FORMULATION OF BACTERIA, VIRUSES AND PROTOZOA TO CONTROL INSECTS

3

H. Denis Burges and Keith A. Jones

CONTENTS

Formulation of Microbial Biopesticides: Beneficial microorganisms, nematodes and seed treatments. Edited by H. D. Burges. Published in 1998 by Kluwer Academic Publishers, Dordrecht ISBN 0 412 62520 2.

3.1 INTRODUCTION

Three major groups of insect pathogens – bacteria, viruses and Protozoa – have one feature in common: they normally infect or poison the insects perorally when they contaminate insect food. Thus to control an insect infestation they must usually be eaten, and need to be spread evenly over the food environment and be hardy enough to stay alive whilst waiting for the insect to eat them. Formulation is vital to ensure the efficiency of these processes. The first task is to make the pathogen-treated food palatable and able to cover the food evenly. On ingestion, the pathogens invade the haemolymph and target tissues, grow within and kill the insects; then in nature survival stages of the pathogen are protected inside the cadavers, which eventually disintegrate. In contrast, survival stages of pathogens produced for insect control have to be applied as sprays or solids spread thinly over the surface of the insects' food, and so are more exposed to the environment. Thus, formulation must replace the natural protection afforded by insect cadavers. The chosen pathogens need to be highly virulent to kill by the smallest possible dosages, a requirement also facilitated by formulation. The modes of action and the main features of the three peroral groups of pathogens are summarized in Table 3.1.

Among the spore-forming bacteria, the crystal-formers also produce durable crystals of toxic protein, which make them virulent and rapidly lethal (Table 3.1). From the formulation aspect, these crystals behave in some respects similarly to stomach poison insecticides. Spores and crystals are eco-

Table 3.1 Mode of action of bacteria, viruses and Protozoa that infect perorally

Main pathogens and hosts	*Mode of action*
Bacteria	
Lepidoptera, mosquitoes, blackflies (*Simuliidae*), some beetles	Proteinous toxin crystals (0.5 × 1.0μm) of *Bacillus thuringiensis* and *B. sphaericus* dissolve in gut, destroy epithelium, and stop insect feeding, while spores (0.8 × 1.7μm) germinate as pH falls, replicate, penetrate gut wall, cause septicaemia, eventually sporulate, each spore with a crystal
Protozoa	
Lepidoptera, beetles, locusts and other Orthoptera	Typical genus *Nosema*. Spores (3 × 5μm) germinate in gut and live in cells of gut, fat body and other tissue, replicate; eventually sporulate as organs are destroyed. Often chronic, debilitating. Transmitted by fouled food, cannibalism and transovarially
Viruses	
Lepidoptera, a few beetles	Mainly baculoviruses with virions protected in protein to form occlusion bodies. Comprise nuclear polyhedrosis viruses (with many virions/large polyhedral body 0.5–1.5 μm in diameter) and granulosis viruses (with one virion/small body, 0.5 μm). Infection and reproduction as for Protozoa but often virulent with acute infections

nomically manufactured by fermentation. For all these reasons, the spore- and crystal-forming bacterium *Bacillus thuringiensis* is used in the leading products now available, accounting for more than 90% of the microbial insecticides currently used. In 1990 its annual sales worldwide were estimated to be up to US$80 million (van Frankenhuyzen, 1993). Sales are projected to more than double by the year 2000 (Bernhard and Utz, 1993). Rigby (1991) lists 12 manufacturers and 55 current commercial products and their uses. Compared with most chemical pesticides, the toxin is somewhat unstable, but it is much more stable than the distributive stages of most pathogens used for pest control.

The biology and ecology of *B. thuringiensis* and its toxins, their mode of action and current status of research and application, have been reviewed in a book edited by Entwistle *et al.* (1993) and the economics have been outlined by Baker and Henis (1990). Other entomopathogenic bacteria have only minor uses, including *B. sphaericus* for mosquito control in developing countries (Jones *et al.*, 1993b).

Some Protozoa have durable spores, producible only in living insects, but their virulence is relatively low (Table 3.1). There has been little practical application, using mainly *Nosema locustae* to control grasshoppers (Capinera and Hibbard, 1987).

Baculoviruses are highly virulent and produce a tough inclusion body, a survival stage comparable to a spore (Table 3.1). In these bodies, virions are protected in an inert protein matrix. Viruses are used much less than *B. thuringiensis*, but are applied to over a million hectares of land annually (Jones *et al.*, 1993b). Winstanley and Rovesti (1993) list 31 commercial viral products produced by 1992 and able to give 85% control of 18 different pest species; all are baculoviruses used largely due to their safety for humans and the environment. The biology, production and application of baculoviruses have been described in two volumes edited by Granados and Federici (1986a, b) and in a recent book by Hunter-Fujita *et al.* (1997a). A few other viruses with protective inclusion bodies have been employed for insect control, but not commercially (Tiong and Munroe, 1976; Katagiri, 1981; McGuire *et al.*, 1991).

The current dominance of *B. thuringiensis* in the microbial insecticide market has resulted

in an advanced and diverse liquid and solid formulation technology, which will be used as the leading example in the present chapter. Production of the viruses in the living insect, and the moderately robust nature of the virus protected in the proteinous inclusion bodies, have resulted in data that extend this technology. There is little information about the Protozoa.

The survival stages and toxin crystals of entomopathogens are particulate (Table 3.1), so formulation must be designed to handle particles, not solutions. Their targets are mainly insects that feed on open leaf surfaces on land or that filter out fine particles of food from water. Thus, single pathogen bodies, or clumps of many in an optimum size range of 5–200 μm, need to be spread evenly over leaves or suspended evenly in water. They can be formulated dry, or as a suspension in a liquid. More information about the target insects and their feeding environments will be given in section 3.3 (Table 3.4 in section 3.3.1 and Table 3.19 in section 3.6.1 below), where the different types of formulations made with pathogens are described – after a consideration of production technology.

3.2 OPTIMIZATION OF PRODUCTION AND STABILIZATION FOR FORMULATION

The active ingredient in a formulation – the pathogen – needs to be produced either *in vitro* by fermentation, as with *B. thuringiensis*, or *in vivo*, as for Protozoa and viruses. An exception is the case of transgenic plants, which form the toxin of *B. thuringiensis* systemically within their tissues (section 3.5). Within the limits of the biological requirements of the microorganism, as well as the economic reality of achieving the most efficient production for the lowest cost, it is necessary to tailor the production process to suit the needs of subsequent formulations. This tailoring aspect will be the main theme of this section. *B. thuringiensis* is used as an example of production by *in vitro* fermentation. The baculoviruses are used as an example of *in vivo* production.

3.2.1 FERMENTATION OF *BACILLUS THURINGIENSIS*

At the end of a *B. thuringiensis* fermentation, the formulator is faced with a complex mixture of toxin crystals, spores, medium solids and solutes with a high moisture content. There are two alternative technologies, semi-solid and deep liquid.

In semi-solid fermentation, bacteria are grown on a sterilized, wetted, aerated, friable, solid substrate, illustrated by Dulmage and Rhodes (1971). This may be inert, e.g. vermiculite, or itself a nutrient, e.g. wheat bran. When most cells have completed sporulation, although a few vegetative cells will remain capable of further growth, fermentation is stopped and the technical product stabilized by drying with hot air to a moisture content of 5% or less (section 2.2.1); this is critical for good storage. Caking of the substrate creates problems during fermentation, hinders even aeration of the fermenting mass and makes drying difficult. After drying, the solid substrate itself acts as a carrier–extender and the technical product is ground – an energy intensive process – to the required particle size. Grinders that do not produce excessive heat must be used. Substrates that swell when wetted for spraying, e.g. bran, must be ground fine enough to prevent swollen particles from blocking spray nozzles. Additives are mixed with the technical product to make dusts and granules (sections 2.3.1a, 2.3.1b, 3.3.1) and wettable powders (sections 2.3.1c, 3.3.4). The physical problems of aeration, drying and grinding make scale-up of semi-solid fermentation difficult. Prevention of nozzle blocking is an awkward problem and technical products made from semi-solid media are more suitable for the manufacture of dusts than wettable powders. Little modification to semi-solid fermentation can be made to improve formulation. The method is now

Table 3.2 Production of a *Bacillus thuringiensis* technical powder by centrifugation and spray-drying fermenter broth (adapted from Lisanky *et al.*, 1993)

Ingredients (Appendix I)	*Percentage (w/w)*	*Function*	*Cost ($/kg product)*
Centrifuged broth	97	Agent	28.00
Aqueous 40% Bevaloid 211	2	Dispersant	0.03
Gum arabic	1	Drying protectant	0.03

Preparation

1 Stabilize broth by adjusting pH to 4.1 with 5 M H_2SO_4
2 Spin sample of broth in bench centrifuge at $\geq 8000\,g$; supernatant should be clear*
3 Sample for later tests on pH, solids, microbiological purity and cell count
4 Centrifuge at $\geq 8000\,g$ and $< 35\,^{\circ}C$ with continuous stirring/agitation of the feed
5 Sample concentrate as for step 2†
6 Add 2% dispersant and 1% gum arabic or 5% lactose slowly while stirring for 5 min, ensuring that no lumps remain
7 Spray-dry‡, using a rotary atomizer, to 6–7% water content; lower levels may cause loss of potency; higher levels will allow caking and contaminants to grow
8 Sieve to $< 50\mu m$ and mill oversize material at $< 35\,^{\circ}C$
9 Bioassay potency of product. The relationship between potency of a 20 g/l solids broth and that of the technical powder is about ×5 for ssp. *kurstaki* and ×3 for ssp. *israelensis*

* If cloudy, cool sample to 5 °C to clear possible interference by antifoam and check microscopically. If there are > 1–2 spores and crystals per field, add 4 ml/l Superfloc C 577 to bulk broth, but with ssp. *israelensis* dilute bulk broth to 1.5% with cold water, then add 6 ml/l of Superfloc to flocculate.
† If the concentrate is not to be spray dried immediately, stabilize by adding 2 g/l potassium sorbate and adjust pH to 4.1 ± 0.1 with 5 M H_2SO_4, or cool to 5 °C.
‡ Spray driers vary. Check residence time of product in drier as over-exposure to heat will lower potency. Typical temperatures are inlet 180–210 °C and outlet 85 °C. Experiment with temperatures and throughput rates.

used for *B. thuringiensis* only in developing countries on a relatively small scale.

Aerated deep liquid, in contrast, is easy to scale-up in large fermenters of 60 000 l capacity or more, of which some 97% is water (Dulmage and Rhodes, 1971). At the end of fermentation, this is concentrated by centrifugation or filtration through bags to a slurry containing about 80% water. Centrifugation may result in some loss of the smallest crystals. The technical fermenter slurry may be stabilized as a suspension concentrate (section 3.3.3b).

The usual alternative method of stabilizing the technical product is energy-intensive spray drying (section 4.6.2). The whole process of making a technical powder is illustrated in Table 3.2. A cook's recipe style has been used to list the ingredients and bring the process to life by describing how to use them, including some detailed but critical points gleaned from experience, so essential to practical success. Many other tables in the same style have been used in this book to illustrate formulation processes. Despite high air temperatures in the drier, spores and crystals survive due to short exposure and the cooling effect of water evaporation, although typically there is a small loss of activity. Lactose (5%) is a common alternative to the gum arabic (Table 3.2) as a protectant against the heat and may have the advantage of partial screening against sunlight in the field (Lisansky *et al.*, 1993).

As another alternative after centrifugation, a technical slurry may be stabilized and dried by lactose–acetone co-precipitation (Dulmage and Rhodes, 1971). Aqueous lactose solution and acetone, which is miscible with water, are added to the slurry and filtered. The resulting powder is washed twice with acetone, then air-dried. On a laboratory scale this is a

valuable alternative harvest technique that avoids exposure to heat and washes away water-soluble metabolites. It provides a check on possible heat damage during spray drying or on the presence of soluble toxic factors, whose action may be confused with that of the δ-endotoxin crystal in spray-dried preparations. In many fermentation media, Ca^{2+} ions precipitate β-exotoxin, so much of this toxin is removed by centrifugation. In conjunction with the right fermentation medium, the co-precipitation method of harvest usually gives a friable powder easy to formulate, but it has not been used on a commercial scale.

After optimization of media to obtain maximum yield, there are sometimes formulation problems that may require modifications to be made. For example, it is sometimes difficult to grind spray-dried powders down to particles less than 20 μm diameter and friability may need improvement. Also, some powders are very hygroscopic, which may require changes of ingredients. All dry products absorb water from moist air and should be stored in water vapour-proof packs. Aleshina *et al.* (1986) claim that addition of KCl to the fermentation medium not only improves insecticidal activity but also physico-chemical properties (wetting and stability of the formulation).

3.2.2 PRODUCTION OF BACULOVIRUSES

The most cost-effective method of producing baculoviruses is in living insects. They can be produced in cell culture on a small scale, but there are problems in scaling-up production. Currently, cell culture is at least $\times$ 10 more expensive than *in vivo* production (Monnet *et al.*, 1994; Weiss *et al.*, 1994), but it would have the great advantage of virtually eliminating contamination.

An example of producing virus in insect larvae is given in Table 3.3, an *in vivo* process also described by Shapiro (1986) and others. A major problem that affects formulation is limitation of the bacterial content arising from the gut of dead larvae (Podgwaite *et al.*, 1983; Grzywacz *et al.*, 1997). This must be below the upper limits set by registration requirements, but with no primary human pathogenic species present and a limit of 10^7 colony-forming units (c.f.u.)/g in some countries [and in the USA before 1983; the Environmental Protection Agency now accepts more, recently as many as 2×10^8 (N. R. Dubois, US Forest Service, Hamden, Connecticut, personal communication)]. Whilst insect debris and contaminant microorganisms can be separated from the virus occlusion bodies by techniques such as differential gradient centrifugation (Harrap *et al.*, 1977), this increases costs by $\times$4 (McKinley *et al.*, 1989). Modifications at the virus replication stage aimed at improving formulation are mainly curbs on spore-forming bacteria, such as use of antibiotics in larval food, good hygiene and stabilization of larval cadavers as soon as possible after maximum virus production or death of larvae, e.g. by deep-freezing. Bacterial invasion can be minimized by harvesting virus-infected larvae just before death (McKinley *et al.*, 1989). Ignoffo and Shapiro (1978) found that virus harvested from live insects was less virulent than that harvested from dead insects; however, D. Grzywacz (Natural Resources Institute, Chatham, UK, personal communication) found no evidence of this. Bacteria might be curbed by low-temperature incubation of large larvae, so that virus replication and cell lysis continue with much less bacterial growth (McKinley *et al.*, 1989). Vegetative bacteria can be killed by manipulation of subsequent spray-drying (Huber, 1986; A. J. Cherry, Natural Resources Institute, Chatham, UK, personal communication).

Freeze-drying, although expensive, has been the commonest method of stabilizing virus (Table 3.3; Martignoni, 1978; Shapiro, 1982; Young and Yearian, 1986). It gave the most active and most stable technical concentrate out of a number of methods compared by Ignoffo *et al.* (1976b) for *Heliothis virescens* nuclear polyhedrosis virus (NPV). Clumping during freeze-drying may create storage and

Table 3.3 Production of a technical powder (Gypchek) of gypsy moth nuclear polyhedrosis virus (LdMNPV) from *in vivo* culture in larvae (J.D. Podgwaite, USDA, Hamden, Connecticut, personal communication)

Ingredients (Appendix I)	*Percentage (w/w)*	*Function*
LdMNPV*	14–18	Pathogen
Insect body parts	82–86	Inert

Preparation

1 Egg masses of a laboratory strain (New Jersey) of the gypsy moth are held for 150 days at 6 °C to complete diapause
2 Eggs are dehaired (Cosenza *et al.*, 1963; Tardif and Secrest, 1970, modified by J. D. Podgwaite), surface-treated for 1 h (10% formalin, v/v), exhaustively rinsed and mechanically placed onto diet† (10–15 per 6 oz cup)
3 Larvae hatching from eggs are reared for 14 days at 26 °C in HEPA-filtered air chambers
4 When larvae reach early instar IV, cups are inoculated with 1 ml of a suspension containing 5×10^6 viral occlusion bodies (OB) per ml and reared at 29 °C
5 Larvae ($\geq$ 70% mortality) are harvested 14 days after inoculation and held at −20 °C until processed
6 Frozen larvae are thawed for 24 h at 4 °C and then blended (1.0 g larvae: 5 ml sterile water) at high speed for 10 s to release OB
7 Blended cadavers are poured through a 100-mesh vibrating separator (Sweco Inc., Florence, Kentucky) and two layers of cotton cheesecloth (Type II, American Fiber & Finishing Inc., Colrain, Massachusetts) to remove large body parts and urticating hairs‡
8 The concentrate is centrifuged (Sharples AS-16VB, Alfa Laval Separation Inc., Warminster, Pennsylvania); continuous flow, 60 l/h at 15 500 rev/min
9 Solids are removed, layered onto trays and frozen at −35 °C
10 Frozen solids are freeze-dried (Tri-philizer, FTS Systems, Stone Ridge, New York) for 24–36 h and then ground (Braun Model KSM2, Braun Inc., Lynnfield, Massachusetts) to a fine powder (3–4% moisture) that contains *ca* 15% (w/w) OB
11 The powder is subjected to microbiological quality assurance testing (standard plate counts, mouse injection) before packaging in Scotchpak heat-sealable water vapour-proof pouches (Kapak Corp., Minneapolis, Minnesota)
12 The technical powder is formulated (tank-mixed, Table 3.15) at the application site in either a lignosulphonate–molasses§ carrier or a commercial spray adjuvant (Carrier 038)¶

* Connecticut USA isolate; mixture of 12–15 closely related genotypes.
† Ingredients per litre final mixture: raw wheat germ (Mennel Milling, Fostoria, Ohio), 120 g; casein, industrial grade (New Zealand Milk Products, Petaluma, California), 25 g; Vitamin Mixture 26862 (Hoffman–LaRoche, Fresno, California), 10 g; Wesson Salt mixture (United States Biochemical, Cleveland, Ohio), 8 g; methyl *para*hydroxybenzoate (Kalama Chemical, Seattle, Washington), 1 g; sorbic acid (Dirigo, Boston, Massachusetts), 2 g; agar (Moorehead and Company Agar Products, Van Nuys, California), 15 g; water, 800 ml.
‡ Hairs passing the screen and cheesecloth (< 1%) float to the surface of the concentrate and are removed by aspiration prior to centrifugation.
§ Lignosite AN (Table 1.12) sunscreen 6% w/v; Mo-Mix (Table 1.7) phagostimulant/antievaporant, 12.5% v/v; Bond (Table 1.6) sticker 2% w/v; water, 85% v/v.
¶ Carrier 038 (Table 1.1).

tank-mixing difficulties, but these can usually be prevented by suitable additives (McKinley *et al.*, 1989).

The NPV cream can be mixed with attapulgite clay or other diluent and spray-dried to yield a microencapsulated formulation (Bull, 1978) as in the product Elcar (Shieh, 1989). Clay increased activity of *Spodoptera littoralis* NPV. Drying conditions are critical. Spray-drying destroyed most of the activity of *Choristoneura fumiferana* NPV (Young, 1989), while with *S. littoralis* NPV under optimum

conditions, no adverse effect has been observed (A. J. Cherry, personal communication). It reduced the shelf-life of *Cydia pomonella* granulosis virus (GV) (Huber, 1986) and a commercial product (Sandoz 406) was less stable than NPVs, even when refrigerated (Young and Yearian, 1986).

Air-drying has been used in some developing countries. For example, powders of *Anticarsia gemmatalis* NPV are made by pouring a thin layer of virus suspension onto large tables and leaving it to dry at room temperature for several hours, then scraping it off. Bacteria obviously multiply during drying, but speed of drying can be increased by fans and warm air (Moscardi, 1989; F. Moscardi, EMBRAPA–CNPSO, Londrina Paraña, Brazil, personal communication).

Lactose–acetone co-precipitation (section 3.2.1) has been used experimentally with viruses (Ignoffo and Shapiro, 1978). NPV and GV formulations mixed relatively easily into water, but some activity was lost during the process and shelf-life was poor (McGaughey, 1975; Hunter *et al.*, 1977; Ignoffo and Couch, 1981; M. A. Parnell, Natural Resources Institute, Chatham, UK and K. A. J., unpublished results).

Filtration can produce semi-purified virus and microfiltration a more purified product (M. A. Parnell and K. A. Jones, unpublished results) as can Sodium Omadine (Dubois, 1976). A recent, inexpensive, novel and experimental method of decontamination uses high pressure and temperature. A pressure of 550–580 MPa for 30–120 min at 50 °C reduced microbial contamination of an aqueous suspension of codling moth GV from 8×10^9 to 4×10^3 c.f.u. without lowering potency of the virus, with similar results with *B. thuringiensis* (Butz *et al.*, 1995); later work on GV was less promising (G. Zimmermann, Institute for Biological Control, Darmstadt, personal communication). This process may be feasible industrially, but would still need inclusion of microbial suppressants in liquid aqueous formulations.

Thus the virus technical concentrate most often needing formulation is a mixture of virus active ingredient, contaminant microorganisms which need to be prevented from multiplying, and insect debris consisting of fats, proteins, carbohydrates, etc.

3.3 FORMULATION TYPES

Bacteria, viruses and Protozoa attack perorally and are applied to control pests feeding in four habitats: foliage, soil, food stores and water. These environments present different major formulation problems. On foliage, the most intractable problem is young foliage growing rapidly – Jones and McKinley (1987) report a doubling of leaf area of cotton within a week, and even greater rates of growth were noted for cotton in Thailand (K. A. Jones, unpublished results) – so effectively diluting a surface spray coverage and necessitating frequent costly resprays. In soil, many pests feed below the surface, a difficult position to reach with particulate microorganisms because the soil is an efficient filter of suspensions applied to its surface. In stored bulks of food, it is similarly hard to place pathogens much below the surface of the bulk. In water, it is hard to maintain the microorganisms suspended for long in the feeding zone of pests such as mosquito and blackfly larvae. By far the most effective ploy that solves these major problems in all environments is to apply the active ingredient systemically to the pests' food plants, especially by means of transgenic plants (section 3.5.1). Here, we will discuss the various alternative approaches taken to provide optimum formulation of products for use in land environments. Treatment of water bodies will be described in section 3.6. The pest insects on land and key behaviour factors that influence control are illustrated in Table 3.4.

Larval behaviour, summarized in Table 3.4, determines the amount of feeding on pathogen deposits, which are greatest on upper surfaces of the leaves highest on the plants.

Table 3.4 Target insects on land, grouped primarily by habitat as it affects control by *Bacillus thuringiensis*, baculoviruses and Protozoa, and secondarily by host plant

Pest insect	*Key ecological features*
Lymantria dispar, gypsy moth, forest defoliator; *Plutella xylostella*, diamondback moth; *Pieris rapae, P. brassicae*, white butterflies, cabbage worms; *Bombyx mori*, moth, domestic silkworm	Young larvae feed singly or clustered mostly on lower surfaces of open leaves (e.g. oak, brassica), leaving upper epidermis intact. Older larvae eat through leaves, but do not burrow
Archips pomonella, small moth, leaf roller	Weaves tree leaves together with silk, also feeds between touching fruit
Choristoneura fumiferana, spruce budworm	Larva I spins silken tunnel and feeds on the surface of conifer needles until instar III, when it bores into buds
Trichoplusia ni, Sabulodes aegrotata, moths, looper larvae	Young larvae feed on under-surfaces of open cotton and vegetable leaves. Older larvae eat through the leaves. Only the largest larvae bore into tomato fruits and cabbage heads
Spodoptera spp., *Anticarsia gemmatalis, Agrotis* spp., *Mamestra brassicae, Lacanobia oleracea, Pseudaletia* (= *Mythimna*) *unipuncta* noctuid moths, cutworms, armyworms	Pests of cotton, vegetables, field crops and cereals. Young larvae as *Pieris* spp. (above). Older larvae eat through leaves, bore into growing points, hearts, fruits and stems, often at soil surface. Hide in daylight
Heliothis (=*Helicoverpa*) spp., noctuid moths, budworms, bollworms, fruitworms	Young larvae browse on under-surface of leaves, as they move up to young cotton bolls, tomato fruits and growing tips of tobacco and other crops, into which they bore while still in the early instars
Cydia (*Laspeyresia*) *pomonella*, codling moth	Larva I feeds briefly on open foliage and surface of young fruit on apple, pear and nut trees before boring into the fruit, eventually emerging to find a pupation site
Ostrinia nubilalis, moth, European corn borer	Young larvae feed on under-leaf surfaces and soon migrate to spaces within leaf whorls, silks and young corn cobs. Older larvae bore into stems and growing corn seed
Leptinotarsa decemlineata, Colorado potato beetle	Adults and larvae feed on open potato foliage and on tubers below ground
Galleria mellonella, wax moth	Larva I very mobile, settles after *ca* 6–12 h on bee-comb and forms silken tunnel. Larger larvae span many comb cells, harming brood and leaking honey

Newly hatched larvae (neonates), the most susceptible larval stage, are too delicate to chew completely though leaves. They browse mainly on the softer lower surfaces, behaviour which shields them from the heaviest pathogen deposits on the upper surfaces. These they do not reach until later in life, when they chew through the whole thickness of the leaves. Some species spin silken tunnels or weave leaves together with silk. This prevents the pathogen reaching part of the larval feeding area. Boring and tunnelling abruptly takes many species away from pathogen deposits. Some species browse extensively before tunnelling, others escape deposits by tunnelling very soon after hatching, leaving

only a short window of opportunity for these pathogens that act perorally.

Application techniques aim to maximize deposits in the areas where the most susceptible stages are found (Appendix II). All formulation types likely to be used with microorganisms are defined in Appendix III.

3.3.1 FORMULATIONS APPLIED DRY: DUSTS, GRANULES AND CAPSULES

Products for application undiluted, in a dry state, are used much less than sprays because of the difficulty in handling and generally less effective methods of application (Young and Yearian, 1986; section 2.3.1).

Details of the preparation of a dust from a technical powder are illustrated in Table 3.5. Dust can be mixed with local carriers on site to avoid transport and storage of bulky carriers. Formulation involving a sunscreen is most effective if the screen is mixed in at the liquid concentrate stage before drying to ensure that it is as close as possible to the organisms; this makes the required concentration of screen independent of the eventual degree of dilution with the carrier (section 3.7.2b). Dust is the method of choice in applying certain pathogens in particular environments. Thus, in nature, cadavers of soil-dwelling beetle larvae killed by *Bacillus popilliae* become virtual bags of spores which disintegrate and wait for fresh larvae to eat the spores. The first commercial microbial product on sale – and still used – for insect control contains this bacterium and mimics nature by formulating spores in clay to form a dust for spreading in small heaps spaced over the soil surface (Dutky, 1963). Protozoa (*Nosema*) and *B. thuringiensis* have been mixed into attractive carriers as dry baits broadcast over the soil surface against other pests, but were only briefly available commercially (part 5 of Table 3.14 in section 3.4.3,). In the storage environment, commercial dusts of *B. thuringiensis* in a wheat flour carrier are applied to the top 10 cm of stored bulk grain to control surface-dwelling lepidopteran pests (McGaughey, 1985). Newly hatched larvae feed selectively on such fine soft food particles and the germs of wheat grains in preference to the harder epidermis (H. D. B., personal communication). Dusts of granulosis virus were also applied experimentally to potato tuber stores to control *Phthorimaea operculella* (A. Farghaly, Plant Protection Research Institute, Cairo, personal communication). Nuclear polyhedrosis virus (NPV) technical powder can be mixed with a carrier for hand application to maize leaf whorls, to control pests such as *Spodoptera frugiperda.* Early work reported superior control of loopers, semi-loopers and cabbage pests with *B. thuringiensis* dusts when compared with conventional sprays (Hall,

Table 3.5 Production of a *Bacillus thuringiensis* dust (500 IU/mg)*

Ingredients (Appendix I)	*Percentage (w/w)*	*Function*	*Cost ($/kg product)*
Technical powder	0.6	Pathogen	0.18
Talcum powder	98.9	Carrier	0.18
Wessalon S	0.5	Free-flow agent	0.03

Preparation

1 In the ratio 5:4 mix technical powder (80 000 IU/mg) and silica powder, e.g. Wessalon S
2 Add mixture to carrier and mix in a powder mixer, e.g. Morton, Z-Blade or Paddle, or in a cement mixer, until all aggregates have dispersed. Preferably mixture is delivered to the carrier in an airstream while mixing. To avoid bulk transport, the above technical powder mixture (1) can be marketed for use with a cheaper local carrier, e.g. another clay or gypsum

* From Lisarshy *et al.* (1993).

1964; Sundara-Babu and Krishnan, 1970). This was believed by Yearian and Young (1978) to be due to more uniform coverage in optimum conditions, especially on the underleaf surface where young larvae generally feed and where the virus is protected from sunlight. In contrast, under windy conditions sprays perform better.

By far the greatest commercial development of dry products has been for application by specially equipped tractors (Table 2.2) to the moist environment of leaf whorls of corn plants, in order to kill corn borer larvae before they burrow into stems. *B. thuringiensis* was more effective and persisted longer when applied dry than in sprays (Lynch *et al.*, 1980). Granules are larger than dust particles and are preferred for corn borer control because they are easier to handle and apply. Clay granules can be made by spraying or mixing with technical concentrates, preferably with the aid of a sticker (Table 3.13 in section 3.4.2). This leaves the pathogens largely exposed on the granule surface. For greater protection they can be incorporated within granules by agglomeration (section 7.7.3a) and compaction (section 7.7.3c). Granules are also made by mixing into a liquid carrier, which gels and can be dried. Since this embeds the pathogens into a matrix, these products are usually termed capsules. The distinction between granules and capsules is a confused area. Logically a capsule has a protective layer around the pathogens but often matrices, which leave some pathogen exposed on the surface, are called capsules. The various terms are defined formally in the glossary (Appendix III); the critical distinctions can be regarded as:

Capsule. An inner core containing active ingredient, surrounded by a coat (e.g. alginate capsule).

Matrix. Formed by a gelling process entrapping active ingredient, but leaving some exposed on the surface, e.g. pregelatinized starch.

Granule. Carrier treated on the outside with an active ingredient, e.g. clay, sand, corn grit.

Water-dispersible granule. Made to disintegrate rapidly in water to form a spray (section 3.3.4).

In this book, we usually use the term adopted by the authors of the work cited, with the result that capsules are sometimes included in paragraphs discussing primarily granules, and *vice versa.* The embedding materials must be soluble or freely permeable in the insect gut to release the pathogens. Early work by Raun and Jackson (1966) with capsules of undisclosed composition gave good control of corn borers but not substantially better than other formulations. This work was not followed up industrially, probably due to the high cost of manufacture.

Recent work with corn starch preparations has been much more promising. Starch consists of amylose, which has a linear structure with film-forming properties of great strength and flexibility, and amylopectin, a highly branched polymer. Cooking in water causes starch to gelatinize, then to become insoluble on cooling. Food-grade starch is freely available and inexpensive, *ca* US$1.32/kg for the pregelatinized (soluble) cornflour Miragel in the USA, and US$0.12/kg for nixtamalized cornflour in Mexico. Early processing methods involved harsh chemical or temperature conditions, lethal to microorganisms. These conditions were avoided by simply mixing one part of pregelatinized starch and technical *B. thuringiensis* with two parts of cold water, drying the resultant gel, grinding and sieving, or passing through an extruder then drying (Dunkle and Shasha, 1988; Bartelt *et al.*, 1990; McGuire *et al.*, 1990; McGuire and Shasha, 1995). On the shelf, no deterioration was detected in 4 months. Benomyl was included to prevent fungal growth in the moist conditions within the corn leaf whorl (Dunkle and Shasha, 1988). On a large scale, so much water would make processing demanding. Water

can be reduced to an equal part by mixing it with substances such as isopropanol (30%) which prevent or delay gelling, producing friable granules that eliminate the need for grinding and sieving. By altering the percentage of alcohol, granule size can be controlled (McGuire and Shasha, 1992). Adding high concentrations of a salt, e.g. $CaCl_2$, or a sugar solution, e.g. molasses, allows further water reduction and so less drying (Shasha and McGuire, 1992). The sugar improves palatability and addition of Coax improves it even more (section 3.4.3), so that the bacterial content can be reduced by 75% without loss of insecticidal activity (Bartelt *et al.*, 1990). These developments tentatively push starch capsules toward commercial feasibility.

In field tests, corn starch capsules preserved *B. thuringiensis* activity in wet years better than corn grit granules and gave $\geq$ corn borer control; both gave $\geq$ control than the normal chemical insecticide (McGuire *et al.*, 1994b). While Coax greatly improved palatability and control (section 3.4.3; part 4 of Table 3.14 in that section), control was not improved by a sunscreen (sections 3.4.4b and 3.4.4e; Table 3.16 in section 3.4.4). Both types of additive were used successfully in an experimental formulation of starch-encapsulated entomopox virus against grasshoppers (part 5 of Table 3.14 in section 3.4.3).

Capsules can be made adherent to wet foliage. On drying they serve as effective bait for corn borer larvae with considerable rainfastness (McGuire and Shasha, 1992). In a comparative study, capsules were made by mixing *B. thuringiensis* powder with the surplus or waste agricultural materials gelatin, pectin, colloidal chitin, alginate or corn starch, involving a variety of reagents and additives in an aqueous phase, then drying, grinding and sieving. Chitin capsules were not acceptable, while gelatin, pectin and corn starch capsules stored well at room temperature (*ca* 26 °C) for 12 months, although no account was taken of storage moisture content. A phagostimulant (section 3.4.3) is desirable to improve palatability. Gelatin and pectin capsules resisted wash-off by rain better than the others (Morales-Ramos *et al.*, 1998). Biotrol 2, an early granular *B. thuringiensis* bait, gave control of budworms and hornworms as good as or better than conventional pesticide sprays (Creighton *et al.*, 1961).

3.3.2 SPRAY TECHNOLOGY

Spraying is the commonest method of application of bacteria and viruses. Since these pathogens act perorally, the spray target is not the insects themselves, the surfaces over which they move or the air they breathe, but rather the insects' food, for which the optimum droplet diameter is 40–100 μm (Table 2.4 in section 2.2.2; Appendix Table II.3). The different types of formulation and application equipment are described in Chapter 2; here only their efficiency and their effects on the pathogens are considered.

Most early attempts to obtain good spray cover used high-volume (HV) sprays, formulated with water as carrier. Spores, toxin crystals, insect debris, fats etc. in unpurified virus preparations are all hydrophobic, while virus occlusion bodies have pH-dependent hydrophobicity, so wetters are needed to obtain efficient mixing (sections 3.4.1, 4.3.6). The pathogens are not damaged by the high pressures in sprayers. Chlorinated water should be avoided or aired for at least 8 h before use because of its disinfectant properties. After mixing, the spray must not be left to stand for long periods before use because then the pathogens deteriorate (section 3.3.6). Since they are particulate they soon settle in spray tanks, a problem solvable only by frequent shaking or continuous agitation because HV sprays are dilute, although additives slow down settling (sections 2.3.2, 3.3.6).

A wide variety of HV sprayers have been used, the choice being dictated by the acreage, terrain and crop involved, as well as by what machines are already available locally, particularly in developing countries (section

2.2.2 and Table 2.2 in that section; Topper *et al.*, 1984; Jones, 1993). Target stages of foliar pests mostly feed on the undersurfaces of leaves, necessitating upward-and side-directed nozzles and presenting severe problems in dense canopies, particularly where sprays must be applied from above (Appendix Table II.7). This stimulated the use of low-volume (LV) and ultra low-volume (ULV) with controlled droplet application (CDA) technology, particularly for large inaccessible areas, or where water was in short supply (section 2.2.2; Cunningham *et al.*, 1975; Lewis and Yendol, 1981; Topper *et al.*, 1984; Renou, 1987; Reardon, 1991). Novel machines have been used experimentally, e.g. *Spodoptera littoralis* NPV has been sprayed through electrostatic spray machinery without harm to the virus (Jones, 1994).

Fogs have been used with viruses and *B. thuringiensis.* There are two types of fogging machines, cold and thermal. With a cold aerosol generator, cottonseed oil was a superior carrier to water containing skimmed milk for treating cotton with *Heliothis* NPV (Falcon, 1974). On single ash trees, an oil–water (1:2) carrier, applied through air-shear nozzles (11.7 μm mass mean diameter) with fan-assistance, gave better deposits of *B. thuringiensis* and control of tent caterpillars (*Malacosoma disstria*) than water alone (Johnson and Morris, 1981). With thermal fogging machines, *B. thuringiensis* has been used in pulse-jet type foggers without significant heat damage (Burges *et al.*, 1979), but early exhaust-type foggers were harmful. Water can be successfully used as carrier in pulse-jet foggers, at least in greenhouses. With water, addition of the VK2 carrier, used with chemical pesticides, should be avoided because of phytotoxicity above certain spray volumes and because it contains methanol and ethoxyethanol (P. Jarrett, Horticulture Research International, Wellesbourne and H. D. B., personal communication).

Reduction of spray volume with conventional sprayers also reduces droplet size (Appendix II, Fig. II.7), which in turn increases the importance of evaporation after the droplet leaves the sprayer (section 2.2.2). Evaporation can reduce droplet size too much, making the spray prone to drift (Appendix Table II.3), with poor impaction and settling on target foliage. Formulation with humectants and anti-evaporants may be used to slow evaporation of droplets of LV or even ULV water sprays, e.g. 0.5% of a hydrolysed polyvinyl alcohol, a refined algin material (Keltose) (section 2.2.2; Appendix Table I.4). More frequently, the rate of evaporation is reduced by oil or oil-in-water emulsions as carriers (Table 3.10 in section 3.3.3 below). Entrapment of pathogens in emulsion droplets in sprays of water has the additional advantage of reducing settling in spray tanks because the buoyant oil partly counteracts the high density of the pathogen particles. Oils used for chemical pesticides tend to be suitable because they are selected as having only low phytotoxicity, a quality which reduces the likelihood of harm to the pathogens. No harm has been experienced during the short exposure period involved in the spraying operation, but some oils tended to inactivate NPV if used for long storage of products (Table 3.6; sections 3.3.6, 3.3.3b). Some workers have observed that viruses and *B. thuringiensis* tend to move to the outside of oil drops, which could lead to undesirable clumping of the pathogens and more exposure to sunlight. Some solvents, used to lower the viscosity of spray in oil, damaged NPV but this did not show in field trials (Table 3.7).

