THE ROLE OF MICROBIAL INSECTICIDES IN FOREST PEST MANAGEMENT

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ABSTRACT

Microbial insecticides differ in several fundamental respects from chemical insecticides and this is reflected in their mode of use which requires careful matching of spray droplet distribution with the feeding sites of target insects. They also act more slowly than chemical insecticides while the pathogen replicates in the host. These characteristics can be seen as constraints but, in management of forest insects, they are compensated for by many of the ecological attributes of the trees themselves; area, size, plant architecture, stability, all of which combine to keep insects mainly at endemic levels. Natural infection helps to retain this endemicity. When pest outbreaks do occur, microbial insecticides play an increasing role in their management, the market being dominated by *Bacillus thuringiensis* products. This is illustrated by reference to major defoliating species - pine beauty moth, spruce budworm, gypsy moth and European pine sawfly. Trends in use of insecticides in forestry have moved from chemical to microbial so that current usage is mainly *Bacillus thuringiensis* in both North America and Europe.

INTRODUCTION

It is probably true to state that, among the crop types that have been the subject of pest management involving microbial insecticides, forests have supported the most successful long term use of these agents and have been important in development of improved methods of application and formulation. This paper explores some of the reasons for this success and considers, with examples, the key attributes that must be considered in their continuing future use.

Early history

Insect pathogens have a long history as key mortality factors affecting insect populations, a feature that was later used to develop microbial insecticides for combating insect pests. The most notable early examples concern baculoviruses which were described by the Chinese as a result of problems in rearing disease-free silkworms, *Bombyx mori* (Steinhaus, 1956). Although not recognised to be viral diseases, the symptoms and frequency of disease incidence were compatible with both baculoviruses and cytoplasmic polyhedrosis viruses. Fungal diseases were the earliest to be described consistently, notably through the work of Agostino Bassi who first described the fungus *Beauveria bassiana* as the "white muscardine disease" of the silkworm. Use of fungal pathogens for pest control was pioneered by Louis Pasteur in France (see Ferron (1985) for review).

Diseases of forest insects have also been noted and have given rise to descriptive names, linked to the symptoms exhibited by infected insects. One of the earliest examples was the German description of baculovirus disease of nun moth, *Lymantria monacha*, as *wipfelkrankheit* or *tree top disease* which is a graphic description of the habit of infected larvae to migrate to the tops of trees (Hofman, 1891). Although the significance of baculoviruses for pest control in forestry has been large, it is the discovery of *Bacillus thuringiensis* (*B.t.*) (Berliner, 1915) that has had the greatest impact on use of microbial insecticides, not only in forestry but in virtually all other crop systems. The first commercial preparation of *B.t.* was "Sporeine" which was developed in France during the late 1930s, although the current range of commercial *B.t.* preparations have essentially resulted from the discovery of the variety *kurstaki* in France and the HD1 strain in the USA (Dulmage, 1970). The ability to scale up production in industrial fermenters has meant that *B.t.* can be produced at competitive prices and to high quality control standards. Efficacy in the field, improvements in formulation and its perceived environmental safety have also enhanced its market share.

ATTRIBUTES OF FORESTS THAT ENCOURAGE USE OF MICROBIAL INSECTICIDES

In considering why use of microbial insecticides has proved to be a viable alternative to chemical insecticides, especially in management of forest insect pests, a number of the characteristics of microbial agents that determine potential sphere of use must be included. These are summarised in Table 1 where both benefits and constraints to use are summarised.

The combination of particulate active ingredients and their ability of replicate in host populations in the field defines the major differences between microbial and chemical insecticides. These differences have practical implications for their use in pest management programmes.

Firstly, unlike the majority of chemicals, microbial insecticides cannot be diluted below a given concentration because of their particulate nature. This places some constraints on the amount of active ingredient that can be contained in a given droplet, particularly in very small droplets where the volume may be insufficient to contain large quantities of pathogen inoculum. This has proved to be a constraint in determining optimal use of *B.t.* for forest pest management and was elegantly demonstrated by Van Frankenhuyzen & Payne (1993). In studies on *B.t.* control of spruce budworm, *Choristoneura fumiferana*, they showed that each droplet had to contain a full lethal dose to be effective. Sub-lethal doses in individual droplets tended to result in only a partial effect upon ingestion which, although giving a brief cessation of feeding, allowed the affected individuals to recover and resume feeding. By this stage the remaining *B.t.* was insufficient to cause mortality and, thus, damage to trees was unacceptable.

