Control of Insect Pests with Entomopathogenic Viruses

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Abstract

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Entomopathogenic viruses, particularly baculoviruses (BV) have been shown to be highly effective against major insect pests, however their use is still limited suggesting a need to identify the factors that limit their adoption. A number of BV insecticides have been successfully developed and examining these cases closely may help to identify key factors. One important factor in successful BV adoption has been where chemical control is not feasible, either because of pest resistance, residue problems or environmental considerations. BV tend to be more successful on high value crops. This is linked to the often higher costs of producing BV. Thus developing more cost effective production and consistent quality control remain important research goals. While safety has been seen as a major advantage for biological agents such as BV, in practice this probably plays little part in promoting adoption. There are also still significant technical constraints such as: poor efficacy or persistence on some key target crops, shelf life, speed of action and limited host range. Although, genetic manipulation has been seen as a key to overcoming some of these, it is not yet certain that any key breakthroughs are in sight. In conclusion the study of these factors may assist in identifying a research agenda that will facilitate the development of better BV pesticides.

Introduction

Insect viruses have been seen as entomopathogenic agents that have considerable potential for development as biological pesticides. Active development programmes are underway in many countries including the Middle East, Asia and Africa. However their use as practical biopesticides is still only a fraction of that seen for *Bacillus thuringiensis* (Bt) products (14). While they can be said to have made significant progress in specific niche markets they have yet to establish a major presence. The baculoviruses (BV), of the genus nucleopolyhedrovirus (NPV), but also some granuloviruses (GV) are the most studied of the viruses. The BV have the greatest potential of the 14 or so groups of viruses that are known to infect insects and alone have been developed into commercial biopesticides. These have been developed in Europe, USA, and Asia (4), however we should remember that they still represent significantly less than 1% of the world-wide insecticide use and probably represent only 20% of the total market for microbial biopesticides. To understand the reasons both for the successes of BV and their limited overall impact it is necessary to look at the biology of these viruses and development process for biopesticides.

The Baculoviruses-mode of action

All baculoviruses are obligate pathogens killing insect hosts by initiating systematic tissue infections. In this they contrast with entomopathogenic bacteria such as Bt whose primary mode of action is toxin mediated. BV primarily infects the larval stages though in hymenoptera infections can also occur in adults. To initiate BV infection the viruses need to be ingested and leaf feeding Lepidoptera, where a route of infection is apparent, host many BV. The tendency of many Lepidoptera to lay many eggs in clusters and complete feeding on a single plant also contributes to the ease with which infection can spread from larva to larva. In highly mobile pests such as locusts or sucking pests such as aphids the difficulty of establishing consistent transmission may help to account for the failure of BV to become evolutionarily established in this group. Also it may be noted that among Lepidoptera that feed by boring BV are less common and often more difficult to utilise as control agents.

BV once ingested quickly enters the cells of the midgut epithelium to initiate a primary cycle of multiplication. The infectious stage of the virus is an occlusion body (OB), also called the polyhedral occlusion body or polyhedra. In NPVs this is a large protein crystal up to 15 microns in size in which many (up to 200) individual infectious viral particles, or virions, are embedded. In GVs a different morphology is seen, here there is a single virion embedded in a much smaller OB up to 0.4 microns. NPV are clearly identifiable under a light microscope and GV just visible. The OB is a protective structure that confers on the virion a high degree of stability that gives the NPV an ability to persist in favourable environments, such as soil, for many years. In the insect midgut the alkaline soluble OB crystal protein (polyhedrin) dissolves releasing the virions to attack the host. Once the virions have initiated a primary infection in the midgut cells the virus undergoes a cycle of multiplication in the nuclei of these cells. The progeny of this primary replication are naked virions and no OBs are produced in the midgut cells during this primary phase of infection. These progeny virions appear 12-24 hours post infection and pass through the basal lamellae of the midgut into the body of the insect to infect other tissues. In Hymenoptera the picture is different as here infection is confined to the midgut cells and OBs are produced in these cells. The virions produced in the midgut cells pass into the heamocoel initiating widespread secondary infections in tracheole cells, hematocytes, hypodermis, fat body etc. In infected insects viral replication is widespread and >90% of susceptible cells may be involved. In these secondary sites of infection there is massive production of the OB form of the virus with up to 4 $x10^7$ OBs per mg body weight being recorded. In an insect such as *H. armigera* the OB production is up to 4 $x10^9$ OBs per larvae and OBs may represent 25% dry matter weight in dead larvae. The comprehensive infection of body tissues invariably results in the death of the host 5-7 days after infection. During the latter part of this cycle 4-5 days after infection the host stops feeding, infective OBs start to appear in the faeces. In the last stage of infection virally coded enzymes such as chitinases and proteases that help to digest the hosts tissues and skin are often produce. The larva thus develops a fragile skin that ruptures easily releasing OBs onto the host plant to infect new larvae.