The effects on efficacy of drop size reaching the forest canopy and of particle size within drops, which disintegrate by splashing on impact with foliage, are described by Entwistle and Evans (1985), Fast *et al.* (1985) and Fast and Reginiere (1984). With virus, less active ingredient is required in a drop than with *B. thuringiensis*, because the lethal dosage is lower. In an extensive USDA effort to develop spray machines, nozzles and specifications for agriculture, NPV caused the highest

Table 3.6 Oils (Table I.1) used with *Bacillus thuringiensis* (Bt) and nuclear polyhedrosis virus (NPV)

Oil	*Effects*
Actipron	Oil used at 20% in water for spraying NPVs of *Heliothis* spp. and *Spodoptera littoralis* and for aerial NPV sprays[1,2]
Bivert (emulsifiable crop oil)	Has been mixed with baculovirus spray[3]
Span 80 and No. 2 fuel oil compared with water + latex + dried blood	Oils better for low-volume aerial sprays of NPV, atomized more readily and effectively controlled sawflies[4]
Mineral oils: Isopar M, Isopar V, Norpar 12, Norpar 13, Norpar 15, BP mineral seal oil, BP light paraffinnic mineral oil, BP 50 spindle oil, BP 150 solvent neutral, Actipron	Storage over 18 months at 4 °C resulted in *ca*×5 reduction in potency of *S. littoralis* NPV in all except Arachis oil, in which all activity was lost in 32 months but some retained in Risella.
Vegetable oils: Seaton rapeseed oil refined and deodorized, Seaton rapeseed oil refined, Seaton soya oil refined and deodorized, Seaton cottonseed oil refined and deodorized, Shell Risella oil (L), Shell Risella oil (EL), BP Arachis oil (Seaton)	Vegetable oil mostly palatable to insects. Mineral oils, except Norpar 12, generally reduced feeding[5]. Arachis oil/Shellsol T mix used successfully in field trial (Shellsol required to reduce viscosity)[6]
Top oil (contains spreader penetrant)	Used at 45% in sprays[7]. Can be phytotoxic[8]
Volck Spray Oil (Chevron): water (1:2) in fan-assisted cold fogger	Better deposits of Bt and caterpillar control on ash trees than water alone[9]

References

1 Entwistle, 1986; Barnett, 1992; Topper *et al.*, 1984
2 Jones, 1994
3 Couch and Ignoffo, 1981
4 Smirnoff *et al.*, 1962
5 Cherry *et al.*, 1994, 1996
6 McKinley, 1985
7 Weinberger and Greehalgh, 1984
8 Smith *et al.*, 1977a
9 Johnson and Morris, 1981

Note: Vegetable oils are better than mineral oils, but need to mix mineral oil with vegetable oil in order to reduce viscosity: quality of vegetable oils can be variable.

mortalities of *Heliothis* spp. in the combination of small drop size, high drop density and high NPV concentration, particularly with increased viscosity caused by thickeners, such as 0.5% polyvinyl alcohol (Table 3.8; Appendix Table I.4; Smith *et al.*, 1977a, 1978a, b). Similar conclusions were drawn from a laboratory study of *Heliothis* NPV (Barnett, 1992).

However, lowering the spray volume has often reduced efficacy. This is attributed to poor coverage, particularly of underleaf surfaces where the most susceptible larval stages feed (Appendix Table II.7). For example, control of *S. littoralis* was poor on cotton treated with NPV by a hand-held spinning disc sprayer, compared with a knap sack sprayer with a tail boom, which directs the spray underneath the leaves (Topper *et al.*, 1984). Yearian and Young (1978) report that with NPV against *Heliothis* spp., optimum spray volumes were in the

Table 3.7 Solvents used with nuclear polyhedrosis virus (NPV)

Solvent	*Effect*
Ethyl acetate	Solvent for polymer did not harm NPV[1]
Petroleum spirit, Shellsol T, isophorone, ethyl acetate	Petroleum spirit, Shellsol T inactivated *Spodoptera littoralis* NPV, variable results with ethyl acetate. Isophorone inactivated NPV at low (LD_{10}), not at high doses (LD_{90})[2], successful in field trials[3]
Toluene, petroleum distillate, methyl–ethyl ketone combinations	Reduced activity of NPV by ×1.4 to ×8, results erratic[4]

References
1 Bull *et al.*, 1976
2 McKinley, 1985
3 Jones, 1994
4 Ignoffo and Batzer, 1971

Note: ethyl acetate and isophorone are best, but all can inactivate NPV.

range 90–400 l/ha, and yields were better (though not significantly) with increasing droplet size from 50 to 200 μm, partially controllable with thickeners (Table 3.8). Yet inaccessibility of targets and the need to reduce operator costs make volume reduction essential, thus much research has been directed to improving LV and ULV formulation (section 3.3.3).

Tracer dyes, selected for their lack of harm to microorganisms and insects (Appendix Table I.9), are added to microbial formulations in trials to assess spray coverage (Morris and Moore, 1975).

Table 3.8 Thickeners (Table I.4) used with aqueous *Bacillus thuringiensis* (Bt) and nuclear polyhedrosis virus (NPV) sprays

Thickener	*Effects*
Cargill Insecticide Base Concentrate (stabilized molasses), Dowanol TPM	Increased drop deposition[1]
Carboxymethylcellulose, Kelzan (xanthan gum)	Deposits of Bt and control of spruce budworm increased[2]
Hydroxypropylmethylcellulose, sorbitol	Compatible with *Spodoptera littoralis* NPV, no anti-feedant effect[3]
Molasses and sugar solutions (thickener, anti-evaporant)	In NPV tank mixes. Increase deposits on row crops and forests[4]
Nalcontrol, Bivert	Deposit and drop size increased with Bt and NPV. Form polymers or invert emulsions which reduce evaporation. Effect of Nalcontrol minimal[5]

References
1 Maksymiuk and Neisess, 1975
2 Morris, 1977a,b
3 Jones, 1988a
4 Young and Yearian, 1986
5 Couch and Ignoffo, 1981

Note: *Best, molasses.*

3.3.3 LIQUID FORMULATIONS FOR SPRAYS

3.3.3a Efficacy About half of the present proprietary *B. thuringiensis* products on the market are oil-in-water emulsions of ever-increasing efficacy. Larvae of some insect species vomit after rapidly eating a high dose, resulting in an interruption of feeding and possible escape from death after the first poisoned meal. Spruce budworm are less likely than these species to escape from large doses in this way. van Frankenhuyzen (1990) and Payne and van Frankenhuyzen (1995) concluded that the key to the success of *B. thuringiensis* treatment of the spruce budworm in the forest environment is to use ULV with highly concentrated formulations to deliver a lethal dose in the first spray droplet encountered by a feeding larva. A lethal droplet for larva VI on coniferous (*Pinopsida*) leaves has a diameter of 80 μm at 95 billion international units (IU) per litre, stronger than any concentrated flowable yet on sale (van Frankenhuyzen and Payne, 1993). The droplet should be lethal because the crystal toxin rapidly inhibits feeding and makes larvae irritable so that they wander. It is thus some time before a sub-lethally dosed larva can feed again and eat more toxin. The residual toxicity half-life of high potency products was 1–2 days under rainy conditions (van Frankenhuyzen and Nystrom, 1987). Thus additives that increase persistence (sections 2.2.3, 3.4) not only increase the chance of further dosage and death when larvae start to feed again, but also render timing of *B. thuringiensis* sprays less critical in terms of bud and insect phenology. Additives also reduce adverse effects of post-spray precipitation, two constraints believed to contribute to inconsistent efficacy.

The lethal dose for a larva has been measured by placing precision droplets individually with single larvae on leaves in

Table 3.9 Production of *Bacillus thuringiensis* water-based flowable concentrate (adapted from Lisansky *et al.*, 1993)

Ingredients (Appendix I)	*Percentage (w/w)*	*Function*	*Cost ($/1 product)*
Fermentation solids	10	Pathogen	2.80
Bevaloid 211	3	Dispersant	0.03
Veegum	0.45	Suspender	0.05
Xanthan gum	0.06	Suspender	0.03
Surfynol TG-E	0.5	Dispersant	0.06
K sorbate	0.2	Fungistat	0.03
5 M H_2SO_4 to pH 4		Bacteriostat	0.03
Water to 100%		Carrier	0

Preparation

1 Centrifuge broth as in steps 1–4 of Table 3.2, concentrating enough to ensure meeting required potency of marketed product
2 Stabilize concentrate by adding 2 g/l potassium sorbate, adjusting the pH to 4 with 5 M H_2SO_4 and adding 3% aqueous Bevaloid 211, mixing for at least 10 min in a paddle stirrer until sorbate dissolves
3 Bioassay stabilized concentrate and adjust with water to desired concentration for market
4 Dry mix enough Veegum and Xanthan gum powders in the ratio 7.5:1 to make a 10-fold strength pre-gel, then slowly add to water while homogenizing with a Silverson high shear mixer at high shear and $< 60\,^{\circ}C$ for 30 min or until fully hydrated
5 Add pre-gel to concentrate (3). Mix at low shear
6 Check pH is 4. Adjust with 5 M H_2SO_4 or NaOH
7 Add 0.5% Surfynol TG-E last. Stir well, avoiding incorporation of air
8 Dispense into containers with continuous gentle stirring

Table 3.10 Production of oil-based flowable concentrate with *Bacillus thuringiensis* ssp. *kurstaki* for ULV application undiluted (Lisansky *et al.*, 1993)

Ingredients (Appendix I)	*Percentage (W/W)*	*Function*	*Cost ($/1 product)*
Technical powder	24.0	Pathogen	6.72
Edelex 13 oil	74.4	Carrier	1.14
Bentone 38	1.2	Suspender	0.09
Propylene carbonate	0.4	Activator	0.05

Preparation

1 Mix suspender into a ×10 strength pre-gel for 45 min in 10% of the oil at maximum shear in a high-shear mixer. Add activator and suspender (ratio 1:3, neat) rapidly while mixing at high shear. A very thick gel forms in 5 min. Move head of mixer around in mixing tank to ensure uniform mixing
2 Transfer gel to main mixing tank
3 Use remaining oil to wash residual pre-gel into main mixing tank. Mix oil and pre-gel for 10 min to ensure even mixture
4 Re-grind technical powder if necessary to achieve a particle size of $< 50\mu$m. Mix in powder of minimum potency of 80 000 IU/mg in batches until all lumps disperse
5 Filter through 125 μm sieve
6 Dispense into containers

the laboratory and retaining only the larvae that eat all their droplet. Calculations from these data to give droplet sizes for the field are only a guide, which must be tested in field trials. There are a number reasons for this uncertainty. Oil droplets impinge better onto leaves than water droplets (Appendix II, Fig. II.6), possibly because evaporation from water creates a hard wall on the droplet making it more prone to bounce-off (Sundaram *et al.*, 1993). Aston (1989) found a disproportionate partitioning of toxin crystals into droplets formed by spinning disc rotary atomizers in proportion to the square of droplet diameter, rather than the cube as might be expected, an effect also reported with NPV inclusion bodies by Killick (1990). In addition, 40–70% of the crystals appeared to be lost from a droplet during its formation (Aston, 1989), while increasing pressure through hydraulic nozzles in the field decreased biological effectiveness – even though plant coverage increased – possibly as a result of a greater loss of crystals as shear forces increased (Smith *et al.*, 1977b). Aston (1989) reported yet another factor, feeding avoidance, which increased as the closeness of droplets decreased. A consensus of literature on the biological effectiveness of *B. thuringiensis* spray deposits suggests an optimal guideline of 90 mm volume median diameter droplets of high potency product at 20 droplets/cm^2 on flat, as opposed to needle-shaped, leaves.

Suspension concentrates are ideal for preparing concentrated products and they can be formulated ready to use (illustrated in Tables 3.9 and 3.10). This started a remarkable chain of cost reductions and increase in use of *B. thuringiensis* for forest pest control. Viscosity, kept high by the particulate pathogen and accompanying solids, is a limiting factor. This must be low enough for easy pumping and application, *ca* 1500 centipoises for all storage and use temperatures. Any increase in volume should be made by adding proprietary formulant carrier to avoid altering the product viscosity. In the 1980s, potency of products was at least doubled by minimizing the solid content of fermentation media to reduce viscosity. In 1985–87, trials with ULV for aerial application against the spruce budworm achieved good control of moderate infestations using only 21 (final volume) of

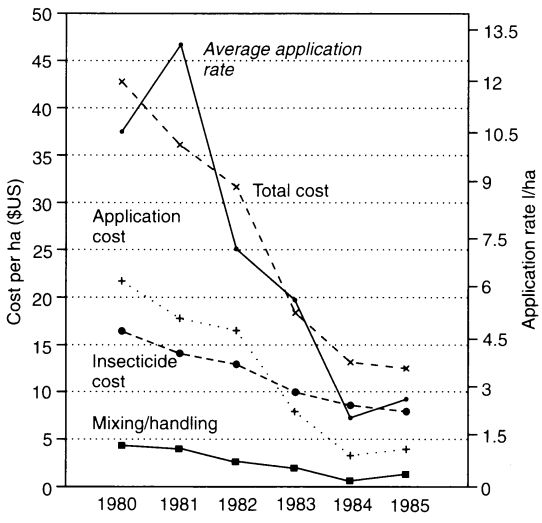

Figure 3.1 Cost components for *Bacillus thuringiensis* control of spruce budworm in forests in Maine, USA (from Cibulski *et al.*, 1993).

spray/ha (at least 22 × 109 IU/ha). Some products were supplied ready formulated to spray, requiring only the addition of the Chevron sticker (0.06%) at the airfield. This minimized spraying costs by eliminating mixing time and maximizing payload of the spray aircraft (Valero, 1989). Costs have been further reduced by transport ready-to-use in bulk tankers. The net result of these improvements arising from advances in formulation has been much lower costs, the component for application falling from highest to second highest (Fig. 3.1). In 1990, more than 70% of all North American forest Lepidoptera control sprays were made with *B. thuringiensis* compared with less than 5% in 1981 (Cibulsky *et al.*, 1993).

3.3.3b COMPOSITION

B. thuringiensis liquid suspension concentrate is formulated from ex-fermenter slurry (section 3.2.1). It avoids the cost of spray-drying and the measurable loss of activity that occurs at that time. Minimal solids need to be added to the slurry, so it has the advantage of low bulk and small particle size, because particles are not aggregated by drying.

The formation of emulsions from the aqueous concentrate improves stabilization of the physical state of a product. It reduces sedimentation of particles during storage and in the spray tank, because the buoyancy of the oil counteracts the high relative densities of the particles. In general, stabilization of activity in aqueous emulsions (Table 3.9 in section 3.3.3a) is more difficult and shelf-life is shorter than with dry products. It mainly involves the prevention of microbial growth and the action of enzymes, most of which have alkaline or near neutral optima. It is achieved by lowering the pH of raw ex-fermenter *B. thuringiensis* material to *ca* 4, and by adding preservatives such as xylol (now discontinued, section 3.7.2c, Appendix Table I.8), sugar concentrates or antibiotics, and preservatives common in the food and cosmetics industries (sections 4.6.6b, 7.6.3; Appendix Table I.8). Suitable preservatives include sodium benzoate, benzalkonium chloride, sorbic acid and proprionate. On a small scale, Ejiofor and Okafor (1991) added only molasses and palm olein. They claimed no loss of activity after 2 years in storage at 34 °C. Xylol was successfully used in early *B. thuringiensis* products, and no deterioration was detected by bioassay with *Galleria mellonella* in an exceptionally stable ssp. *galleriae* oil-in-water emulsion during storage at 2–5 °C for 18 years (H. D. B. and P. Jarrett, personal communication). Lacey (1985a) exposed *B. sphaericus* in buffer solutions to pH levels from 3 to 10.8. The best preservation of insect activity and spore viability, 308 days, was at 4 °C and neutral pH. At 4 °C, both values remained relatively high for 3 months at pH 3–10. At pH 10.8, activity was lost in 1 week but spore count remained high. Decline was faster at 21 than at 4 °C, the difference being significant for activity in 2 months at neutral pH (see also section 3.3.6).

In general, with both species of bacteria, manufacturers have difficulty in maintaining

good storage of formulated products containing water for longer than 18 months without refrigeration. Generally, oil-based concentrates are more stable than those containing water, e.g. 2 as against 1.5 years at 25 °C (Devisetty, 1988).

Occluded viruses can be very stable in water when purified. For example, *S. littoralis* NPV was stored at room temperature in darkness for 8 years without loss of activity (McKinley, 1985). However, water-based formulations have rarely been marketed, except in developing countries, because of the difficulty of curbing growth of contaminants in less pure preparations. Growth is easily arrested by drying in wettable powders (section 3.3.4). However, currently there is more interest in developing flowable concentrates and two are at present on the market (R. Georgis, ThermoTrilogy, Columbia, Maryland, personal communication).

In contrast to emulsions, formulation of a concentrated bacterial or virus product by suspension of finely ground dry technical powder in oil alone (Table 3.10 in section 3.3.3a) increases sedimentation because of the relatively larger size of particles and the low density of the oil carrier. However, this problem can be reduced by grinding the particles very finely, so that they form a colloid when suspended. Oil has the advantages of low viscosity and minimal evaporation during spraying, as well as easier stabilization of activity due to low moisture content, the moisture content of the oil itself being removable by heating before use. Table 3.6 and Couch and Ignoffo (1981) describe some oils that have been used with both *B. thuringiensis* and viruses. Vegetable oils are preferable to mineral oils for spraying because they are less phytotoxic and possibly more palatable. However, freeze-dried *Spodoptera littoralis* NPV stored equally well at 26 °C in a mineral oil alone and a plant (Arachis) oil for 15 months, then rapidly deteriorated in the Arachis oil to only <1–16% activity after 32 months, compared to >90% in the mineral oil (Cherry *et al.*, 1998).

3.3.4 WETTABLE POWDERS AND WATER-DISPERSIBLE GRANULES

Prototype and early *B. thuringiensis* and NPV commercial products were wettable powders, because they are relatively easy to produce. It was soon realised, however, that these formulations have two disadvantages: difficulty with mixing into water and comparatively large particle size.

Wettable powders consist of technical powders (Table 3.2 in section 3.2.1 and Table 3.3 in section 3.2.2) plus additives to make them readily miscible with water and stable during storage on the shelf. Details of formulation with *B. thuringiensis* are illustrated in Table 3.11. Up to 80% of the product can be technical powder (section 3.2), the remainder being fillers (Appendix Tables I.1.2, I.1.3), surfactants (Appendix Table I.5) and dispersants (Appendix Table I.4) included to improve application and handling. The ratio of pathogen to filler is critical. The filler maintains flowability and prevents agglomeration of hydrophobic pathogen particles during storage, which would reduce wettability and clog spray nozzles (Couch and Ignoffo, 1981). The filler (Table 3.11) can be the same as that in powders applied dry (section 2.3.1) but it must be hydrophilic and disperse quickly in water as a uniform suspension; *ca* 33% lactose, e.g. in skimmed milk (which contains 50% lactose), has been used very successfully as filler. The insecticidal activity of any toxin that becomes bound or adsorbed to filler clays is retained and sometimes enhanced (Tapp and Stotzky, 1995). With virus, a wettable powder of *S. littoralis* NPV contained 50% freeze-dried virus-infected insects, 30% Speswhite china clay (English China Clays Ltd, Stoke-on-Trent, Derbyshire) plus 20% of a 1:1 mix of a synthetic silica (Neosyl) as dispersant/filler and a surfactant (Etocas 30). The silica was included to prevent clumping as well as to aid flowability (McKinley *et al.*, 1989). Formulation as a wettable powder improved short-term (15 month)

Table 3.11 Production of wettable powder with *Bacillus thuringiensis* (adapted from Lisansky *et al.*, 1993)

Ingredients (Appendix I)	*Percentage (W/W)*	*Function*	*Cost ($/kg product)*
Technical powder	20	Pathogen	5.60
Kaolin China Clay	75.25	Carrier	0.44
Wessalon S	0.75	Free-flow agent	0.03
Bevaloid 116	2	Dispersant	0.09
Surfynol 104S	2	Wetter	0.26

Preparation

1 From the bioassayed potency of the technical powder batch, calculate percentage, *P*, of powder needed for required potency of marketed powder
2 To *P*% technical powder* add 0.75% (by weight of final product) silica powder, e.g. Wessalon S, 2% dispersant and 2% wetter †in a powder mixer (e.g. Morton, Z-Blade or Paddle). Mix at $< 35\,^{\circ}C$ until all silica aggregates are dispersed
3 Add (100-*P*-4.75)% carrier. Mix thoroughly at $< 35\,^{\circ}C$

* If the fermentation ingredients have not conferred enough stickiness to the product, a sticker (e.g. maize dextrin) is desirable.
† Alternative wetters are Air Products Acetylenic Surfactant S485, Tween 20 and Montanox 80.

survival of the virus in store but had no long-term advantage over unformulated technical powder (Cherry *et al.*, 1998). When mixing a spray, it is desirable to wet the powder with a little water, then pre-mix to make a smooth paste or cream before adding to the spray tank. Surfactants should normally be either a solid incorporated in the on-shelf product, or a solid or liquid added to the tank-mix. Exceptionally they can be adsorbed to the surface of one of the other ingredients, as was done with Etocas 30 by McKinley *et al.* (1989). However, adsorption to a solid will increase the amount of time and agitation required to wet and suspend the powder in water. Dispersants ensure that particles of the technical product do not attract each other and tend to distribute uniformly in a water column without settling.

Water-dispersible granules have been recently introduced to improve handling and mixing, and to reduce the amount of fine dust that puffs up into the air. Particles are held together by binders (Appendix Table I.4), which may also serve as stickers during application. In contrast to powders, the granules mix instantaneously into water where dispersants (Appendix Table I.4) in the granule rapidly release particles on stirring. Dispersants include low-foam surfactants (which may later function as wetters) and water-soluble additives such as sugars (which later act as phagostimulants). At the end of processing a free-flow material such as Supernat 22 is added to ensure that the granules pour easily and do not compact. Finally, light sieving may be needed to remove dust. The extra processing and additives may increase manufacturing time and costs, but this may be offset by the useful dual functions of some of the additives and time savings when mixing spray.

During storage, *B. thuringiensis* and baculovirus have the great advantage of being the most stable of the biocontrol agents. Shelf-life of dusts, wettable powders and water-dispersible granules with satisfactory moisture content exceeds 18 months, the absolute minimum time required for trouble-free commercialization. For example, most commercial leaflets quote a shelf-life of 2 years for dry *B. thuringiensis* products at a yearly average temperature not exceeding $25\,^{\circ}C$. At lower temperatures, experience has shown that powders lose little activity even after many years in storage, provided that they have been processed and stored correctly.

With viruses, most species of NPV remain infective for several years at room temperatures (Huger, 1963; Lewis and Rollinson, 1978), and tussock moth NPV for at least 5 years in a cool, dry place (Martignoni, 1978). In contrast to NPV, the GV of *Pieris brassicae* survived better as an aqueous suspension than a dry powder (David, 1978). Loss of efficacy occurred with *Erinnyis ello* GV after being stored frozen for 3 years (CIAT, 1987).

At higher temperatures, activity is soon reduced. The crystal of *B. thuringiensis* is stable for 8 h at 80 °C but is inactivated by 15 min at 120 °C (Gaugler and Finney, 1982). As long as containers were moisture-proof, 40 °C and 90% RH had no effect on the viability of spores for 12 weeks (Pinnock *et al.*, 1977). With baculovirus, at 50 °C or above activity is lost in hours or minutes (Jaques, 1977). At 38–42 °C, significant loss in activity can occur in a few months or even weeks (David and Gardiner, 1967; Hunter *et al.*, 1973; Jaques, 1977).

3.3.5 IMPROVEMENT OF SPRAYS BY ENCAPSULATION

B. thuringiensis can be tank mixed with starch powder and sugar to form a spray formulation in water that autoencapsulates on drying. The best of seven mixtures tested by McGuire and Shasha (1990) was equal parts sucrose and a commercial cold water-dispersible pregelatinized corn starch, Mirasperse. In the spray water, it did not clump to block nozzles and viscosity was satisfactory. After drying on leaves, the capsules held molecules in juxtaposition to spores and crystals, completely surrounding most of them. The sugar acted as a dispersant while in solution and, together with the starch, as a sticker and phagostimulant after drying. On exposure to simulated rain in a greenhouse, 70% remained on cotton leaves after 2 weeks, compared with 4 days or less for the six other mixtures. In assays after 7–8 days, *ca* 80% of larvae were killed compared with *ca* 20% by non-encapsulated *B. thuringiensis*. Autoencapsulating starch-based sprays are not yet on the market and have the disadvantage that they need to be applied in a specified volume of spray to achieve the correct concentrations.

This disadvantage can be overcome by drying into granules as a formulated product, either to apply dry (section 3.3.1) or to use in a spray. Fine products, encapsulated by spray-drying, can be sprayed in any volume because the protective matrix tightly holds the pathogen in close juxtaposition to additives such as sunscreens, i.e. in the optimal position, to avoid waste. The functional and economic significance of this is discussed in section 3.7.2b. Tamez-Guerra *et al.* (1996) spray-dried a mix of sugar (48%), pre-gelatinized cornflour (24%), corn starch (24%), technical *B. thuringiensis* powder (3%) and citric or lactic acid (0.3%). Insecticidal activity was increased *ca* ×2 compared with technical powder, probably as a result of improved palatability. Shasha *et al.* (1995) spray-dried *B. thuringiensis* in kraft lignin solution, cross-linked with $CaCl_2$ to form insoluble capsules, without reducing insecticidal activity. The process of Tamez-Guerra *et al.* (1996) has been adopted for use with baculoviruses. Multiple-embedded nuclear polyhedrosis viruses (MNPVs) including *Autographa californica* MNPV (AcMNPV) have been spray-dried without loss of activity, and preliminary laboratory and field experiments demonstrated improved solar stability (M. R. McGuire, National Center for Agriculture and Utilities Research, USDA–ARS, Peoria, Illinois, personal communication). Nuclear polyhedrosis virus, encapsulated in the water-insoluble polymer styrene maleic anhydride half ester with sunscreens, was 4–10–fold less active in bioassays than the non-encapsulated virus. This is believed to be due to inactivation by ethyl acetate (Table 3.7 in section 3.3.2) during encapsulation and not to failure of gut juices to release the virus in the insect gut. During encapsulation, the NPV was dispersed in an ethyl acetate

solution of the polymer in a high-shear stirrer and spun into predominantly 10–30 μm diameter capsules with a high velocity rotating disk (Bull *et al.*, 1976; section 2.3.2c). Additional materials for trial for encapsulation included polycationic biopolymers, chitosan, gelatin types A and B, and polyglycosamine.

Epiphytic microorganisms, transformed with *B. thuringiensis* toxin genes, offer another approach to protect the toxin from factors that cause it to deteriorate. A number of organisms have been investigated as carrier capsules, e.g. *Bacillus subtilis*, *B. sphaericus*, *Pseudomonas cepacia*, *Agrobacterium*, *Erwinia*, *Klebsiella*, *Flavobacterium* and *Alcaligenes* (Rigby, 1991). Some are aggressive colonizers of foliage. For example, toxin-carrying *Pseudomonas* can protect lettuce (Rigby, 1991) and toxin-carrying *Bacillus megaterium* can protect cotton for several weeks, far longer than conventional *B. thuringiensis* sprays (Bora *et al.*, 1994). Jacobs (1989) reported on a programme to insert *B. thuringiensis* toxin genes into a fluorescent pseudomonad to control the sugarcane stem borer, *Eldana saccharina*. The aim was to increase persistence and coverage through the ability of the carrier organism to colonize plants; unfortunately the organism was rapidly lost to the soil. Even if these organisms are technically successful, they have yet to pass the hurdle of registration of transgenic microorganisms that can multiply in the environment.

With CellCap products the registration problem has been solved by killing the encapsulating carrier bacterium (*Pseudomonas* spp.) and fixing the cell wall and cytoplasm by a physical and chemical process (Barnes and Cummings, 1986; Gelernter and Schwab, 1993). These capsules are formulated in the same ways as conventional mixtures of spores and crystals. In field tests the activities of the resulting products were × 2–3 that of conventional products and the persistence of activity on the crop was extended, e.g. to give 76% mortality after 7 days compared with 44% for Dipel and 32% for Javelin, neither of which contained sunscreens (Soares *et al.*, 1993). Activity in CellCap is due to the protein toxin alone and, presumably, any loss of activity due to the absence of live *B. thuringiensis* spores was compensated by synergism with phylloplane bacteria (section 3.5.1) and outweighed by protection afforded by the capsule. The CellCap products are a commercial success, marketed mainly as liquid concentrates containing many of the major toxins of *B. thuringiensis*.

3.3.6 CAUSES AND TIMING OF DETERIORATION

There are many causes of deterioration of bacteria, baculovirus and Protozoa because they have live components and complex proteins, such as crystal toxin and polyhedrin (Table 3.1 in section 3.1.1). This section compares deterioration at the different stages of production, storage and use of the pathogens. It highlights places where formulation can help. The principal causes of deterioration are high temperature, length of time of exposure to causative factors, presence of free water (as opposed to molecularly bound water), adverse pH, enzymes (particularly proteases), surfactants and combinations of these factors. Some substances, e.g. certain sugars and amino acids, stimulate bacterial spores to germinate and lose their innate resistance to adverse factors.

During its passage from production through formulation to ingestion by target pests, a pathogen passes through a number of critical points. At different times, particular deteriorating factors assume significant importance. Some time elapses between stopping the production process and preservation by drying. Deterioration in fermenter liquors can be minimized by cooling and lowering pH to a minimum of 4 to curb not only dissolution of the *B. thuringiensis* by high pH and the action of proteases and autolysis, but also the growth of both contaminants and the remaining vegetative *B. thuringiensis* cells. To

avoid brief extremely acid conditions during the concentration involved in drying, the pH of the liquors must not be lowered too much, or must be partially raised again. For viruses, near neutral pH is best (Ignoffo and Garcia, 1966). It protects the polyhedrin and those virions that lie at or near the surfaces of occlusion bodies, and also inhibits alkaline protease (of insect origin) associated with the viruses at harvest and capable of attacking occlusion bodies. However, Guillon (1995) recommends buffering at pH 4–4.5 by addition of food grade stabilizers, e.g. ascorbic acid, to simultaneously prevent growth of contaminant microorganisms.

During spray-drying, substantial time at critically high temperature must be avoided, e.g. the time particles adhere to drum surfaces, and high temperature in the mass of particles accumulating in the collecting vessel.

For grinding the product after stabilization by drying below a moisture content of 7%, a machine must be selected that does not generate excessive heat, e.g. one with a chopping action. Alternatively a 'wet' mill can be used before drying.

The above conditions established during stabilization and harvest allow stable shelf storage of *B. thuringiensis* for 18 months to many years, depending on formulation type (sections 3.3.3b and 3.3.1). With viruses impurities are important. Purified spray-dried or freeze-dried *Spodoptera littoralis* NPV lost no activity during 32 months at 26 °C, but unpurified virus lost 87–97% activity. This deterioration was reduced by storage under vacuum or mineral oil but not under a plant (Arachis) oil. It was probably due to autoxidation of fats as a result of exposure to oxygen, not to the presence of bacteria (Cherry *et al.*, 1998). Moisture content was not measured, a factor critical to the survival in store of many organisms. Dempah and Coz (1980) observed sharp decreases in activity of dry preparations of *B. thuringiensis* var. *israelensis* stored under humid tropical conditions in open containers. Jones *et al.* (1991) reported that a wettable powder of *S. littoralis* NPV stored unsealed in the laboratory in Egypt lost 22% activity in 1 month, 52% in 3 months and 90% in 1 year. High humidity caused premature germination of *B. thuringiensis* spores and autolytic spoilage of products (Couch and Ignoffo, 1981), the effects being more rapid the higher the temperature. Thus, water vapour-proof containers are essential for storage of dry products which absorb moisture rapidly when the containers are opened. Hygroscopic additives are best avoided if possible.

Before application, when a wettable powder is mixed into water to form a spray fluid, soluble substances are dissolved. These include enzymes, bacterial nutrients, additives such as sugar (which may stimulate spore germination), and surfactants (section 3.4.1). If the spray is allowed to stand, deterioration is significant in 1–2 days and may be accelerated by some surfactants that may slowly solubilize the toxin crystal and polyhedrin at ambient temperatures. Standing should therefore be minimized.

On application to foliage, deterioration is accelerated by a number of factors. The water evaporates, concentrating all the substances present and accentuating pH. Further substances are dissolved from the leaf surface (section 3.4.5) and the pathogens are exposed to sunlight, rainfall, etc. (sections 3.4.4 and 3.4.2).

Formulation counteracts most causes of deterioration by using a series of additives, which are the subject of the next section.

3.4 ADDITIVES

Additives help a spray to reach its target and improve performance once it is there. Wetters facilitate leaf coverage, phagostimulants improve palatability, and synergists increase the effect on larvae. Of the post-spray hazards, rain is countered by stickers, sun by sunscreens and allelochemicals in leaves by phagostimulants and neutralizing additives. The following sections assess the importance

of each factor and then the efficiency of each type of additive in functionally increasing the potency of a spray or decreasing the adverse effects of the hazards. Available data have been assembled in tables to indicate the order of magnitude to be expected of each type of improvement. For example, a synergist may lower the LC_{50} up to 1000–fold in the laboratory, but less in the field, where the required pathogen dosage to achieve pest control may still be reduced. This advantage has to be assessed against availability, cost and any disadvantages of adding the synergist. Finally, indications are given of where and when each additive is best used – is it of general applicability and value as an insurance to cover the worst-case usage, or should it be applied just to tank mixes in varying amounts for different pests, and sprayed only on some crops and at some times only?

The units of quantification need some explanation. Ratios are used. The effect of a factor such as rain is expressed as the persistence ratio of a pathogen with and without rain. Similarly, the effect of an additive, such as a sticker, is given by the ratio of persistence with and without the sticker. The most valuable ratios are obtained from LC_{50} values. Frequently, only percentages of mortality at single dosage points are available. These are much less useful, whether expressed as ratios or just as differences between two percentages.

3.4.1 WETTERS

Wetters are used for three reasons.

They improve spray coverage on hydrophobic leaf surfaces.

They facilitate mixing into water of hydrophobic spores and toxin crystals, as well as NPV occlusion bodies which have pH-dependent hydrophobicity (section 3.4.2).

They are also used to form emulsions between oil and water by reducing interfacial tension and surface tension (see also sections 2.3.1c, 2.3.2b, 4.3.6 and 4.6.6b).

Table 3.12 summarizes uses, qualities and performances of many wetters, as well as the concentrations at which they are applied. They are used at 0.01–0.5% in tank mixes (Table 3.12; Table 3.15 in section 3.4.3 below) and at 2–18% as dispersants in commercial products subject to storage, including use at 4–5% as emulsifiers (Table 3.12, also Table 3.2 in section 3.2.1, Table 3.9 in section 3.3.3, Table 3.11 in section 3.3.4, Table 4.7 in section 4.6.3). Dispersants in the products double up as wetters on reaching the spray tank.

The best wetters can be judged by a general assessment of the varied data in Table 3.12 (see Note at foot of Table) and the frequency with which they have been used in practice. Briefly, the best include the Tritons, Tweens and the new organosilicone superspreaders: non-ionic wetters are preferable. Liquid Tritons and Tweens are unpopular with spray operators because they are difficult to mix into water.