Secondly, bacterial, protozoal and viral insecticides must be ingested to be activated while fungal and nematode agents can either be ingested or penetrate the host through the cuticle. In effect, there is no rapid contact or fumigant action and thus encounter between host and pathogen depends on host feeding activity rather than direct contact of droplets at the time of spraying. Sprays must, therefore, be directed to the feeding sites of the target hosts, rather than to the precise distributions of the host individuals themselves at the time of spray application. This requires detailed knowledge of host feeding behaviour over time (Evans, 1986).

Thirdly, all pathogens require time for replication from initial infection to eventual death of the host. This is dependent on inoculum load, stage of development of the target insect and on temperature but, in all cases, the speed of kill tends to be longer than for chemical insecticides, the latter usually acting directly on some characteristic of host physiology leading to rapid mortality. Insect growth regulators, such as diflubenzuron, are exceptions because, in general, they require ingestion and moulting of the host before the effect is manifested.

Table 1: Characteristics of microbial insecticides in relation to their benefits and constraints.

Attribute	Benefits	Constraints
Particulate entity (spore, polyhedron, crystal)	Some protection against environmental degradation; can be quantified for experimental and practical use.	Cannot be diluted below particular concentration - some droplets will have no active ingredient.
Specificity	Most have restricted host range giving high environmental safety.	Narrow spectrum of activity restricts usage in field; lower commercial potential if crop attacked by range of pests.
Sustainability	Produced *in vivo* or *in vitro* with little reliance on non-renewable resources.	Reliance on non-synthetic production methods can constrain scale-up and increase costs.
Ability to replicate	Replication in hosts produces secondary inoculum which can lead to further cycles of infection in new hosts.	If host range is wide can increase chances of infection in non-target hosts.
Persistence	Particulate inocula can persist outside host between generations; this may be sufficient to initiate infection in other host populations.	May be regarded as constraint if host range is wide and effects on non-targets are likely to be a problem.

Although the above three characteristics are common to all crop systems, analysis of the characteristics of forests reveals that there are positive attributes that favour the use of microbial agents relative to their use in other crops. Notably, microbial insecticides tend to kill more slowly than chemical insecticides, although this may be partially compensated by early cessation of feeding. Relatively slow kill can, therefore, result in unacceptable damage to the crop and, where the damage threshold is low, can rule out the use of microbials as the sole means of control. Forests, however, tend to have a high damage threshold, reflecting a number of their ecological attributes (Table 2).

Table 2: Ecological attributes of forests in relation to potential for supporting microbial control of insect pests.

Attribute	Characteristic	Potential for microbial insecticides
Area occupied	Forests tend to be climax vegetation and occupy greater areas than other plants. Species-area relationships predict that greater areas result in greater numbers of insect species.	Large, contiguous, areas can favour persistence of inoculum and wider availability of hosts. Aerial application is favoured, giving economies of scale and speed.
Size	Greater availability of feeding resources. In most cases, food availability (≡ damage) is not limiting	High tolerance of damage partially offsets slow speed of kill. Cosmetic damage is usually not a problem
Plant architecture	Size results in complex plant architecture and many niches supporting a wide range of herbivore and natural enemy species.	Targeting of droplets may be difficult but tolerance to damage may compensate. Microbials and natural enemies act together.
Stability	Trees are long lived and, thus, tend to accrue insect species, both herbivores and natural enemies. This increases opportunity for pathogens to evolve with hosts.	Microbial pathogens tend to be relatively specific to hosts; enhanced use poses little threat to non-target organisms. Microbial insecticide action is augmented by other natural enemies.
Tendency to the endemic state	The above attributes tend to keep herbivores at endemic levels	Microbial agents are often associated with high insect populations and are a major factor in returning insect populations to endemic levels.