Understanding this life cycle helps us understand some of the advantages and limitations of BV. As an infectious agent it multiplies after being ingested and the first generation of infected insects produces more BVs that can initiate secondary infections increasing the impact of sprays and increasing levels of BV on the crop. The drawback is that the infection process takes time and so the speed of kill of BVs is slower than with chemicals or a toxin mediated biopesticide such as Bt. The killing time of 5-7 days is closer to that seen in some selective chemicals such as IGRs. The need for ingestion and the absence of contact action (unlike entomopathogenic fungi) also limits its effectiveness to those situations where BV can be applied effectively to the site of feeding.

Specificity and safety

An important characteristic of the baculoviruses is their high degree of host specificity. BVs as a genus only infect insects and a few other arthropods. Most of the 370 recognised NPV species infect only a few closely related host species at most (11, 16). This specificity confers the important property of making them safe for man, domestic animals, non-host insects and plants but this also limits the spectrum of target pests (1, 6). Research has shown that the chance that BV, even mutated BV, being able to replicate in a plant or vertebrate is effectively zero (11). BV do not attack or replicate in the insect predators or parasitoids that are important in the natural control of many pest species. The majority of NPV affect Lepidopteran species, though a few effect Hymenoptera and Diptera, so that no BV have been identified in many major insects pests. Fortunately BV do infect some of the most damaging insect pests that insecticide resistance has made very difficult to control using conventional pesticides. Key species n this respect are the Heliothines, including *Helicoverpa armigera*, *Heliothis zea*, *Spodoptera* species and *Plutella xylostella* all of which are globally important pests of fibre crops, cereals, legumes and vegetables. The BV of these pests are very specific, PxGV only attacks *P. xylostella*, Heliothine NPVs are cross infective to most members of the group while *Spodoptera* BV, *S. littoralis* NPV, *S. exigua* NPV and *S. litura* NPV have variable host specificity. Where a single species is the key pest this is not a major limitation, however where several of these species are pests on the same crop at the same time it presents a problem.

Efficacy

One of the most attractive properties of NPVs is that they are effective against species that have become highly resistant to chemical insecticides. It is this factor that has led to their use in IPM programmes in India, Thailand and Australia to control *H.armigera and Spodoptera* Spp. that have become increasingly difficult to control using conventional insecticides.

Many trials on a number of NPVs have been carried out indicating that NPV can produce results equivalent or better than those with insecticides. NRI in collaboration with national scientists, have carried out such trials in Egypt on cotton with *S.littoralis* NPV, in Thailand with *S.exigua* NPV and *H.armigera* NPV on cotton and vegetables, and in India on legumes, cotton and vegetables. An even larger body of work, mainly in the USA has shown similar results (5). However, significant progress in promoting the uptake of NPVs by farmers has mainly occurred where chemical resistance has been a major factor. One reason for this is that the slower speed of kill of NPVs makes them less immediately appealing to farmers than the fast acting chemical insecticides that have an instant and very visible impact on pests. The rapid kill of chemicals makes them more compatible with the practice of many farmers who leave pests untouched, hoping for the best, until they see large larvae or damage and only then spray. Indeed against pest populations showing no resistance chemicals will almost always prove to be simpler to use and more effective than a BV in the short term. However once insecticide resistance develops to a significant level, as invariably seems to happen with pests such as *H.armigera*, *P.xylostella* and *Spodoptera* species, the balance alters in favour of the BV.

One approach to overcome slower kill of BV has been to stress the need to incorporate BV into holistic IPM systems that maximise natural enemies and move away from the chemical insecticide use paradigm. This stresses farmer education, scouting, and integration of multiple biotic and cultural methods for pest control (21).

An alternative approach, favoured by the commercial sector, has been to look to genetic manipulation to improve the speed of kill and widen the host range of NPVs (9). Considerable research has been carried out on incorporating invertebrate toxin genes into BV to improve speed of kill to approach that of chemical pesticides. However while research progress has been reported as yet no commercial products based upon GM viruses seem likely in the near future.

Persistence

The persistence of NPV on the crop once applied has been the subject of some research. It is a drawback of NPVs that their persistence can be reduced by two major factors; ultraviolet light and plant surface chemistry. The UV in sunlight, especially high intensity tropical sunlight is a potent inactivator of viral DNA. Work on the *S.littoralis* NPV in Egypt showed clearly the rapid deactivating effect of sunlight on unshaded NPV (2, 11). It also showed that of a range of UV protectants tested none were superior to a simple unpurified suspension of insect derived NPV. Since then more effective chemical UV protectants have appeared but their very high cost makes their inclusion in NPV formulations currently uneconomic.