Generally, *B. thuringiensis* and baculoviruses are compatible with many commercial surfactants (Table 3.12). Little information is available on the effect of surfactants on Protozoa. Some wetters harm or inhibit pathogens (Appendix Table I.5). In the only truly critical study to mimic the effect of wetters during spraying, *B. thuringiensis* was exposed to wetter solutions in buffer for 24 h, then accurately bioassayed on leaf or sheet beeswax (second group in Table 3.12). Only Teepol L reduced potency during this severe exposure. The non-ionic Triton X-100 and Tween 80 have been widely and successfully used in sprays, insect bioassays and viable spore counts (Table 3.12; Burges and Hussey, 1971). Morris (1975) reported that Triton X-100 impaired germination and/or bacterial growth when added to broth in shake flasks inoculated with *B. thuringiensis* spores. However, it is not phytotoxic on plants at operational spray concentrations (H. D. B., personal communication) and it has been included in experimental application of *Helicoverpa* (*Heliothis*) *armigera* NPV in Thailand (K. A. J.,

Table 3.12 Wetters tested in aqueous preparations of *Bacillus thuringiensis* (Bt), *B. sphaericus* and nuclear polyhedrosis virus (NPV) in alphabetical order of the first entry in each group

Surfactant (composition, Appendix Table I.5)	*Effects*
Acetylenic Surfactant S485, Tween 20, Montanox 80	Used in commercial dry Bt products[1]
Agral; casein; Manoxol OT; Plyac; Sovix; Spreader; Spreadite; Teepol L; Tenac; Triton GR5, B-1956, X-100; 1% exposed to Bt (Thuricide, serotype V) 24 h, 30 °C, pH 7.2 in buffer	Accurate assays on cabbage against *Lacanobia oleracea*, *Pieris brassicae* and on beeswax against *Galleria mellonella*. Only Teepol[2] increased LC_{50} (× 6.5)
AL-1246, 1280, 1364, 1403; Atlox 848, 849, 3404/849; Atplus 300, 448; Plurafac A-24; Triton GR7M; Triton X-35, X-45, X-363M, X-N60; Witconol H-31A	Satisfactory with Bt[3]
Alkylphenols; Arlacel 'C'; Colloidal X-77; Igepal CO-630; Later's surfactant; Novemol; Petrol AG; Sandovit; Triton X-45, X-100, X-114, X-155; Tween 20; Vatsol OT	Good results with Bt, virus and fungus. No indication that additives reduced effectiveness of the pathogens[4]
Atlox 848, 849 and 3404/849, anionic and non-ionic; Triton X-100, non-anionic; 0.1%	Inhibited Bt fermentation and growth[5]
Cargill Insecticide Base Concentrate	More acidic than polyethylene glycol; did not decrease evaporation or improve sticking;[3] consistent drop spectra[3]
Crodofos N3N (4%)	Compatible with *Heliothis armigera* and *Spodoptera exigua* NPVs for formulation in Arachis oil[6]
Cyclamines, hexylamine, butyl-, octyo-, de-, dodecylamine hydrochloride and cetyltrimethylammonium bromide; cationic	0.03% enhanced and 0.1% depressed NPV infection of *Pseudaletia unipuncta* larvae, possibly by attachment to NPV surfaces, so neutralising their negative charges at pH 8 and overcoming repulsion by negative charges on membranes of midgut cells[7]
Lovo 192, 0.4%; Later's surfactant, 0.1%; Igepal CO-630, 0.1%; Triton B195b, 0.1%; Plyac, 0.4%; Pinolene 1882, 4%; Folicote 351; Hi-Spread-Casein 10% + lime 90%, 4%	Did not affect germination of Bt spores, nor prevent growth in media[8]
Maywood surfactant	Increased spread of Bt on foliage[3]
Maywood surfactant; whey; corn oil surfactant (corn oil emulsified with Maywood surfactant)	Thickened and physically stabilised Bt tank mix; did not decrease evaporation or improve sticking[3]; consistent drop spectra[3]
Multifilm Buffer-X, 0.04%; Triton X-100, 0.04%, X-152, 0.02%, X-172, 0.02%, B-1956, 0.03%	*Heliothis* NPV unharmed by 24 h storage at 30 °C[9]
Organosilicones in tank-mixes	Excellent spread of Bt to cryptic parts of leaves. No harm to Bt[10]
Petro Morwet EFW, 1%	With 10% lactose in *B. sphaericus* slurry during spray drying, an effective product concentrate[11]
Span-85, Biofilm, Triton B-1956, 0.1%; non-ionic	No adverse effects on Bt fermentation and growth[5]

Table 3.12 (*Contd.*)

Surfactant (composition, Appendix Table I.5)	*Effects*
Teepol, Triton X-100, Pitsulin, Etocas 30	Compatible with *S. littoralis* or *H. armigera* NPVs, no reduced effectiveness of NPV[12]
Surfynol TG-E, 0.5%; Bevaloid 211, 3%	Used as dispersants in Bt flowable formulations[1]
Triton AG-98	Recommended in tank-mix with Collego[13]
Triton CS-7 spreader-binder	Decreased activity of aqueous NPV tank-mix in laboratory[14]
Triton CS-7; polyvinyl alcohol	Compatible with *Heliothis* NPV[15]
Tween 80	3% for water-based and 18% for oil-based Bt flowable formulations[1]
Tween 80 (5%), emulsion in oil	Successful with *H. armigera* and *S. exigua* NPVs in Arachis oil[16]
4% Tween 80 + Span 80 emulsions of 6% water in no. 2 furnace oil, 7 days storage with Bt	No loss of toxicity, germination and growth on agar not inhibited, sprayed foliage readily eaten by budworm larvae[17]

References

1 Lisansky *et al.*, 1993
2 P. Jarrett, personal communication
3 Ignoffo and Couch, 1981
4 Angus and Luthy, 1971
5 Morris, 1975
6 K. A. J., personal communication
7 Yamamoto and Tanada, 1978a, 1980
8 Morris, 1969
9 Ignoffo and Montoya, 1966
10 Green *et al.*, 1996; C. F. Green, personal communication
11 Lacey *et al.*, 1988
12 Topper *et al.*, 1984; Jones, 1988a; K. A. J., unpublished data
13 Industrial literature Ecogen, Langhorne, Pennsylvania 1990
14 Smith *et al.*, 1978a
15 Smith *et al.*, 1978b
16 K. A. J., unpublished data
17 Angus *et al.*, 1961

Note: Best, organosilicone. Non-ionic wetters preferable. Triton X-100 and Tween 80 tested more than any other wetter (section 3.4.1), but difficult to mix into sprays in the field. Side effects listed in Appendix Table I.5.

personal communication). It is not regarded as harmful to fungal spores, which are less well protected than those of bacteria (sections 4.3.6, 4.6.6b). There is some evidence of the possibility of harm by Triton CS-7, as well as the Altox and cyclamine groups of wetters (Table 3.12). Organosilicone superspreaders have been used with microbials only recently, but no relevant studies on bacteria and viruses comparing them with other wetters have appeared. Experience of use suggests that they do not harm microbials when added at the spray tank stage but they may cause on-shelf deterioration if included in proprietary microbial formulations. There is some evidence that Silwet L-77 damages fungal herbicides (section 6.5). Organosilicones are believed to carry water-soluble pesticides into open stomata (Green *et al.*, 1996), so they may do the same for pathogens. They

should carry particles very effectively even to the feeding areas of cryptic larvae, e.g. those that feed under silken tubes (Table 3.4). Manufacturers of microbials will advise on suitable wetters for their products.

In practice, wetters are best incorporated into commercial products in quantities that give adequate dispersion into the spray carrier and good cover of tractable foliage. More should be added to tank mixes for very hydrophobic, waxy or hairy foliage.

3.4.2 STICKERS

Measurement of physical persistence of unprotected pathogens has produced conflicting results. Burgerjon (1989) reports that the activity of preparations of *B. thuringiensis* spores and toxin crystals is not rapidly lost when subjected to artificial rain; however, Krieg (in Huber, 1989) concluded that rain rapidly removed crystals from foliage. This is much the commoner effect, e.g. 3 mm of rain removed 46–72% of the original deposits on leaves from 12 Dipel products (Sundaram *et al.*, 1993).

Of the baculoviruses, GVs and to a lesser extent NPVs adhere strongly to leaf surfaces, and are not easily washed off by rain (David and Gardiner, 1966; Richards and Payne, 1982; Richards, 1984; Entwistle and Evans, 1985; Young and Yearian, 1986; Burgerjon, 1989). Acquisition of polyhedral inclusion bodies (PIBs) of NPV from a spray involves long-range forces such as electrostatic forces, van der Waals' forces and hydrophobic interactions. Retention of PIBs involves shorter-range forces, i.e. various types of chemical bonds (Small, 1985). At neutral pH in most conditions, PIBs are negatively charged and the hydrophobicity is inversely related to pH, thought to be due to a charge-masking effect (Small *et al.*, 1986; Small and Moore, 1987). These relationships are likely to be similar for GVs. Attachment will be weaker if products contain particles large enough to impair contact between inclusion bodies and the leaf surface (Appendix II, Fig. II.6). Entwistle and Curruthers (1989) report that cabbage leaves did not readily retain PIBs because of epicuticular wax and that *Neodiprion sertifer* NPV was acquired more readily by pine and cotton leaves than was *S. littoralis* NPV. Also, *S. littoralis* NPV was acquired more readily by cotton than pine.

Jones and McKinley (1987) and Jones (1988a) reported that *S. littoralis* NPV was physically lost from cotton leaves in Egypt. This happened also with *H. armigera* NPV in Thailand (K. A. J., unpublished results). Overall, loss of NPV was greater from the upper surface of leaves than from the lower surface. In both countries, this loss occurred during periods of no rain and was attributed to the abrasive action of wind and dust or sand, which can remove epicuticular waxes. It is unlikely that any sticker could prevent this, or could adhere so strongly to pathogens as to prevent release in the insect gut. Purification of virus suspensions reduces adhesion to the leaf, because the insect protein and debris acts as an effective sticker (Jones, 1988a; Entwistle and Curruthers, 1989).

Stickers (Appendix Table I.6) improve adherence of pathogens to foliage. They reduce wash-off by $\times 2$ to $\times 10$ after up to 13 cm of rain (Table 3.13). Specialist stickers are usually used at rates of 0.1–2% and the multifunctional molasses up to 25% (Table 3.13; Table 3.15 in section 3.4.3 below). Effectiveness varies from the delaying action of water-soluble materials, such as molasses, to the fastness of materials such as resins, which dry to become insoluble. Jones (1988a) tested 13 stickers on viruses, including celluloses, vegetable gums and molasses. Gum Guar was the most effective. None of the gums inactivated the virus or inhibited feeding. Stickers may double up as thickeners, i.e. additives to increase spray viscosity and increase the size of spray drops, e.g. gums and molasses, or as phagostimulants, e.g. molasses. Details of the characteristics and performance of individual stickers are given

Table 3.13 Stickers tested with *Bacillus thuringiensis* (Bt) and nuclear polyhedrosis virus (NPV) sprays in alphabetical order of first entry in each group

Sticker (composition, Appendix Table I.6)	*Effects*
Acrylic polymer, Rhoplex AC33NP, all 2% + tracer dye Erio Acid Red XB 400, 0.2%. Efficient ULV cover of leaves	Superior to Chevron Sticker, no effect on toxicity of Bt, stable on pine needles to manual handling and heavy rain (dye leached out of drops)[1]
Biofilm; High Tack Fish Glue; Nacrylic X4260, X4445; NuFilm 17; Plyac; X-Link 2873	Best protection of Bt among 18 stickers in 2.5 cm rain while tank-mixes without stickers lost *ca* 30% activity[2]
Bond, 2% on lettuce with no pathogen	No effect on feeding of gypsy moth larvae[3]
Bond. Recommended for Bt Dipel 8L and Thuricide 48LV by air, not for Foray	At 2%, retained 88–100% original activity after 6 mm rain, c.f. 29% without Bond on oak[4,5]
Bond, 5%; Acrylocoat, 3%	Highly effective with Bt and NPV against gypsy moth on oak seedlings[6]
Carboset	Depressed Bt activity[2]
Casein 0.05% cross-linked enhanced mortality by ×3.8, 0.05% casein ×2.8 and 1% gluten ×2.2 with Bt	With *Ostrinia nubilalis* after 13 cm of simulated rain on pre-washed cotton plants in spray chamber[7]
Chevron spray sticker in shaken flasks of broth	Did not impair germination and growth of Bt[8]
Chevron sticker, 0.6% in ULV aerial spray, 2 l/ha on moderate spruce budworm populations	Effective control with ready-to-use Bt suspension concentrates requiring only mixing in of sticker[9]
Chevron, 0.6–0.1%; Rhoplex B60A, 2%; Dupont spreader-sticker, 0.06%; Biofilm, 0.1% with Bt; Biofilm, 0.1% with NPV	Good results in forest against spruce budworm and gypsy moth; Rhoplex initially blocked flow meter and spinning disc cage with Bt[6]
Chevron spray sticker with Cargill Insecticide Base concentrate (stabilized molasses) 25%	Results of LV sprays of Bt generally favourable[10]
Chevron spray sticker with sorbitol and chitinase	Acceptable spruce budworm control with Bt; sorbitol improved spray deposit[11]
Chevron sticker (with Shade), 6%; molasses, 25%	NPV effectively controlled gypsy moth larvae[12]
Corn starch (pregelatinized) + sucrose 1:1 in spray	Some evidence 4% solids improved Bt field rainfastness, 1–2% did not, nor 4% under 6 cm artificial rain[13]
Dextrin (maize)	Used as sticker in some commercial wettable powders[14]
Geon Latex (colloidal vinyl chloride polymer), 10% on mulberry foliage	Insoluble in water. Palatable to silkworm larvae, protected Sudan IV for 6 weeks outdoors, Bt from 3 min daily hosing for 2 weeks, or 12 h in moving water, or 12 h washing under a shower (toxicity retained)[15]
Gluten (1%) needs pH > 10 or < 5 for dispersion in water	Lower bulk replacemant for sugar and starch (2–4%). Enhanced mortality c.f. commercial fomulation (20% against 90%)[16]
Gluten (1.3% at pH 10) in spray on young, washed cotton plants	Enhanced mortality of *0. nubilalis* due to Bt by ×10.1 after 5 cm simulated rain in greenhouse[17]
1% gluten ×1.53 = 0.5% casein ×1.49 > 2% flour/sucrose ×1.23	Increased mortality of *0. nubilalis* due to Bt after 3.2 cm simulated rain on cabbage in field[18]

Lignin, kraft (0.5%) crosslinked with $CaCl_2$, exposed to 5 cm simulated rain on cotton.	Did not harm Bt and NPV and protected 52–98% original activity from wash-off[19]
Encapsulated, or not cross-linked	No reduction of Bt wash-off[19]
Lignosulphonate, 0.5%	No reduction of Bt wash-off[19]
Lysine KKL increased persistence ×7; polyglucine ×6: citric acid by-product ×4; molasses of peat ×3	3 days after spraying apple trees with *Malacosoma neustria* NPV[20]
Methyl cellulose. Water soluble	In field 4 cm rain reduced spore count of Bt by 80%[15]
Molasses, egg albumen, whole milk, larval extract	Compatible with NPVs in field trials[21]
Plyac	Recommended for Bt in Foray[4] and Dipel[5] by air. Compatible with *Heliothis* NPV[22]
Polyvinyl sticker	Use abandoned as it adhered semi-permanently to car finishes[23]
Rhoplex B60A, ×2.1; Acrylocoat, ×1.9; NuFilm 17, ×1.3	Improved rainfastness of Bt on oak seedlings after 2.5 cm rain[24]
Skimmed milk, 0.5%	Aqueous NPV (2.4 l/ha), excellent control of pine sawfly[25]
Skimmed milk, 2%; methyl cellulose, 2%	NPV controlled *Lymantria dispar* on hardwood trees[26]
Sutro (25%) with molasses (25%)	Rain washed off more Bt than with water plus Sutro alone[2]
Topwet spreader sticker, 0.1%	No effect on germination of Bt spores, good control of *Heliothis punctiger* in field test[27]
Sorbitol, 1%; gum guar, 1%; gum xanthan, 1%; gum tragacanth, 1%; gum karaya, 1%; acacia gum, 1%; locust bean gum, 1%; gelatin, 1%; molasses, 0.1–10%; casein, 1%	Compatible with *S. littoralis* NPV. Gum guar best in field tests, but still some physical loss of virus. All palatable to larvae. None more effective than unpurified virus[28]

References

1 Fast *et al.*, 1985
2 Neisess, 1979
3 Farrar *et al.*, 1995
4 McLane, 1991
5 Devisetty, 1988
6 Morris, 1985
7 Behle *et al.*, 1996
8 Morris, 1975
9 Valero, 1989
10 Lewis *et al.*, 1974
11 Smirnoff, 1977
12 Yendol *et al.*, 1977
13 McGuire *et al.*, 1996
14 Lisansky *et al.*, 1993
15 Angus, 1959
16 McGuire *et al.*, 1994a
17 Behle *et al.*, 1997a
18 Behle *et al.*, 1997b
19 Shasha *et al.*, 1995
20 Jankevica and Zarins, 1997
21 Jones, 1994
22 K. A. J., unpublished data
23 Nichols, 1985
24 Cibulsky *et al.*, 1993
25 Bird, 1953
26 Magnoler, 1974
27 D. J. Cooper, Waite Agricultural Research Institute, Australia, personal communication, 1984
28 Jones, 1988a

Note: best, acrylic polymers, Biofilm, Chevron sticker, skim milk, gum guar, cross-linked lignin.

in Table 3.13; the best are listed in a Note at the foot of the Table. Lignin (very good), casein, flour, albumen, gluten, milk and molasses are also sunscreens (Table 3.16 in section 3.4.4 below).

Thus, degrees of acquisition and retention of pathogens are likely to be related to differences in pathogen species, formulations and environmental conditions (plant species, type of leaf surface, position on leaf, precipitation, wind, etc.). A formulated product should contain enough sticker for most treatment situations, unless specialist products are formulated to retain strong natural adhesion on tractable foliage, e.g. codling moth GV on apple. Extra sticker should be added to the tank mix for water-repellent foliage. Also, a sticker may benefit a pathogen in other ways, e.g. 4 days after application, *B. thuringiensis* alone on red oak seedlings without rain killed 89% of test gypsy moth larvae, whereas applied with 1 or 3% Bond it killed 97–100% larvae, whether or not exposed to 0.6 or 1.2 cm of rain (McLane, 1991).

One sticker, Carboset, harmed a pathogen. Other reasons for unsuitability have also been reported (Appendix Table I.6). A polyvinyl sticker was withdrawn because it fouled car paintwork, and some stickers are incompatible with individual formulations because precipitation from suspension blocks on-line filters and nozzles (Table 3.13).

3.4.3 PHAGOSTIMULANTS

In the field, phagostimulation is a combination of attracting an insect to an area bearing the stimulant and encouraging it to eat more once it is there. The latter is the effect usually measured in the laboratory by placing insects on food either with or without the stimulant. A few experiments allow the insects to make a choice (Table 3.14).

Phagostimulants (Appendix Table I.7) encourage pests to eat a maximum amount of pathogen before it deteriorates on the foliage or, with *B. thuringiensis*, before the crystal toxin arrests feeding (section 3.3.3a). Researchers sought gustatory recipes for cotton pests (Table 3.14, part 1), later incorporated into commercial adjuvants (Appendix Table I.7), and then tried each recipe on other pests and crops. Some principles are evident from the data assembled in Tables 3.14 and 3.15. The most stimulatory materials were plant extracts and materials made from seeds, particularly cottonseed flour and oil, cornflour, corn oil and soybean flour. Combinations of these materials were more stimulative than single materials. Although stimulating many pest insects, no single material (Table 3.14, part 1) or combination (Table 3.14, part 2) does so universally. These principles are not surprising: plant pests have evolved to fill niches created by the defences of individual plant species, mainly in the form of allelochemicals (section 3.4.5) and mechanical difficulties presented to feeding insects. The adult insects choose the niche plants for oviposition and the resulting larvae choose where and on what to feed, often in a crop monoculture. The attractive materials described above are almost free from allelochemicals and are easy to eat.

Some pitfalls are apparent in experiments with phagostimulants, partly explaining the variability of the data in Table 3.14. In the laboratory, it is not easy to mimic the insect in natural free-ranging competition between micro-areas hit or missed by a spray or bait on fresh plant material of its chosen food plant. The dose of pathogen eaten on evenly treated leaf is proportional to the amount of leaf eaten, but the resultant mortality is not always so. With *B. thuringiensis*, food intake was generally lower in four insect species given treatments causing the highest mortality, possibly a result of stimulants speeding up ingestion of a lethal dose before the antifeeding action of the crystal toxin stopped further feeding (Yendol *et al.*, 1975; Farrar and Ridgway, 1995; section 3.3.3a). Effective concentrations of stimulants tend to be high, altering the physical performance – and hence

Table 3.14 Phagostimulants used with microorganisms *Bacillus thuringiensis* (Bt), nuclear polyhedrosis virus (NPV) and Protozoa

Phagostimulants and ranking (composition, Appendix Table I.7)	*Effects and activity*
1. Cotton insects on cotton	
Corn, water extract with NPV	Increased *Heliothis* feeding on cotton leaf, also mortality by ×2.4[1]
Corn and other plant extracts impregnated in paper	Increased feeding of *Heliothis* spp. on the paper up to ×24 to ×30[2]
Corn extract with NPV	Increased mortality in leaf assay ×1.7. In field increased cotton yield ×1.2, reduced damage ×1.5–3.6[2]
Cottonseed oil, sugars, extracts of parts of cotton plant mixed in agar	Pink bollworm neonates fed on cottonseed oil, sucrose, extracts except leaf extract, galactose, raffinose agars[3]
Cottonseed flour (Pharmamedia and Proflo), soya flour, dried extract of corn seed and extracts of various vegetables all at 5%, or cottonseed oil 10% in agar	*Heliothis virescens* neonates favoured Pharmamedia agars most, and mixtures more than single materials[4]
Cottonseed oil sprayed bait with NPV in the field	*Heliothis* spp. control better than NPV alone and equivalent to cotton standard chemical control[5]
Soybean oil and soybean flour > corn oil and corn flour	Mortality with NPV in laboratory[6]
Grassmeal > wheatgerm > 'groundbait' (grassmeal, Celacol M2500, bran, wheatgerm, molasses) > molasses	Compatible with *Spodoptera littoralis* NPV. Field tests: all except molasses increased feeding of *S. littoralis* larvae, grassmeal by 32%[7]
Citrus pulp bait (Griffin Corp.); Coax; Gustol; soybean adjuvant (8% soybean flour, 5% soybean oil, 1% sucrose)	Soybean adjuvant and Coax gave higher mortality with *Heliothis armigera* NPV than citrus pulp and Gustol, all gave better control c.f. no adjuvant[6]
Lactose, maltose, glucose, sucrose in laboratory soybean baits	No significant difference in mortality between sugars with NPV[6]
Molasses (10%) added to cottonseed meal–Shade bait sprayed on leaf	×1.1 potency with NPV in *Heliothis zea* under simulated sun[8]
Coax 1.2–12%; cottonseed oil 1%; molasses 2% in spray with Bt	Only Coax significantly increased mortality (by 13–25%) of *Heliothis* spp. on cotton leaf[9]
Coax, 3.4 kg/ha in trials with NPV and Bt	Decreased *Heliothis* damage (×2.1) on cotton and increased yield (×1.3)[10]
Molasses (e.g. 10%) 0.6% + NPV	×2 potency with NPV against *H. armigera* on cotton[11]
Sugar + *Heliothis* NPV	Increased yields of cotton in some tests[12], but not others[13,14]
2. Commercial adjuvants compared	
Pheast, Coax, Gusto and Entice with *Lymantria dispar, H. zea, Ostrinia nubilalis* and *Plutella xylostella*	Stimulants variably (0–8%) increased mortality with Bt on leaf in Petri dish and greenhouse with all species; stimulants were not consistently different in all insects and host plants[15]
3. Gypsy moth and other species feeding on open leaf	
Molasses (12.5%) on lettuce leaf discs	Increased gypsy moth larval feeding ×1.7 and ×2.3[16]

Table 3.14 (*Contd.*)

Phagostimulants and ranking (composition, Appendix Table I.7)	*Effects and activity*
Coax and 16 spreader stickers, phagostimulants, oils, etc.	Only Coax increased control of the Colorado potato beetle by Bt ssp. *tenebrionis*[17]
Sugar (10%) added to Keltose–Shade or PVA–Shade sprays of Bt	×1.6 and ×2.6 potency in *Trichoplusia ni* on leaf under simulated sun, c.f. no sugar[8]
Molasses	×10 more deaths of *P. xylostella* GV in greenhouse tests[18]
Heliothis NPV on potted gram (legume); stimulant unspecified	Instar III larvae ate ×2.1 LC_{90} dosage with stimulant, c.f. ×1.5 without[19]
4. Corn borer	
Molasses > fresh corn leaf = $CaCl_2$ + Coax > $CaCl_2$, all at 17–18%, except Coax, 8%. In granules in corn whorls	Significant differences with corn borer in both greenhouse and field. Molasses improved potency of Bt by ×1.3[20]
Coax (4.7%) in granules > plain granules by ×320 and leaf by ×12; corn oil 1% < leaf by ×0.22, sugar = leaf	Feeding; stimulants in starch granules with *O. nubilalis* and fresh untreated corn leaf in Petri dishes without pathogens[21]
Coax (25%) > homogenized fresh leaf (16%) > $CaCl_2$ 35% + Coax (15%) > $CaCl_2$ (18%) in flour granules	Mortality of corn borer on cotton leaf and preference in feeding choice tests. Coax increased potency of Bt ×4. Larvae rejected granules with $CaCl_2$[22]
Coax (1–10%) in corn starch granules	Reduced corn borer tunnel length in field by ×2.2 to ×3.0 with 400 IU Bt and by 0 to ×2.4 with 1600 IU[23]
Coax > corn oil by ×5, corn oil = plain capsules	In capsules on corn in greenhouse with Bt at the LC_{50} for *O. nubilalis*[21]
5. Dry baits	
Wheat bran, Nolo Bait with *Nosema locustae*	Improved grasshopper suppression[24,25]
Wheat bran bait with Bt	Successful control of *Agrotis ipsilon*[26]
Molasses (16%) in corn starch granules with entomopox virus	Increased grasshopper mortality ×1.1 to ×1.2 in assays with rye seedlings[27]

References

1 Allen and Pate, 1966
2 Montoya *et al.*, 1966
3 Bell and Kanavel, 1975
4 Bell and Kanavel, 1978
5 Andrews *et al.*, 1975
6 Smith *et al.*, 1982
7 Jones, 1990
8 Smith *et al.*, 1980
9 Luttrell *et al.*, 1982
10 Bell and Romine, 1980
11 Roome, 1975
12 Stacey *et al.*, 1977, 1980
13 Bull *et al.*, 1976
14 Pfrimmer, 1979
15 Farrar and Ridgway, 1995
16 Farrar *et al.*, 1995
17 Riethmüller, 1990
18 K. A. J., personal communication
19 Ignoffo and Couch, 1981
20 McGuire *et al.*, 1994
21 Bartelt *et al.*, 1990
22 Gillespie *et al.*, 1994
23 McGuire *et al.*, 1990
24 Caudwell, 1993
25 Johnson and Henry, 1987; Meneley and Sluss, 1988; Lockwood and Debrey, 1990
26 Salama *et al.*, 1990c
27 McGuire *et al.*, 1991

Note: best, Coax and some plant extracts (with or without sugars).

Table 3.15 Tank-mixes used in forest and field with *B. thuringiensis* (Bt) and nuclear polyhedrosis virus (NPV)

Tank-mix (ingredient details, Appendix I)	*Effects and insect control*
Forest	
Molasses 25% + Shade 3–6% + spreader-sticker 0.1% with Bt	Effective against sawfly (*Neodiprion lecontei*)[1,2,3]. Tank-mix superior to commercial formulation (Sandoz 285WP) against spruce budworm[4]
Molasses 25% + Shade 3% against *N. lecontei*	Effect of NPV in the mixture equalled effect in water alone[5]
(1) 19.3% Molasses + 2.3% or 4.6% Shade + 1.7% Chevron oil + 76.7% water; (2) 12.3% Protec and 87.7% water. Both 20 l/ha	NPV (Gypchek wettable powder) gave ×2 better gypsy moth larva reduction with Beeconmist than with flat fan nozzles with (1); *vice versa* with (2)[6]
Molasses (ProMo, feed grade) 12.5% + Orzan LS 6 or 10% + Rhoplex B60A 2% + stream water (pH 5–8) for aerial sprays	Standard in North American tests with Gypsy moth NPV (Gypchek) in 1987–92. Handling on site slow. Frequent superior results c.f. Gypchek alone or in simpler mixes[7,8]
Molasses (MO-MIX) 12.5% + Lignosite 6% + Bond 2% + stream water (pH 5–8) for ground spray	Gypsy moth larvae fed ×1.56 c.f. NPV alone on lettuce leaf discs[9]. Standard in field tests of Gypchek from 1993. Frequent superior results of Gypchek alone or in simpler mixes[8]
Carrier 038 95%, water 5% + Gypchek (NPV) against gypsy moth	Recommended 1996, 25–60 $\times 10^9$ OB/ha is ×2 or more the dosage in the molasses mixes to minimize the volume per ha. Has better sun screening and less droplet evaporation[10]
Carboxymethylcellulose 0.2% + polyvinylpyrrolidone 1% + Erio Acid Red XB 0.1% + Chevron Spray sticker 0.1% ± acephate 0.5% (organophosphate insecticide, 6% of normal rate)	With Dipel 36B liquid (Bt). Some clogging of Micronair emission system[11]. Strong effect of evaporation. High population reduction and very good foliage protection due to superior deposit rates and resistance to weathering[11]
(1) Uvinul DS49 1% + Uvitex ERN-P 1% + Chevron Spray Sticker 0.1% + acephate 0.6% (10% of normal rate)	With Dipel 16B liquid (Bt). Clog-free spraying[11]; superior retention of activity[11]; high population reduction and very good foliage protection due to superior deposit rates and resistance to weathering[11]
(2) Xanthan gum 0.02% + (1) above	With Dipel 36B liquid. Clog-free etc as (1) above[11]
Latex + dried blood in water, or fuel oil + magnabentonite + Span emulsion	NPV effective against small sawfly larvae (Neodiprion swanei), not against older larvae[12]
Field crops	
Molasses 10%, Teepol 0.5%, Tinopal RBS 200 0.001%	Best formulation of purified *S. littoralis* NPV of a number tested in the field[13]
Nutrisoy 7B soybean flour 8% + crude soybean oil 0.5% + sucrose 1% + Triton CS-7 0.01%	Suggested after studies on individual additives[14,15]
Cottonseed oil + sucrose + Dacagin hydroxycellulose	Has several desirable properties but spray qualities poor against *Heliothis* on cotton[16,17]
Cottonseed oil bait modified with sucrose + Dacagin hydroxycellulose + glycerin	With NPV attracted bollworms and reduced population[16]

Table 3.15 (*Contd.*)

Tank-mix (ingredient details, Appendix I)	*Effects and insect control*
Polyvinyl alcohol 99% hydrolysed + Shade	Best of several tank-mixes with NPV for *Heliothis* control[18]
Speswhite china clay 30%, Neosyl 10%, Etcos 30 10%	Wettable powder with freeze-dried *S. littoralis* NPV. Effective field control of larvae[19]
Egg albumen 0.3%, Teepol 0.01%, soybean flour 5%	Increased mortality by NPV in lab, but not in field c.f. NPV + Teepol[20]

References

1 Cunningham *et al.*, 1975
2 Knapp and Cunningham, 1977
3 de Groot *et al.*, 1979
4 Cunningham *et al.*, 1978
5 de Groot and Cunningham, 1983
6 Lewis and Yendol, 1981
7 Reardon *et al.*, 1992
8 Reardon and Podgwaite, 1994
9 Farrar *et al.*, 1995
10 J.D. Podgwaite, USDA, personal communication
11 O.N. Morris, Agriculture Canada, personal communication
12 Ignoffo *et al.*, 1976b
13 Topper *et al.*, 1984
14 Smith *et al.*, 1978a
15 Smith *et al.*, 1982
16 McLaughlin *et al.*, 1971
17 Andrews *et al.*, 1975
18 Smith *et al.*, 1978b
19 Jones *et al.*, 1994
20 Pawar *et al.*, 1992

effectiveness – of sprays in the field. Stacey *et al.* (1977) found the most effective sprays had the largest drops (235 μm diameter) and the most deposit on the upper zone of cotton plants. These results were not repeatable later, even with larger drops of (600 μm (Stacey *et al.*, 1980). It is debatable whether to regard bulky, stimulant-laced sprays as sprays or as baits. Stacey *et al.* (1977) suggested that increases in cotton yield recorded in the presence of sugars may have been due to physiological effects of the sugar on the plant. Sucrose is routinely used with *B. thuringiensis* on grape against grape berry moth.

The value of a phagostimulant with NPV was clearly shown with gypsy moth on lettuce leaf discs by Farrar *et al.* (1995). Technical virus had no effect on feeding, but a commercial wettable powder was strongly deterrent (× 0.10), as was the synergist Tinopal LPW (= Blankophor BBH) (× 0.43). Molasses strongly stimulated feeding (Table 3.14, part 3) and its use with the wettable powder in a tank mix (NPV+12.5% molasses+2% Bond +6% Lignosite AN), with or without 1% Tinopal LPW, restored feeding to the level with water alone. Concurrently with the feeding, potency (LD_{50}) of virus alone was less than that of the tank-mix (by × 20), but the synergism of the Tinopal was so great that the LC_{50} ratio, (with/without it; × 42 in the tank mix and × 214 in the wettable powder) was remarkable, as was the LC_{50} ratio (× 1671) of Shapiro and Robertson (1992); the synergism swamped the feeding inhibition. Stimulants improved potency of both virus and *B. thuringiensis* in other insects feeding on expanded leaves (Table 3.14, part 3).

Cotton pests have responded to the best phagostimulants, which partially masked the cotton leaf surface alkalinity and allelochemicals (Table 3.14, part 1). Coax, developed specially for cotton, is the most widely used and successful stimulant adjuvant. Results with viruses and single stimulants (including sugars) with *Heliothis* spp. have varied. Molasses did not stimulate *S. littoralis* (Table 3.14).

Starch is being developed as a carrier for corn borer control. Alone, it is less attractive

than fresh corn leaf. The difference disappears on addition of sucrose, and starch+Coax is more attractive than fresh leaf (Table 3.14, part 4). Starch can be formulated as a dry granular corn borer bait (section 3.3.1) or as a self-encapsulating spray (section 3.3.5).

Dry baits for other pests commonly consist of pathogen-treated cereals or cereal products. These are shown to be attractive and stimulatory to pests by virtue of successful pest control. Bran, corn meal, cottonseed meal and wheat stimulated various insects, and molasses made corn starch palatable to grasshoppers (Table 3.14, part 5).

There has been no evidence that any of the phagostimulants (Table 3.14) have been harmful to, or incompatible with, pathogens. Comparing results in different publications is difficult, but a general view of the literature in these tables gives the impression that the most effective phagostimulants have been Coax, molasses and plant extracts.

3.4.4 SUNSCREENS

Data on field persistence of microbial insecticides reveal that sunlight is the most destructive of the environmental factors (Ignoffo and Hostetter, 1977; Ignoffo *et al.*, 1977; Jones and McKinley, 1987; Jones, 1988a). A subjective assessment of the value of different sunscreen additives is presented in Table 3.16. Factors involved in this assessment are elaborated below.

3.4.4a Damaging wavelengths in sunlight Of light reaching the earth's surface, that with wavelengths mainly up to 380 nm, with a peak at *ca* 300 nm, kills *B. thuringiensis* spores, while light of mainly 300–380 nm damages crystals (J. Mitchell, Cranfield Biotechnology Centre, Cranfield University, UK, personal communication; Pusztai *et al.*, 1991). However, Griego and Spence (1978) found that the greatest kill of spores occurred at 400 nm in the visible range because of its much greater amount of energy compared with light of shorter wavelengths. The medium-wave or erythermal UV band (UVB, 280–320 nm) is the most important photoinactivator of baculovirus, with considerable but slower effect in the near-UV region (UVA 320–360 nm), and in some cases some effect above this (David, 1969; Timans, 1982; Griego *et al.*, 1985; Martignoni and Iwai, 1985; Killick, 1986; Jones *et al.*, 1993b). Light of some longer wavelengths, however, may be beneficial to microorganisms (Jones *et al.*, 1993b). The wavelengths involved in experiments have been taken into account for assessments in Table 3.16.

3.4.4b Effect of sunlight on different pathogens in different situations Insect bioassay showed that a laboratory simulation of the UV radiation in sun affected the potency of *B. thuringiensis* less than six other types of pathogen. Exposure to UV for 4 h, that reduced the original activity of *B. thuringiensis* to 46%, reduced the activity of entomopox virus to 18%, of *Nomuraea rileyi* conidia to 13%, of NPV and cytoplasmic polyhedrosis virus (CPV) to 8%, and of GV and spores of the protozoan *Vairimorpha necatrix* to 4% (Ignoffo *et al.*, 1977). Work on protection of two types of pathogen, *B. thuringiensis* and baculovirus, is distinguished in Table 3.16.