The combination of attributes in Table 2 will tend to keep insect populations at endemic levels, particularly bearing in mind that some species of tree support wide insect diversity (for example oak (*Quercus* spp) supports 423 of insects in Britain (Kennedy & Southwood, 1984)) and yet are not usually subject to critical damage from insect herbivore populations. Some insect species will, of course, occasionally build to damaging levels, although in many cases this will be a single species, making targeting of the host a relatively simple matter, thus favouring the selectivity of microbial insecticides. Many of the successful microbial agents currently used in forestry were, in fact, isolated from epidemic insect populations where the pathogen was instrumental in returning populations to endemic levels. Notable examples are the baculoviruses of gypsy moth, *Lymantria dispar*, isolated at the turn of this century (Jones, 1910), of European spruce sawfly, *Gilpinia hercyniae*, which has proved to be an effective, long term regulator of populations in Europe and Canada (Cunningham & Entwistle, 1981) and of Douglas fir tussock moth, *Orgyia pseudotsugata* in Canada and the USA (Cunningham, 1995). An interesting recent example involving a fungal pathogen is the appearance of *Entomophaga maimaiga* in gypsy moth populations in the north eastern USA (Hajek *et al.* 1990). This fungus was previously only recorded in Japan but, in 1989, was found to be causing widespread infection in gypsy moth populations in several States in NE USA. It is now regarded as having considerable potential as a microbial insecticide (Hajek *et al.* 1996b).

As with chemical insecticides, the critical feature of successful use microbial agents is the necessity to deliver the correct dosage to the appropriate target insect stage with minimal contamination of non-target areas in the immediate environment. Great progress has been made in application technology during the past 20 years, much of the impetus coming from the need to improve efficacy of *B.t.* (Van Frankenhuyzen, 1993). Notable among the contributory factors has been an increase in potency of *B.t.* products used in forestry, so that preparations with over 30 BIU/l are now routinely available. The major benefit of these high potency preparations is the ability to apply them undiluted in ultra low volumes down to 1 l/ha (J Sanders, Abbott Laboratories, personal communication). Further refinement in spray application techniques have come from matching the distribution of droplets to the feeding sites of the target host insects and ensuring that each droplet contains a lethal dose (Van Frankenhuyzen & Payne, 1993). Similar approaches were advocated by Entwistle *et al* (1990) in optimising aerial application of baculovirus for control of pine beauty moth, *Panolis flammea*, in Scotland.

CASE STUDIES

Pine beauty moth in Scotland: Baculovirus (Nuclear Polyhedrosis Virus)

Pine beauty moth, *P. flammea*, presents an interesting example of an insect that is normally innocuous on its native host plant Scots pine, *Pinus sylvestris*, but has become a major pest on the introduced lodgepole pine, *P. contorta*. Early outbreaks, which resulted in extensive tree mortality, were treated successfully with the organophosphate insecticide fenitrothion and also led to the first aerial applications involving ULV, CDA technology against a forest pest in Britain (Holden & Bevan, 1979; Holden & Bevan, 1981). A baculovirus (Nuclear Polyhedrosis Virus (NPV)) was isolated from the epidemic insect populations observed in the late 1970s and, following research into dosage-mortality relationships and larval behaviour and distribution in the field, led to field trials of both homologous pine beauty moth NPV and the NPV of the closely related cabbage moth (*Mamestra brassicae*) (Entwistle & Evans, 1987). Using the Control Window concept developed by Evans (1994), research into the distribution of feeding sites of the target first instar larvae, the loading of droplets with the minimum lethal dose and the distribution of droplets through the canopy, provided information on the parameters necessary for successful use of the NPV. Using rotary atomisers (modified Micron sprayers X15 applicators mounted on a self contained hanging boom system) to generate droplets with VMD between 50 µm and 80 µm, it was demonstrated that up to 95% of droplets were captured on the top 35% of the canopy, coinciding precisely with the preferred oviposition and early feeding sites of the larvae (Entwistle *et al.* 1990). This enabled a reduction in spray volume to between 3 and 5 l per ha and of dosage to 2.2 x 10^{11} NPV polyhedra per ha (Entwistle & Evans, 1987). Preparations of both PfNPV and MbNPV were tested successfully in the field and used in spray operations from 1986 to 1988. Although the areas treated were relatively small in comparison with use of *B.t.* against other forest pests, the example is significant because it demonstrates the value of matching spray technology and host biology to give increased efficacy in the field.

