (Photo Viridaxis)*

Ephedrus cerasicola *inserting egg into aphid. (Photo Viridaxis)*

Team from Viridaxis at their facilities. (Photo Viridaxis)

In the January/February 2012 (Issue 122) of *PH&G*, we reported on our attendance at a meeting of the IOBC Protected Crops IPM group in the UK. We visited Double H Nurseries, a greenhouse potp lant operation in New Milton, Hampshire, to observe biocontrol in practice, and were intrigued by a new delivery mechanism for aphid parasitoids in their poinsettia crop. The product was called Aphidsure mix™ and it was being marketed by BCP Certis based in the UK. It turns out there is much more to this product than meets the eye. The supplier of the parasitoid delivery units is a Belgium-based company called Viridaxis, a relatively new player that markets its range of products through several biocontrol companies, principally in Europe. But let's first digress to the basics.

Mode of action of aphid parasitoids

The units we observed dispense aphid parasitoids, tiny wasps, which target aphids. Each female parasitoid is capable of laying many eggs during her short lifetime. She lays the eggs singly into the target aphids. The egg hatches into a larva, which uses the aphid as a convenient lunch box, understandably killing it in the process. The white, legless grub completes its development through a pupal stage to the new adult within the aphid 'skin', mummifying the aphid in the process. Silvery or even black mummies among aphid colonies are familiar to most growers on IPM programs. The wasp then escapes its confines by chewing a circular emergence hole in the aphid. After mating, females search the crop for the right species of aphid to parasitise. Some parasitoids will only successfully tackle large aphid species, others only small.

Traditional commercial use of aphid parasitoids

For several years, all parasitoids have been sent as single species, mummified aphids in vermiculite for sprinkling or placing in containers in crops. Although this can work well with correct timing and attention to rates, it is a preventive treatment, which can sometimes be hit and miss. Monitoring the crop to determine which aphid species are present is necessary, and that takes time and expertise. It is also a tricky and expensive process for the provider of the parasitoids, because single species aphids must be reared on plants (this means a secure greenhouse and all this entails), exposed to the parasitoids, extracted as mummies off the leaves, packaged and stored. Removing the need for greenhouses and plants is a huge step forward in any invertebrate production system.

Commercial production

An off-plant rearing system has largely been achieved in recent years for predatory mites, with the notable exception of *Phytoseiulus persimilis*, which will only eat spider mites, for which there is presently no sustainable artificial diet (not for want of trying). For biological control to be competitive with chemicals, production of beneficials has to be on an industrial scale. Viridaxis parasitoid production is based on the adoption of new technologies, which arose from research into artificial rearing of cereal aphids at the Louvain Catholic University (UCL) in Belgium in the 1990s. The journey has taken several years but the process can hardly be faulted. We were truly overwhelmed by the dream collaboration between Universities, government and investment companies in Belgium. Every level

works together to turn intellectual capital into tangible and profitable outcomes. It is a huge achievement for a small country, and one that Australia could well emulate.

A short history of Viridaxis

In 1998, in response to a call for bids by the Federal Ministry of Agriculture, the Biogeography Unit of Louvain Catholic University[1], headed by Prof. Thierry Hance, launched a 6-year project into control of aphids in cereal crops. Vincent Cambier, a PhD student, developed the first artificial media for aphid rearing while doing his doctoral thesis (1996-1999). With financial backing from Wallonia's[2] Department of Technologies, Research and Energy, Prof. Hance and his team developed an innovative solution for parasitoid production using diet-filled biopolymer capsules in place of the aphids. The project's promoters (Hance and Cambier) formed the company Viridaxis SA in 2004 in Charleroi, Belgium, with start-up capital of 475,000 Euros. In 2005, the Regional Government of Wallonia granted the company a recoverable advance of 754,250 Euros to improve scaling-up and to automate insect production. Then in 2007, they obtained a capital increase of 960,000 Euros, the shareholders being the Vives fund[3], Viridaxis' majority shareholder, and six new investors, namely S.R.I.W.[4] and five Business Angels in the BAMS[5] network. This allowed them to industrialise insect production, expand production capacity and develop new products for aphid control in outdoor crops, notably strawberries and apples. The company recently moved to new premises in Gosselies.

Viridaxis has maintained a cooperative link with the Ecology and Biogeography Laboratory of UCL. The Viridaxis team presently consists of over 30 people with specialised scientific expertise in chemistry, biology, biotechnology and automation. The CEO is Vincent Cambier. The company holds an exclusive patent for the use of biopolymer capsules containing powdered crustacean and algal extracts that reproduce the physiology of the aphid. You can read all about it at www.wipo.int/pctdb/en/wo.jsp?wo=2003000047. It makes very interesting reading, and illustrates the enormous complexity of such an undertaking. It is underpinned by ongoing research conducted by several individuals at various universities studying many aspects of both aphid and parasitoid physiology and behaviour, which can vary substantially between species of aphids and parasitoids. One size does not fit all. A great deal of painstaking basic research over several years has gone into the successful development of the artificial aphid and artificial diet. You may think of an aphid as a sac of fluid with not much brain power, but the range of behaviours it is capable of exhibiting would shame the average teenager. The complexity of producing the diet and inducing the correct physiological and behavioural responses is perhaps an explanation as to why products currently on the market do not actually contain parasitoids in biopolymer capsules, but contain parasitoids in aphid mummies. The aphids are clearly being reared on artificial diet, which is still a substantial achievement as it takes out the expensive greenhouse phase of production. We did endeavour to obtain an explanation of this from the company but it is all firmly commercial-in-confidence so we must await developments. This is a long-term project, which has made great strides, but is progressing according to a strategic vision, no doubt with an eye on the bottom line.