In the field, the half-life of *B. thuringiensis* and baculovirus varies greatly, from as little as 10 h to 10 days (Entwistle and Evans, 1985). In general, the half-life in full sunlight without protective screens centres at *ca* 24 h. Thus there appears to be much scope for improvement. This scope varies in different situations, being greatest where the pest feeds on the upper surface of foliage and in the tropics, and minimal on lower leaf surfaces deep in a foliage canopy under cloud, where UV radiation penetrates least. For example, the level of UV radiation reaching undersurfaces of leaves in the lower canopy of the cotton crop in Egypt was only 1% of that at the top of the plants (Jones, 1988a). In contrast, UV is not an important degrading factor deep in cryptic

Table 3.16 Effect* of sunscreens on *Bacillus thuringiensis* (B) and nuclear polyhedrosis or granulosis virus (V) exposed to UV light

Sunscreens (composition, etc. Appendix Tables I.9 – I.12)	*Percentage*	*Effect**	*References (orders as under effect)*
I ABSORBENTS			
Amino acids			
Adenine	1.0	++	1V
Glutamic acid	1.0	+	2V
Histidine	1.0	++	3V
Lysine KK1	0.5	**p+++**	**4V**
Phenylalanine	1.0–10.0	++	3V
	0.1	+	3V
Proline	1.0	++	3V
Soya hydrolysate	5.0	**a++++,**	**5V**
		a+++	**5V**
Tryptophan	10.0	++++	3V
	0.01–1.0	+++	3V
	0.001	++	3V
Tyrosine	10.0	++++	3V
	1.0	+++	3V
	0.1	++	3V
	0.01	+	3V
B Vitamins			
p-aminobenzoic acid (PABA)	5.0	+++	6V
	1.0	+, s++	2V, 7B
	0.1	s++	7B
Amyl-dimethyl-*p*-aminobenzoic acid	5.0	+++	6V
Ethoxylated *p*-aminobenzoic acid	5.0	+++	6V
iso-Octyl *p*-aminobenzoic acid	5.0	++	6V
Choline chloride	1.0	++	2V
Folic acid	1.0	++++,	2V,
		s+++, +++	7B, 7B
	0.1	++, s++,	2V, 7B,
		++	7B
Xanthopterin	1.0	+++	2V
Inositol	1.0	+	2V
Nicotinic acid	1.0	++	2V
Pantothenic acid	1.0	++	2V
Pyridoxine	1.0	++	2V
Riboflavin	2.0	0	8V
	1.0	+++	2V
	0.25	++	2V
Thiamine	1.0	+	2V
Cosmetic sunscreens			
p-Aminobenzoic acid and derivatives; see under B vitamins			
Benzilidine sulphonic acid	5.0	++++	6V
Benzyl cinnamate	3.0	0	9B
2-Ethoxyethyl-*p*-methoxycinnamate	5.0	+++	6V
2-Ethylhexyl salicylate	5.0	++	6V
Homomenthyl salicylate	5.0	–	6V

Eusolex 4360	5.0	+	6V
	0.1	s++++, +++, **++**, **+++**	10B, 10V, **10B**, **10V**
Eusolex 6300	5.0	++	6V
	0.1	s+++, ++	10B, 10V
Eusolex 8021	0.1	++++	10V
Uvinul DS49	1.0	s+, **+++**, ++	9B, **9B**, 11V
Uvitex ERN-P	0.1	s+++	9B
Dyes			
Acid Black 48	1.0	++	12V
Acid Orange 8	1.0	++	12V
Acridine Yellow	1.0	++++	12V
Acriflavin	0.42 mmol/g	++++	13B
	0.06 mmol/g	+++	13B
Alcian Blue 8gx	1.0	++	12V
Alizarin Yellow R	1.0	++	12V
Alkali Blue	1.0	++++	12V
Astrazone Orange	1.0	++	12V
Astrazone Yellow	1.0	++	12V
Azocarmine	1.0	++	12V
Benzopurpurin 4B	1.0	+++	12V
Bismark Brown	1.0	++	12V
Brilliant Blue g	1.0	++	12V
Brilliant Blue R	1.0	+++	12V
Brilliant Yellow	1.0	++++	12V
Buffalo Black	2.0	c+++	14V
Chrome Axurol	1.0	++	12V
Chrysophenine	1.0	+++	12V
Cibachron Blue	1.0	++	12V
Cibachron Yellow	1.0	+++	12V
Congo Red C.I. 22120	1.6	c++++	15V
	1.0	++++, ++++, s++++, ++++	16V, 12V, 7B, 7B
	0.5	++++	16V
	0.25	+++	16V
	0.1	++, s+++, ++	16V, 7B, 7B
	0.04	**0**	**17B**
Curcumin	1.0	++	12V
Direct Red 81	1.0	++	12V
Direct Yellow 8, 17	1.0	++	12V
Disperse Blue 14	1.0	+++	12V
Disperse Orange II	1.0	+++	12V
Erio Acid Red XB100	0.1	s++++	9B
Fast Blue	1.0	+++	12V
Haematoxylin	1.0	++	12V
Indigo Carmine	1.0	+++	12V
Lissamine Green	1.0	++++	12V
Mercurochrome	1.0	++++	12V
Methyl Green	0.53 mmol/g	++	13B

Table 3.16 (*Contd.*)

Sunscreens (composition, etc. Appendix Tables I.9 – I.12)	*Percentage*	*Effect**	*References*
Methyl Orange	1.0	+++	12V
Methyl Red	1.0	+++	12V
Mordant Brown 1,33	1.0	+++	12V
Neutral Red[1]	1.0	++	12V
Nigrosin	1.0	+++	12V
Orange IV	1.0	++	12V
Orcin	1.0	++	12V
Reactive Blue 4	1.0	++	12V
Rhodamine B	0.1 mmol/g	+	13B
20 stains	1.0	+	12V
20 stains	1.0	0	12V
Miscellaneous			
Aesculin	0.1	s+	10B
Bentonite	?	**p+++**	**4V**
Carbon, carbon Rb, carbon black, charcoal, India Ink	5.0	++, **+++**, **+++**	6V, **5V** **18V**
	2.0	c++++, ++++, **y+**	14V, 14V, **19V**
	1.6	c++++	15V
	1.0	**0, ++, ++, 0, ++, 0, 0, c++++, 0,**	**20B, 21V, 21V 21V, 21V, 21V, 21V, 19V, 21V,**
	0.42	**c++++**, **yco**, +++,	**19V**, **19V**, 19V
	0.1	s++++	10B
	?	**y0**, **+++**	**22V**, **22V**
Citric acid by-product	0.5	**p+++**	**4V**
Coax	6.0	++++	6V
	5.0	++	11V
Corn starch	carrier	s0, 0	7B, 7B
Corn starch/flour/sucrose	carrier	c++++, +++	23B, 23B
Flour 961/sucrose	2.0	**+**, ++++, +++	**24B**, 25B, 26V
Flour, corn pre-gelatinized	0.5–4.0	+++	17B
Lignin, Kraft, + $CaCl_2$	0.5	++++, s++++, +++	26V, 26B, 26V
Lignin, Indulin, + $CaCl_2$	0.5	s++++	26B
Lignin, REAX, + $CaCl_2$	0.5	s+++	26B
Lignin, Kraft, + $CaCl_2$	43.5 (carrier)	c++++, c++++	26B, 26V
Lignin, Kraft, + no $CaCl_2$	0.5	s++++	26B
Lignosulphonate, Na	2.0	0	8V
	0.5	s++	26B
	0.1	s++	10B
Orzan LS	6.0	**p0**, ++++, **++++**	**27V**, 11V, **11V**
Raymix L_3	12.15	+++	11V

Raymix powder	6.28	++++	11V
	1.05	++	11V
Melanin	0.0003	s++++,	28B,
		++++	28B
Molasses	25.0	++++	11V
	5.0	++, +++,	6V, 6V,
		+++	6V
Dri-mol	5.0	+	6V
Sucrose	5.0	++	6V
Molasses of peat	0.5	**p+++**	**4V**
Polyglucine	0.4	**p++++**	**4V**
Protec-100	5.0	++	6V
Shade	6.0	+++, **py++**,	11V, **29V**,
		py0	**29V**
	5.0	+++, ++,	6V, 15V,
		0, +++	**19V**, 18V
	2	++, ++++,	30B, 30B,
		0, ++	31V, 31V
	1.6	c++++	15V
	1	+++	32V
	0.5	**+, +y**	**32V, 26V**
	0.25	+++	32V
	0.1	s0, 0	10B, 10V
	?	+	33V
	?	**+, y+**	**21V**, **22V**
Sulisobenzone	0.25	−−,	32V,
		+, +y	**32V, 32V**
Talc	carrier	+	18V
Yeast, brewer's	5.0	**+++**	**5V**
Nitrogenous metabolic products			
Allantoin	1.0	++	1V
Guanine	1.0	++	1V
Hypoxanthine	1.0	0	1V
Urea	1.0	0	1V
Uric acid	10.0	++++	1V
	5.0	+++	1V
	1.0	++	1V
Xanthine	1.0	+	1V
Optical brighteners			
Aclarat 8678	1.0	+++	34V
Calcofluor LD, RWP	1.0	+ to ++	34V
Columbia Blue	1.0	0	34V
Intrawite ABL	1.0	0	34V
Intrawite CF	1.0	++++	34V
	0.1	+++	34V
	0.01	+	34V
Intrawite EBF, ERN	1.0	+++	34V
Leucophor BS	1.0	+++ to ++++	34V
	0.1	+++	34V
	0.01	+	34V
Leucophor BSB	1.0	++++	34V

Table 3.16 (*Contd.*)

Sunscreens (composition, etc. Appendix Tables I.9 – I.12)	*Percentage*	*Effect**	*References*
	0.1	+++	34V
	0.01	+	34V
Leucophor EHB	1.0	+	34V
Leucophor EFR, KNR, PAB, PAL, PAT, WGS	1.0	+++	34V
Phorwite AR	1.0	+++ to ++++, **p+++**	34V, **35V**
	0.1	++ to +++	34V
	0.01	+++	34V
	0.001	++	34V
Phorwite BLK, BRU	1.0	+++ to ++++	34V
	0.1	++ to +++	34V
	0.01	++	34V
	0.001	0	34V
Phorwite C1	1.0	+++	34V
	0.1	++	34V
	0.01	+	34V
Synacril White	1.0	0	34V
Tinopal CBS-X	2.0	–	**8V**
	1.6	c+++	15V
	1.0	++	11V
Tinopal DCS	5.0	++++	11V
Tinopal LPW	1.0	++++	34V
	0.5	**p++++**, **py+++**, **p++++**, **pyo, pyo**, **pyo**	**36V**, **37V**, **35V**, **38V, 38V**, **38V**
	0.1	+++, s+++, **py+++**	34V, 9B, **37V**
	0.05	**p++++**	**35V**
	0.02	**py+**	**37V**
	0.01	+++	34V
	0.001	++	34V
Proteins			
Casein	0.5	**++**, ++	**24B**, 25B
Egg albumen	5.0	**a+++**, +++	**5V**, 39V
Gluten, wheat	1.0	**+++**, +++	**24B**, 25B
Milk, peptonised	5.0	**++++, a+++**	**5V, 5V**
Milk, skimmed	5.0	++	5V
	1	++	32V
	0.25	++	32V
II REFLECTORS			
Aluminium powder	2.0	c+	14V
Titanium dioxide	0.84	c++++, **c++++, yc0**	19V, **19V, 19V**
	0.084	+++	19V

III ANTIOXIDANTS AND OXIDATIVE ENZYMES			
Ascorbic acid	10.0	++++	40V
	1.0	+++	40V
	0.1	++	40V
Na ascorbate	3.0	**0**	**9B**
Catalase	10.0	++++	40V
	1.0	+++	40V
	0.1	++	40V
Peroxidase	10.1	++++	40V
	1.0	0	40V
Phenylthiocarbamide	0.1	++++	40V
	0.01	++	40V
Propyl gallate	0.1	++++	40V
	0.01	+++	40V
	0.001	++	40V
Superoxide dismutase	10.0	+++	40V
	1.0	++	40V

References

1 Shapiro, 1984
2 Shapiro, 1985
3 Ignoffo and Garcia, 1995
4 Jankevica and Zarins, 1997
5 Jaques, 1971
6 Shapiro *et al.*, 1983
7 Dunkle and Shasha, 1989
8 Killick, 1990
9 Morris, 1983
10 Krieg, 1975
11 Martignoni and Iwai, 1985
12 Shapiro and Robertson, 1992
13 Cohen *et al.*, 1991
14 Ignoffo and Batzer, 1971
15 Ignoffo *et al.*, 1991
16 Shapiro, 1989
17 McGuire *et al.*, 1996
18 Ignoffo and Garcia, 1996
19 Bull *et al.*, 1976
20 M. R. McGuire and B. S. Shasha, USDA, personal communication, 1994
21 Jaques, 1972
22 Ignoffo *et al.*, 1972
23 Tamez-Guerra *et al.*, 1996
24 Behle *et al.*, 1997b
25 Behle *et al.*, 1996
26 Shasha *et al.*, 1995
27 Webb *et al.*, 1993a
28 Liu *et al.*, 1993
29 Stelzer *et al.*, 1977
30 Smith *et al.*, 1980
31 Smith *et al.*, 1978a
32 Richards, 1984
33 Ignoffo *et al.*, 1976a
34 Shapiro, 1992
35 Webb *et al.*, 1994b
36 Webb *et al.*, 1994a
37 Webb *et al.*, 1996
38 Reardon and Podgwaite, 1994
39 J. W. Klijnstra, Brueren and T. A. de Vlieger, Agricultural University, Wageningen, personal communication
40 Ignoffo and Garcia, 1994

* Symbols: standard type, laboratory studies; bold type, field studies; −−, statistically significant adverse effect; −, non-significant adverse effect; 0, no effect; +, non-significant protection; ++, small but significant protection; +++, good significant protection in studies with a series of screens; ++++ best protection; s, only spore viability studied; c, encapsulated; **p**, field population study; **y**, field yield or defoliation; bold type without appended letter, laboratory assay of field-exposed pathogen.

Note: best in rough order of merit, melanin, insect remains, Tinopal LPW and other optical brighteners, Orzan LS, Coax, molasses, carriers (e.g. clay, flour), lignin, carbon, titanium dioxide, milk, albumin, Eusolex 4360, Uvinal DS 49, Congo Red CI, Shade (in general, tests disappointing).

positions for corn borer control, exposure being reduced by 93% in corn whorls and 97% in leaf axils, where the insects normally feed (McGuire *et al.*, 1994b).

3.4.4c Mode of action of sunlight Pusztai *et al.* (1991) believe that toxin crystals are photosensitized by exogenous (and possibly endogenous) chromophores picked up from fermentation broth after the lysis of bacterial cells. The chromophores create singlet oxygen species on irradiation by light. Decreasing the exposure of crystals to oxygen, e.g. by use of glycerol as a humectant, reduces photodamage. Pozgay *et al.* (1987) found that tryptophan in the crystal was destroyed. Cohen *et al.* (1991) suggest that photoprotection is attained with cationic chromophores such as acriflavin, by transfer of energy from excited tryptophan moieties to the chromophore molecules. The mechanisms of baculovirus inactivation are not fully known, but are most likely to be similar to those reported for mammalian viruses. With these, inactivation is primarily due to the formation of DNA cross-links through pyrimidine dimers. This may be related to the formation of peroxide radicals (Ignoffo and Garcia, 1994). In Table 3.16, part I, screens that function by preventing damaging wavelengths from reaching the pathogens are distinguished from the antioxidants and enzymes that act by scavenging hyperactive oxygen.

3.4.4d Effect of conditions of test Test conditions have great effects on assessments with sunscreens. These effects are minimized in Table 3.16 by making direct comparisons between screens only within studies, i.e. experiments within which conditions are identical. However, differences between studies involving the same screen are often great. These differences are partially due to the parameters measured: studies with spores alone (assigned as 's' in the table) and the whole pathogen complex of *B. thuringiensis* (no letter assigned) are identified in the table. Field studies (bold type) are more stringent than laboratory work, because the effects of screens are least likely to show up when insect populations (p) or yield and defoliation (y) are measured. Consequently, when using the table different weights must be given to the different types of results. In particular, effects on *B. thuringiensis* spores are less important than effects on the whole spore–crystal complex and – even more pertinent – effects demonstrated in the field, especially on yield or foliage protection, are more meaningful than laboratory results.

A number of studies have been undertaken in the field where microorganisms have been exposed to direct sunlight on glass slides or Petri dishes. Under tropical conditions, Jones *et al.* (1993a) found that glass surfaces on which NPV samples were placed reached 60 °C within a few minutes; the temperature alone would be sufficient to inactivate the virus (section 3.3.4). This problem was avoided by placement of samples on the surface of a refrigerated tray. The same problem occurs with some laboratory artificial sunlight equipment and UV lamps. Again, heat should be dissipated, particularly since light inactivation is influenced by temperature.

Different results may be attributed to less obvious technical factors. For example, dusts exposed on the surface of agar plates are wetted, but those exposed on glass are not; wetting and redrying increased the susceptibility of *Heliothis* NPV in a talc-based dust by ×28 (Ignoffo and Garcia, 1996). Martignoni and Iwai (1985) reported that the sunscreen Coax on dry, non-wettable Teflon pads gave 61% of the protection obtainable by the same concentration of Shade, in contrast to 113% reported by Shapiro *et al.* (1983) on moist, porous agar-based insect diet. Shade is soluble in water but Coax is not, which may result in considerably different distributions on the two surfaces. Also, moisture on the agar surface would allow diffusion of the singlet oxygen species created by the irradiation, facilitating their absorption by Shade, but the water-soluble

screen would dissolve and diffuse away into the agar (Table 3.16). Silicobenzone inactivated 70% of codling moth GV as it dried on a slide, probably because a 1% solution has an acid pH of 2.5, but it did not increase the speed of inactivation on apple trees, possibly because of the buffering action of chemicals (Richards, 1984). It is essential that final conclusions on the efficiency of UV protectants be based on field tests.

A sunscreen may have other benefits in addition to photoprotection. Thus it may be difficult to apportion the cause of an observed improvement. For example, molasses also alters viscosity of a spray and hence spray drop size and distribution, which in turn influence the amount of the pathogen that target larvae eat. Also, spray composition determines the spread of a drop after impact, which influences the thickness of the dried deposit and so the amount of protective screen above the particles (Appendix II, Fig. II.6). As another example of multiple action, milk has moderate sun-screening capacity (Table 3.16), is used as a wetter–sticker (Table 3.13 in section 3.4.2; Richards, 1984) and humectant (section 4.3.4), and also acts as a feeding and growth stimulant to larvae of the torticid moth, *Archips pomonella*, causing increased damage to apple foliage treated with codling moth GV since *Archips* is not susceptible to this virus (Glen *et al.*, 1984; Richards, 1984).

The position of a pathogen in a drop is important (Appendix II, Fig. II.6). Pathogens have been observed to float on the surface of an oil drop, where they would gain little protection (Killick, 1986). This must be remedied by use of surfactants. In photographs of deposits, some pathogens were virtually unshielded by a screen, while others could be totally hidden under bigger particles. On drying, some deposits of lignosulphonate developed cracks (Killick, 1986). Molasses, on the other hand, appeared to cover all particles in a deposit (K. A. J., unpublished results).

The efficiency of any sunscreen depends on the depth of the layer of screen covering the pathogen (Appendix II, Fig. II.6), which, in turn, depends on drop size. Functional optimization of spray design requires a balance between larger drops to achieve the best photoprotection and smaller droplets to achieve the best spray cover.

The effectiveness of a screen covering the organisms depends on the concentration of both the screen and the organisms in a product. For example, 5% (of final weight) Shade added to a technical concentrate of *Heliothis* NPV, before freeze drying and grinding, improved survival after exposure to UV radiation (peak at 365 nm) by × 1.9 (ratio of OARs with and without Shade: an OAR is an original activity ratio), but by × 5.7 in a talc-based dust (Ignoffo and Garcia, 1996). This difference is not surprising because the amount of Shade per polyhedral inclusion body (PIB) in the dust was × 25 that in the concentrate due to the different PIB concentration.

3.4.4e Effectiveness of different sunscreens

Sunscreens act by selectively absorbing, blocking or reflecting UV radiation, or by negating active oxygen radicals. These materials are classified by mode of action in Table 3.16 and Appendix Table I.9–12, and further described in section 4.3.3c. Absorbents convert damaging UV light to harmless visible wavelengths.

Natural absorbents that accompany the pathogens in microbial products confer variable UV protection. This is explained by the demonstrated protective action of amino acids, B vitamins, nitrogenous metabolic products, oxidative enzymes and proteins (Table 3.16). These materials occur in residues of fermentation solids in *B. thuringiensis* products and in insect remains in unpurified or partially purified virus. Many materials are protective at 1% or more, and tyrosine, tryptophan, folic acid, ascorbic acid and catalase give some protection at low concentrations of 0.1% and less. Remarkably, the insect pigment melanin is highly effective at only 0.0003% (Table 3.16; discussed in section 10.9) and it

is the active pigment in a UV-resistant *B. thuringiensis* mutant (Patel *et al.*, 1996). Unpurified NPV has frequently proved to be more photostable than purified NPV (e.g. Manjunath and Mathad, 1982; Shapiro, 1984). Surprisingly, Jones (1988a) found that none of 12 recognized screens tested gave better protection than the debris in unpurified NPV. Some of the naturally occurring absorbents in Table 3.16 can be considered as candidate screens in practice. None of the nitrogenous metabolic products is suitable. Although tryptophan and tyrosine are good protectants, an amino acid would not be economically feasible to use (Ignoffo and Garcia, 1995). Some B vitamins protect by absorbing UV (Table 3.16). The best are folic acid and riboflavin, which also function as biochromes in insects and other animals. Some other fluorochromes are also effective (Table 3.16). The commercially available xanthopterin, which is chemically close to pteridine, a constituent of folic acid, was almost as effective as the vitamin. Among the proteins (Table 3.16), albumin and milk products are already used as spray stickers (section 3.4.2) and sometimes as additives during harvest of pathogens (section 3.2.1). Gluten makes sprays autoencapsulate (section 3.3.1), as well as being a feeding stimulant (section 3.4.3) and sticker (section 3.4.2). Their protection from sun is a valuable additional asset.

Three good natural products, used as screens in practice, are listed under 'miscellaneous' in Table 3.16. Molasses is a multi-purpose additive which was once popular and is still used at high concentrations, at which it is a good sunscreen. However, it is cumbersome and cannot be easily incorporated for product storage; while freeze-drying or spray-drying could make this possible, these processes are difficult with molasses. Carbon products have been tested extensively as blocking screens with good results. This is thought to be due, at least partly, to the ability of carbon to act as an oxygen sink, preventing the formation of free active radicals (Ignoffo and Garcia, 1978). The phagostimulant Coax is also an excellent sunscreen and – being a dry powder – can be mixed into dry stored products; however, it can be difficult to suspend in water (Jones, 1994). All three materials are bulky, a big disadvantage for aerial application.

The need for an easy-to-handle water-soluble protectant, effective at low concentration and miscible with most products during storage, led to the special marketing of Shade, a polyflavanoid (Table 3.16, miscellaneous; Appendix Table I.12). This absorbs radiation maximally at 285–290 nm (Krieg *et al.*, 1980b), as well as peroxide radicals (Ignoffo and Garcia, 1978). Also, it was an effective buffer on cotton, and reduced foliar pH below 8.6 which may have reduced inactivation of virus by high pH (Young and Yearian, 1976). As Shade was taken off the market, many other materials were evaluated from the late 1980s.

The new materials include cosmetic sunscreens, dyes, lignin, lignosulphonate (by products of wood pulping) and optical brighteners (Table 3.16). Of the cosmetics, Uvitex ERN-P and the Eusolex series at 0.1%, also benzilidene sulphonic acid at 5%, were rated best. Both water- and oil-soluble dyes include good protectants; Congo Red is the best. Some, e.g. the alkaloid berberine, are best avoided due to mammalian toxicity.

A number of studies directly compared the new with the older materials. Shapiro *et al.* (1983) and Jones (1988a) found the best from both to be Coax, benzilidene sulphonic acid, Eusolex, Indigo Carmine, molasses and clays (Table 3.16). One of the best comparative laboratory studies is that of Martignoni and Iwai (1985), who took cost into account. They rated two lignosulphonates, Orzan LS and Raymix Powder, as best, and Tinopal DCS considerably behind as second best on grounds of effectiveness and cost. Orzan and Raymix Powder have excellent properties as spray additives: they easily dissolve in cold water and the solution is free from insolubles, with low surface tension and pH near neutral. Both substances were less expensive than many cosmetic sunscreens.

Over the last 10 years, most progress in field testing sunscreens has been made with NPV, the type of pathogen more in need of protection. In 1986, Orzan LS was demonstrated to be effective in North American gypsy moth NPV field tests (Podgwaite and Shapiro, 1986; Table 3.16). It was used in the standard Gypchek formulation in subsequent trials. Recently, a number of stilbene optical brighteners have proved effective at low concentrations (Table 3.16). They have been most effective with some moderately active viruses due primarily to their spectacular synergistic effects (section 3.4.6). Tinopal LPW (= Blankophor BBH) at only 0.05% was outstanding in the laboratory and promising in the field (Table 3.16). It has been possible to lower the current recommended dose rate for ground application of Gypchek using brightener by ×10 (Reardon *et al.*, 1996). Other superior brighteners include Intrawite CF, Leucophor BS and BSB, and Phorwite AR (Table 3.16).

User-friendly commercial gypsy moth NPV products now being developed in trials from 1992 are expected to contain a sunscreen. Although Orzan LS is no longer commercially available, the main choices are likely to be from the lignosulphonates and optical brighteners.

Use of UV protectants at 1–10% in low-or high-volume tank-mixes is wasteful, and they can be more efficiently used in microcapsules (section 3.3.5). Carbon black and titanium dioxide gave good protection in the laboratory and on field cotton when mixed with NPV, and excellent protection when encapsulated in a polymer (Table 3.16). McGuire *et al.* (1991) incorporated Congo Red into matrices of starch (the matrix itself is a blocker); incorporation may partly solve the dye's unfortunate property of staining one's skin, as experienced by one operator who finished brilliant red all over after applying an experimental field spray containing the non-encapsulated dye. However, after storage without exposure to UV it decreased grasshopper mortality, possibly by toxicity to the virus, altering the infection cycle or deterring feeding.

Both Shade and carbon are believed to reduce free oxygen radicals, an alternative mode of action to absorption and reflection. Among possible antioxidants and oxidative enzymes that scavenge or catalyse the degradation of reactive radicals (Table 3.16, part III; Appendix Table I.12), low concentrations of propyl gallate might be used in tank mixes because of low cost and common use as a food additive (Ignoffo and Garcia, 1994).

Taking an overall view, the best sunscreens are listed in approximate order of merit in the Note at the foot of Table 3.16.

A few sunscreens have been critically examined for possible harm to pathogens (Appendix Tables I.9–12). Sulisobenzone inactivated GV on slides.

3.4.5 ADDITIVES TO COUNTER FOLIAGE FACTORS

The chemical composition of plant leaves may influence insect pathogens in various ways. The cotton phylloplane is highly alkaline, with pH values as high as 11. High pH dissolves crystals of *B. thuringiensis* and harms baculoviruses, making them less stable on the leaf surface. Standing NPV in phosphate buffer at pH 4–9 for 24 h at 30 °C had no effect but, at pH 10, virus was inactivated even when encapsulated in the water-insoluble polymer SMA-2625A (Appendix Table I.3; Bull *et al.*, 1976). Occlusion bodies of several NPVs are solubilized – and hence inactivated – at pH values close to 10 (Griffith, 1985). Addition of Shade and molasses reduces the pH of sprays (Young and Yearian, 1976) and the pH can be made slightly acid with >0.06% Sorba Spray Zip (Vail *et al.*, 1977, 1980). Buffered sprays have had negative or indifferent effects (Falcon, 1971; Young and Yearian, 1976). Elleman and Entwistle (1985a, b) suggested that, rather than the effect of alkalinity, free Mg^{2+} ions in cotton dew prevented the dissolution of polyhedra within the insect gut,

an effect reversible by the addition of a chelating agent, e.g. ethylenediaminetetra-acetic acid (EDTA) (Appendix Table I.7).

The surface of some leaves is acid, e.g. pH 1 on chickpea due to maleic acid, which might directly inactivate an organism, and also lower the insect gut pH and impair the activity of both the crystal and spore of *B. thuringiensis.* Thus Gringorten *et al.* (1992) found that reducing the alkaline pH of enzyme-activated crystal toxin reversibly lowered toxicity in assays on lawns of IPRI-CF-1 cells, and in force-feeding assays with silkworm larvae. Also, the principal activator of spores in the gut of tobacco hornworm larvae is alkaline pH (Wilson and Benoit, 1993). Dew of <pH 4 on leaves could denature the crystal or NPVs.

Allelochemicals, part of a plant's natural defence against herbivores (an increasing area of study) influence insect pathogens in various ways. Some plant extracts inhibit bacterial growth (e.g. Morris, 1972; Morris and Moore, 1975). Nicotine, an alkaloid, reduces the activity of *B. thuringiensis*, probably that of the crystal (Barbosa, 1988; Krischik *et al.*, 1988). Tannins (mixtures of polyphenols) are general precipitators of proteins and inactivate the toxin, as shown by a reduction of larval mortality after pre-reacting tannin with crystals in various stages of activation (Lüthy *et al.*, 1985). The presence of tannin depends on the plant species: for example, it depressed the activity of *B. thuringiensis* in gypsy moth larvae on tannin-rich trees more than on aspen, a tree low in tannin (Appel and Schultz, 1994).

The amount of insect feeding on plants is reduced by many allelochemicals, e.g. nicotine and tannins (Navon, 1992, 1993; Morris *et al.*, 1995). This decreases the amount of bacterium or virus ingested, and may be partly responsible for differences in LC_{50} values of the same pathogen on different plants observed by Richter *et al.* (1987), Santiago-Alvarez and Ortiz-Garcia (1992). Allelochemicals probably also have an effect in other ways (Jones, 1988a; Navon, 1992, 1993). Richter *et al.* (1987) suggested that differences in the susceptibility to NPV of larvae reared and dosed on different plant species was due to stress. It is well established that a protein present in mulberry leaves combines with a protein in the gut of silkworms to form an anti-viral protein (Uchida *et al.*, 1984; Hou and Chui, 1986). Although no similar mechanism has been identified for other insect or plant species, this example does illustrate that such effects are possible. Felton *et al.* (1987) found that two ortho-hydroxy phenolic compounds, rutin and chlorogenic acid, which are present in tomato plants and are potential sources of insect host-plant resistance, reduced the infectivity of *Helicoverpa zea* NPV in tissue culture, but rutin had no effect on *Manduca sexta* NPV on leaf (Krischik *et al.*, 1988).

Specific attempts to neutralize antibacterial, antiviral and allelochemical substances have been unsuccessful (the above example of EDTA against free Mg^{2+} ions is one exception, although probably not a practical solution). However, these substances may be partially counteracted by the use of phagostimulants (sections 2.2.4, 3.4.3). Also, additives regarded primarily as stickers, sunscreens and synergists (Table 3.13 in section 3.4.2, Table 3.16 in section 3.4.4, Tables 3.17 and 3.18 in section 3.4.6 below) may owe part of their ability to prolong the activity of organisms to anti-allelochemical action. Problems associated with extremes of leaf surface pH might be addressed through encapsulation of the organism in a polymer insoluble in either acid or alkali, or with good buffering capacity, e.g. the sugar–starch formulation (McGuire *et al.*, 1996). Alternatively, protection from both low and high pH may be given by formulation in oil.

3.4.6 SYNERGISTS

Additives have a bewildering array of interactions with pathogens, ranging from great increases in activity to nearly total inhibitions

of host deaths. Benz (1971) describes eight types of effect within this range. An additive may be innocuous to the insect on its own, but within the same host–pathogen system it may have a spectacular effect with the pathogen as a classical synergist, or its lethal effects may do no more than add to the mortality caused by the pathogen, a transition that can be dosage dependent. For simplicity in this chapter, all increases in mortality in combinations will be called synergy.

All pathogens detailed in this chapter attack perorally, so the dosage taken up by the insect depends on the amount of food eaten. Thus phagostimulants (section 3.4.3; Appendix Table I.7) synergize and feeding depressants antagonize the pathogens, while malnutrition due to feeding depression may itself kill, giving a synergistic increase of mortality. Table 3.17 relates the effects of synergists to their suggested modes of action. Some synergists may combine more than one mode of action, e.g. acetamide, caffeine, tannic acid (a variable mixture of complex phenolic acids) and simpler phenolic compounds, e.g. chlorogenic acid and polyphenol oxidase (Ludlam *et al.*, 1991), may synergize by reducing feeding, while direct poisoning may also be a factor.

B. thuringiensis is synergized by protease inhibitors, although MacIntosh *et al.* (1990) have evidence that they do not prevent release of toxins from the crystal in the gut. Activities of all three main strain groups, active in Lepidoptera, Diptera and Coleoptera, were synergized.

B. thuringiensis synergists may act at any stage of the pathogenic process (Table 3.17). At the very beginning, seed extracts stimulate feeding (section 3.4.3) and increase the amount of pathogen ingested, while some also contain trypsin inhibitors (see above). Alkalis increase gut pH, aiding protoxin solubilization by breaking disulphide bonds. Ca^{2+}, K^{+}, Na^{+} and Zn^{2+} ions (Table 3.17, part III) are cofactors of proteolysis, which cleaves protoxins into active toxins. Additives that damage the epithelium and impair secretion of alkaline gut juices lower gut pH, enabling spore germination and bacterial growth, although some synergistic additives are known germination inhibitors – an activity that would have the opposite effect. Ascorbic acid is a common ingredient in insect diets and influences insect health; larval mortality of the codling moth was increased by sub- and supra-optimal proportions (Pristavko and Dovzhenok, 1974). Abrasion by boric acid and erosion by chitinase may make the peritrophic membrane more permeable to the toxins. Detergents are lipid emulsifiers and may increase the permeability of the epithelial cells themselves to these toxins. Without direct proof, ascribing causes to observed effects, these suggestions are tentative. Some of the above synergistic additives may, of course, have other effects which may be concurrent, active in different circumstances, or functional in different insects. Thus, Ca^{2+} is a K-channel blocker that inhibits the action of toxin on the apical membrane of epithelial cells and causes inhibition. Tannic acid, a strong synergist (Table 3.17, part VI), can also inactivate the crystal toxin (section 3.4.5). After the toxin disrupts epithelial permeability, the blood is polluted and the presence of extra amino acids may alter the vital amino acid balance in the blood, which could explain both observed synergisms and inhibitions (Table 3.17, part I).

Whatever their modes of action, some synergists appear exciting in practice. With *B. thuringiensis* in pests of only limited susceptibility, i.e. species of *Spodoptera* and *Agrotis*, Salama's group found that over 18 additives in insect diets gave $\geq$ 10–fold synergism (Table 3.17, part III, references 4, 1, 5). In these and other insects, the most promising additives were $CaCO_3$ and $CaO, CaSO_4$, $CuCO_3.Cu(OH)_2$, K_2CO_3, Na_2CO_3, $ZnSO_3$ and $ZnSO_4$, also the amino acids arginine, asparagine, glutamine, ornithine, proline, serine, tryptophan and valine, as well as acetamide, caffeine, tannic acid, Tween 60 and 80, trypsin inhibitor, EDTA, sodium thioglycolate

Table 3.17 Synergists of *Bacillus thuringiensis* (Bt) and of nuclear polyhedrosis virus (NPV) in assays on artificial insect diet and, in bold print, in leaf assays and field tests

Synergist (Appendix Table I.7)	*Concentration (%)**	*Effect (× fold)†*	*Reference*	*Suggested mode of action*
I AMINO ACIDS AND AMIDES WITH Bt				
DL-Alanine	?	4.6	1	Bt germination
D-Alanine + D-serine	0.12–0.48	**R**	**2**	inhibitors
L-Arginine	0.01	3.5	3	Many amino acids
	0.05	3.5	3	affect Na^+ and K^+
	0.1	1.8, 3.6, 6.5, **14**	3, 4, 5, **5 leaf**	transport in gut. May alter toxin activity.
	?	40	1	Balance in blood important
L-Asparagine	?	26	1	
DL-Aspartic acid	?	4.8	1	
D-Cycloserine	0.02	1	2	Germination and protease inhibitor, bactericide
L-Glutamic acid	?	3.1	1	
DL-Glutamine	?	29	1	
Guanosine	0.05	**1**	**2**	Germination inhibitor
DL-Ornithine	?	22	1	
L-Proline	?	31	1	
L-Serine	0.05	1.7	3	
	?	7–40	1	
DL-Serine	?	7.1	1	
L-Tryptophan	0.5	13	4	
DL-Tryptophan	?	16	1	
L-Valine	0.05	2.3	3	
	0.1	4.2, **20**	5, **5 leaf**	
	?	7–40	1	
DL-Valine	?	7.3	1	
D-Methionine, L-phenylalanine, DL-phenylalanine, L-tryptophan	0.1	0.52–1.19	5	
L-Cystine, L-histidine, DL-isoleucine, L-leucine, L-lysine, DL-methionine, DL-threonine, DL-tyrosine	?	0.57–1.5	1	
II SURFACTANTS WITH Bt				
Cetyltrimethylammonium bromide	0.025	3.6	4	Damage lipid membranes of gut
Tween 40	0.5	3.1	4	epithelial cells
Tween 60	0.05	1.6, 9.3	3, 4	
Tween 80	0.05	0.3, 6	3, 4	
Sodium dodecyl sulphate	0.01	1.8	3	
	0.05	3.5	3	
	0.1	1	3	

III INORGANIC SALTS AND ACIDS WITH Bt				
$(NH_4)_2S_2O_8$	0.05	0	3	
$(NH_4)_2HPO_4$	0.05	1.4	3	
	0.5	**7.3**	**5 leaf**	
Boric acid	0.01	1.1	3	Abrasive, damages
	0.05–0.1	3.5, 3.5	3, 3	peritrophic membrane
	1	**1.3–1.8, 4.0–7.0**	**6, 6**	and epithelial
Borax	0.1	**6.6**	**5 leaf**	membrane
	0.5	27	5	
$CaCO_3$	0.05	1.6, **10.1**	3, **3**	
	0.1–0.25	4.6, 6.0, 4.3	1, 4, 5	
	0.5	**1.2, 1.2, 1.4, 5.7**	**7, 7, 7 all yield, 15 leaf**	
CaO	0.05	1.7, 7.6	3, 4	
	0.1	15, **15**	5, **5 leaf**	
	0.5	**0, 1.2**	**7, 7**	
$Ca(OH)_2$	0.5	**1, 1.2**	**7, 7**	
$Ca(NO_3)_2$	0.01	1	4	
$CaSO_4$	0.05	1.9, **2.0,**	3, **3,**	
	0.1	3.5, 4.6	1, 1	
	0.5	**1.1, 1.3**	**7, 7 both yield**	
	1	5.2	4	
$CuCO_3 \cdot Cu(OH)_2$	0.05	1.5, 14.6, 20	3, 1, 4	
	0.5	**1.1**	**7 yield**	
CuO	0.05	1, 13.5	1, 4	
Copper phosphate	0.05	6.7	13	
$CuSO_4$	0.05	3.0	4	
$MgCl_2$	0.01	3.5	3	
$MgSO_4$	0.05	2.1	3	
	0.01	1.9	3	
	0.05	3.5, **2.8**	3, **3**	
	0.1	1.5	3	
	0.2	**R**	**2**	
K_2CO_3	0.05	2.2, **4.1**	3, **3**	
	0.075	**1.3, 1.4, 1.1**	**8, 8, 7 all yield**	
	0.5	8.8; 4.8; **4.4**; **1.1, 1.2, 1**	5, 5, **5 leaf**, **8, 8, 7 all yield**	
$KHCO_3$	1	18	4	
K_2HPO_4	0.05	1.8	3	
	1	25, 2.5	1, 5	
Na_2CO_3	0.05	1.8, **7.6**	3, **3**	
$NaNO_3$	1	3.9	4	
$NaNO_2$	0.1	**R**	**2**	
$ZnSO_4$	0.05	2.2, 20	3, 4	
	0.1	16	5	
	1	10	1	

Table 3.17 (*Contd.*)

Synergist (Appendix Table I.7)	*Concentration (%)**	*Effect (× fold)†*	*Reference*	*Suggested mode of action*
$ZnSO_3$	0.1	4.7, **8.9**	1, **5 leaf**	
	0.5	**1, 1.2, 1.2**	**7, 7, 7 all yield**	
IV MISCELLANEOUS WITH Bt				
Ascorbic acid	0.05	2.3	3	Excess reduced haemocytes and phagocytes, increased susceptibility
Caffeine	0.1	3.5, **9.2**	9, **9**	Feeding inhibitor
Dimethyl sulphoxide	0.05	1.4	3	
Dodecyclamine	0.05	1	3	Some acylamines increased NPV infection
Dipicolinic acid	0.02	**R**	**2**	Germination inhibitor
Enhancin protein from GV	various	0.7–3.4	10	
Neemazal-T	0.001	1.0–2.9	10	
Salicylic acid	0.5	0.83	5	
p-amino salicylic acid	0.1–0.2	**2.9**	**2**	
	0.5	1.6	3	
Sodium salicylate	0.5	0.50	5	
Sorbic acid	0.05	1.6	3	
V OPTICAL BRIGHTENERS WITH NPV				
Tinopal LPW	0.01	1, **1–1.6**	11, **11**	Causes virus maturation in gut cells
= Blankophor BBH	0.02	**1–4.3**	**12**	
= Brightener 28	0.05–0.1	15,	11,	
= Calcofluor White M2R		1584,	11,	
		0.7–461	10	
		1–2.7	**11,**	
		2.1–5.7,	**12**	
	0.5	**5.5–11.7**	**12**	
	1.0	**1.7–2.3**	**11,**	
		42, 214	13, 13	
	0.25	4–16	14	
	1.0	41–214	15	
VI ORGANIC ACIDS AND THEIR SALTS WITH Bt				
Acetamide	0.01	1.5, **5.0**	3, **5 leaf**	Reduces feeding
	0.05	3.5	3	
	0.10	2.3, 21	3, 5	
	1.0	24, 25	1, 4	
Calcium acetate	0.05	1.7, 3.7, 8.8	1, 3, 4	
Fumaric acid	0.5	3.9	1	
Lauric acid	0.05	1.5	3	
Malic acid	0.05	1.8	3	
	0.5	3.8	1	
K tartrate	0.1	3.8	1	
Sodium acetate	1.0	3.4	1	
Na formate	0.5	2.6	1	
Uric acid	0.5	1.9	1	

VII PHENOLIC COMPOUNDS WITH Bt				
NH_4 benzoate	0.1	**1**	**2**	Feeding inhibitors, direct poisons
Methyl-*p*-hydroxybenzoate	0.88	1.83	16	
	1.76	0.82	16	
Sodium benzoate	0.5	2.5, 0.75	4, 5	
Gallic acid	0.001	0–1.4	16	
	0.01	0.66–0.75	16	
	0.72	3.4	17	
Phenylacetic acid	0.1	1.1	5	
Resorcinol	0.27	3.6	17	
Tannic acid	0.0025	7.1, 0	18, 18	
	0.05	1.8	3	
	1.0	20	5	
VIII PROTEASE INHIBITORS WITH Bt				
20 seed extracts	< 20	1.4–6.9	19	Phagostimulants
Metallo, sulphydryl, carboxyl protease inhibitors, amylase	1.0	1	19	
Trypsin inhibitors	2×10^{-6}–0.6	2–40	19	
	0.05	1.8	3	
IX PROTEIN SOLUBILIZING REAGENTS WITH Bt				
EDTA	0.25	**1**	**2**	Germination inhibitor, chelating agent, protease inhibitor
	0.5	12	1	
Na_2 – β-glycerophosphate	0.05	6.0	4	
	0.1	2.0	5	
	1.0	**12**	**5**	
Sodium thioglycolate	0.05	1.9, 16	3, 4	
	1.0	3.4, 3.0	1, 5	
Potassium phosphate, K_2HPO_3	1.0	5.6	4	
Urea	0.5	2.7, 2.3	1, 4	

References

1 Salama *et al.*, 1989
2 Burges, 1977
3 Morris *et al.*, 1995
4 Salama *et al.*, 1985
5 Salama *et al.*, 1986
6 Doane and Wallis, 1964
7 Salama *et al.*, 1990b
8 Salama *et al.*, 1990a
9 Morris *et al.*, 1994
10 El-Salamouny *et al.*, 1997
11 Zou and Young, 1996
12 Webb *et al.*, 1996
13 Farrar *et al.*, 1995
14 Vail *et al.*, 1996
15 Dougherty, 1996
16 Dimetry and Matter, 1990
17 Sivamani *et al.*, 1992
18 Gibson *et al.*, 1995
19 MacIntosh *et al.*, 1990

* Percentage (wt/vol) in diet or spray.