Spruce budworm in North America; *Bacillus thuringiensis*

Both eastern spruce budworm, *C. fumiferana* and western spruce budworm, *C. occidentalis* are major defoliating lepidopterous pests of coniferous forests in Canada and northern USA where they affect various species of *Abies, Picea* and Douglas fir, *Pseudotsuga menziesii* (Dahlsten & Dreistadt, 1984). Repeated defoliation can weaken and, eventually, kill trees or make them susceptible to attack by other biotic agents. Large areas have been sprayed by both chemical and, in recent years, microbial insecticides (Cunningham, 1995; Van Frankenhuyzen, 1993). Budworm larvae are difficult to target precisely because the most susceptible first instar larvae only feed for a short time on the expanding buds before entering, in the second instar, a needle mining phase and it is generally more efficient to time spray application for the bud break phase (Cadogan & Scharbach, 1993). Increasingly successful spray programmes have been carried out as techniques of application, allied to high potency formulations of *B.t.*, have developed through the 1980s. This was well reviewed by Van Frankenhuyzen (1993) who described the change from volume application rates of up to 10 l per ha to current usage of around 1 l per ha. Costs of such application dropped as the potency of the commercial preparations was increased, thus optimising both droplet loading with *B.t.* and reducing aircraft spray times. Similar operational requirements apply to the NPV of spruce budworm which, although providing significant population reduction, has been inconsistent in reducing damage, even with dosages as high as >8 x 10^{12} polyhedra per ha (Cunningham, 1995).

Gypsy moth in North America and Europe; *B.t.*, NPV and fungus

Gypsy moth is the most serious pest of deciduous trees in temperate regions. A native of Europe, the moth has become a major pest in North America following its introduction during the 19th century. It attacks a very wide range of host plants, although preferring oak and other broadleaved trees, causing severe defoliation, loss of growth and increased susceptibility to other pests and diseases. An additional problem, especially in urban environments, is the presence of urticating hairs on the larvae. These can cause severe skin irritation in susceptible individuals and there is, thus, considerable public pressure to manage populations of this moth in both Europe and North America. As noted in the introduction, the NPV disease of gypsy moth has been known for most of this century. Other microbial agents include microsporidia and fungi, both offering potential as microbial insecticides. The recent finding of the fungus *E. maimaiga* and its early field testing in north east USA provides some promise for future use, particularly as the agent appears to induce natural epizootics and extensive larval mortality (Hajek *et al.* 1996b). It is also significant to note that comparisons of laboratory and field host ranges have shown that the ecological host range is much narrower than the physiological host range that was indicated in laboratory study (Hajek *et al.* 1996a).

A facility for mass production of NPV has been established by USDA Forest Service at their Otis Airforce Base in Maryland. Here they produce the registered viral product Gypchek® sufficient to treat approximately 1800 ha per annum (Lewis *et al.* 1979). The virus has proved effective at dosages of up to 1.25 x 10^{12} polyhedra per ha applied approximately 10 days apart for control of second instar larvae (Cunningham, 1995). A recent advance in formulation using stilbene optical brighteners resulted in an increase in efficacy by increasing both absolute mortality and speed of kill (Webb *et al.* 1993).

Although virus has proved effective, the wide availability of *B.t.* and improvements in application technology and formulations have ensured that the bacterium has become the dominant microbial agent for practical field use. Reflecting the greater susceptibility of early instar larvae and the need to target their feeding sites, considerable effort has gone into optimising spray delivery (Dubois *et al.* 1994). Within the droplet size 110 µm to 163 µm, there was no difference in coverage of leaves or efficacy of applied *B.t.*. However, Dubois *et al* (1994) confirmed the need to target first and second instar larvae. Recent work by Payne *et al* (1996), comparing NPV and *B.t.* application using different droplet sizes indicated that more effective control was achieved with smaller droplets and that further improvements could be expected if sprays were applied in higher wind speed conditions with greater air turbulence in the canopy. Overall, gypsy moth continues to pose problems in both Europe and North America and is subject to spray applications, predominantly with *B.t.* It is also clear that the improvements in spray application and formulation, including the use of optical brighteners, will continue to increase the efficacy of microbial insecticides against this major forest pest.