However the limiting effects of UV inactivation should not be overestimated. If the BV is infectious enough then even BV with short persistence can produce acceptable results. One should also remember that short persistence times also characterise many chemicals and other biopesticides like Bt. Plant chemistry can also have a significant impact on BV efficacy. Results from a series of field trials have shown that BV performs better on some crops such as tomato than on others like cotton and chickpea. On cotton the plant surface chemistry has been implicated as a factor and chemicals in the glandular exudates have been shown to cause inactivation of NPV. It is probable that absence of such inactivating factors may be one reason why BV has been adopted more widely by farmers on crops such as tangerine, okra, grapes and onions than on cotton.

BV Production

BV mass production is exclusively through *in vivo* replication in whole insects. BVs like all viruses need host cells to replicate in. Cell lines have been developed for a number of NPVs and GVs but are currently only suitable for small-scale production (1). Mass production in insects is conceptually simple. Larvae are grown to an appropriate size, usually on artificial diet; they are then fed diet sprayed with the BV. The insects are incubated for 5-7 days on this diet to allow the BV infection to develop and the OBs to multiply, they are then harvested at, or just prior to death (3, 7, 20). The infected cadavers are subsequently macerated in water to release the NPV, then the suspension filtered to remove skin and hard body parts that could jam sprayers. The resultant suspensions can then be used directly or formulated further. Such is the efficiency of viral replication in host insects that enough virus to apply to a hectare of crop ($0.5 – 2 \times 10^{12}$ OBs) can be produced from 200-500 infected larvae.

The equipment and facilities needed for such *in vivo* production are relatively simple and cheap to set up in comparison with chemical pesticides. This has attracted researchers in a number of developing countries to explore the use of NPV as a locally produceable biopesticide (18). In my own work NRI itself has helped establish the local production of NPV in India and Thailand. Similar programmes by other organisations such as the International Potato Center (CIP) has set up pilot plant production of *Phthorimaea operculella* GV for control of potato tuber moth in Bolivia, Tunisia and Egypt (13, 17). An outstanding example of such a programme is that in Brazil where the *Anticarsia gemmatalis* NPV is now produced and used to control that pest on more than 1,000,000 hectares of soybean (15). This is an especially noteworthy programme in that the commercial mass production of NPV utilising field production techniques has enabled the product to be produced at a cost lower than that of chemical pesticides.

However while production is in principal simple. in practice, maintaining an adequate supply of disease free insects, preventing contamination by unwanted micro-organisms and maintaining production output and quality is demanding (12). Strict attention must be given to quality control. Many attempts to develop BV production have foundered due to inability to prevent contamination of the product or preventing pathogens from destroying the insect culture. Failure to control quality can also result in products that have grossly inadequate NPV content; these are also often grossly contaminated with bacteria and other microbes (8). Such products can be a real problem as they are ineffective and if sold to farmers will damage the reputation of NPV (12). One factor in the successful establishment of mass production in India and Thailand may have been that both countries had established silkworm industries so that there was already commercial experience with the mass rearing Lepidopteran larvae.

However with good practice small to medium enterprises producing NPV can be established and sustained. These enterprises can be started up with only a fraction of the capital needed for conventional pesticide production units. In India many such small NPV producers appeared after 1990 in response to the Indian Governments very active promotion of NPV and other biopesticides in the wake of severe crop failures due to chemical pesticide resistance. There are at least 33 commercial and state sector producers of NPV reported as active in Indian (19). The quality of NPV products has improved after 1995-97 when NRI in collaboration with the Indian Council of Agricultural Research and ICRISAT ran training courses to help producers improve quality standards and production methods (8). These producers are mainly commercial companies and include a variety of enterprises including existing pesticide manufacturers, biotechnology companies, seed companies and state institutes. Most are small to medium enterprises whose total production of NPV products ranges from 5,000-15,000 hectares per annum but several are now expanding this by an order of magnitude with at least two new mass production plants under construction. These are not only expanding to meet local demand but also to meet the potential for exporting NPV products to the rapidly expanding market in other countries.

In India village level production by farmers themselves has been started by ICRISAT and some NGO's but its long-term sustainability will be dependent on maintaining acceptable quality.

Here one should mention that success in developing biopesticide products is crucially dependant on involving not just academic researchers but in involving expertise on production scale-up, quality control, formulation, and marketing that is mainly found outside the academic sector (10). Success requires a truly multi-disciplinary approach and often a multi-institutional team to bring the skills needed for success. Future successful promotion of biopesticides will be dependent upon developing just such collaborative projects including the private sector.