† Statistically significant ratios of LC_{50} or of percentage mortality for pathogen + synergist to LC_{50} or percentage mortality for pathogen alone. Non-significant ratios indicated by zero. Where actual values are not given in the orginal publications, significant reduction in potency is shown by R, and significant enhancement by E.

and sodium glycerophosphate. These results were obtained with a range of *B. thuringiensis* strains, including one of the newer strains, that are more active than *kurstaki* against *Spodoptera* spp.

Effects were even more impressive on leaf (bold values in Table 3.17). Seven additives gave greater synergism on cotton leaf than in diet and one *vice versa* (Morris *et al.*, 1995). Most diets contain no inhibitory allelochemicals, so these differences may reflect reactions on leaf-to-plant allelochemicals (sections 3.4.5). With a virus, Jones (1988b) found the LC_{50} of NPV against *S. littoralis* to be lower on diet than on cotton or lucerne, possibly due in part to the presence of inhibiting allelochemicals in the leaves.

Synergists could improve some uses of *B. thuringiensis* from marginal to highly economic. With var. *kurstaki* against *S. littoralis* on cotton leaf, for example, an LC_{50} of 1222 μg/ml (0.1% by weight) could be upgraded to 122 or even 12 μg/ml (Salama *et al.*, 1986). In this species, rapid direct poisoning by the toxin may be synergized to replace slow death by attrition, due to starvation and prominent involvement of the spore. Many of the synergists are inexpensive, e.g. individual salts would increase the cost of sprays by only 2.6–7.0 cents/l (Morris *et al.*, 1995). In field tests, synergistic effects of some of the salts have been demonstrated as $\times$1.1 to $\times$5.7 increases in crop yields (Table 3.17).

Combining synergists sometimes enhances the synergism (Table 3.18). The two most effective combinations both involved K_2CO_3, one with borax and the other with $ZnSO_4$; their collective synergisms were $\times$3 the expected sums of individual synergisms. Six combinations increased synergisms by >20%, five remained similar, but four combinations resulted in less synergism by at least 20% (Table 3.18).

Some of the synergisms are probably specific to certain plant and insect species, since results in Table 3.17 vary and refer to many different insects, crops and trees. For example, the addition of tannin might inhibit *B. thuringiensis* activity in some insect species not adapted to feeding on tannin-rich plants (section 3.4.5). Also, allelochemicals in some leaves may prevent synergism of some substances active on diet. Thus a note of caution is sounded, and these materials are likely to be best used in specific tank mixes, rather than added to the on-shelf products. Some effects appeared to be *B. thuringiensis*-dosage specific. For instance, sodium dodecyl sulphate and arginine showed significant synergisms at the LD_{50}, but not at the LD_{90} level (Morris *et al.*, 1995). This suggests further caution.

Viruses have even more exciting synergists. Recent patented work by Shapiro and coworkers with stilbene optical brighteners on virus (Table 3.17, part V) has been spectacular (Shapiro *et al.*, 1992). These are also sunscreens (section 3.4.4e). Tinopal LPW (= Blankophor BBH) enabled NPV to infect midgut cells, a tissue where virus production of polyhedral bodies does not normally occur. Within 24 h of application on insect diet, infection was irreversible and within another 24 h, feeding stopped prematurely and the larvae eventually died – exhibiting most of the effects of starvation (Hamm and Shapiro, 1992; Shapiro, 1992; Shapiro and Robertson, 1992; Sheppard and Shapiro, 1994; Sheppard *et al.*, 1994).

The stilbene effect occurs on diet and leaf (Table 3.17, part V), giving enhanced mortality and early oak foliage protection in 48 h (Sheppard *et al.*, 1994). The synergism is most pronounced with the less active NPVs (Dougherty *et al.*, 1996; Vail *et al.*, 1996) and with low concentrations of virus (Vail *et al.*, 1996). There was no mortality in hosts not susceptible to the virus alone (Adams *et al.*, 1994; Hunter-Fujita *et al.*, 1997b). Remarkable enhancements were obtained, $\times$118 at 0.1% Tinopal LPW and $\times$1670 at 1% against gypsy moth larvae (Shapiro and Robertson, 1992) and $\times$164 to $\times$303 000 at 0.1% against *S. frugiperda* (Hamm and Shapiro, 1992). Less

Table 3.18 Effect of combinations of synergists on the potency of *Bacillus thuringiensis* against *Spodoptera littoralis*, compared (in parenthesis) with the sums of the effects of the synergists individually (Salama *et al.*, 1986)

Synergist combinations	*Concentrations (%)*	*Effect (× fold)**
K_2CO_3+ borax	0.5 + 0.5	122(36)
Borax + tannic acid	0.5 + 1.0	23(47)
K_2CO_3 + tannic acid	0.5 + 1.0	12(29)
Borax + tannic acid + K_2CO_3	0.5 + 1.0 + 0.5	60(56)
L-arginine + L-valine	**0.1 + 0.1**	**17(34)**
L-arginine + β-glycerophosphate	**0.1 + 1.0**	**26(33)**
L-arginine + L-valine + β glycerophosphate + acetamide	**0.1 + 0.1 + 1.0 + 0.1**	**24(51)**
$ZnSO_4$ + $CaCO_3$	**0.1+0.5**	**16(15)**
$ZnSO_4$ + $(NH_4)_2HPO_4$	**0.1 + 0.5**	**20(21)**
$ZnSO_4$ + K_2CO_3	**0.1 + 0.5**	**45(17)**
$ZnSO_4$ + borax	**0.1 + 0.1**	**39(15)**
$ZnSO_4$ + K_2CO_3 + CaO + $(NH_4)_2HPO_4$ + borax	**0.1 + 0.5 + 0.1 + 0.5 + 0.1**	**60(47)**
$CaCO_3$ + $(NH_4)_2HPO_4$	**0.5 + 0.5**	**11(13)**
$CaCO_3$ + borax	**0.5 + 0.1**	**27(12)**
$CaCO_3$ + $ZnSO_4$ + $(NH_4)_2HPO_4$ +borax	**0.5 + 0.1 + 0.5 + 0.1**	**42(28)**

* LC_{50} without synergist/LC_{50} with synergist, on artificial diet and on (bold) cotton leaf.

spectacular results were obtained in forest trials, from which 0.1% Tinopal LPW and one tenth the normal rate of NPV has been recommended for its ability to halve defoliation with a visible but not unsightly Tinopal deposit, speculatively estimated to reduce the commercial materials cost of US$8 per large shade oak tree for virus alone to US$3 (Webb *et al.*, 1996; Table 3.17, part V, reference 12). Tinopal is stable at pH 3.0–10.4, at 121 °C for 5 min and under UVA, UVB or UVC for 7 days (Shapiro and Argauer, 1995).

Among other synergists, early work showed a 10– to 100–fold enhancement of NPV by 0.03% hexylamine (C_6) and dodecylamine (C_{12}), while higher concentrations were inhibitory (Yamamoto and Tanada, 1980). These may alter the charge on virus particles and hence their attraction to cell membranes. Cationic detergents (cetyltrimethylammonium bromide and dodecylamine hydrochloride) enhanced infection; anionic detergents did not (Yamamoto and Tanada, 1978b). Boric acid and sodium tetraborate increased NPV potency (McKinley, 1985). In present industrial practice, the only synergists (excepting chemical insecticides) tentatively employed with viruses are stilbenes, which permit a ×10 reduction in amount of NPV applied against gypsy moth (see above).

Numerous workers have tested combinations of chemical insecticides with pathogens in the field. The overall impression is that the effect of these combinations is variable and dependent on doses used, age of insect tested and whether the chemical and microbial insecticides were administered simultaneously or separately (e.g. reviews by Benz, 1971; Jones, 1994). This is particularly true for chemicals with antifeedant activity either before or after poisoning occurs. The most useful results, therefore, are from field tests against natural pest populations. These have mostly been with tank mixes. However, some products have been formulated to include chemical pesticides, for example, Mamestrin+ includes deltamethrin at 10% of the normal field rate and the normal concentration of *Mamestra brassicae* NPV, synergism having

been demonstrated in both laboratory and field.

Both synergism and antagonism been noted between different pathogens. Two NPVs were synergistic in *Trichoplusia ni* (Lara-Reyna *et al.*, 1996). In four Lepidopteran species, GV capsules contain synergistic factors that aid the fusion of NPV with the cell membrane of the midgut brush border (e.g. Tanada and Hukuhara, 1971; Derksen and Granados, 1988). However, caution is needed, because Hunter-Fujita *et al.* (1992, 1997b) found that another species, *S. littoralis* GV, antagonizes the homologous NPV, although the effect appears to be dose-related. This GV has been found as a contaminant in batches of NPV produced in Egypt and the UK. Variable results have also been reported with mixtures of NPV and *B. thuringiensis* (Chancey *et al.*, 1973; Lipa *et al.*, 1975; McVay *et al.*, 1977).

3.5 SYSTEMIC ACTION

To date, systemic action of insect pathogens has been achieved only with the δ-endotoxin of *B. thuringiensis.* There are two methods of making the endotoxin act systemically.

The toxin gene can be transformed into plants to produce the toxin in plant tissue, which has already had a huge impact on the use of *B. thuringiensis* for pest control.

The plant may be inoculated with commensal endophytic microorganisms, themselves transformed with the toxin gene.

3.5.1 TOXIN-PRODUCING PLANTS

Transformation of *B. thuringiensis* toxin genes into plants has been reviewed by Ely (1993). Levels of toxin expression have been progressively increased to 0.1% of the total soluble plant protein (Hickle and Fitch, 1990), which is equivalent to about 0.003% toxin in plant tissue, well above the LC_{99} of highly susceptible insects and sufficient to kill at least some larvae of less susceptible Lepidopteran pests normally targeted with *B. thuringiensis* insecticides, such as *Spodoptera* spp.

Once the transformed plant has been perfected, no further action is necessary from the user except possibly resistance management (section 3.7.4). The systemic toxin protects the plant in the manner of a systemic formulation, often for the whole life of the crop, and afterwards biodegrades with the plant remains. The gene is transmitted in the seed. In 1996, transgenic varieties of corn, cotton and potato were grown commercially on over 2 million acres in the USA. Gelernter (1997) states

'overall performance of the crops has been superlative. By overcoming most of the problems that have plagued microbial insecticides – most importantly, delivery to the target insect – *B. thuringiensis* plants have taken the quantum leap necessary for the transformation of *B. thuringiensis* from a novelty to a mainstream product.'

Other transformed strains and crops will be produced. For cotton alone, it is projected that the number of planted acres will increase to 2.4 million in 1997. In many Lepidoptera, pure crystals entirely without spores applied to insect artificial growth medium are less active than spore–crystal mixtures (Li *et al.*, 1987). Luckily, this does not impair toxin activity in transgenic plants, because phylloplane flora assume the function of the spores: 14 out of 15 phylloplane bacterial species tested were effective synergizers if formulated with pure crystal toxin (Dubois and Dean, 1995).

Systemic action can be achieved in the aquatic environment by transforming *B. thuringiensis* toxin genes into algae in the food chain of filter-feeding mosquito larvae (Gelernter and Schwab, 1993; Yap *et al.* 1994; Anon., 1995). Use of an ammonium-secreting host organism adds the additional function of a nitrogen fertilizer (Boussiba *et al.*, 1992; Ziniu *et al.*, 1996). These have not been developed commercially.

3.5.2 TOXIN-PRODUCING ENDOPHYTES

Endophytic micro-organisms, which live inside plants, can be transformed with toxin genes. These were being developed as Incide products by Crop Genetics International, but were later dropped. A transformed maize endophyte, *Clavibacter xyli* var. *cynodontis*, gave some control of the European corn borer (Rigby, 1991; Gelernter and Schwab, 1993). The endophyte did not survive outside the host plant or in plant debris, was not seed-transmitted and did not spread from inoculated maize to adjacent non-inoculated plants. There was a minimal 4% reduction in yield (Rigby, 1991). The endophytes did not affect substantially the extent of pest–crop residue decomposition (Tester, 1992).

Endophytes can be applied as a spray to plants or as a seed dressing. They require formulation technology for delicate organisms. Application to seed is the most convenient and efficient method. They are forced into seed in a pressure chamber through microscopic cracks that develop in the seed coat during the seed-drying process. Very low quantities are needed per acre compared with a typical 1–5 lbs/acre/season for traditional *B. thuringiensis* products. Other advantages are as for transformed plants, except for the lack of seed transmission.

Strains of *Rhizobium* expressing the *cry III* gene significantly reduced damage by *Sitona* species feeding on root nodules of pea and lucerne. These transformed strains competed with wild strains but were less effective at increasing plant biomass (Bezdicek *et al.*, 1994). They could presumably be formulated for soil inoculation (section 7.3).

3.6 APPLICATION TO WATER

B. thuringiensis and *B. sphaericus* are used extensively for mosquito and blackfly control (Table 3.19) in a variety of different water masses, ranging from fast-flowing rivers in West Africa to public drinking containers in the Far East (section 2.2.2c). Many different formulations have been developed. The main commercial products are suspension concentrates, followed by wettable powders and much lesser quantities of large-grained formulations. Both species of bacteria behave similarly and can be regarded as interchangeable from the formulation viewpoint. Neither viruses nor Protozoa have been commercially applied to water.

3.6.1 PROBLEMS OF THE AQUATIC ENVIRONMENT AND ITS TARGET INSECTS

Unique problems are presented to the formulator by water as a target surface (section 2.2.2c) and mosquitoes and blackflies as target insects (Table 3.19). For control of mosquito larvae, formulated bacteria are sprayed or spread over the surface of static

Table 3.19 Target aquatic insects and feeding behaviour that influences control by *Bacillus thuringiensis*

Insect	*Mode of feeding*
Blackfly larvae: inhabit fast-running water	
Simuliidae, *Simulium* spp	Larvae adhere to static objects and filter passing water in streams and rivers
Mosquito larvae: inhabit static and slow-moving water	
Aedines, *Aedes* spp	Filter feed and scavenge along the bottom, vertical surfaces and objects
Anophelines, *Anopheles* spp	Sweep under the upper water surface and filter feed downwards relatively weakly
Culicines, *Culex* spp	Filter feed strongly throughout a water mass

or slow-moving water into which they sink at a rate determined by the design of the formulation. Blackfly larvae live in fast-moving water courses and are controlled by pouring bacterial suspensions into the water at consecutive points, from which they are carried downstream.

With mosquitoes, different feeding habits of larvae of different species (Table 3.19) influence the effectiveness of the bacteria. *Culex* larvae filter-feed up and down the depth of the water; they are often termed column feeders. *Aedes* larvae tend to scavenge along substrate surfaces, particularly the bottom. *Anopheles* larvae feed on buoyant material trapped at or just below the water surface and filter-feed downwards relatively poorly, depending on availability of food at the surface. In comparable conditions, two *Anopheles* species filtered water at the rate of 33–34 and 49–55 μl/larva/h, respectively, while *Culex quinquefasciatus* filtered 490–590 and *Aedes aegypti* 590–690 μl/larva/h (Aly, 1988). *Anopheles* larvae were most susceptible to *B. thuringiensis* held near the surface, e.g. formulated in flour or lipid capsules. In wheat flour +5% corn oil in 100–ml volumes of water in plastic cups, *B. thuringiensis* was ×2–4 more effective against three species of *Anopheles* than suspensions of very fine particles containing the same amount of *B. thuringiensis* applied to the surface and allowed to diffuse downwards. The larvae ingested the buoyant formulation in a short time (maximum 20 min). In larger volumes (175 l), the differences were much greater, ×39–68 (Aly *et al.*, 1987). When confined in buoyant lipid capsules in test tubes 4.5 cm deep, purified crystals were ×20 more effective than in suspensions against *Anopheles* larvae, but only ×2–3 more effective against *Aedes* and *Culex* larvae. Rather than a position effect, the latter result was probably due to the capsules being of a more amenable size for feeding than free crystals (Cheung and Hammock, 1985). When assayed with dissolved crystal, toxicity was less than with intact crystals in all three types of larvae because these larvae are filter-feeders and do not ingest the protein efficiently in soluble form (Schnell *et al.*, 1984); the observed toxicity was similar in larvae of all three types (Cheung and Hammock, 1985). These observations accord in showing that larval feeding habits partly explain why species of *Anopheles* have consistently appeared less susceptible to *B. thuringiensis* suspensions than the column- and bottom-feeding *Culex* and *Aedes* larvae in laboratory assays and field tests (for review see Lacey, 1985b).

Thus differently formulated products are required for mosquito larvae of different feeding types. Buoyant products are required for anophelines, but products should stay in suspension below the surface for column- and bottom-feeders. In natural waters, rapid sinking should be avoided because steady deposit of debris would soon cover the particles.

The content of crystal toxin in products is measured by bioassay with *Aedes aegypti* and expressed in IU. The use of small volumes of water, soon filtered clear of particles by the assay larvae, largely negates the effect of particle size, texture and formulation to give a true measure of toxin content. However, in the field, even when comparing suspensions, the effectiveness against target mosquito and blackfly larvae depends more on the rate of settling than on the IU (for review see Guillet *et al.*, 1982; Molloy *et al.*, 1984; Lacey, 1985b; Lacey and Heitzman, 1985). Lack of agreement between potency measurements with *Aedes aegypti* larvae and field performance of products against blackflies in West Africa has not caused serious difficulties. There is some confusion about the quantitative value of the IU according to different definitions [1 international unit (ITU) = 2.5 *Aedes aegypti* units (AAU)], discussed by Wassmer (1995) in relation to an excellent list of current formulated proprietary products, their costs per IU and application rates. Universal acceptance of the World Health

Organisation's recommended *Aedes* assay technique and definition by industry – and users also – would be a great advantage (Guillet *et al.*, 1990).

The effectiveness of many bacterial formulations against both mosquitoes and blackflies is short-lived in the field, often only 1–2 days. This is due to rapid settling, adsorption to plants and other substrates (which also filter particles out of the water), denaturing of the crystal by sunlight and engulfment by filter feeding fauna (for reviews see Lacey, 1985b; Lacey and Undeen, 1986). A major objective of formulation is to extend the effective period. The UV component of sunlight is much less important in water, where particle settling is the key factor, than on land foliage; partly because water filters out much of the UV radiation. With *B. thuringiensis* only the effect of sunlight on the crystal reduces larval mortality, since the spore is unimportant in mosquito and blackfly larvae. In water 2.5 cm deep, the equivalent of 6 days natural sunlight inactivated *B. thuringiensis* ssp. *israelensis* (Ignoffo *et al.*, 1981), but shorter exposures did not (Garcia and Des Rochers, 1979; Mulligan *et al.*, 1980). In much less than 6 days, particles are mostly filtered out of fast-running water or largely sink out of reach of a damaging dose of radiation in still water. *B. sphaericus* is more susceptible to sunlight, being inactivated in clear water a few centimetres deep in full sun (Mulligan *et al.*, 1980), while strong sunlight reduced its effectiveness several-fold (Skovmand and Bauduin, 1998). A sunscreen might be beneficial with *B. sphaericus*, particularly in formulations designed to float. However, as with many additives used in water, the screen must be insoluble in water and must strongly adhere to the particles. This would be facilitated by encapsulation (section 3.6.5). Probably no present proprietary product for water incurs the expense of a sunscreen.

Sprays do not need to be fine for application to water. Without this constraint the range of product types has become wide.

3.6.2 SUSPENSION CONCENTRATES AND WETTABLE POWDERS

Suspension concentrates (= flowables; section 3.3.3b) extend the effective period that the product remains freely suspended in water by attaining minimal particle size. This is achieved best in aqueous flowables by maintaining harvested fermentation residues in a wet state (Table 3.9 in section 3.3.3). In the production of wettable powders, the drying process aggregates the material and it is costly to grind particles down to less than 10 μm in diameter.

The impact of particle size is well illustrated by the work of Molloy *et al.* (1984). In the laboratory, the ideal particle size for ingestion by blackfly larvae was 35 μm diameter (Molloy *et al.*, 1984). Larvae of mosquitoes ingest particles ranging from <0.5 μm (i.e. the size of small toxin crystals) to a bit above 100 μm (if soft or flocculent) (Dahl, 1988). However, in water courses the critical factor for blackfly control (Table 3.19 in section 3.6.1) is the distance that particles are carried downstream from each application point. A suspension concentrate with finer particles than wettable powders gave the greatest number of kills because small particles held up best in the water for the longest distance, although mortalities were similar for both types of formulation near the application points. The mean particle size in the suspension concentrate was 2.1 μm in diameter (range 0.5–28.0, usually single spores and crystals), compared with 5.2 (0.5–122.0) and 4.0 (0.5–98.8) for two wettable powders. Several other workers have shown that the rate of sinking of particles in different products is positively correlated with particle size (Guillet and Escaffre, 1979; Guillet *et al.*, 1980; Hinkle, 1983; Lacey and Undeen, 1984; Guillet *et al.*, 1990). Efficacy against early instar larvae decreased sharply as particles increased in size above their feeding-size range (Guillet *et al.*, 1985a). Products with large particles lost efficacy in turbid water during the rainy season or after floods

due to competition with natural particles for capture and ingestion (Guillet *et al.*, 1985b). Products with fine particles suffered from these problems much less.

Incorporation of wet fermentation solids as a water-in-oil emulsion has the advantage of combining the slow sinking rate of fine particles with the buoyancy of oil. However, an early emulsion of *B. thuringiensis* ssp. *israelensis* inhibited feeding of *Simulium* larvae above 1 p.p.m. for 15 min; a mineral oil, kerosene, had a similar effect (Molloy *et al.*, 1981). Double-strength Teknar (Teknar 2X) flowable aqueous concentrate improved blackfly control in USA streams. Less satisfactory results were achieved with a flowable of equal potency, based on dry solids in oil to try to keep particles afloat to improve carry (Table 3.10 in section 3.3.3a, using dry solids in ssp. *israelensis* and modified method) (Lacey and Heitzman, 1985). The reaction of mosquito larvae was not affected adversely by vegetable oil added to an inert carrier (Aly and Mulla, 1986).

A difficulty lies in the assessment of viscosity. Viscous products have poor dispersibility and a high rate of settling, as well as creating pumping problems when refilling aircraft and spraying, although sprays need not be fine as with control of epigeal forest pests. An improved method of measuring viscosity has been developed in the West African onchocerciasis programme, although a visual assessment provides valuable first-hand information – a product is suitable if the drops break up when they hit the water and disperse well into clouds of fine particles (Guillet *et al.*, 1990).

For most applications to water, suspension concentrates have been the basic products of choice, particularly for use by air with ULV technology. They gave good control of container-inhabiting mosquito species by ULV treatment of piles of tyres (Lee *et al.*, 1996). They can be used to treat rice fields at the position of water inlet during flooding (McLaughlin and Vidrine, 1984). They are particularly useful for point applications against blackflies to rivers, being easy to apply, e.g. typical streamside time for handling, mixing and application was *ca* ×3 less (5–10 min) than for powders (20–25 min), also they were miscible and poured adequately at freezing temperatures ((9 °C) (Molloy and Struble, 1989). They are bettered by wettable powders only when long shelf-life is at a premium, although suspension concentrates have been sufficiently stable in the heat of West Africa if stored in the open (Guillet *et al.*, 1990). Suspension concentrates have the commercial disadvantage of being heavy to package and transport, as well as needing preservatives to prevent bacterial activity in storage (section 3.3.3b); however, they are less expensive to apply (Knepper *et al.*, 1991).

3.6.3 PENETRATION OF FOLIAGE FOR MOSQUITO CONTROL

Although they are the products of choice for most applications against blackfly, high- or low-volume sprays of suspension concentrates are impracticable for treating many mosquito habitats under foliage canopies because sprays do not readily penetrate dense foliage. However, successful mosquito control under canopies has recently been reported using aerial ULV sprays with droplets with volume mean diameters of 150–200 μm (Cyanamid, 1992); presumably because enough spray drifts through the foliage into the water.

For the consistent penetration of foliar canopies, e.g. forest, rice field or salt marsh, granule-sized products need to be heavy enough to roll down leaves into the water. Successful carriers include corn cob grits (Lacey and Inman, 1985, 1 mm diameter; Lacey, 1986; Lacey *et al.*, 1988, 12/14 mesh; Wilmot *et al.*, 1993, 5–8 mesh), clay (Lacey and Inman, 1985, 1 mm diameter), sand (Becker and Margalit, 1993; Table 3.20) and polymers (Table 3.22 in section 3.6.4 below). Except for the polymers, the bacteria are applied to the surfaces of the

Table 3.20 Production of heavy, fast-release granules of *Bacillus thuringiensis* ssp. *israelensis* (Bti) suitable for preparation by the user for control of mosquito larvae (Lisansky *et al.*, 1993)

Ingredients	*Percentage (w/w)*	*Function*	*Cost ($/kg product)*
Technical powder	3.88	Agent	1.09
Blasting sand	94.2	Carrier	0.03
Wessalon S	0.075	Free-flow agent	0.02
Energol WT-1	1.88	Sticker oil	0.18

Preparation

1 Mix technical Bti powder (80 000 IU/mg) and silica powder (e.g. Wessalon S) in the ratio 49:1 (w/w). Market in separate container
2 Thoroughly mix 94% dry sand, 100–500 μm, with sticker in rotary (e.g. cement) mixer 5–10 min. The sand can be local to avoid bulky transport and the oil can be marketed with the Bti in a separate container
3 Stop mixer, add 4% Bti–silica mixture (1), cover mouth of the machine, mix for 10 min
4 Use product immediately at 5 kg/ha to penetrate foliage canopy. The sticker is designed to release Bti on entry into water so that the Bti floats. Many stickers, such as Golden Bear oil, do not readily release the Bti. For open water, other carriers can be used, such as corn grits, which float and so should not be used in windy weather

granules. Granules can be delivered by air or ground equipment (Table 2.2).

3.6.4 FLOATING AND SLOW-RELEASE PRODUCTS

Products can be made to float, suspend (Table 3.21) or sink (Table 3.20), according to the feeding types of target mosquitoes (section 3.6.1). They can release the bacteria rapidly or slowly. Gustatory stimulants can be added to optimize larval feeding.

Rapid-release effervescent tablets have been designed for easy transport and use in small water masses. These float and dispense particles into the water from the water surface. To be economic, a high unit activity was used (750 ITU/mg). From each tablet, the bacteria were spread over 1–3 m^2 of water if not obstructed by vegetation, and gave nearly 100% control of susceptible *Aedes* (Most and Quinlan, 1986; Skovmand and Eriksen, 1993).

Granules with rather slower release, for protection of water masses of all sizes, have been formed by adhering as much technical powder as possible to the surface of inert carriers. The high concentration minimizes the amount of product to be applied and, hence, the weight to be carried by aircraft. The cost of transporting and packaging heavy, bulky granules can be avoided by keeping granule composition simple to enable mixing with local materials near the site of use (Table 3.20). Lacey *et al.* (1988) reported 5% powder content by weight, and Lacey and Inman (1985) 5–17%. As soon as they are wetted, granules dehisce, releasing particles of variable size. Crystals of *B. thuringiensis* have a density of 1.40 and spores 1.32 (K. Bernhard, Lörrach, Germany, personal communication), and low density carriers such as corn cob grits readily form floating granules; these gave excellent control of *Psorophora columbiae* in re-flooded rice fields when applied by air before or after flooding (Lacey, 1986). However, rice hulls treated with *B. thuringiensis* spp. *israelensis* wettable powder adhered with gelatin gave poor control compared with the wettable powder applied as a spray, because the water carried them away from mosquito larvae in rice fields (McLaughlin and Billodeaux, 1983). In unobstructed water bodies, wind bunched light, floating products to windward. These

Table 3.21 Manufacture of heavy, storable, quick-release granules of *Bacillus thuringiensis* ssp. *israelensis* for control of mosquito larvae* (Sjogren, 1996)

Ingredient (Appendix I)	*Percentage (w/w)*	*Function*
Technical powder, 10 000 IU or more/mg	4.7	Larvicidal powder
Dicaperl HP 920 (perlite powder)	3.7	Flotation agent
Water	1.2	Solvent
Morwet EFW	0.01	Surfactant
Glycerol	1.4	Plasticizer
Fish gelatin (45%)	2.0	Adhesive
Blast sand, Texas 12/20, heat dried	86.7	Heavy core carrier
Sipernat 22	0.3	Free-flow drying agent

Manufacture of 1000 lb batch

1 Mix technical powder and Dicaperl thoroughly in a sealed powder blender (air, ribbon or rotary type). Can be stored
2 Blend water, Morwet, glycerol and gelatin at 55–60 °C (do not exceed 65 °C) in heat-jacketed mixer with a stirring paddle to form a viscous brown blend
3 Rotate sand in a rotary mixer (e.g. Munson or Continental), while lightly spraying in the blend, using a flat-fan nozzle (e.g. Spray Systems 8001), and simultaneously adding the powder with a vibratory unit, both additions timed to extend over 5–7 min
4 Rotate for 1 min
5 Add Sipernat over 1 min, still rotating. The granules should be free-flowing, 0.5–1.0 mm in diameter. The amount of Sipernat can be varied according to ambient humidity and moisture content of the granules. The final total moisture content after manufacture should not exceed 7% of the ingredients, including sand to ensure good storage
6 Sieve to remove excess powder. Retain a sample in a water vapour-proof container for reference
7 Pack in bags with water vapour proof-liner

* Mortality of *Aedes vexans* larvae was 92.4–100% in 17 out of 19 field tests treated at 5 kg granules/ha.

problems can be avoided by using heavy sand granules (Table 3.20; Becker and Margalit, 1993). After impact, the oil helps to suspend the bacteria in the water. A particulate flotation additive can be used and a surfactant to improve release of bacteria from the granule (Table 3.21); these avoid any risk of sticker oils (Table 3.20) inhibiting larval feeding. A commercial product, LarvXSG, based on Table 3.21, releases the bacteria over 0.1–72 h, economizes application to 5 kg/ha and is suitable for storage and transport. Some additives have an adverse effect, e.g. a cement (Guillet *et al.*, 1985a) and some oils.

Sustained-release granules can be made by altering the ingredients in Table 3.21, viz: technical powder, 15.0%; flotation agent, Propyltex (polypropylene powder), 12.7%; water, 10.8%; sticker, phagostimulant, Technical Protein Colloid 90014, 2.7%; cross-linking aldehyde to reduce water solubility of dry colloid, 40% glyoxal, 0.1%; sand, 58.2%; free-flow drying agent, Sipernat 22, 0.5%. The bacteria are released over a period of 10–30 days.

Floating slow-release granules containing *B. sphaericus* doubled the control period of *Anopheles gambiae* in clear water pools or of *Culex* in sewage water containers and – at the high dosage of 30 kg/ha – in cesspools. The granules initially floated and spread on the surface, then sank slowly while disintegrating into small particles, 10–20% of which still floated after several days. They were compared with a flowable concentrate of equivalent potency. Big granules, 1–2 mm in diameter, were effective for much longer than high doses of flowable concentrates (Skovmand and Bauduin, 1998).

Slow-or sustained-release pellets and briquettes, for easy hand treatment of small water masses, can be made by compounding bacterial powder with fine inert additives and dispersants (section 7.7.3c). These can be made buoyant by choosing light-weight additives. Cork, polypropylene powder (Accurel, details in Appendix Table I.4) and moulding plaster have been used (Lacey *et al.*, 1984; Kase and Branton, 1986). Compounding *B. sphaericus* in pellets with partially hydrogenated vegetable oil, talc and a starch-based super-absorbent polymer (Supersorb, details in Appendix Table I.4) extended residual activity against *Culex* in large and small plots, including pollufed water. When pellets were applied to dry artificial larval habitats 5 days before flooding, *Psorophora columbiae* hatching at flooding was eliminated, while the technical powder was ineffective as a pre-flood treatment, apparently due to solar inactivation of the toxin (Lord, 1991). As a dispersant, powdered sucrose has the disadvantage of encouraging growth of fungi and microorganisms (Lacey *et al.*, 1984). Sucrose–polypropylene pellets provided limited control of *Culex* for 3 weeks with *B. thuringiensis* ssp. *israelensis* (Ragoonanansingh *et al.*, 1992) and good control for 8 weeks with *B. sphaericus* (Lacey *et al.*, 1984). Briquettes of both species gave effective control for 3 weeks when the experiment had to be terminated (Lacey *et al.*, 1988). A relatively large amount of bacteria per unit area was used, but this is balanced out by residual control and ease of use. In an attempt to obtain season-long control of *Aedes albopictus* in domestic containers, higher-than-label dosages of Bactimos pellets were used; complete control lasted only 60 days, and significant levels of control were found up to 360 days (Nasci *et al.*, 1994). Spread of inoculum from pellets and briquettes is restricted, especially in environments with emergent vegetation in small water bodies. A delay in the release of sufficient toxin for immediate kill of older larval instars is a disadvantage that can be remedied by surface-coating with wettable powder that would release more bacteria initially (Lacey *et al.*, 1988). Rose (1989) suggested that inclusion of bread yeast or baking powder in a floating matrix would increase flotation by producing CO_2. One product containing 10% *B. thuringiensis*, the Bactimos Briquet, is available commercially.