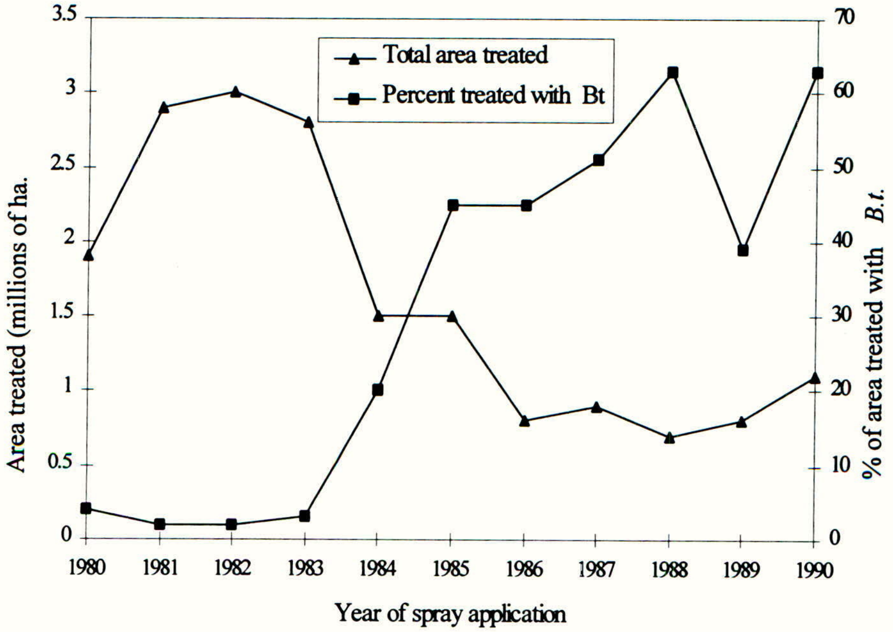
European pine sawfly in Europe and North America; Baculovirus

Use of NPV against European pine sawfly, *Neodiprion sertifer*, provides the benchmark against which all other microbial insecticides should be measured. *N. sertifer* is widespread in northern temperate pine forests and causes extensive defoliation of mature needles, especially on younger pine trees. Tree mortality is, however, extremely rare. A NPV disease, which typically for sawflies affects only the midgut epithelial cells, is often naturally associated with epidemic populations of the sawfly and usually results in disease epizootics (Cunningham & Entwistle, 1981). The principal characteristics that favour use of this microbial agent for management of the sawfly are the rapid production of secondary inoculum arising from breakdown of infected gut cells and their transfer during defensive regurgitation by the larvae, combined with the gregarious larval feeding habits. This facilitates very rapid development of secondary infections and, hence, early mortality of whole colonies of the sawfly. Research in both USA and Europe, especially in the UK, has indicated that field dosages of only 5 x 10^9 polyhedra will result in 100% kill, provided the virus is applied efficiently to the foliage (Evans, 1990). This remarkably low dosage is equivalent to the virus productivity from only 50 larvae, in marked contrast to lepidopteran NPVs that are generally at larval equivalent rates of from 100 to several hundred. In addition, Doyle & Entwistle (1988) demonstrated that it was possible to apply the NPVs of pine beauty moth and pine sawfly in the same tank mix, even though the numbers of polyhedra per average sized droplet for the two viruses was 20 and 0.5 respectively, indicating that many droplets did not contain sawfly virus at all.

TRENDS IN USE OF MICROBIAL INSECTICIDES IN FORESTRY

The attributes of forests described earlier have certainly aided development of microbial insecticides for specific forest use and, through technology transfer, for wider application in other crop types. Indeed, Navon (1993), in reviewing the use of *B.t.* for control of lepidopterous pests, concluded that it is *generally more effective in forest than in agriculture* and cited several of the attributes in Table 2 in support of this contention.