Regulation

An important factor in promoting the development of biopesticides is the regulatory environment. Where countries have decided to support the promotion of biopesticides and have set in place registration systems that help potential manufacturers such as in Brazil, India and Thailand, producers have felt able to invest in production and registration. However in many countries lack of expertise in pathogen registration has created a climate where registration is uncertain or involves unnecessarily complicated, inappropriate or expensive regulation and this is a major discouragement to local production or registration of biopesticides. Currently there are promising initiatives to develop appropriate harmonised registration packages in Africa so that products can attain registration in several countries with minimal need to duplicate expensive efficacy trials or safety tests (L Vaughan, A C Cherry, personal communications).

Formulation and application

One of the great advantages of BV as compared to some other biological control approaches is the ease with which they can used by farmers. They are sprayed and applied in the same way and using the same equipment as existing chemical pesticides. They can be used as direct replacements for chemical pesticides, though work better if incorporated into a properly developed IPM system. The OB of BV is an inherently stable particle that can be formulated easily and retain infectivity for a long time without any special formulation. BV can be formulated as simple aqueous suspensions, wettable powders and oil formulations

using standard techniques (2). Trials have shown that simple unpurified NPV is more effective than purified specially formulated products as purifying NPV of insect derived material removes many components that help to preserve NPV activity in the field (11). However research on improved formulation could help to improve the efficacy limitations of BV on problem crops reducing costs.

Current use of viral biopesticides

Currently BV use is largely restricted to niche markets, where resistance and or residue problems make chemicals ineffective or unacceptable. In Thailand uptake has primarily been on vegetable and fruit crops, often for export, where both factors operate. The drive to reduce chemical residues on crops for export to developed countries may indeed create major new opportunities for BV products. This will undoubtedly be further increased as older chemical pesticides are banned for use on these export crops by importers responding to public pressure.

BV use has grown in Australia to control *H. armigera* as a key tool in a resistance management program with the 50,000 litres used in 1999-2000 meeting only 30% of the estimated demand (C Hauxwell personal communication). In India use is growing to overcome *H.armigera* on cotton as part of IPM programmes but also on other crops. In China *H.armigera* NPV products equivalent to 100,000 ha are produced for use, primarily on cotton (J M Vlak personal communication).

The future scale of BV use will be effected by many factors, including competition from new, safer chemicals, however the banning of older chemicals and more stringent residue regulations will create significant new opportunities. In addition the introduction of insect resistant GMO crops may open new sectors either as supplementary sprays for controlling secondary pests or for treating refugia of non-GMO crops.

الملخص

جريفاكس، ديفيد. 2000. مكافحة الآفات الحشرية باستخدام الفيروسات الممرضة للحشرات. مجلة وقاية النبات العربية. 18: 128-132.

تعدّ الفيروسات الممرضة للحشرات وبخاصة فيروسات الباكيولو (BV) ذات فعالية عالية في مكافحة الآفات الحشرية، ولو أن استخدامها في المكافحة ما زال محدوداً مما يشير إلى أهمية تحديد العوامل التي تحدّ من تبنيها. لقد تم بنجاح تطوير عدد من مبيدات الحشرات (BV) وقد ساعد فحص هذه الأمثلة عن قرب في تعريف العوامل المحددة. كان أحد العوامل المهمة في تبني BV هو في الحالات التي لا يمكن فيها استخدام المكافحة الكيميائية، إما لظهور المقاومة في الآفة أو تفادياً لمشكلة الآثار المتبقية للمبيد أو لاعتبارات بيئية. وينجح استخدام BV في المحاصيل ذات القيمة العالية، نظراً للكلفة العالية لإنتاجه. وعليه يعدّ تطوير إنتاج هذا الفيروس الممرض مجدي اقتصادياً ومن نوعية ثابتة من الأهداف البحثية المهمة. وفي حين يؤخذ موضوع الأمان كحسنة رئيسية لعوامل المكافحة الأحيائية ومن بينها BV، إلا أن لهذه الناحية دوراً ثانوياً في تحفيز التبني من الناحية العملية. ولا تزال هناك معوقات تقنية أيضاً مثل الفعالية الضعيفة أو الثباتية على بعض المحاصيل المستهدفة، وفترة الحفظ القليلة، وسرعة التأثير والمدى العائلي المحدود. وينظر إلى التحوير الوراثي كمفتاح للتغلب على بعض من هذه المعوقات على أنه لم يتم تحقيق تقدم في هذا المجال حتى الآن. وبالنتيجة، فإن دراسة هذه العوامل قد تساعد في تحديد منهج بحثي قد يسهل تطوير مبيدات BV أفضل.

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