Floating controlled-release briquettes, pellets and granules (Tables 3.22 and 3.23) were made from Culigel polymers capable of absorbing over $\times 100$ to *ca* $\times 5000$ their weight of water (Levy, 1989). Rate of release of both bacterial species from these super-absorbent polymer granular matrices depended on the inert ingredients admixed with a technical bacterial powder during fabrication of the insecticidal granules by aqueous microsponging or entrapment techniques (Tables 3.22, 3.23). Bacterial powders and formulants were shaken with preformed granules (2–3 or 4–5 mm diameter) in water for several hours, strained, washed and air-dried at 27 °C on screen trays for 1–4 days. The granules may absorb over 50% by weight of powder and additive. The rate and percentage of pesticide loading within Culigel granules were related to the super-absorbency, porosity and size of the granules, as well as to the bacterial and inert formulants added to the water (Tables 3.22 and 3.23). Culigel water-insoluble components are non-toxic, biodegradable/erodible, and protectant against oxidation and sun (Levy *et al.*, 1992a, b). The rate and duration of the effective sustained release were affected by the water quality of a mosquito habitat. Release kinetics could be adjusted for a specific water quality, or a range of water qualities, by altering the type and/or concentration of inert dispersant or dispersant complex (Table 3.23). Bacteria can be applied with other insecticides as oil- or water-based, variable viscosity Culigel sprays. Pellets and briquettes can be made by agglomeration techniques (section 7.7.3a; Levy *et al.*, 1993a–c). Two proprietary delivery systems, Matricap and Gelgrade, were based on similar

Table 3.22 Slow-release Culigel granules with bacteria for application to water to control mosquito larvae

Granule	*Data*
Culigel with *Bacillus thuringiensis* ssp. *israelensis* (Bti) and *B. sphaericus* against *Aedes* and *Culex* spp.	90–100% control lasted 68–133 days: type II granules = III > I > IV (Table 3.23) in water with 10% sea water at 16–22 kg/ha, giving equal or better control to chemical alternatives[1]
Culigel with Bti (34%) + 3.8% dispersant B or *B. sphaericus* (16%) + 16% dispersant A (Table 3.23) at 14–16 kg/ha	Granules (type IV, Table 3.23) control 6–7 larval generations of *Culex quinquefasciatus* in fresh and brackish water for 2–3 months. Shelf life > 124–224 days[2]
Culigel 3 with Bti (18%) + dispersant A (18%) or an aliphatic alcohol + salts, or *B. sphaericus* (11%) + dispersant B (11%) (Table 3.23)	Granules (type III) control *C. quinquefasciatus* for >4 months at 20 kg/ha in 10% seawater[3]
Culigel cross-linked modified polyacrylamide type and cross-linked acrylic types with Bti and *B. sphaericus*	Granules, loaded by shaking with aqueous technical powders in the presence of a non-ionic liquid heterocyclic dispersant of ethoxylated fatty alcohol surfactant, control *Culex* for 3–4 months. Ionic dispersants produced poor release profiles[4]

References

1 Levy *et al.*, 1993a
2 Levy *et al.*, 1993b
3 Levy *et al.*, 1993c
4 Levy *et al.*, 1992b

Table 3.23 Dispersant complexes used in Culigel polymers (Levy *et al.*, 1993c)

Types of granular Culigel superabsorbent polymers evaluated as controlled-release matrices

Type	*Description*
I	Cross-linked copolymer of acrylamide and sodium acrylate
II	Lightly cross-linked potassium polyacrylate
III	Partial sodium salt of a lightly cross-linked polypropenoic acid
IV	Cross-linked potassium polyacrylate/polyacrylamide copolymer

Types of inert ingredients used in dispersant complexes to regulate pesticide release from Culigel granules

Type	*Description*
Alcohols	Surfactants
Surfactants	Binders
Emulsifiers	Suspending agents
Solvents	Compatibility agents
Salts	Wetters
Diluents	Oils

Inert dispersant complexes used to regulate pesticide release from Culigel type III granules

Dispersant complex	*Description*
A	2-Ethyl hexanol + magnesium chloride
B	2-Ethyl hexanol + acrylic acid, copolymer
C	Sulphated alkyl carboxylate and sulphonated alkyl napththalene, sodium salt + acrylic acid, copolymer
D	Sodium alkyl aryl sulphonate + acrylic acid, copolymer

materials. With proprietary coating adjuvants, they provided controlled delivery compositions of comparable performance, depending on the incorporated formulants (Levy, 1997; Levy *et al.*, 1996, 1997).

Formulated bait can be used to increase feeding and to attract mosquito larvae to a product, whereas inert materials may depress feeding. *Anopheles albimanus* larvae found bait by random locomotion, not by directional movement (Aly and Mulla, 1986). On contact with floating food, larvae stopped swimming and fed rapidly, resulting in aggregation of larvae at the food source. Inert materials did not influence swimming and diving in search of food; filtering larvae expelled collected inert particles. *Aedes vexans* gathered food particles (wheat flour, fishmeal or yeast) $\times 3$ faster than inert particles (kaolin, pumice or synthetic cellulose). Aqueous fishmeal extract accelerated ingestion of inert particles to equal the ingestion rate of food particles, demonstrating gustatory stimulation of larvae (Aly, 1983). The presence of food in particles probably also regulates feeding by larvae of *Culex pipiens* (Dadd *et al.*, 1982). Aggregations of mosquito larvae have been observed feeding under decomposing carcasses of larger animals (G. M. Roberts, Wolfson Mosquito Control Project, University of Southampton, personal communication). Aggregation of mosquito larvae was induced by carbohydrates, nucleotides and proteins, as well as a variety of foods including corn cob flour. It was not influenced by cellulose and a range of inert materials. Vegetable oil added to an inert material (kaolin) did not change larval reaction (for review see Aly and Mulla, 1986), but mosquito larvae aggregated around latex beads treated with yeast extract (Aly, 1988). Oils had an adverse effect on blackfly control (section 3.6.2). Satiation caused mosquito larvae given unlimited food to reduce feeding and larvae stopped feeding in the presence of unlimited inert materials. Larvae given small amounts of food (dried flour) fed at a steady rate. Becker *et al.* (1991) found tablets containing wettable bacterial powder, flour and inert material (1:1:1) were no more attractive than tablets without the food, and suggested that spent fermentation ingredients in the powder were already a strong attractant. Many technical powders contain additives such as lactose to facilitate harvest and spray drying (e.g. Lacey *et al.*, 1988; section 3.2). Low density and softness of particles might be more important factors for ingestion than absolute size ranges; flat, flocculent particles, a little above 100 μm, have been observed being ingested by fourth instar larvae (Dahl, 1988), but flocculent particles sink rapidly.

The concentration of bacteria used in baited products depends on whether maximum dispersion across the habitat for short-term control is intended. If so, the minimum to achieve complete kill is 0.2% technical powder (Aly *et al.*, 1987). Otherwise much higher quantities are desirable, e.g. 5% technical powder in corn cob grit granules, 5–13% in briquettes and 30% in sustained-release pellets (Lacey *et al.*, 1988).

Formulated baits have been used in pellets to attract bottom-feeding species. Fishmeal increased effectiveness (Aly, 1983). Becker *et al.* (1991) designed tablets to sink to the bottom of domestic water reservoirs from which amounts of water were continually taken and the reservoirs periodically topped up. The turbulence thus caused shortened the effective life of the pellets. Incorporation of 33% flour caused pellets to break up sooner, possibly by encouraging growth of microorganisms.

3.6.5 ENCAPSULATION

Formulated matrices (section 3.3.1) offer organisms some protection from environmental conditions, and the distinct skin around capsules offers more, as well as greater, opportunities of improving suspendability in water. Margalit *et al.* (1984) made two types of capsule: (1) by stirring a mixture of *B. thuringiensis* ssp. *israelensis* and dried

yeast or yeast extract in a solution of low density polyethylene in cyclohexane, and (2) by stirring the bacteria into a slowly cooling, fine emulsion of a fatty acid (decanoic, palmitic or stearic). The capsules were filtered and dried. Both types increased the flotation coefficient and improved the insecticidal activity against *Culex* and *Aedes* larvae in glass containers with mud on the bottom.

Cheung and Hammock (1985) microencapsulated pure crystals in lipid droplets. These were mostly 3–12 μm in diameter, ideal sizes for larval feeding. After application to the surface, over 50% of the capsules remained in the top 0.2 inches of water for 9 h; *ca* 30% were still there after 24 h, while the rest dispersed evenly down to 12 inches. Efficacy improved $\times$2–3 against *Culex* and *Aedes* larvae and $\times$20 against *Anopheles.* Lipid capsules of different buoyancy can be formed.

Prill (Table 5.2) were formulated by thoroughly mixing *B. sphaericus* into aqueous aluminium carboxymethylcellulose (CMC), a medium-viscosity polymer with a degree of substitution of 0.7 (Sigma), and dropping into aqueous 0.05–M $Al_2(SO_4)_2$ at pH 3.4 and 4 °C (Elçin *et al.*, 1995). The best release profile, over 14 days, was given by a CMC concentration of 1%. Compared with free bacterial preparations in water, prill protected germination after exposure at 50 °C for 7 days, at pH 3 to some extent for 45 days, and under UVC radiation slightly for 24 h, while potency in *Culex* spp. was well protected from these extreme conditions for 45 and 60 days and 48 h, respectively.

A microencapsulated formulation of *B. thuringiensis* had little advantage over the wettable powder product, Bactimos, for control of the surface-feeding *Anopheles stephensi*, but extended the effective period from 2 to 8 days for the bottom-feeding *Aedes aegypti* (Vorgetts and Buescher, 1985).

Of all the above large-grained formulations, only corn grit, clay and sand-and-oil granules, as well as various pellets and briquettes, have been marketed to date.

3.6.6 MONOMOLECULAR SURFACE FILMS

Monomolecular surface films (monolayers) combine many of the objectives of formulation for aquatic use. They float, spread, persist, resist movement by wind (except on large water areas, although larvae tend to move too), and are easily used in both small and large water bodies, while being innocuous to most non-target insect groups and to vertebrates (Levy *et al.*, 1984; Roberts and Burges, 1984; Roberts, 1989a, b; 1991).

Monolayers spread instantaneously on application to a point on the surface of water and distribute technical bacterial products suspended in them. The bacteria are released into the water, decreasing in quantity with distance from the point of application for at least 15 m (Roberts and Burges, 1984). When the whole water surface has been covered by a single layer of molecules, excess monolayer remains as a reservoir globule at the surface and retains many suspended particles. Molecules stretched across the surface are slowly degraded and are replaced from the globule, carrying particles along with them. As molecules spread across the water surface, some particles are released immediately, others are retained at the surface and released later (Roberts, 1989a, b). In clear tropical waters, the effective period of mosquito control was extended from 9 to 15 days before adults started to emerge again from the regenerated population. The extension was less in polluted waters and more in temperate conditions (Roberts and Burges, 1984; Roberts, 1989a).

Monolayers interrupt the breathing of larvae and pupae at the water surface and prevent egg laying by adults of species that alight on the water, often destroying them by drowning (Reiter and McMullen, 1978). Generally the monolayer kills larvae more slowly than *B. thuringiensis* ssp. *israelensis*, and also kills pupae. The lethality of monolayers to insect stages not attacked by the toxin adds complementary mortality to its many

advantages as a formulation ingredient. The toxin deteriorates during long-term storage in monolayers, e.g. Monoxi, so the two ingredients are packaged separately. They are stable in the short term and can be held together briefly after mixing on site, potency being maintained for 35 days at 5 °C, 21 at 25 °C and 10 at 35 °C, after which it slowly declines and is lost in 60 days at 35 °C (Roberts, 1989a). Good suspension can be maintained by an insoluble gelling additive such as 5% fumed silica (Cab-O-Sil; G. M. Roberts, personal communication). Spreading of monolayers from point sources enables coarse sprays to be used or, in small water masses, drops from a dropping bottle. Both ingredients are innocuous and can be used safely by anyone, including children in a community mosquito control programme in the tropics (Roberts, 1989a, b). Tested monolayer chemicals include egg lecithin (Reiter and McMullen, 1978); Liparol (soybean lecithin and C_{12}–C_{14} isoparaffins; Becker and Ludwig, 1983) and Arosurf nonionic surfactant, the 2M ethoxylate of isostearyl alcohol (Levy *et al.*, 1984). Monoxi, a 1:1 v/v mix of oleyl alcohol monoethoxylate suspended at 15% by high-speed mixing in water, is an effective but bulky product. This, together with two mixtures not requiring pre-suspension in water, (1) oleyl alcohol monoethoxylate mixed with 10% v/v cetyl stearyl diethoxylate, and (2) oleic acid monoethoxylate mixed with 10% v/v cetyl stearyl diethoxylate, were superior to Arosurf, which has the disadvantage of being more miscible in water (Roberts, 1989a; 1991).

There are some conflicting data on the relative efficacies of bacteria and monolayers. The LC_{50} of *B. thuringiensis* ssp. *israelensis* over 1–2 days was greater in the presence of a monolayer (Nugud and White, 1982), while the reverse was found with the monolayer, Monoxi (Roberts, 1989a). Aly *et al.* (1987) reported that mixtures of bacteria and Arosurf in field tests did not kill *Culex* larvae at concentrations of bacteria effective without the monolayer. They found that 1–5% Arosurf, mixed with bacteria and flour, impaired larval feeding and hence ingestion of bacteria. This is probably an effect of the monolayer wetting the setae on the breathing siphons and reducing larval activity, as well as holding bacteria at the surface as a slow-release mechanism. However, slow-release products typically contain high concentrations of bacteria in order to extend mortality over a protracted period. It can be concluded that both control methods are effective and that comparisons depend on the monolayer in use. Over an extended period, mixtures of Monoxi and related compounds (Roberts, 1989a; 1991) are most effective, giving a valuable extension of the period between treatments, also with overall reduced costs. Monolayers have been marketed for use with bacteria on a small scale.

3.7 FUTURE TRENDS AND RESEARCH IN FORMULATION TECHNOLOGY

3.7.1 PATHOGEN PRODUCTION

Production of *Bacillus thuringiensis* worldwide is mainly confined to deep liquid fermentation for maximum efficiency and ease of handling and of scale-up. At harvest, water is removed by centrifugation or filtration and the liquid slurry stabilized by spray drying or preservatives. For products marketed dry (dusts, granules, capsules, wettable powders and water-dispersible granules), universal formulation requirements continue to need attention, i.e. to avoid caking and improve palatability as well as sunscreening. Thus, avoidance of hygroscopic nutrients and stabilizers for spray-drying would prevent caking. Use of multipurpose additives could be more economical than a series of individual additives. For example, during spray-drying the use of heat protectants with sticking, phagostimulant and sunscreening properties (section 3.7.2c) would reduce the need for additives with these properties later during formulation, particularly since the stickers among

them are incorporated at relatively high percentages, e.g. skimmed milk powder and starch products. Some of the newer additives, e.g. starches and lignin (alone and in combinations), need investigation for a variety of useful qualities including heat protection during spray-drying and – before drying – preservation (e.g. by acidity) and suspension in aqueous concentrates. In addition, growth of the vegetative *B. thuringiensis* cells remaining at harvest and of possible small numbers of contaminating bacteria and fungi must be prevented, as well as enzymatic breakdown of the protein toxin crystals. Residual proteinases remain at harvest and there are interstitial proteinases in the crystal itself, with marked variation between different strains. There is little published work on preservatives, an area that should reward further research; a specialist study of enzymes and their inhibitors would probably lead to valuable improvement in stability. An option is pH manipulation, although different pathogens have different optimal pH requirements. *B. thuringiensis* tolerates strongly acid conditions to inhibit alkaline proteases that attack the crystal toxin; *B. sphaericus* and viruses store best at neutral pH, which inhibits alkaline proteases in occlusion bodies (section 3.3.6).

Formulations of bacteria as aqueous suspensions avoid the cost of drying and are easier to apply, but have the continuous problem of preservation in the presence of water. These key contrasting features should maintain a roughly equal market share for dry and liquid bacterial products.

With the other two pathogen types, *in vivo* production is at present the only method for Protozoa and the most cost-effective method for many wild-type viruses, particularly in developing countries (Jones, 1988b). Virus is harvested as a slurry and most commonly stabilized by freeze-or spray-drying, the relative success of the two methods being likely to depend on careful research and attention to the physical and chemical conditions. Trends are greater use of formulation additives, as described for *B. thuringiensis*, with some potential for dual function of additives as filtration aids at harvest and as stabilizers during drying.

There is an increasing move towards *in vitro* production of viruses. This is largely driven by the development of genetically engineered products (Hawtin and Possee, 1993). Many of these are producible only *in vitro* due to the formation of transformed insect-specific toxins during viral replication (Vlak, 1994). Compared with *in vivo* products, the relative absence of contaminants has the formulation advantage of easier stabilization; however there is no insect debris or melanin, etc., which are collectively effective sunscreens, stickers and phagostimulants. Hence, there is likely to be a greater trend towards use of relevant additives, many with a potentially dual action. At present, some viruses are being engineered for short environmental persistence to ease regulatory problems (Vlak, 1994; Wood, 1994). However, as genetically engineered and formulated products become more acceptable, it is likely that products will be engineered and formulated for greater persistence. Already a number of studies have been directed toward selection of more UV tolerant strains, with some – albeit limited – success in the laboratory (e.g. Brassel and Benz, 1979).

The trend in formulation and development of peroral insect pathogens has been towards sophistication. However, only limited sophistication has been adopted by industry. This is mainly due to the increased cost that it would incur, because the biologicals are competing with the less expensive chemicals. The early microbials had two important disadvantages – difficulty of use and high cost. Formulation research has greatly improved user friendliness and is beginning to target the most cost-efficient options/compromises. Basic studies on the lethal pathogen content of individual droplets/particles have helped, and need more emphasis.

Shelf-life of dry products during storage is good and comparable with that of chemicals. This gives them a competitive edge over liquid concentrates, which tend to store well for only 18 months in ambient conditions for *B. thuringiensis*, and for impracticably short times for viruses and Protozoa.

Whilst there are some common aspects to formulation for application to land and water, the differences in these environments cause different approaches to be taken.

3.7.2 TRENDS IN FORMULATION FOR USE ON LAND

3.7.2a Products applied dry Corn borer control is representative of the specialist uses of products applied dry, and indicates the most likely direction of their future development. In leaf axils of the corn plant where most of the material lodges, the most important environmental factor is moisture, not sunlight. The new starch-based granules show a promising level of protection. Sucrose is incorporated to make the starch easier to handle, but fungi grow in moist leaf axils, normally curbed by using a fungicide. Continuation of the present very active research should be rewarding; an alternative sugar not usable by fungi might be worth investigating. When a phagostimulant, Coax, is added to make the granules more palatable they become more attractive than fresh leaf and act as bait, so that dosage of *B. thuringiensis* can be reduced by 75%. Because information on cost of manufacturing the granules is unavailable, it is unclear whether it is economical to replace part of the bacteria with Coax (McGuire and Shasha, 1995). However, land pests are mostly controlled by sprays.

3.7.2b Products applied as sprays In the short term, the types of spraying machines available on site are unlikely to be changed for use with microbial insecticides, except possibly in forestry. Therefore, the main avenues of progress are formulation and machinery modifications, such as nozzle changes. The two must go together because formulation and delivery systems are inextricably interrelated. Research on sprays is being guided by the environmentally friendly nature of insect pathogens. The main considerations are the biological effect on the insect pests and the economy of active ingredients. There are virtually no concerns about hazards to man, vertebrates and non-target organisms. For example, if there is spray drift – reduction of which lowers crop protection efficiency (Taylor *et al.*, 1993) – the real problem is loss of pathogen reaching the feeding area of the insect within the spray target zone, not toxic hazard to man, animal and plant in the zone reached by the drift.

The three prime requirements of pest control by peroral pathogens are to place them as evenly as possible where they are most likely to be eaten, to make them palatable and to protect them once they are there. Thus, the target is the insects' food, not the insects themselves. The problem is complex, depending on the species of pathogen, insect and food plant, as well as on the interactions of their ecologies and of weather. Recently, formulation research has been aided by modelling (Taylor *et al.*, 1993; Cooke and Régnière, 1996). For example, modelling suggested avoidance of *B. thuringiensis* by gypsy moth larvae (Hall *et al.*, 1995). Modelling optimizes research effort and permits large numbers of theoretical simulations, but is not a substitute for experiment and observation. Notoriously, however, effects demonstrated in the laboratory often do not show up in field tests, but models are valuable in erecting hypotheses for testing in the field, although the models are only as good as their databases and the assumptions made within them. The field environment introduces another echelon of variation and ecological imponderables. For example, in a multi-instar larval population in which instars I and II feed only on the lower surface of the leaf without breaking

though to the upper surface, while instar III chews through the leaf, should a model concentrate on effects on the very susceptible instars I and II, even though less deposit reaches the lower leaf surface; or should attention be directed to the refractory instar III because they are most likely to survive anyway? Field tests quantify variation in pest control levels, allowing calculation of dosage levels needed to reduce the probability of failure to a reasonable proportion of treatments.

In response to the above considerations, here have been three major formulation trends, each with strengths and weakness.

Highly efficient suspension concentrates of bacteria have been developed.

Wettable powders have been improved and made into user-friendly water-dispersible granules.

The effectiveness of products for low- to high-volume sprays has been increased by the use of capsules/granules. These trends should continue to dominate use of these pathogens and their research requirements.

The main motive for the trend towards suspension concentrates has been to obtain good coverage of inaccessible environments, such as forest canopies with aerial ULV, CDA sprays (section 3.3.3a). *B. thuringiensis* ready-to-use oil-in-water emulsions have been effectively formulated directly from newly harvested fermenter slurries. This may not always be industrially convenient and some suspension concentrates are formulated from spray- or freeze-dried technical powders. These powders have the advantage that they can be bulk stored in a very stable state until required for formulation, which facilitates formulation of specialist products for relatively small markets. It also gives the option of formulating the concentrate using oil as the carrier. It has the twin disadvantages of (1) the cost of spray-drying, plus the small decrease in activity that is often incurred, and (2) the cost of grinding the powder without producing a damaging degree of heat and, if a ULV product is to be made, the need to grind very fine. Recent developments with and without anti-evaporants provide another option – the formulation of products for ULV application in water as the carrier (Parnell *et al.*, 1996). This is a probable area for future studies. The formulation of various liquid products from technical powders is likely to continue.

The most concentrated *B. thuringiensis* products contain in the order of 2–4% crystals per g or per ml of technical material. Increases obtainable by improvement of fermentation and strain would be useful, but use of a crystal extraction technique to increase concentration would be too expensive. However, the interaction of a larva of some insect species with *B. thuringiensis* requires yet more concentration of products to obtain the highest mortality from the first larval meal after spraying (section 3.3.3a). Spray bulk can be reduced by fermenting spore-free mutants, thus eliminating the spore. These mutants are unlikely to be used for strains applied against land pests, because many of these pest species are less susceptible in the absence of spores. CellCap products have no spores in order to benefit from the protective action of the cellular capsule (section 3.3.5), which appears to compensate for the absence of the action of spores, probably because the infective and destructive septicaemic roles of vegetative *B. thuringiensis* cells are partly taken over by insect gut flora, which originates from phylloplane epiphytes (Li *et al.*, 1987; Dubois and Dean, 1995). However, further research may show that this compensation may be inadequate in some insect species for which some spore-containing technical concentrate could be formulated into specialist products. The addition of only a small proportion of spores may be effective (Li *et al.*, 1987). In contrast, spore-free products are widely used against target aquatic insects, which do not have reduced susceptibility in the absence of spores.

Development of liquid products for viruses is in its infancy. If stabilization of contaminants can be mastered without harming the

occlusion bodies, an aqueous product would utilize the good storage qualities of some viruses in water. Oil-based flowables, which facilitate ULV and CDA application, have been produced (section 3.3.3.b), but improvement of shelf-life beyond 15 months is desirable. Because the lethal dose of a baculovirus by weight is usually very low, the first larval meal should be lethal.

The second of the three trends, the improvement of *B. thuringiensis* wettable powders, flourished because they have the best stability in storage. This advantage is balanced by the cost of spray-drying. Grinding can be minimized by ensuring that a friable technical product is produced by fermentation and harvest. Long shelf-life will always ensure a market place for wettable powders, even though they are more difficult and time-consuming to tank mix than suspension concentrates, sometimes requiring extra mixing machinery. High potency water-dispersible granules are just coming onto the market. They are easier to handle and tank mix than powders. Unlike granules of chemical pesticides, production of less atmospheric dust during their production and use has little atoxic safety advantage, because the pathogens themselves are not hazardous, although additives may cause irritation. Whether or not granules supersede wettable powders is likely to depend not only on the value placed on decreased operator time against the extra cost of processing, plus the cost of binder and dispersant, but also on whatever develops as convention in the pesticides industry.

The third trend, development of capsules/granules for low- and high-volume sprays, arises from the need to use additives at these volumes. When applied to tank mixes, wetters, stickers, sunscreens, phagostimulants and allelochemical masks become increasingly uneconomic the greater the application volume. For example, the cost of a sunscreen at 5% in 500 l/ha may be prohibitive. Most additives, particularly sunscreens, function best when in close juxtaposition to pathogen particles. One answer to these problems is to microencapsulate the additives with the pathogens, making the product independent of spray volume, minimizing the quantity of additives and maximizing their efficiency (section 3.3.5). Recent studies have produced starch and lignin microcapsules which screen organisms effectively from the sun but are not rainfast (Shasha *et al.*, 1995; Tamez-Guerra *et al.*, 1996). More work is needed to produce capsules that stick to leaves and resist washoff (e.g. Morales Ramos *et al.*, 1998). With *B. thuringiensis*, each microcapsule is more than large enough to contain sufficient toxin to kill a young larva before feeding is depressed, as already discussed in section 3.3.3a for droplets of CDA and ULV sprays. Empty capsules can be avoided by choosing appropriate production methodology. Success of the capsule system has been proven by the commercially profitable CellCap products: obstacles for fabricated capsules/granules are probably economic.

The economics of incorporating additives is largely determined by the ratio of the expensive technical pathogen products to the additives chosen for their low cost. This ratio affects efficiency of the additives, but bulk may necessitate compromise. For example, Tamez-Guerra *et al.* (1996) varied the ratio of the *B. thuringiensis* technical powder to the carrier/sunscreen, flour+cornstarch+sucrose powder, by 100:0, 3:97, 10:90, 25:75 and 50:50, then exposed the microcapsules to sunlight and obtained 19, 96, 86, 64 and 42% kills, respectively, with test larvae. Thus the greater the proportion of carrier, the greater the protection from sunlight. Applying 0.5 kg of *B. thuringiensis*/ha at a ratio of 3:97 in a spray to obtain a nearly complete kill would need 16.6 kg of formulated product. The cost of the carrier is relatively low, *ca* US$0.5/kg, and would not push up product cost extensively, but it would increase the cost of making the capsules. To obtain a similar kill, only 2 kg/ha of product would be needed at 1:1, which is more representative of a commercial

formulation. Further knowledge of manufacturing costs would permit a benefit analysis of the extra protection of juxtaposition against the cost of encapsulating ingredients and processing, plus the slight loss of potency that occurs during some processing.

S. H. Bọk (Korea Research Institute of Bioscience & Biotechnology, Kist, Yusong, Taejon, South Korea, personal communication) made rice-soybean-based capsules with added nutrients and *ca* 10% of technical *B. thuringiensis*. Sprayed at rates similar to wettable powders, insect activity was greatly extended, postulated as being due to demonstrated bacterial replication in the field. However, any effect due to replication is not likely to be reliable as it would occur only in conditions humid enough to increase the water activity sufficiently in the capsules.

Formation of formulated microcapsules by spray-drying might be a breakthrough in their economical manufacture, if the normal drying process at fermentation harvest can be used. This would depend on the stabilization and handling qualities of the encapsulating material and formulants in commercial driers. Hopefully, they might replace the usual stabilizers, so that the only extra cost of encapsulation would be the cost of its inert components. Whole or part fermenter loads might be used for batches of different products merely by changing the ingredients.

3.7.2c Additives Molasses is one of the most useful additives, and one of the few that almost always shows positive benefits in both laboratory and field. This is probably because it is multifunctional as a sunscreen (as effective as five other good screens; Martignoni and Iwai, 1985), a thickener, a phagostimulant and a mask for adverse foliage factors. In one experimental product, it also functions as one of two preservatives present during storage. It is also widely available and cheap, making it ideal for use in developing countries. However, it has the disadvantages of being bulky and messy as a spray tank additive, and sometimes causes problems with filtration equipment e.g. clogging during spraying. Attempts to replace it by a combination of more user-friendly materials illustrate an increasingly common trend in formulation development. It is desirable to find more multifunctional additives. Examples are starch products (encapsulation matrix, sunscreen, buffer and sticker) and Tinopal LPW (powerful synergist for virus and sunscreen). A watchful eye should be kept for bonus benefits, such as the enhancement by Tinopal of natural virus already causing cyclic disease in forest insect populations or of partially inactivated virus, and also for adverse effects, such as slight repulsion of some larvae by Tinopal LPW.

Provided that they do not impair release of peroral pathogens in the insect gut, stickers cannot overstick this type of pathogen to plants, in contrast to the contact-acting fungi for which pick-up of spores from the plant onto the external parts of the insect body is important for infection (section 10.8).

Sunscreens protect peroral pathogens while they are on leaves, but other possible actions, both in storage and in the insect gut, should also be considered. Some protectants against adverse plant factors exert their effects while on the leaf, but many also have important oxidative or alkylative activity in the insect gut. Attempts to neutralize adverse effects of these foliage factors on pathogens in the field have had limited or no success, even when possible modes of action of factors have been identified. Additives that mask the effects of foliage, e.g. phagostimulants, have had more success. Possibly much benefit could still be gained by identifying more factors specific to particular crops and additives that might neutralize them. For example, exceptionally high synergism multiples of $\times 15$ to $\times 1584$ (part V of Table 3.17 in section 3.4.6, references 11 and 13) for Tinopal LPW with NPVs in diet bioassays translate to large significant field multiples of $\times 2.1$ to $\times 11.7$ for gypsy moth on oak (part V of

Table 3.17 in section 3.4.6, reference 12) but only to ×1–2.3 for armyworms (*Pseudoletia unipuncta*, Table 3.4 in section 3.3.1) on cotton and soybean (part V of Table 3.17 in section 3.4.6, reference 11) at concentrations between 0.1 and 1.0%. The industrially viable improvement on oak compared with the modest improvement on row crops may have been due to plant-specific allelochemicals and to insect/plant interactions. More investigation of masking/neutralizing plant factors on cotton and oak may be rewarding.

Synergistic plant allelochemicals and other known synergists need more investigation as potential formulation additives. For instance, piperonyl butoxide – used with pyrethrins and a known inhibitor of mixed-function oxidase activity in insects – might synergize certain of the allelochemicals (Hedin *et al.*, 1988) and, possibly, have direct action on the pathogens as well. Whether allelochemicals and synergists react with spores, crystals or both, requires study. Those reacting with crystals may be particularly important in genetically engineered plants that express this toxin, even to the extent of selecting candidate plant varieties with a low content of possible toxin inhibitors such as tannin, or with a high content if the action of an inhibitor in the insect supplements that of the toxin. A continued search for counteractors of pathogen inhibitors is required; modes of action of synergists (section 3.4.6, Table 3.17) need more study to try to enhance this search.

Some synergists may have general activity applicable to all the pest–plant systems for which a product is recommended. Others, or more particularly counteractors of pathogen inhibitors, may be limited to certain systems. These particular systems can be important, e.g. tannins in oak impairing the activity of *B. thuringiensis* on oak in stands of mixed tree species (Appel and Schultz, 1994) or on tannin-rich cotton. Such limited synergists/counteractors would be best added to the tank mixes only for the relevant plants.

Additives may also harm biocontrol agents. Those inflicting severe harm are soon weeded out, but those causing slight harm over an extended period present a problem of recognition. This problem becomes more difficult with mixtures of additives and with proprietary adjuvants. The best remedy is to build up an assemblage of data, such as that presented in the tables and in Appendix I.

Many of the so-called inert, non-insecticidal additives formulated with chemical pesticides are toxic or potentially dangerous to man and environment. The US Environmental Protection Agency has listed 'inerts' in four categories: (1) high toxicological concern (57 compounds, no new approvals are being granted for products containing these); (2) high priority for testing (62); (3) to be reviewed later (800); (4) no concern. With microbial pesticides, the high degree of safety and the absence of phytotoxicity of the microbes themselves should be backed up by use of additives only in category 4, preferably of food-grade quality. The use of xylol (category 2) has been discontinued in suspension concentrates of *B. thuringiensis*.

Trought (1989) lists companies specializing in conventional encapsulation systems. Costs and the limitations of developing for niche markets are likely to determine the commercial future of these systems for *B. thuringiensis*. Lignin and starch are probably the materials nearest to industrial use, particularly after the recent economies and streamlining in manufacture (section 3.2 and 3.7.1). This technology has been licensed by the US Department of Agriculture to the Biotechnology Research Development Corporation, Peoria, Illinois (Anon., 1992). If this type of encapsulation can be made competitive costwise, this powerful research organisation stands a good chance of success.

The insect pigment melanin, a very powerful photoprotectant (Table 3.16 in section 3.4.4), has been produced by *Streptomyces lividans* transformed with a tyrosinase gene (Liu *et al.*, 1993). Similar transformation of

B. thuringiensis cells may enable CellCap type (section 3.3.5) capsules highly protected from solar radiation to be produced (section 10.9).

More emphasis should be given to developing formulations for control of stored grain pests, soil insects and nematodes. A continual stream of new additives and new chemical groups, such as organosilicone super-wetters, are being applied with chemical pesticides. These are ready-made candidates for use with pathogens. They must first be examined for harmful effects on the pathogens, after assessing clues to be gained from chemical structure, general toxicity, phytotoxicity and known environmental effects. This should be followed by comparisons with formulants already in common use, structured so as to pinpoint effects specific to the test substances and also to unexpected activities, such as synergism and dual action. The search should of course be continued for additives for pathogens *de novo* – another Tinopal LPW (section 3.4.6) may be around the corner.

Improvement of formulation is playing an increasingly complex role in the development of commercial products. Both research organizations such as the Canadian Forest Service, and primary producers of microbial insecticides, are forging confidential links with companies having formulation experience to gain access to their skills and technology.

3.7.3 PRODUCTS FOR USE IN WATER

There is great scope for diversity of formulations for application to water, which has inspired research. Industry, to date, has incorporated only a limited selection of the findings in marketed products.

3.7.3a Suspension concentrates and wettable powders Because of the overriding requirement to use small particles that stay suspended as long as possible, flowable suspension concentrates should remain the dominant products for treatment of both large and small water bodies. Concentrates can be manufactured ready-to-spray by ULV without dilution to minimize loading time, a critical feature for aerial application. Research is important to increase potency and to reduce the aircraft load, which can be accomplished, among other ways, by using spore-free mutants to dispense with the weight of the spore (sections 3.7.1, 3.7.2b, 3.7.3e). Surfactants are necessary as dispersants in the products to maintain suspension in storage and to ensure good mixing when the product reaches the water.

When limited shelf-life of suspensions is a problem, the more stable wettable powders can be used, despite their more rapid settling. Light-weight materials added during or after drying would increase suspendability or make particles float. They would also increase particle size and improve the efficiency with which the target larvae filtered them from the water. Particles of <45 μm diameter could be made highly suspendable. Although these are near optimum laboratory sizes for feeding, they may be disadvantaged in flowing water by more entrapment in vegetation. Oil is probably the easiest material to add. Although the balance of evidence shows that at least vegetable oils are palatable to mosquito larvae, there is some evidence that mineral oil inhibits the feeding of blackfly larvae. Research on different oils should be rewarding. An additive that produces gas in water is an alternative, aiming to form on each particle a very small bubble that remains attached, although bubbles would probably be dislodged by fast-flowing water.

3.7.3b Granules, pellets and briquettes The practical problems encountered during mosquito larva control converge to suggest the use of formulations of granule size in preference to dry powders. Size and weight are needed to make granules roll down foliage cover into the water. Particles must float or suspend in water to be maintained in the various larval feeding zones (Table 3.19 in

section 3.6). Controlled release of these particles from granules is needed to extend the short life typical of suspension concentrates and wettable powders in the relatively static water bodies where mosquito larvae live. Blowing of floating granules by wind into limited surface areas must be avoided. Perhaps the ideal is a 2 mm granule, heavy enough to roll down and penetrate the water surface, yet buoyant enough to bob up and float just below it; initially, sufficient filterable particles should be released to kill all larvae including the least-susceptible late instars, then particles should be dispensed at a steady rate to kill the highly susceptible larvae emerging over time from eggs; however, particles must not be too heavy to increase, due to high weight, the cost of transporting them to the site of use and applying them by air. Technical powder, coated on to the granule surface, could release particles rapidly; it could be combined with a phagostimulant to entice larvae to accumulate below the granules and hasten their eating a lethal meal. A spate of research in the 1980s produced much data addressing these factors individually. This led to products on the market based on corn grits, clay and sand, each designed primarily for a specific purpose, but subject to either the problem of wind drift or that of sinking too fast. However, a start was made towards a multipurpose formula by adding 25% heavy particles to a buoyant corn grit granule (Sutherland, 1990). Manufacture was limited to the simplest procedures of spraying a technical product on to the granules or mixing it with them. Progress has been curbed by the cost of more sophisticated processing and materials, as well as lack of assurance that improved efficiency and less frequent application will repay any extra cost (section 10.4). Encouragingly, a patent for a complex granule (LarvXSG) has recently been filed (Sjogren, 1996), but more research on these granules is required. More urgently, comparative field trials with granules of known composition are needed to assess the advantages of sophisticated granules, varying the composition to examine the value of each formulation aspect, since most of these values are still based mainly on theory. Or, will non-target fauna eat up such tasty phagostimulant morsels before the full benefit of prolonged controlled-release can be obtained?