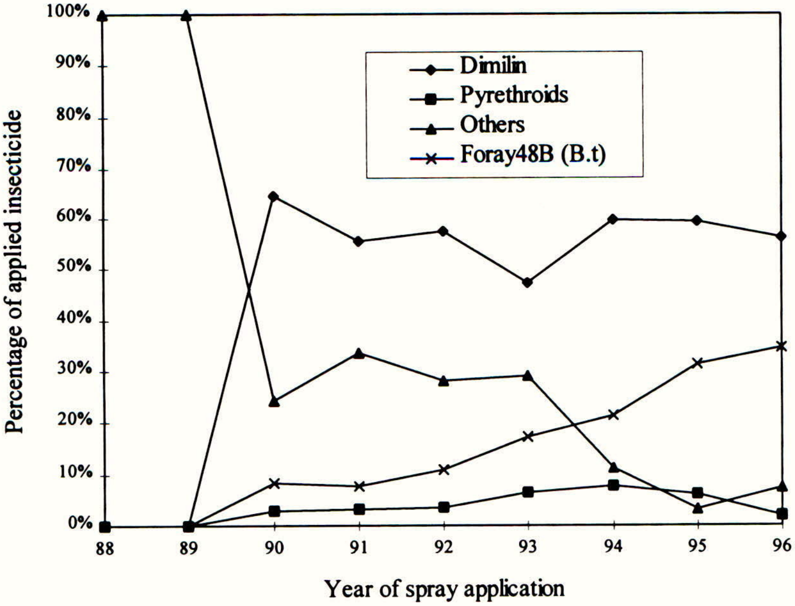
Figure 1: Trends in use of *Bacillus thuringiensis* against spruce budworm, *Choristoneura fumiferana* in eastern Canada (from Van Frankenhuyzen (1993))



Although advances in technology have undoubtedly made microbial insecticides more competitive with chemical insecticides for forest pest control, it is the environmental benefit that has had the greatest impact on their increased use. Specificity and the ability to use the products in both rural and urban "forests" has brought wider public acceptance of microbial insecticides, even though there may be a financial premium arising from their use (Navon, 1993). The coincidence of improved production and formulation techniques with the rise of the green movements in the 1980s has resulted in a major shift in usage patterns of insecticides in North America and, more recently, in eastern Europe, the two areas with the greatest areas requiring active intervention.

These trends are illustrated in Figure 1 for eastern Canada (Van Frankenhuyzen, 1993) and in Figure 2 for eastern Europe (Belarus, Lithuania and Poland) (Mr J Sanders, Abbott Laboratories, personal communication).

Figure 2: Trends in use of *Bacillus thuringiensis* (Foray 48B®,Abbott Laboratories) versus chemical insecticides in Eastern Europe (percentage share of insecticides applied).



In both regions there has been a major increase in the proportion of areas treated with *B.t.* at the expense of chemical insecticides such as fenitrothion (Canada), diflubenzuron and pyrethroids (eastern Europe). This trend is expected to continue in the future with at least 50% of areas being treated with microbial insecticides, of which *B.t.* will remain dominant.

Further improvements in technology of spray application allied to matching the formulation of the microbial agent to the specific biology of the target pest and its feeding substrate will continue to favour the forest environment, where the relatively high tolerance of damage remains one of the most potent attributes. Evans (1994) has combined the characteristics necessary to ensure success in use of microbial insecticides in a *Control Window* that summarises the principal characteristics that must be considered in their successful use. It is pertinent to note that the model was developed in studies of defoliating Lepidoptera and Hymenoptera on coniferous forest crops.

CONCLUSIONS

Although there are many successes in use of microbial insecticides in forestry, there are still constraints, common to all crop types, in their use. Costs are still relatively high and, even in forestry, the slow mode of action in certain situations may prove a problem. However, the overall conclusion must remain that application of microbial preparations in forestry remains a viable, environmentally friendly method of pest management. *B.t.* will continue to dominate the forestry market in the immediate future because of wide availability, competitive price and increasing reliability. Some further work is needed to improve consistency of results over wide ranges of forest canopies and target insect biologies. Viruses will continue to be successful in forestry, having the major advantages of specificity and, at least in temperate zones, a considerable degree of persistence between generations. Constraints in production will continue, with reliance on rearing host insects in laboratory conditions, but, as shown by gypsy moth, economies of scale can be achieved. Fungi have been under-exploited but with increased knowledge and the realisation that their specificity in the field is much higher than suggested by laboratory studies, may have considerable prospects.

The future, therefore, remains bright for use of microbial insecticides in forestry and there will continue to be a place for these agents in the spectrum of tools for pest management in the forestry environment.

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