Aphidsure mix™ inserted in plastic pipe at Double H Nursery, UK. (Photo M. Steiner)

BerryProtect in raspberries, clipped to string in the canopy. (Photo Viridaxis)

OrnaProtect unit showing attachment to stake. (Photo Viridaxis)

BerryProtect unit showing emerging parasitoids and central honey feeder. (Photo Viridaxis)

The Viridaxis range of products

Viridaxis decided to concentrate on the needs of specific crops (www.viridaxis.com/home_en.html), in particular market garden and ornamental greenhouse crops. Information was collected on all aphids likely to be of concern in those crops, and which parasitoid species would be effective. The list of parasitoids able to be reared artificially expanded to finally provide the six core species determined as necessary for an effective parasitoid cocktail: *Aphidius colemani* in 2005, *A. matricariae*, *A. ervi* and *Ephedrus cerasicola* in 2006, *Praon volucre* in 2007, and *Aphelinus abdominalis* in 2009. The five crops selected, with Viridaxis product names in brackets, were strawberries (FresaProtect™), first commercialised in 2010, basil (BasilProtect™), berries (BerryProtect™), vegetables (VerdaProtect™), and ornamentals (OrnaProtect™). The present target is field crops, which will be a serious scaling up of production and require delivery through aerial or field sprayer or blower application. Steps in this process included harvesting and rearing of the parasitoids, selection of parasitoids with necessary host range, cage trials, semi-field trials and field trials. The field trials necessitated testing in a variety of growing conditions, at many locations, on several cultivars to ensure a robust result. Commercial development certainly didn't happen overnight, but considering the complexities, the ingredients for successful commercialisation came together remarkably quickly.

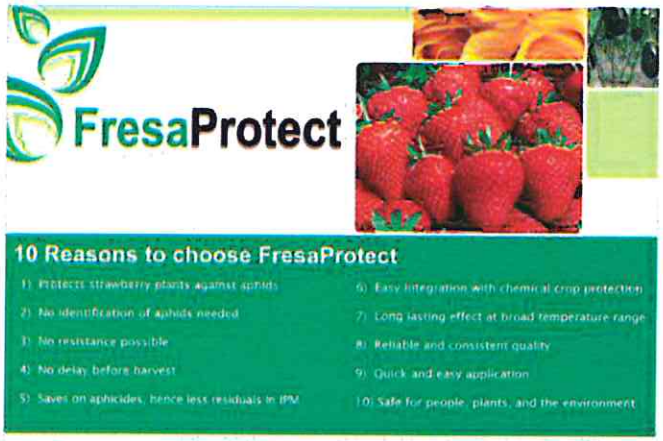
The six parasitoids and their primary aphid hosts, all sourced from Belgium, are:

1 *Aphelinus abdominalis* (for foxglove aphid [*Aulacorthum solani*]), potato aphid [*Macrosiphum euphorbiae*], and rose aphid [*M. rosae*]);
2 *Praon volucre* (for foxglove aphid, potato aphid, rose aphid and green peach aphid [*Myzus persicae*]);
3 *Aphidius matricariae* (for cotton aphid [*Aphis gossypii*]), green peach aphid, foxglove aphid and black bean aphid [*Aphis fabae*]);
4 *Aphidius colemani* (for cotton aphid, green peach aphid and tobacco aphid [*Myzus nicotianae*]);
5 *Aphidius ervi* (for potato aphid, foxglove aphid and green peach aphid), and
6 *Ephedrus cerasicola* (for foxglove aphid, green peach aphid, and lily aphid [*Aulacorthum circumflexum*]).

See www.viridaxis.com for details on individual parasitoids.

Biological Services in Loxton, South Australia, markets *Aphidius colemani*, *A. ervi* and *Aphelinus abdominalis* in the Australian market. The aphid species detailed are not the only species, which may be attacked by a specific parasitoid. Some parasitoids, such as *A. abdominalis* and *A. ervi*, are more adept at parasitising larger aphid species. OrnaProtect contains a high proportion of *Ephedrus cerasicola*. This species changes the behaviour of aphids attacked so that they hide for mummification, a necessity for an ornamental crop where leaves must be visibly clean of bugs, good or bad, at point of sale. Several other parasitoid species are being investigated for future use. This is an ambitious company.