A multipurpose formulation may not always be needed, for example, for single-species populations. Great flexibility could be obtained by packaging different granules/capsules separately with the intention of using them either singly or roughly mixed on site for multispecies populations. Granules intended to float could incorporate a sunscreen, which would also be valuable for granules marketed for application to dry ground prior to expected flooding (section 3.6.4). Possible advantages of capsules over granules might be better control of buoyancy and better protection of the toxin crystals from deterioration, but the comparison is essentially that of comparing carrier materials and manufacturing processes.

Zaritsky *et al.* (1991) have bioencapsulated *B. thuringiensis* ssp. *israelensis* by feeding it to the ciliate *Tetrahymena pyriformis*, which in turn is eaten by mosquito larvae. Although such transient entrapment does not seem a practical method of biocontrol, it does demonstrate that filter-feeding organisms trap *B. thuringiensis* ssp. *israelensis* applied for mosquito control. Manasherob *et al.* (1996) obtained higher mortality of larvae with bioencapsulated bacteria than with the bacteria alone.

Pellets and briquettes are used mainly in small water masses for convenience and for minimum frequency of application. Their technical requisites are much the same as those for granules. Their use may increase in the domestic market, but putrefaction may be a problem in domestic water supplies.

Although application to water is currently restricted to bacteria, future trends may include other microorganisms. Williams *et al.* (1992) conducted an initial study on the impact of an iridescent virus on blackflies,

which has the advantage of being able to spread through the population.

3.7.3c Monomolecular layers Monolayers combine so many of the objectives of formulation for aquatic use, as well as complementing the action of the bacterial toxins on larvae, that they surely are prime subjects for more development for the formulation of bacteria. Compounds are available that persist longer than Arosurf (section 3.6.6; Roberts, 1989a). A method is required to prevent long-term deterioration of toxin when formulated in monolayer on the shelf, possibly by lowering the moisture content. The cost needs reducing and marketing needs improvement.

3.7.3d Bacillus sphaericus B. sphaericus (Table 3.1 in section 3.1) is more persistent than *B. thuringiensis* ssp. *israelensis*; control can remain substantial up to 4 weeks, although reasons for the difference are not understood (for review see Lacey and Undeen, 1986). Because of its greater durability, it may benefit more from formulation, and thus may be the better candidate for use in slow-release products. While being more active against species of *Anopheles* and *Culex*, but less against *Aedes*, it has the slight disadvantage of being more susceptible to solar radiation (section 3.6.1). The spore and crystal of *B. sphaericus* do not separate and the complex settles in water less rapidly than free crystals of *B. thuringiensis* ssp. *israelensis*, possibly because the less dense spore acts as a float (Karch and Hougard, 1986). Perhaps production of an oil globule beside the crystal could be obtained by genetic engineering to improve flotation. However, greater flotation would increase the importance of sunlight, which could be countered by an insoluble sunscreen or by inserting a gene for production of melanin, a very powerful natural screen (section 3.7.2c).

3.7.3e Products without live spores When a formulated product free from live spores is needed to treat water, spores can be killed by UVC or gamma radiation. 'Dead' products are as effective as 'live' products in controlling mosquito and blackfly larvae (sections 3.7.2b, 3.7.3a; Lacey *et al.*, 1978; Engler *et al.*, 1980; Krieg *et al.*, 1980a; Burke *et al.*, 1983). There are, however, a few reports of reduced potency after radiation: a small reduction after UVC treatment (Li *et al.*, 1987) and after gamma radiation from 172 to 137 ITU (Beck and Becker, 1992) with *B. thuringiensis* ssp. *israelensis*, also small reductions after dosages of gamma radiation up to 1800 kR, statistically significant with *B. thuringiensis* ssp. *israelensis* but not with *B. sphaericus* (Lacey and Smittle, 1985). It can be concluded that the radiation has only an unimportant effect on the crystal and that infection caused by the spore exerts virtually no effect on the control of target aquatic insect larvae.

Better products are obtained from spore-free mutants. Spore weight is absent and there is no risk of radiation damage to the crystal. Fermentation could be made even more efficient if some of the cell resources normally used to produce a spore could be diverted to production of larger crystals.

3.7.4 SYSTEMIC EXPRESSION OF MICROBIAL FACTORS

The challenge of maintaining the presence of *B. thuringiensis* endotoxin in new, rapidly growing foliage has been met in two ways:

crop plants are transformed directly with toxin genes;
toxin genes are transformed into endophytic microorganisms that can be applied as microbial insecticides.

Both have the key advantage of being self replicating.

Transgenic plants have greater commercial potential. However, crop varieties have to be transformed individually, a considerable commercial limitation. Also, in near-monoculture situations, there is the spectre of intense selection pressure causing insects to develop

resistance to the toxin. This can be countered by various strategies (Marrone and MacIntosh, 1993), the present favourite being the planting of refugia not involving *B. thuringiensis* (mandatory for cotton in the USA) combined with crops producing high concentrations of toxin, e.g. >25 times the LC_{99} for the target insect (Gelernter, 1997). Some suppliers sell seed conditional on certain anti-resistance steps being used as a part of cultural practice. Plant varieties resistant to diseases and/or pests, and tailored by traditional genetic techniques, are collectively an outstanding success in control programmes. When the disease organisms and pests resistant to these genetic varieties have appeared, either the operational scope of the varieties has been reduced, or the varieties have been superseded by new re-tailored resistant varieties. There appears to be no reason why plant varieties genetically engineered with *B. thuringiensis* toxins should not become as successful as, or even more widely used than, microbial insecticides. Registration requirements for transgenic plants have been eased in the USA and should not inhibit commercialization. Use during 1996 against *Heliothis* spp. met some problems (Gelernter, 1997). Predictably, damage was sometimes caused by pests secondary to *H. virescens*, such as *H. zea* and *Spodoptera exigua*. This could be countered in future by transforming more toxins into the plants, a ploy that would also reduce the likelihood of toxin-resistant insects. No induced resistance was found in 1996 in *H. virescens* due to commercial growing of transgenic plants (Gelernter, 1997). Naturally resistant wild strains of *Plodia interpunctella* occur in food stores. Outdoors, resistant strains of *Plutella maculipennis* have occurred in response to the long-term use of *B. thuringiensis* spp. *kurstaki* insecticides, including cross-resistance to abamectin (Wright *et al.*, 1995).

Despite the potential of transgenic plants, in many situations, particularly in developing countries, spray application of microbes is likely to be the method of choice due to the cost and unavailability of seed supply; even the supply of normal certified seed is problematic in many developing countries.

In water, algae transformed to express the *B. thuringiensis* ssp. *israelensis* toxin will be limited by the range of water masses favourable to growth of the species of alga used. Rice areas may be good targets. Additional features in the alga, such as nitrogen fixation (Boussiba *et al.*, 1992) would increase the chances of industrial success.

The use of endophytes, which are delicate organisms vulnerable both during storage and when infecting the plants, will need sophisticated formulation required for organisms that do not form tough spores. Epiphytic organisms are also relatively delicate and will face shelf-life problems. The commensal microorganisms will have the problems of genetically manipulated organisms in obtaining regulatory permission for release into the environment. Use of commensals has two advantages: (1) they would be applied only once to a crop; and (2) they would function as living microcapsules protecting the toxin from deteriorative factors. However, in tests on maize to date, crop damage reduction has been variable and obtainable only in maize hybrids compatible with the endophyte (Anon., 1991). This suggests that future use will depend on further technical improvements.

Although transgenic genes from insect pathogens used to protect organisms are currently limited to *B. thuringiensis* endotoxin genes, a wider range may be used in future. For example, Hanzlik *et al.* (1995) produced infectious particles of *H. armigera* stunt virus in non-insect cells, and report on the possibility of developing transgenic plants or other non-insect hosts that produce this virus. Genes from sources other than insect pathogens can also be included.

The genetic engineering of baculoviruses is now well established. It is aimed mainly at increasing the speed of kill or stopping insects

feeding by the insertion into the virus of genetic sequences that express specific toxins, or by the deletion of some sequences from the baculovirus genome (Hawtin and Possee, 1993; Vlak, 1994). Transformed virus products should be commercially available in the near future, with an increasing variety and function.

In the future, from the formulation viewpoint, the outstanding success should continue to be the insertion of *B. thuringiensis* toxin genes into plants, subject to the curbs of resistance development in insects and limitation to only some pests in a pest complex. Resistance can be delayed by anti-resistance strategies and use of different combinations of toxin genes. A wider range of pests can be controlled by co-insertion of other types of genes.

3.7.5 NOVEL STRATEGIES FOR CONTROL

A wider range of control strategies, involving novel approaches with different formulations, is likely to be employed in future. For example, traps may be used to attract insects and infect them with a pathogen (e.g. Tatchell, 1981). A sunscreen would not be needed in the formulation, because the pathogen is in a protected environment. Also, insects may be reared for release and infection with a pathogen to induce an epizootic, as has been done with the rhinoceros beetle infected with its non-occluded baculovirus; the virus is likely to be formulated only for storage because healthy beetles are immersed in a simple virus suspension to infect them (Bedford, 1981).

There should be an increasing range of products available for a larger range of pests. For example, the range of *B. thuringiensis* toxins being isolated is rapidly expanding. Crickmore *et al.* (1995) list 50 different sequenced crystal protein genes. Bravo *et al.* (1995) report that toxins are now known with activity against Lepidoptera, Diptera, Coleoptera, Hymenoptera and Acarina, as well as Nematoda, Trematoda and Protozoa. As a result targets such as nematodes and blowflies will be included in product development research. These new targets will involve new environments, e.g. dried fish for blowflies (Turner *et al.*, 1994), which present new challenges to the formulator.

Probably more sophisticated models of the interaction of spray volume, droplet size, product type, persistence and effect of additives will be developed to facilitate assessment of the effect of changes in formulation on product action. This should be very important for predicting the action of genetically altered products where the cost of safety testing prior to field experimentation is high.

3.7.6 RESEARCH PRIORITIES

From the summary above, a number of priorities become clear.

A stage has been reached when the true practical field value of recent formulation innovations needs better comparative study. Sufficient innovations have been taken up by industry to encourage work to be limited to marketed products, which is largely research in the dark because product compositions are proprietary. It is so frustrating to read of significant trial differences between proprietary products of equal potency and not be able to find reasons for these differences due to absence of formulation data. More comparative work is needed with formulations of published composition, so that the value of known material combinations can be assessed and combinations improved. Inclusion of some proprietary products would help to show the consequences of unknown industrial compromises that presumably were thought necessary, for instance to limit costs.

Costs are crucial. They largely determine the degree of use made of microbial insecticides and the opportunity for economies of scale. Cost reductions and streamlining in manu-

facture, distribution and user strategy need priority integrated study. Aspects of the outstanding example of manufacturer–user cooperation in the development and use of ULV application of pathogens in North America (Fig. 3.1; section 3.3.3a) could well be applied to other fields.

Research should be concentrated on some obvious weak points. One weak point is limited shelf-life of *B. thuringiensis* flowables and even of dry virus products; some outstanding improvements in harvest and storage of fungal pathogens (Chapter 4) may be applicable to viruses. Another weak point is the limited post-application life of the pathogens; this mainly translates into putting and preserving pathogens where they are eaten most on land foliage, to maintaining them in the feeding zones of mosquito larvae, and to improvement of carry in running water for blackfly larvae.

Scope for formulation improvement has recently been most obvious in the design of experimental granules for use both on land and in water. This momentum must be maintained and coupled with studies of how far granule complexity and cost can be increased before being negated by uncontrollable natural forces, such as dilution of cover on new foliage by plant growth, and the removal or masking of products in water by physical forces (e.g. trapment) and by non-target biota.

The search for new additives must continue. The rapid increase in our knowledge-base should enable this search to be better guided.

To achieve viable market size, manufacturers must often make products broad spectrum. User flexibility can be improved by research on tank mix technology for locally available types of spray machine, and by manufacturer research on making products more compatible with the requirements of different tank mixes for different situations.

More attention should be given to optimizing concentration and particle/droplet size for individual pest scenarios. These vary according to whether only young larvae are to be targeted because older ones have poor susceptibilities, or whether it is feasible to kill all larvae. With *B. thuringiensis*, the objective is rapid acquisition of a lethal dose before the toxin stops the insect feeding, a matter of good cover near the initial feeding sites of neonate or instar II larvae and sufficiently concentrated cover to lethally poison older larvae during their diurnal feeding bouts.

There is one area of unfulfilled promise in mosquito larviciding: the combination of *B. thuringiensis* with monolayers. Early experience with Arosurf has been disappointing. Some of the newer materials need testing, with thought being given to the most compatible bacterial products and to combined marketing of bacteria and monolayer. Experiments should be designed to illustrate advantages to be gained by reduced application frequency.

REFERENCES

Adams, J. A. R., Sheppard, C. A., Shapiro, M. and Thompkins, G. J. (1994) Light and electron microscopic investigations on the histopathology of the midgut of gypsy moth larvae infected with LdMNPV plus a fluorescent brightener. *J. Invertebr. Pathol.* **64**, 156–9.

Anon. (1991) CGI's InCide tests successful. *AGROW* 149, 20.

Anon. (1992) USDA licences starch encapsulation. *AGROW* 166, 25.

Anon. (1995) Cyanotech to sell *Bt* mosquito agent. *AGROW* 236, 18.

Aleshina, I. M., Evpak, A. N. and Lomovskaya, T. F. (1986) Preparation of bacterial entomopathogenic preparate by cultivation of *Bacillus thuringiensis* adding specified salt compounds to increase yield and improve properties. Patent SU1 510 813 A890 930.

Allen, G. E. and Pate, T. L. (1966) The potential role of a feeding stimulus in connection with the nuclear polyhedrosis virus of *Heliothis*. *J. Invertebr. Pathol.* **8**, 129–31.

Aly, C. (1983) Feeding behaviour of *Aedes vexans* larvae (Diptera: Culicidae) and its influence on

the effectiveness of *Bacillus thuringiensis* var. *israelensis*. *Bull. Soc. Vector Ecol.* **8**, 94–100.

Aly, C. (1988) Filtration rates of mosquito larvae in suspensions of latex microspheres and yeast cells. *Entomol. Exp. Appl.* **46**, 55–61.

Aly, C. and Mulla, M. S. (1986) Orientation and ingestion rates of larval *Anopheles albimanus* in response to floating particles. *Entomol. Exp. Appl.* **42**, 83–90.

Aly, C., Mulla, M. S., Schnetter, W. and Xu, B.-Z. (1987) Floating bait formulations increase effectiveness of *Bacillus thuringiensis* var. *israelensis* against *Anopheles* larvae. *J. Amer. Mosq. Control Ass.* **3**, 583–8.

Andrews, G. L., Harris, F. A., Sikorowski, P. P. and McLaughlin, R. E. (1975) Evaluation of *Heliothis* nuclear polyhedrosis in a cottonseed oil bait for control of *Heliothis virescens* and *Heliothis zea* on cotton. *J. Econ. Entomol.* **68**, 87–90.

Angus, T. A. (1959) Potential usefulness of vinyl latices as stickers. *Can. Entomol.* **91**, 254–5.

Angus, T. A. and Luthy, P. (1971) Formulation of microbial insecticides, in *Microbial Control of Insects and Mites* (eds H. D. Burges and N. W. Hussey), Academic Press, London, pp. 623–38.

Angus, T. A., Heimpel, A. M. and Fisher, R. A. (1961) Tests of a microbial insecticide against forest defoliators. Spray formulation, toxicity, diagnosis and bacteriological assay of spray deposit. Bi-monthly Progress Report, Department of Forestry, Forest Entomology and Pathology Branch, No. 17, pp. 1–2.

Appel, H. M. and Schultz, J. C. (1994) Oak tannins reduce effectiveness of Thuricide (*Bacillus thuringiensis*) in the gypsy moth (Lepidoptera: Lymantriidae). *J. Econ. Entomol.* **87**, 1736–42.

Aston, R. P. (1989) The use of *Bacillus thuringiensis* (Berliner) for the control of *Heliothis armigera* (Hubner) (Lepidoptera: Noctuidae) on cotton. PhD thesis, Cranfield Institute of Technology, UK.

Baker, C. A. and Henis, J. M. S. (1990) Commercial production and formulation of microbial biocontrol agents, in *New Directions in Biological Control: Alternatives for Suppressing Agricultural Pests and Diseases*, Proceedings of a ULCA Colloquium, Frisco, Colorado, January 20–27 1989 (eds R. R. Baker and P. E. Dunn), Alan R. Liss, New York, pp. 333–4.

Barbosa, P. (1988) Interactions among herbivora, allelochemicals, and insect pathogens, in *Novel Aspects of Insect–Plant Interactions* (eds P. Barbosa and D. K. Letourneau), Wiley, New York, pp. 215–9.

Barnes, A. C. and Cummings, S. E. (1986) US Patent 4 695 455.

Barnett, A. L. (1992) An investigation into the encounter of a baculovirus deposit on a cotton leaf by *Heliothis virescens* (Lepidoptera: Noctuidae) larvae. MSc thesis, University of London.

Bartelt, R. J., McGuire, M. R. and Black, D. A. (1990) Feeding stimulants for the European corn borer (Lepidoptera: Pyralidae): additives to a starch-based formulation for *Bacillus thuringiensis*. *Environ. Entomol.* **19**, 182–9.

Beck, M. and Becker, N. (1992) The use of *Bacillus sphaericus* in the integrated mosquito control program in the upper Rhine valley, in *Proceedings of the VII European Annual Meeting of the Society for Vector Ecology*, Bologna, Faculty of Agriculture, University of Bologna, p. 21.

Becker, N. and Ludwig, H. W. (1983) Mosquito control in West Germany. *Bull. Soc. Vector Ecol.* **8**, 85–93.

Becker, N. and Margalit, J. (1993) Use of *Bacillus thuringiensis israelensis* against mosquitoes and blackflies, in Bacillus thuringiensis, *an Environment Biopesticide: Theory and Practice* (eds P. F. Entwistle, J. S. Cory, M. J. Bailey and S. Higgs), Wiley, Chichester, pp. 147–70.

Becker, N., Djakaria, S., Kaiser, A. *et al.* (1991) Efficacy of a new tablet formulation of an asporogenous strain of *Bacillus thuringiensis israelensis* against larvae of *Aedes aegypti*. *Bull. Soc. Vector Ecol.* **16**, 176–82.

Bedford, G. O. (1981) Control of the rhinoceros beetle by baculovirus, in *Microbial Control of Pests and Plant Diseases* 1970–1980 (ed. H. D. Burges), Academic Press, London, pp. 409–26.

Behle, R. W., McGuire, M. R. and Shasha, B. S. (1996) Extending the residual insecticidal activity of *Bacillus thuringiensis* with casein based formulations. *J. Econ. Entomol.* **89**, 1399–405.

Behle, R. W., McGuire, M. R., Gillespie, R. L. *et al.* (1997a) Effects of alkaline gluten on the insecticidal activity of *Bacillus thuringiensis*. *J. Econ. Entomol.* **90**, 354–60.

Behle, R. W., McGuire, M. R. and Shasha, B. S. (1997b) Effects of sunlight and simulated rain on the residual activity of *Bacillus thuringiensis* formulations. *J. Econ. Entomol.* **90**, 1506–6.

Bell, M. R. and Kanavel, R. R. (1975) Potential of bait formulations to increase effectiveness of nuclear polyhedrosis virus against the pink bollworm. *J. Econ. Entomol.* **68**, 389–91.

Bell, M. R. and Kanavel, R. F. (1978) Tobacco budworm: development of a spray adjuvant to

increase effectiveness of a nuclear polyhedrosis virus. *J. Econ. Entomol.* **71**, 350–2.

Bell, M. R. and Romine, C. L. (1980) Tobacco budworm field evaluation of microbial control in cotton using *Bacillus thuringiensis* and a nuclear polyhedrosis virus with a feeding adjuvant. *J. Econ. Entomol.* **73**, 427–30.

Benz G. (1971) Synergism of micro-organisms and chemical insecticides, in *Microbial Control of Insects and Mites* (eds H. D. Burges and N. W. Hussey), Academic Press, London, pp. 327–55.

Bernhard, K. and Utz, R. (1993) Production of *Bacillus thuringiensis* insecticides for experimental and commercial uses, in Bacillus thuringiensis, *An Environmental Biopesticide: Theory and Practice* (eds P. F. Entwistle, J. S. Cory, M. J. Bailey and S. Higgs), Wiley, Chichester, pp. 255–67.

Bezdicek, D. F., Quinn, M. A., Forse, L. *et al.* (1994) Insecticidal activity and competitiveness of *Rhizobium* spp. containing the *Bacillus thuringiensis* subsp. *tenebrionis* (-endotoxin gene (*cry III*) in legume nodules. *Soil Biol. Biochem.* **26**, 1637–46.

Biache, G. and Chaufaux, J. (1986) Studies on the conditions for using baculoviruses in the control of noctuids. *Colloques de l'INRA* **34**, 149–65.

Bird, F. T. (1953) The use of a virus disease in the biological control of the European pine sawfly, *Neodiprion sertifer* (Geoffr.). *Can. Entomol.* **85**, 437–46.

Bora, R. S., Murty, M. G., Shenbagarathai, R. and Vaithilingam, S. (1994) Introduction of a Lepidoptera-specific insecticidal crystal protein gene of *Bacillus thuringiensis* subsp. *kurstaki* by conjugal transfer into a *Bacillus megaterium* strain that persists in the cotton phyllopshere. *Appl. Environ. Microbiol.* **60**, 214–22.

Boussiba, S., Thomas, S., Douek, J. *et al.* (1992) Mosquito biocontrol by the (-endotoxin of *Bacillus thuringiensis* var. *israelensis* cloned in an ammonium-excreting mutant of a rice field isolate of the nitrogen-fixing cyanobacterium *Anabaena siamensis*, in *Proceedings of the XXV Annual Meeting of the Society for Invertebrate Pathology*, Heidelberg, p. 154.

Brassel, J. and Benz, G. (1979) Selection of a strain of the granulosis virus of the codling moth with improved resistance against artificial ultraviolet radiation and sunlight. *J. Invertbr. Pathol.* **33**, 358–63.

Bravo, A., Lorence, A. and Ramírez, R. Q. (1995) Biopesticides compatible with the environment: *Bacillus thuringiensis*, a unique model. *Biocontrol* **1**, 41–55.

Bull, D. L. (1978) Formulations of microbial insecticides: microencapsulation and adjuvants. *Misc. Publ. Entomol. Soc. Amer.* **10**, 11–20.

Bull, D. L., Ridgway, R. L., House, V. S. and Pryor, N. W. (1976) Improved formulation of the *Heliothis* nuclear polyhedrosis virus. *J. Econ. Entomol.* **69**, 731–6.

Burgerjon, A. (1989) Persistence to washing with rain of *Bacillus thuringiensis* and viral inclusion body preparations. *IOBC/WRPS Bull.* **12**, 78.

Burges, H. D. (1977) Control of the wax moth *Galleria mellonella* on beecomb by H-serotype V *Bacillus thuringiensis* and the effect of chemical additives. *Apidologie* **8**, 155–68.

Burges, H. D. and Hussey, N. W. (eds) (1971) *Microbial Control of Insects and Mites*, Academic Press, London.

Burges, H. D., Jarrett, P. and Kanagaratnam, P. (1979) Application of *Bacillus thuringiensis* with thermal fogging machines, in *Proceedings of the Second International Colloquium on Invertebrate Pathology*, Agricultural Campus, Prague, Society for Invertebrate Pathology, pp. 27–8.

Burke, W. F. Jr, McDonald, K. O. and Davidson, E. W. (1983) Effect of UV light on spore viability and mosquito larvicidal activity of *Bacillus sphaericus* 1593. *Appl. Environ. Microbiol.* **46**, 954–6.

Butz, P., Fritsch, E., Huber, J. *et al.* (1995) Ultra-high pressure decontamination of insect biocontrol preparations. *Biocontrol Sci. Technol.* **5**, 243–6.

Capinera, J. L. and Hibbard, B. E. (1987) Bait formulations of chemical and microbial insecticides for suppression of crop-feeding grasshoppers. *J. Agric. Entomol.* **4**, 337–44.

Caudwell, R. (1993) Bait formulation of microbial agents for grasshopper control. *Biocont. News Info.* **14**(3), 53–7.

Chancey, G., Yearian, W. C. and Young, S. Y. (1973) Pathogen mixtures to control insect pests. *Ark. Farm. Res.* **22**, 9.

Cherry, A. J., Parnell, M. A., Smith, D. and Jones, K. A. (1994) Oil formulations of insect viruses. *IOBC/WRPS Bull.* **17**, 254–7.

Cherry, A. J., Parnell, M. A., Grzywacz, D. *et al.* (1996) Long-term storage of baculovirus preparations, in *Abstracts of the 29th Annual Meeting of the Society for Invertebrate Pathology*, University of Córdoba, p. 14.

Cherry, A. J., Parnell, M. A., Grzywacz, D. and Jones, K. A. (1998) Long-term storage of baculovirus preparations. *Biocontrol Sci. Technol* (in press).

Cheung, P. Y. K. and Hammock, B. D. (1985) Micro-lipid-droplet encapsulation of *Bacillus*

thuringiensis subsp. *israelensis* (-endotoxin for control of mosquito larvae. *Appl. Environ. Microbiol.* **50**, 984–8.

CIAT (1987) Biological control halts cassava hornworm, in *CIAT Report, 1987*, CIAT, Cali, Colombia, pp. 34–6.

Cibulsky, R. J., Devisetty, B. N., Melchior, G. L. and Melin, B. E. (1993) Formulation and application technologies for microbial pesticides: review of progress and future trends. *J. Test. Eval.* **21**, 500–3.

Cohen, E., Rozen, H., Joseph, T. *et al.* (1991) Photoprotection of *Bacillus thuringiensis kurstaki* from ultraviolet irradiation. *J. Invertebr. Pathol.* **57**, 343–51.

Cooke, B. J. and Régnière, J. (1996) An object-orientated, process-based stochastic simulation model of *Bacillus thuringiensis* efficacy against spruce budworm, *Choristoneura fumiferana* (Lepidoptera: Tortricidae). *Int. J. Pest Manag.* **42**, 291–306.

Cosenza, B. J., Boger, E. A., Dubois, N. R. and Lewis, F. B. (1963) *A Simple Device for Dehairing Insect Egg Masses*, US Forest Service Research Note NE–1, Upper Darby, Pennsylvania.

Couch, T. L. and Ignoffo, C. M. (1981) Formulation of insect pathogens, in *Microbial Control of Pests and Plant Diseases 1970–1980.* (ed. H. D. Burges), Academic Press, London, pp. 621–34.

Creighton, C. S., Kinard, W. S and Allen, N. (1961) Effectiveness of *Bacillus thuringiensis* and several chemical insecticides for control of budworms and hornworms on tobacco. *J. Econ. Entomol.* **54**, 1112–4.

Crickmore, N., Zeigler, D. R., Feitelson, J. *et al.* (1995) Revision of the nomenclature for the *Bacillus thuringiensis cry* genes, in *Abstracts of the 28th Annual Meeting of the Society for Invertebrate Pathology*, Cornell University, Ithaca, p. 14.

Cunningham, J. C., Kaupp, W. J., McPhee, J. A. R. *et al.* (1975) *Aerial Applications of Nuclear Polyhedrosis Virus to Control European Pine Sawfly*, Neodiprion sertifer (*Geoff.*) *at Sandbanks, Provincial Park, Quinte Island, Ontario in 1975*, Canadian Forest Service Information Report IPX-X-7.

Cunningham, J. C., Kaupp, W. J., House, G. M. *et al.* (1978) *Aerial Application of Spruce Budworm Baculovirus: Tests of Virus Strains, Dosages and Formulations in 1977*, Information Report FPM-X-3, Canadian Forest Service, Sault Ste Marie.

Cyanamid (1992) *Acrobe® Biolarvicide: Technical Information, Field Trial Summary*, American Cyanamid Company, Wayne, New Jersey.

Dadd, R. H., Kleinjan, J. E. and Merrill, L. D. (1982) Phagostimulant effects of simple nutrients on larval *Culex pipiens* (Diptera: Culicidae). *Ann. Entomol. Soc. Amer.* **75**, 605–12.

Dahl, C. (1988) Control potentials in feeding mechanisms of mosquito larvae. *Bull. Soc. Vector Ecol.* **13**, 295–303.

David, W. A. L. (1969) The effect of ultraviolet radiation of known wavelengths on a granulosis virus of *Pieris brassicae. J. Invertebr. Pathol.* **14**, 336–42.

David, W. A. L. (1978) The granulosis virus of *Pieris brassicae* L. and relationship with its host, in *Advances in Virus Research* (eds M. A. Lauffer, F. R. Bang, K. Maramorosch and K. M. Smith), Academic Press, New York, pp. 111–61.

David, W. A. L. and Gardiner, B. O. C. (1966) Persistence of granulosis virus of *Pieris brassicae* on cabbage leaves. *J. Invertebr. Pathol.* **8**, 180–3.

David, W. A. L. and Gardiner, B. O. C. (1967) The effect of heat, cold, and prolonged storage on a granulosis virus of *Pieris brassicae., J. Invertebr. Pathol.* **9**, 555–62.

Dempah, J. and Coz, J. (1980) *Etude de la Stabilité de la Poudre Primaire du Serotype H14 de* Bacillus thuringiensis *R 153–78 en Zone Tropicale*, World Health Organisation Document WHO/VBC/80. 767, WHO, Geneva.

Derksen, A. C. G. and Granados, R. R. (1988) Alteration of a lepidopteran peritrophic membrane by baculovirus and enhancement of viral infectivity. *Virology* **167**, 242–50.

Devisetty, B. N. (1988) Microbial formulations – opportunities and challenges, in *Pesticide Formulations and Application Systems*, Vol. 8, ASTM STP 98 (eds D. A. Hovde and G. B. Beestman), American Society for Testing and Materials, Philadelphia, pp. 46–64.

Dimetry, N. Z. and Matter, M. M. (1990) Diversified effects of some chemicals on the pathogenic efficacy of *Bacillus thuringiensis* against the cotton leafworm, *Spodoptera littoralis* Boisduval. *Bull. Fac. Agric. Univ. Cairo* **41**, 191–8.

Doane, C. C. and Wallis, R. C. (1964) Enhancement of the action of *Bacillus thuringiensis* Berliner on *Porthetria dispar* (Linnaeus) in laboratory tests. *J. Invertebr. Pathol.* **6**, 423–9.

Dougherty, E. M., Guthrie, K. P. and Shapiro, M. (1996) Optical brighteners provide baculovirus activity enhancement and UV-radiation protection. *Biol. Control* **7**, 71–4.

Dubois, N. R. (1976) Effectiveness of chemically decontaminated *Neodiprion sertifer* polyhedral inclusion body suspensions. *J. Econ. Entomol.* **69**, 93–5.

Dubois, N. R. and Dean, D. H. (1995) Synergism between Cry IA insecticidal crystal proteins and spores of *Bacillus thuringiensis*, other bacterial spores, and vegetative cells against *Lymantria dispar* (Lepidoptera, Lymantriidae) larvae. *Environ. Entomol.* **24**, 1741–7.

Dubois, N. R., Mierzejewski, K., Reardon, R. C. *et al.* (1994) *Bacillus thuringiensis* field applications: effect of nozzle type, drop size, and application timing on efficacy against gypsy moth. *J. Environ. Sci. Health.* **B29**(4), 679–95.

Dulmage, H. T. and Rhodes, R. A. (1971) Production of pathogens in artificial media, in *Microbial Control of Insects and Mites* (eds H. D. Burges and N. W. Hussey), Academic Press, London, pp. 507–40.

Dunkle, R. L. and Shasha, B. S. (1988) Starch-encapsulated *Bacillus thuringiensis*: a potential new method for increasing environmental stability of entomopathogens. *Environ. Entomol.* **17**, 120–6.

Dunkle, R. L. and Shasha. B. S. (1989) Response of starch-encapsulated *Bacillus thuringiensis* containing ultraviolet screens to sunlight. *Environ. Entomol.* **18**, 1035–41.

Dutky, S. R. (1963) The milky diseases, in *Insect Pathology: An Advanced Treatise*, Vol. 2. (ed. E. A. Steinhaus), Academic Press, New York, pp. 75–114.

Ejiofor, A. O. and Okafor, N. (1991) Formulation of a flowable liquid concentrate of *Bacillus thuringiensis* serotype H-14 spores and crystals as mosquito larvicide. *J. Appl. Bact.* **71**, 202–6.

Elçin, Y. M., Corkmus, C. and Sacilik, S. C. (1995) Aluminium carboxymethylcellulose encapsulation of *Bacillus sphaericus* 2362 for control of *Culex* spp. (Diptera: Culicidae) larvae. *J. Econ. Entomol.* **88**, 830–4.

Elleman, C. J. and Entwistle, P. F. (1985a) Inactivation of a nuclear polyhedrosis virus on cotton by substances produced by the cotton leaf surface glands. *Ann. Appl. Biol.* **106**, 83–92.

Elleman, C. J. and Entwistle, P. F. (1985b) The effect of magnesium ions on the solubility of polyhedral inclusion bodies and its possible role in the inactivation of a nuclear polyhedrosis virus of *Spodoptera littoralis* by the cotton leaf exudate. *Ann. Appl. Biol.* **106**, 93–100.

El–Salamouny, S., Huber, J., Elnagar, S. and El-Sheikh, M. A. K. (1997) Increasing the susceptibility to nuclear polyhedrosis viruses by synergistic additives, in *Microbial Insecticides: Novelty or Necessity?* Symposium Proceedings No. 68 (ed. H. F. Evans), British Crop Protection Council, Farnham, pp. 289–92.

Ely, S. (1993) The engineering of plants to express *Bacillus thuringiensis* (-endotoxins, in Bacillus thuringiensis, *an Environmental Biopesticide: Theory and Practice* (eds P. F. Entwistle, J. S. Cory, M. J. Bailey and S. Higgs), Wiley, Chichester, pp. 105–24.

Engler, S., Morawcsik, J., Schnetter, W. and Kreig, A. (1980) Pilot-versuch zur Bekampfung von Stechmuckenlarven im Freiland mit einem UV-behandelten Praparat von *Bacillus thuringiensis* var. *israelensis*. *Anz. Schadlingskde, Pflanzenschutz, Umweltschutz.* **53**, 181–4.

Entwistle, P. F. (1986) Spray droplet deposition patterns and loading of spray droplets with NPV inclusion bodies in the control of *Panolis flammea* in pine forests, in *Fundamental and Applied Aspects of Invertebrate Pathology, IV International Colloquium on Invertebrate Pathology*, Wageningen (eds R. A. Samson, J. M. Vlak and D. Peters), Society for Invertebrate Pathology, pp. 613–5.

Entwistle, P. F. and Curruthers, W. R. (1989) Acquisition and loss of nuclear polyhedrosis virus inclusion bodies by plant surfaces. *IOBC/WRPS Bull.* **12**(4), 79–80.

Entwistle, P. F. and Evans, H. F. (1985) Viral control, in *Comprehensive Insect Physiology, Biochemistry and Pharmacology* (eds G. A. Kerkut and L. I. Gilbert), Vol. 12, Permagon, Oxford, pp. 347–412.

Entwistle, P. F., Cory, J. S., Bailey, M. J. and Higgs, S. (eds) (1993) Bacillus thuringiensis, *an Environmental Biopesticide: Theory and Practice*, Wiley, Chichester.

Falcon, L. A. (1971) Microbial control as a tool in integrated control, in *Biological Control* (ed. C. B. Huffaker), Plenum Press, New York, pp. 346–64.

Falcon, L. A. (1974) Insect pathogens: integration into a pest management system, in *Proceedings of Summer Institute on Biological Control of Plant Insects and Diseases* (eds F. G. Maxwell and F. A. Harris), University Press of Mississippi, Jackson, p. 618.

Farrar, R. R. Jr and Ridgway, R. L. (1995) Enhancement of activity of *Bacillus thuringiensis* Berliner against four Lepidopterous insect pests by nutrient-based phagostimulants. *J. Entomol. Sci.* **30**, 29–42.

Farrar, R. R. Jr, Ridgway, R. L., Cook, S. P. *et al.* (1995) Nuclear polyhedrosis virus of the gypsy moth (Lepidoptera: Lymantriidae): potency and

effects of selected adjuvants on insect feeding behaviour. *J. Entomol. Sci.* **30**, 417–28.

Fast, P. G. and Reginiere, J. (1984) Effect of exposure time to *Bacillus thuringiensis* on mortality and recovery of the spruce budworm (Lepidoptera: Tortricidae). *Can. Entomol.* **116**, 123–30.

Fast, P. G., Kettela, E. G. and Wiesner, C. (1985) Measurement of foliar deposits of *Bt* and their relation to efficacy, in *Microbial Control of Spruce Budworms and Gypsy Moths*, Symposium of the USDA Forest Service, Broomall, Pennsylvania, pp. 148–9.