The parasitised mummies are mixed in known proportions, which are tailor-made for each crop, depending on the prevalence of particular aphid species in each crop. The brochure for FresaProtect (*see Figure*) exemplifies the strategy of covering all aphid species adequately with the six-parasitoid mix. BCP Certis, after field trials, asked Viridaxis for their own standard mix, which they market as Aphidsure mix (240 mummies/unit) in a broader range of crops. The product contains an increased proportion of *E. cerasicola* and *A. matricariae*. BCP Certis also market a strawberry crop mix, Aphidsure fragaria™. Aphidsure mix was used at Double H greenhouses in chrysanthemums,



Aphid/ parasitoid	Aphidius ervi	Aphidius matricariae	Ephedrus cerasicola	Praon volucre	Aphidius colemani	Aphelinus abdominalis
Acyrtosiphon malvae	xx			xxx		
Aphis craccivora		xx		x	xxx	
Aphis fabae		x		x	(x)	
Aphis forbesi		(x)			(x)	
Aphis gossypii		xx	(x)	x	xxx	
Aphis nasturtii		xx		x		
Aphis rubicola		xx			xx	
Aulacorthum solani	xx	(x)	xxx	xx		xx
Chaetosiphon fragaefolii			(x)	(x)		(x)
Macrosiphum euphorbiae	xxx			xxx		xxx
Macrosiphum rosae	xx			xxx		xx
Myzus ascalonicus		(x)	(x)	(x)		
Myzus persicae	x	xx	xx	xx	xxx	xx
Rhodobium porosum	xx		(x)	(x)		xxx

FresaProtect brochure showing range of aphids controlled by six parasitoids. (Photo Viridaxis)

begonia and poinsettia with good results. According to Ward Stepman, European sales and marketing manager for BCP Certis, problems have only occurred in nurseries where Aphidsure was released too late or in insufficient numbers. Cold temperatures early in the season may also impact on establishment. The parasitoids in the mummies, apart from comprising 5-6 species, are of different ages or cold storage temperature history. The effect of this is to widen the period of their emergence and thus provide a continual presence in the crop. The mummies are packaged in a cardboard tube, which on the occasion we observed was placed within a large piece of white PVC piping attached to a post. It should not be exposed to the sun. The tube can also be placed within the plant canopy. Too easy. The flip top lid opens to reveal six emergence holes for the wasps and a central well which is filled with honey. This ensures the new wasps have food for their virgin flight and for maximum egg production, as well as increased longevity. Mummies can also be purchased as a single species in units of 500-10,000 (check with Viridaxis for availability).

How to use the product

The general recommendation is to release parasitoids preventively before aphids are seen. This is a far more effective strategy than relying on first finding aphids in the crop, which is very subjective and sometimes like looking for the proverbial needle in a haystack. There is no need any more to have the aphid species identified as at least one of the parasitoids will attack it. Distribution is simple and takes little time, far less than for pesticide application. In strawberries, it is recommended to make three releases at intervals of 3 weeks starting 2 weeks after plants start growing. This covers the growing season. For other berries (raspberries, currants, blueberries), releases should start when floral buds appear. The rate for all crops is one unit for every 200m^2. The application interval is 2 weeks for other than berry crops. The units can be stored for 1-2 days at 10°C on receipt but unless environmental conditions are extreme it is always best to make releases on receipt.

Cost relative to existing strategies

No-one could give us a present cost, because it is set by distributors, except to say that it was initially a little more expensive than the existing parasitoid distribution system, but that it was now comparable. If you have an insecticide, which still works, a big 'if', you may find it more expensive, but from all accounts control is extremely good with this system, at least comparable to that with insecticides, with the added bonus that there are definitely no resistance problems, no down-time to schedule pesticide applications, and no-one breathing down your neck about chemical residues. This is a win-win situation.

To become mainstream, integrated pest management needs technologically advanced production and distribution systems to ensure cost-effective, time-efficient, reliable pest management alternatives to chemical pesticides. Globally, the biocontrol industry has made great strides in the last 5 years but still has a long way to go. The industry cannot stay as a cottage industry. It needs serious investment dollars, and these are becoming increasingly scarce, particularly so in Australia where IPM research has been effectively abandoned by the horticultural research agencies and grower organisations in favour of promotion, marketing and political lobbying. We are very pleased to see that with the right type of inter-agency support and encouragement, companies such as Viridaxis can enter the technological age, thrive and prove a worthy adversary in the marketplace. It clearly hasn't arrived there by luck, but through the efforts of a very skilful, hardworking and dedicated team, and by very good management and vision.

Endnotes

1. The UCL supports the creation of spin-off companies to economically exploit knowledge. Viridaxis is one of these companies. Every assistance is given to build innovative companies. The Louvain Technology Transfer Company was set up to associate LCU research teams with Sopartec, the UCL technology transfer company.
2. Wallonia is the predominantly French-speaking southern region of Belgium, with 3.5 million inhabitants.
3. Vives is a private venture capital fund with the objective of stimulating the setting up and growth of spin-offs from Universities and to invest in technological innovation enterprises in the early years. Vives II was launched in 2011 with a first capital round of 43 million Euros and is the largest European investment fund ever launched by a university.
4. S.R.I.W. The mission of this Group is to intervene financially for the long-term with the objective of promoting the economic development of the Walloon Region.
5. BAMS selects projects with high growth potential to give private investors effective access to innovative companies. It comprises over 100 Business Angels.

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