Felton, G. W., Duffet, S. S., Vail, P. V. *et al.* (1987) Interaction of nuclear polyhedrosis virus with catechols: potential incompatibility for host resistance against Noctuid larvae. *J. Chem. Ecol.* **13**, 947–957.

van Frankenhuyzen, K. (1990) Effect of temperature and exposure time on toxicity of *Bacillus thuringiensis* Berliner spray deposits to spruce budworm, *Choristoneura fumiferana* Clemens (Lepidoptera: Tortricidae). *Can. Entomol.* **122**, 69–75.

van Frankenhuyzen, K. (1993) The challenge of *Bacillus thuringiensis*, in Bacillus thuringiensis, *an Environmental Biopesticide: Theory and Practice* (eds P. F. Enwistle, J. S. Cory, M. J. Bailey and S. Higgs), Wiley, Chichester, pp. 1–35.

van Frankenhuyzen, K. and Nystrom, C. W. (1987) Effect of temperature on mortality and recovery of spruce budworm (Lepidoptera, Tortricidae) exposed to *Bacillus thuringiensis* Berliner. *Can. Entomol.* **119**, 941–54.

van Frankenhuyzen, K. and Payne, N. J. (1993) Theoretical optimization of *Bacillus thuringiensis* Berliner for control of the eastern spruce budworm, *Choristoneura fumiferana* Clem. (Lepidoptera: Tortricidae): estimates of lethal and sublethal dose requirements, product potency and effective droplet sizes. *Can. Entomol.* **125**, 473–8.

Garcia, R. and Des Rochers, B. (1979) Toxicity of *Bacillus thuringiensis* var. *israelensis* to some Californian mosquitos under different conditions. *Mosq. News* **39**, 541–4.

Gaugler, R. and Finney, J. (1982) A review of *Bacillus thuringiensis* var. *israelensis* (serotype H14) as a biological control agent of black flies (Simuliidae). *Misc. Publ. Entomol. Soc. Am.* **12**, 1–17.

Gelernter, W. D. (1997) Resistance to microbial insecticides: the scale of the problem and how to manage it, in *Microbial Insecticides: Novelty or Necessity?* Symposium Proceedings No. 68 (ed. H. F. Evans), British Crop Protection Council, Farnham, pp. 201–12.

Gelernter, W. and Schwab, G. E. (1993) Transgenic bacteria, viruses, algae and other microorganisms as *Bacillus thuringiensis* toxin delivery systems, in Bacillus thuringiensis, *an Environmental Biopesticides: Theory and Practice.* (eds P. F. Entwistle, J. S. Cory, M. J. Bailey and S. Higgs), Wiley, Chichester, pp. 89–104.

Gibson, D. M., Greenspan Gallo, L., Krasnoff, S. B. and Ketchum, R. E. B. (1995) Increased efficacy of *Bacillus thuringiensis* subsp. *kurstaki* in combination with tannic acid. *J. Econ. Entomol.* **88**, 270–7.

Gillespie, R. L., McGuire, M. R. and Shasha, B. S. (1994) Palatability of flour granular formulations to European corn borer larvae. *J. Econ. Entomol.* **87**, 452–7.

Glen, D. M., Wiltshire, C. W., Milsom, N. F. and Brain, P. (1984) Codling moth granulosis virus: effects of its use on some other orchard arthropods. *Ann. Appl. Biol.* **104**, 99–106.

Granados, R. R. and Federici, B. A. (eds) (1986a). *The Biology of Baculoviruses, Vol. 1, Biological properties and Molecular Biology*, CRC Press, Boca Raton, Florida.

Granados, R. R. and Federici, B. A. (eds) (1986b) *The Biology of Baculoviruses, Vol. 2, Practical Applications for Insect Control*, CRC Press, Boca Raton, Florida.

Green, C. F., Rimmerp, H. E., Beers, E. H. and Stevens, P. J. G. (1996) Organisosilicone adjuvants to target argrichemicals to their sites of action, in *Proceedings of the Brighton Crop Protection Conference – Pests and Diseases*, Vol. 3, British Crop Protection Council, Farnham, pp. 813–22.

Griego, V. M. and Spence, K. D. (1978) Inactivation of *Bacillus thuringiensis* spores by ultraviolet and visible light. *Appl. Environ. Microbiol.* **35**, 906–10.

Griego, V. M., Martignoni, M. E. and Claycomb, A. E. (1985) Inactivation of nuclear polyhedrosis virus (Baculovirus subgroup A) by monochromatic UV radiation. *Appl. Environ. Microbiol.* **49**, 709–10.

Gringorten, J. L., Milne, R. E., Fast. P. G. *et al.* (1992) Suppression of *Bacillus thuringiensis* (-endotoxin activity by low alkaline pH. *J. Invertebr. Pathol.* **60**, 47–52.

Griffith, I. P. (1985) A new approach to the problem of identifying baculoviruses, in *Microbial and Viral Pesticides* (ed. E. Kurstak), Marcel Dekker, New York, pp. 507–27.

de Groot, P. and Cunningham, J. C. (1983) *Aerial Spray Trials with a Baculovirus to Control*

Red-headed Pine Sawfly in Ontario in 1979 and 1980, Information Report FPM-X-63, Canadian Forest Service, Sault Ste Marie.

de Groot, P., Cunningham, J. C. and McPhee, J. A. R. (1979) Control of Red-headed Pine Sawfly with a Baculovirus in Ontario in 1978, and a Survey of Areas Treated in Previous Years. Canadian Forest Service, Information Report FPM-X-20.

Grzywacz, D., McKinley, D. J., Jones, K. A. and Moawad, G. (1997) Microbial contamination in *Spodoptera littoralis* nuclear polyhedrosis virus produced in insects in Egypt. *J. Invertebr. Pathol.* **69**, 151–6.

Guillet, P. and Escaffre, H. (1979) Evaluation de *Bacillus thuringiensis* israelensis de Barjac pour la lutte contre les larves de *Simulium damnosum* S. II. Efficacité comparée de trois formulations expérimentales, WHO/VBC/79. 735, World Health Organisation, Geneva (mimeo).

Guillet, P., Dempah, J. and Coz, J. (1980) Evaluation de *Bacillus thuringiensis* serotype 14 de Barjac pour la lutte contre des larves de *Simulium damnosum* S. L. III. Données preliminaires sur la sedimentation de l'endotoxine dans l'eau et sur sa stabilité en zone tropicale, WHO/VBC 80. 756, World Health Organisation, Geneva (mimeo).

Guillet, P., Escaffre, H. and Prud'hom, J. M. (1982) *Bacillus thuringiensis* H14, a biocontrol agent for onchocerciasis control in West Africa, in *Invertebrate Pathology and Microbial Control, Proceedings of the III International Colloquium on Invertebrate Pathology*, University of Sussex, Brighton (eds C. C. Payne and H. D. Burges), Society for Invertebrate Pathology, pp. 460–5.

Guillet, P., Escaffre, H., Prud'hom, J. M. and Bakayoko, S. (1985a) Etude des facteurs conditionant l'efficacité des preparations à base de *Bacillus thuringiensis* H14 *vis-à-vis* des larves du complex *Simulium damnosum* (Diptera: Simuliidae). Part 1. Influence de la nature et de la taille des particules. *Cab. ORSTOM, sér. Ent. méd. et Parasitol.* **23**, 257–64.

Guillet, P., Escaffre, H., Prud'hom, J. M. and Bakayoko, S. (1985b) Etude des facteurs conditionnant l'efficacité des préparations à base de *Bacillus thuringiensis* H14 *vis-à-vis* des larves du complexe *Simulium damnosum* (Diptera: Simuliidae). Part 2, Influence du temps de contact et de la quantité de particules naturelles en suspension dans l'eau. *Cab. ORSTOM, sér. Ent. méd. et Parasitol.* **23**, 265–71.

Guillet, P., Kurtak, D. C., Philippon, B. and Meyer, R. (1990) Use of *Bacillus thuringiensis israelensis* for onchocerciasis control in West Africa, in *Bacterial Control of Mosquitoes and Blackflies* (eds H. de Barjac and D. J. Sutherland), Rutgers University Press, New Brunswick, pp. 187–201.

Guillon, M. (1995) Industrial production of insect viruses for biological control. Technical aspects and economical interest, in *Proceedings of a Conference on Microbial Control Agents in Sustainable Agriculture: Field Experience, Industrial Production and Registration*, October 18–19, 1995, St Vincent, Italy, pp. 65–72.

Hall, F. R., Chapple, A. C., Taylor, R. A. J. and Downer, R. A. (1995) Modelling the dose acquisition process of *Bacillus thuringiensis*: influence of feeding pattern on survival, in *Biorational Pest-Control Agents: Formulation and Delivery* (eds F. R. Hall and J. W. Barry), American Chemical Society, Washington, pp. 68–78.

Hall, I. M. (1964) Use of micro-organisms in biological control, in *Biological Control of Insect Pests and Weeds* (ed. P. DeBach), Reinhold, New York, pp. 610–28.

Hamm, J. J. and Shapiro, M. (1992) Infectivity of fall armyworm (Lepidoptera: Noctuidae) nuclear polyhedrosis virus enhanced by a fluorescent brightener. *J. Econ. Entomol.* **85**, 2149–52.

Hanzlik, T. N., Bawden, A., Brooks, E. M. *et al.* (1995) Producing an insecticidal virus in cells of plants and other non-hosts, in *Proceedings of the Vth International Congress on the Impact of Viral Diseases in the Developing World*, Johannesburg, South Africa, July 9–14, 1995, International Comparative Virology Organization, Montreal, p. 36.

Harrap, K. A., Payne, C. C. and Robertson, J. S. (1977) The properties of three baculoviruses from closely related hosts. *Virology* **79**, 14–31.

Hawtin, R. E. and Possee, R. D. (1993) Genetic manipulation of the baculovirus genome for insect pest control, in *Parasites and Pathogens of Insects, Vol. 2: Pathogens* (eds N. E. Beckage, S. N. Thompson and B. A. Federici), Academic Press, London, pp. 179–95.

Hedin, P. A., Parrott, W. L., Jenkins, J. N. *et al.* (1988) Elucidating mechanisms of tobacco budworm resistance to allelochemicals by dietary tests with insecticide synergists. *Pesticide Biochem. Physiol.* **32**, 55–61.

Hickle, L. A. and Fitch, W. L. (1990) *Analytical Chemistry of* Bacillus thuringiensism, ACS Symposium Series 432, American Chemical Soceity, Washington, D. C.

Hinkle, N. C. (1983) Factors affecting the efficacy of *Bacillus thuringiensis* var. *israelensis* as a mosquito larvicide. MSc thesis, Auburn University.

Hou, R. F. and Chiu, C. S. (1986) Antiviral proteins in midgut of the silk worm, *Bombyx mori*, fed on different sources. *Ent. Exp. Appl.* **42**, 3–8.

Huber, J. (1986) *In vivo* production and standardisation, in *Fundamental and Applied Aspects of Invertebrate Pathology, IV International Colloquium on Invertebrate Pathology*, Wageningen (eds A. Samson, J. M. Vlak and D. Peters), Society for Invertebrate Pathology, pp. 87–9.

Huber, J. (1989) Workshop 2: Environmental persistence of pathogens. Second round table discussions. *IOBC/WRPS Bull.* **12**(4), 93–7.

Huger, A. (1963) Granulosis of insects, in *Insect Pathology: An Advanced Treatise*, Vol. 1 (ed. E. A. Steinhaus), Academic Press, New York, pp. 531–75.

Hunter, D. K., Hoffman. D. F. and Collier, S. J. (1973) Pathogenicity of a nuclear polyhedrosis virus of the almond moth, *Cadra cautella*. *J. Invertebr. Pathol.* **21**, 282–6.

Hunter, D. K., Collier, S. S. and Hoffman, D. F. (1977) Granulosis virus of the Indian meal moth as a protectant for stored in-shell almonds. *J. Econ. Entomol.* **70**, 493–4.

Hunter-Fujita, F. R., Dodsworth, J., Jones, K. A. *et al.* (1992) Observations on the relationship between a granulosis virus and a nuclear polyhedrosis virus infecting the Eyptian cotton leafworm, *Spodoptera littoralis*, in *Abstracts of the 25th Annual Meeting of the Society for Invertebrate Pathology*, Heidelberg, p. 312.

Hunter-Fujita, F. R., Entwistle, P. F., Evans, H. F. and Crook, N. E. (1997a) *Insect Viruses and Pest Management*, Wiley, Chichester.

Hunter-Fujita, F. R., Vasiljevic, S., Jones, K. A. and Cherry, A. (1997b) Effects of mixed infections with GV and NPV on the biology of the Egyptian cotton leafworm *Spodoptera littoralis*, in *Microbial Insecticides: Novelty or Necessity?* Symposium Proceedings No. 68 (ed. H. F. Evans), British Crop Protection Council, Farnham, pp. 271–8.

Ignoffo, C. M. and Batzer, O. F. (1971) Microencapsulation and Invertebr. Pathol. p. 312. Heidelberg. ultraviolet protectants to increase sunlight stability of an insect virus. *J. Econ. Entomol.* **64**, 850–3.

Ignoffo, C. M. and Couch, T. L. (1981) The nucleopolyhedrosis virus of *Heliothis* species as a microbial insecticide, in *Microbial Control of Pests and Plant Diseases 1970–1981* (ed. H. D. Burges), Academic Press, London, pp. 330–62.

Ignoffo, C. M. and Garcia, C. (1966) The relation of pH to the activity of inclusion bodies of a *Heliothis* nuclear polyhedrosis. *J. Invertebr. Pathol.* **8**, 426–7.

Ignoffo, C. M. and Garcia, C. (1978) UV-photoinactivation of cells and spores of *Bacillus thuringiensis* and effects of peroxidase on inactivation. *Environ. Entomol.* **7**, 270–2.

Ignoffo, C. M. and Garcia, C. (1994) Antioxidant and enzyme effects on the inactivation of inclusion bodies of the *Heliothis* baculovirus by simulated sunlight-UV. *Environ. Entomol.* **23**, 1025–9.

Ignoffo, C. M. and Garcia, C. (1995) Aromatic/heterocyclic amino acids and the simulated sunlight–ultraviolet inactivation of the *Heliothis/Helicoverpa* baculovirus. *Environ. Entomol.* **24**, 480–2.

Ignoffo, C. M. and Garcia, C. (1996) Simulated sunlight-UV sensitivity of experimental dust formulations of the nuclear polyhedrosis virus of *Helicoverpa/Heliothis*. *J. Invertbr. Pathol.* **67**, 192–4.

Ignoffo, C. M. and Hostetter, D. L. (eds) (1977) Environmental stability of microbial insecticides. *Misc. Publ. Entomol. Soc. Am.* **10**, 117–9.

Ignoffo, C. M. and Montoya, E. L. (1966) The effects of chemical insecticides and insecticidal adjuvants on a *Heliothis* nuclear polyhedrosis virus. *J. Invertebr. Pathol.* **8**, 409–12.

Ignoffo, C. M. and Shapiro, M. (1978) Characteristics of baculovirus preparations processed from living and dead larvae. J. *Econ. Entomol.* **71**, 186–8.

Ignoffo, C. M., Bradley, J. A. R., Gilliland, F. R. *et al.* (1972) Field studies on stability of the *Heliothis* nucleopolyhedrosis virus at various sites throughout the cotton belt. *Environ. Entomol.* **1**, 388–90.

Ignoffo, C. M., Yearian, W. C., Young, S. Y. *et al.* (1976a) Laboratory and field persistence of new commercial formulations of the *Heliothis* nucleopolyhedrosis virus, *Baculovirus heliothis*. *J. Econ. Entomol.* **69**, 233–6.

Ignoffo, C. M., Hostetter, D. L. and Smith, D. B. (1976b) Gustatory stimulant, sunlight protectant, evaporation retardant; three characteristics of a microbial insecticidal adjuvant. *J. Econ. Entomol.* **69**, 207–10.

Ignoffo, C. M., Hostetter D. L., Sikorowski, P. P. *et al.* (1977) Inactivation of representative species of entomopathogenic viruses, a bacterium, fungus

and protozoan by an ultraviolet light source. *Environ. Entomol.* **6**, 411–5.

Ignoffo, C. M., Garcia, C., Kroha, M. J. *et al.* (1981) Laboratory tests to evaluate the potential efficacy of *Bacillus thuringiensis* var. *israelensis* for use against mosquitoes. *Mosq. News* **41**, 85–93.

Ignoffo, C. M., Shasha, B. S. and Shapiro, M. (1991) Sunlight ultraviolet protection of the *Heliothis* nuclear polyhedrosis virus through starch-encapsulation technology. *J. Invertebr. Pathol.* **57**, 134–6.

Jacobs, S. J. (1989) Micro-organisms as potential biological control agents of *Eldana saccharina* Walker (Lepidoptera: Pyralidae), in *Proceedings of the 5th Annual Congress of the African Sugar Technologists' Association*, Vol. 63, ASTA, Natal, pp. 186–8.

Jankevica, J. and Zarins, I. (1997) Biological control of *Malacosoma neustria* L. population with Latvian isolate of nuclear polyhedrosis virus, in *Microbial Insecticides: Novelty or Necessity?* Symposium Proceedings No. 68 (ed. H. F. Evans), British Crop Protection Council, Farnham, pp. 285–8.

Jaques, R. P. (1971) Tests on protectants for foliar deposits of a polyhedrosis virus. *J. Invertebr. Pathol.* **17**, 9–16.

Jaques, R. P. (1972) The initiation of foliar deposits of viruses of *Trichoplusia ni* (Lepidoptera: Noctuidae) and *Pieris rapae* (Lepidoptera: Pieridae) and tests on protectant additives. *Can. Entomol.* **104**, 1985–94.

Jaques, R. P. (1977) Stability of insect pathogenic viruses. *Misc. Publ. Entomol. Soc. Am.* **10**, 99–116.

Johnson, D. L. and Henry, J. E. (1987) Low rates of insecticides and *Nosema locustae* (Microsporidia: Nosematidae) on baits applied to roadsides for grasshopper (Orthoptera: Acrididae) control. *J. Econ. Entomol.* **80**, 685–9.

Johnson, W. T. and Morris, O. N. (1981) Cold fog applications of pesticides for control of *Malacosoma disstria*. *J. Arboriculture* **7**, 246–51.

Jones, K. A. (1988a) Studies on the persistence of *Spodoptera littoralis* nuclear polyhedrosis virus on cotton in Egypt. PhD Thesis, University of Reading.

Jones, K. A. (1988b) The use of insect viruses for pest control in developing countries. *Asp. Appl. Biol.* **17**, 425–33.

Jones, K. A. (1990) Use of a nuclear polyhedrosis virus to control *Spodoptera littoralis* in Crete and Egypt, in *Pesticides and Alternatives* (ed. J. E. Casida), Elsevier, Amsterdam, pp. 131–42.

Jones, K. A. (1993) Simple techniques for the application of *Bacillus thuringiensis* in field crops suitable for developing countries, in *The Biopesticide* Bacillus thuringiensis *and its Applications in Developing Countries* (eds H. S. Salama, O. N. Morris and E. Rached), National Research Centre, Cairo/IDRC, Ottawa, pp. 43–51.

Jones, K. A. (1994) Use of baculoviruses for cotton pest control, in *Insect Pests of Cotton* (eds G. A. Matthews and J. P. Tunstall), CAB International, Wallingford, pp. 477–504.

Jones, K. A. and McKinley, D. J. (1987) The persistence of *Spodoptera littoralis* nuclear polyhedrosis virus on cotton in Egypt. *Asp. Appl. Biol.* **14**, 323–34.

Jones, K. A., Grzywacz, D., Irving, N. S. *et al.* (1991) Production and Application of Nuclear Polyhedrosis Virus to Control the Cotton Leafworm in Egypt: Final Project Report (Project T0061), Natural Resources Institute, Chatham (mimeo).

Jones, K. A., Moawad, G., McKinley, D. J. and Grzywacz, D. (1993a) The effect of natural sunlight on *Spodoptera littoralis* nuclear polyhedrosis virus. *Biocontrol Sci. Technol.* **3**, 189–97.

Jones, K. A., Westby, A., Reilly, P. J. A. and Jeger, M. J. (1993b) The exploitation of microorganisms in the developing countries of the tropics, in *Exploitation of Microorganisms* (ed. D. G. Jones), Chapman & Hall, London, pp. 343–70.

Jones. K. A., Irving, N. S., Grzywacz, D. *et al.* (1994) Application rate trials with a nuclear polyhedrosis virus to control *Spodoptera littoralis* (Boisd.) on cotton in Egypt. *Crop Prot.* **13**, 337–40.

Karch, S. and Hougard, J. M. (1986) Etude comparative au laboratoire du devenir et de materière active et des spores de *Bacillus sphaericus* souch 2362 et de *Bacillus thuringiensis* sérotype H-14 en milieu aqueux. *Cab. ORSTOM, Sér, Ent. Méd. et Parasitol.* **24**, 175–9.

Kase, L. E. and Branton, P. L. (1986) US Patent 4–631–857.

Katagiri, K. (1981) Pest control by cytoplasmic polyhedrosis viruses, in *Microbial Control of Pests and Plant Diseases 1970–1981* (ed. H. D. Burges), Academic Press, London, pp. 433–40.

Killick, H. J. (1986) UV protectants in viral control of insects in relation to spray droplet size, in *Fundamental and Applied Aspects of Invertebrate Pathology, IV International Colloquium on Invertebrate Pathology*, Wageningen (eds R. A. Samson, J. M. Vlak and D. Peters), Society for Invertebrate Pathology, pp. 620–3.

Killick, H. J. (1990) Influence of droplet size, solar ultraviolet light and protectants, and other factors on the efficacy of baculovirus sprays against *Panolis flammea* (Schiff.) (Lepidoptera: Noctuidae). *Crop Prot.* **9**, 21–8.

Knapp, W. J. and Cunningham, J. C. (1977) Aerial application of nuclear polyhedrosis virus against the red-headed pine sawfly, *Neodiprion lecontei* (Fitch). Canadian Forest Service Information Report IP–X–14.

Knepper, R. G., Wagner, S. A. and Walker, E. D. (1991) Aerially applied, liquid *Bacillus thuringiensis* var. *israelensis* (H-14) for control of spring *Aedes* mosquitoes in Michigan. *J. Amer. Mosq. Control Assoc.* **7**, 307–9.

Krieg, A. (1975) Photoprotection against inactivation of *Bacillus thuringiensis* spores by ultraviolet rays. *J. Invertebr. Pathol.* **25**, 267–8.

Krieg, A., Engler, S. and Rieger, M. (1980a) Produktion von Präparaten auf der Basis von *Bacillus thuringiensis var. israelensis mit UV-inaktivierten sporen zur biologischen Bekämpfung von Mückenlarven. Anz. Schadlingskde* **53**, *129–33.*

Krieg, A., Groner, A., Huber, J. and Matter, M. (1980b) Uber die Wirkung von mittel-und langwelligen ultravioletten Straheln (UV-B und UV-A) auf insektenpathogene Bakterien und Viren und deren Beeinflussung durch UV-Schutzstoffe. *Nachrichtenbl. Dtsch. Pflanzensch.* (*Braunschweig*) **32**, 100–6.

Krischik, V. A., Barbosa, A. P. and Reichelderfer, C. (1988) Three trophic level interactions: allelochemical, *Manduca sexta* (L.) and *Bacillus thuringiensis* var. *kurstaki* Berliner. *Environ. Entomol.* **17**, 476–82.

Lacey, L. A. (1985a) Effects of pH and storage temperature on spore viability and larvicidal activity of *Bacillus sphaericus. Bull. Soc. Vector Ecol.* **10**, 102–6.

Lacey, L. A. (1985b) *Bacillus thuringiensis* serotype H-14, in *Biological Control of Mosquitoes* (ed. H. C. Chapman), American Mosquito Control Association, Fresno, pp. 132–58.

Lacey, L. A. (1986) Development of operational formulations of *Bacillus thuringiensis* var. *israelensis* and *Bacillus sphaericus* for vector control, in *Fundamental and Applied Aspects of Invertebrate Pathology, Proceedings of the IV International Colloquium on Invertebrate Pathology*, Wageningen (eds R. A. Samson, J. M. Vlak and D. Peters), Society for Invertebrate Pathology, pp. 497–500.

Lacey, L. A. and Heitzman C. M. (1985) Efficacy of flowable concentrate formulations of *Bacillus* thuringiensis var. israelensis against black flies (Diptera: Simuliidae). *J. Amer. Mosq. Control Assoc.* **1**, 493–7.

Lacey, L. A. and Inman, A. (1985) Efficacy of granular formulations of Bacillus thuringiensis (H-14) for the control of Anopheles larvae in rice fields. *J. Amer. Mosq. Control Assoc.* **1**, 38–42.

Lacey, L. A. and Smittle, B. J. (1985) The effects of gamma radiation on spore viability and mosquito larvicidal activity of Bacillus sphaericus and Bacillus thuringiensis var. *israelensis. Bull. Soc. Vector Ecol.* **10**, 98–101.

Lacey, L. A. and Undeen, A. H. (1984) Effect of formulation, concentration, and application time on the efficacy of Bacillus thuringiensis (H-14) against black fly (Diptera: Simuliidae) larvae under natural conditions. *J. Econ. Entomol.* **77**, 412–8.

Lacey, L. A. and Undeen, A. H. (1986) Microbial control of blackflies and mosquitoes. *Ann. Rev. Entomol.* **11**, 265–95.

Lacey, L. A., Mulla, M. S. and Dulmage, H. T. (1978) Some factors affecting the pathogenicity of Bacillus thuringiensis Berliner against blackflies. *Environ. Entomol.* **7**, 583–8.

Lacey, L. A., Rose, D. H., Lacey, C. M. *et al.* (1988) Experimental formulations of Bacillus sphaericus for the control of anopheline and culicine larvae. *J. Ind. Microbiol.* **3**, 39–47.

Lacey, L. A., Urbina, M. J. and Heitzman, C. M. (1984) Sustained release formulations of Bacillus sphaericus and Bacillus thuringiensis (H-14) for control of container-breeding Culex quinquefasciatus. *Mosq. News* **44**, 26–32.

Lara-Reyna, J., Del Rincon-Castro, M. C. and Ibarra, J. E. (1996) Synergism between two nuclear polyhedrosis viruses: TnSNPV and AcMNPV, in Abstracts of the 29th Annual Meeting of the Society for Invertebrate Pathology, University of Córdoba, Spain, p. 47.

Lee, H. L., Gregorio, E. R., Khadri, M. S. and Seleena, P. (1996) Ultralow volume application of Bacillus thuringiensis s. sp. israelensis for control of mosquitoes. *J. Amer. Mosq. Control Assoc.* **12**, 651–5.

Levy, R. (1989) Insecticidal delivery compositions and methods for controlling a population of insects in an aquatic environment. US Patent 4 818 534.

Levy, R. (1997) Compositions and methods for reducing the amount of contaminants in aquatic and terrestial environments. US Patent 5 679 364.

Levy, R., Powell, C. M., Hertlein, B. C. and Miller, T. W. Jr (1984) Efficacy of Arosurf® MSF (monomolecular surface film) base formulations of Bacillus thuringiensis var. israelensis against mixed populations of larvae and pupae: bioassay and preliminary field evaluations. *Mosq. News* **44**, 537–43.

Levy, R., Nichols, M. A. and Miller, T. W. Jr (1992a) Culigel® superabsorbent polymer controlled-release and pest-management systems: technology review. *J. Florida Mosq. Control Assoc.* **63**, 87–8.

Levy, R., Nichols, M. A. and Miller, T. W. Jr (1992b) Culigel® controlled-release and pest-management systems, in Pesticide Formulations and Application Systems, Vol. 12, ASTM STP 1146 (eds B. N. Devisetty, D. G. Chasin and P. D. Berger), American Society for Testing and Materials, Philadelphia, pp. 214–32.

Levy, R., Nichols, M. A. and Miller, T. W. Jr. (1993a) Pesticide delivery from Culigel® superabsorbent polymers: mosquito and cockroach control studies, in Proceedings of the 1st International Conference on Insect Pests in the Urban Environment, pp. 153–61.

Levy, R., Nichols, M. A. and Miller, T. W. Jr. (1993b) Comparative performance of Culigel® superabsorbent polymer-based pesticide formulations, in Pesticide Formulations and Application Systems, Vol. 13, ASTM STP 1183 (eds P. D. Berger, B. N. Devisetty and F. R. Hall), American Society for Testing and Materials, Philadelphia, pp. 312–34.

Levy, R., Nichols, M. A. and Miller, T. W. Jr (1993c) Encapsulated systems for controlled release and pest management, in Polymeric Delivery Systems. Properties and Applications, ACS Symposium Series No. 520 (eds M. A. El-Nokaly, D. M. Piatt and B. A. Carpentier), American Chemical Society, Cellulose Paper and Textiles Division, Washington, DC, pp. 202–12.

Levy, R., Nichols, M. A. and Opp, W. R. (1996) Bioremediation with Gelgrade® systems, in Proceedings of the 23rd International Symposium on Controlled Release Bioactive Materials, Controlled Release Society, Kyoto, pp. 39–40.

Levy, R., Nichols, M. A. and Opp, W. R. (1997) Targeted delivery of pesticides from Matricap® compositions, in Pesticide Formulations and Application Systems. Vol 17, ASTM STP 1328, (eds G. R. Goss, M. J. Hopkinson and H. M. Collins), pp. 63–93, Am. Soc. Testing and Materials, West Conshohocken, PA.

Lewis, F. B. and Rollinson, W. D. (1978) Effect of storage on the virulence of gypsy moth nucleopolyhedrosis inclusion bodies. *J. Econ. Entomol.* **71**, 719–22.

Lewis, F. B. and Yendol, W. G. (1981) Gypsy moth polyhedrosis virus: efficacy, in The Gypsy Moth: Research Toward Integrated Pest Management USDA Technical Bulletin 1584 (eds C. C. Doane and M. L. McManus), US Department of Agriculture, Washington, DC, pp. 503–518.

Lewis, F. B., Dubois, N. R., Grimble, D. *et al.* (1974) Gypsy moth (Porthetria dispar: Lep. Lymantriidae): efficacy of aerially-applied Bacillus thuringiensis (Bacteria). *J. Econ. Entomol.* **67**, 351–4.

Li, R. S., Jarrett, P. and Burges, H. D. (1987) Importance of spores, crystals, and (-endotoxins in the pathogenicitiy of different varieties of Bacillus thuringiensis in Galleria mellonella and Pieris brassicae. *J. Invertebr. Pathol.* **50**, 277–84.

Lipa, J. J., Slizynski, K., Ziemnicka, J. and Bartowski, J. (1975) Interaction of Bacillus thuringiensis and nuclear polyhedrosis virus in Spodoptera exigua, in Environmental Quality and Safety, Supplementary Vol. III, Pesticides (eds F. Coulson and F. Korte), Georg Tieme, Stuttgart, pp. 668–71.

Lisansky, S. G., Quinlan, R. J. and Tassoni, G. (1993) The Bacillus thuringiensis Production Handbook, CPL Scientific, Newbury.

Liu, Y.-T., Sui, M.-J., Ji, D.-D. *et al.* (1993) Protection from ultraviolet irradiation by melanin of mosquitocidal activity of Bacillus thuringiensis var. israelensis. *J. Invertebr. Pathol.* **62**, 131–6.

Lockwood, J. A. and Debrey, L. D. (1990) Direct and indirect effects of a large-scale application of Nosema locustae (Microsporida: Nosematidae) on rangeland grasshoppers (Orthoptera: Acrididae). *J. Econ. Entomol.* **82**, 377–83.

Lord, J. C. (1991) Sustained release pellets for control of Culex larvae with Bacillus sphaericus. *J. Amer. Mosq. Control Assoc.* **7**, 560–4.

Ludlam, C. T., Felton, G. W. and Duffey, S. S. (1991) Plant defenses: chlorogenic acid and polyphenol oxidase enhance toxicity of Bacillus thuringiensis subsp. kurstaki to Heliothis zea. *J. Chem. Ecol.* **17**, 217–37.

Lüthy, P., Hofmann, C. and Jacquet, F. (1985) Inactivation of (-endotoxin of Bacillus thuringiensis by tannin. *FEMS Microbiol. Letts* **28**, 31–3.

Luttrell, R. G., Young, S. Y., Yearian, W. C. and Horton, D. L. (1982) Evaluation of Bacillus thuringiensis–spray adjuvant–viral insecticide

combinations against Heliothis spp. (Lepidoptera: Noctuidae). *Environ. Entomol.* **11**, 783–7.

Luttrell, R. G., Yearian, W. C. and Young, S. Y. (1983) Effect of spray adjuvants on Heliothis zea (Lepidoptera: Noctuidae) nuclear polyhedrosis virus efficacy. *J. Econ. Entomol.* **76**, 162–7.

Lynch, R. E., Lewis, L. C. and Berry, E. C. (1980) Application efficacy and field persistence of Bacillus thuringiensis when applied to corn for European corn borer control. *J. Econ. Entomol.* **73**, 4–7.

Magnoler, A. (1974) Field dissemination of nucleopolyhedrosis virus against the gypsy moth, Lymantria dispar *L. Z. Pflanzenbr.* **9**, 497–511.

Maksymiuk, B. and Neisess, J. (1975) Physical properties of Bacillus thuringiensis spray formulations. *J. Econ. Entomol.* **68**, 407–10.

Manasherob, R., BenDor, E., Margalit, J., *et al.* (1996) Raising activity of Bacillus thuringiensis var israelensis against Anopheles stephensi larvae by encapsulation in Tetrahymena pyriformis (Hymenostomatida: Tetrahymenidae) *J. Amer. Mosq. Control Assoc.* **12**, 627–31.

Manjunath, D. and Mathad, S. B. (1982) Effect of sunlight on the infectivity of purified and non-purified NPV of the armyworm Mythimna separata Walker. *Ind. J. Agric. Sci.* **51**, 750–6.

Margalit, J., Markus, A. and Pelah, Z. (1984) Effect of encapsulation on the persistence of Bacillus thuringiensis israelensis, serotype H14, a potential mosquito larvicide. *Appl. Microbiol. Biotechnol.* **19**, 382–3.

Marrone, P. G. and MacIntosh, S. C. (1993) Resistance to Bacillus thuringiensis and resistance management, in Bacillus thuringiensis, an Environmental Biopesticide: Theory and Practice (eds P. F. Entwistle, J. S. Cory, M. J. Bailey and S. Higgs), Wiley, Chichester, pp. 221–35.

Martignoni, M. E. (1978) Virus in biological control: production, activity, and safety, in The Douglas Fir Tussock Moth: a Synthesis (eds M. H. Brookes, R. W. Stark and R. W. Campbell), US Department of Agriculture, Washington, DC, pp. 140–7.

Martignoni, M. E. and Iwai, P. J. (1985) Laboratory evaluation of new ultraviolet absorbers for protection of Douglas-fir Tussock moth (Lepidoptera: Lymantriidae) Baculovirus. *J. Econ. Entomol.* **78**, 982–7.

MacIntosh, S. C., Kishore, G. M., Perlack, F. J. *et al.* (1990) Potentiation of Bacillus thuringiensis insecticidal activity by serine protease inhibitors. *J. Agric. Food Chem.* **38**, 1145–52.

McGaughey, W. H. (1975) A granulosis virus for Indian meal moth control in stored wheat and corn. *J. Econ. Entomol.* **68**, 346–9.

McGaughey, W. H. (1985) Evaluation of Bacillus thuringiensis for controlling Indian meal moths (Lepidoptera: Pyralidae) in farm grain bins and elevator silos. *J. Econ. Entomol.* **78**, 1089–94.

McGuire, M. R. and Shasha, B. S. (1990) Sprayable self-encapsulating starch formulations for Bacillus thuringiensis. *J. Econ. Entomol.* **83**, 1813–6.

McGuire, M. R. and Shasha, B. S. (1992) Adherent starch granules for encapsulation of insect control agents. *J. Econ. Entomol.* **85**, 1425–33.

McGuire, M. R. and Shasha, B. S. (1995) Starch encapsulation of microbial pesticides, in Biorational Pest Control Agents: Formulation and Delivery, ACS Symposium Series No. 595 (eds F. R. Hall and J. W. Barry), American Chemical Society, Washington, DC, pp. 230–7.

McGuire, M. R., Shasha, B. S., Lewis, L. C. *et al.* (1990) Field evaluation of granular starch formulations of Bacillus thuringiensis against Ostrinia nubilalis (Lepidoptera: Pyralidae). *J. Econ. Entomol.* **83**, 2207–10.

McGuire, M. R., Streett, D. A. and Shasha, B. S. (1991) Evaluation of starch encapsulation for formulation of grasshopper (Orthoptera: Acrididae) entompox viruses. *J. Econ. Entomol.* **84**, 1652–6.

McGuire, M. R., Gillespie, R. L. and Shasha, B. S. (1994a) Survival of Ostrinia nubilalis (Hübner) after exposure to Bacillus thuringiensis Berliner encapsulated in flour matrices. *J. Econ. Entomol.* **29**, 496–508.

McGuire, M. R., Shasha, B. S., Lewis, L. C. and Nelsen, T. C. (1994b). Residual activity of granular starch-encapsulated Bacillus thuringiensis. *J. Econ. Entomol.* **87**, 631–7.

McGuire, M. R., Shasha, B. S., Eastman, C. E. and Oloumi-Sadeghi, H. (1996) Starch-and flour-based sprayable formulations – effect on rainfastness and solar stability of Bacillus thuringiensis. *J. Econ. Entomol.* **89**, 863–9.

McKinley, D. J. (1985) Nuclear polyhedrosis virus of Spodoptera littoralis Boisd. (Lepidoptera: Noctuidae) as an infective agent in its host and other related insects. PhD thesis, University of London.

McKinley, D. J., Moawad, G., Jones, K. A. *et al.* (1989) The development of nuclear polyhedrosis virus for the control of Spodoptera littoralis (Boisd.) in cotton, in Pest Management in Cotton

(eds M. B. Green and D. V. Lyon), Ellis Horwood, Chichester, pp. 93–100.

McLane, W. (1991) Spray stickers, in Aerial Spraying for Gypsy Moth Control: a Handbook of Technology (ed. R. Reardon), USDA Forest Service, Morgantown, pp. 92–6.

McLaughlin, R. E. and Billodeaux, J. (1983) Effectiveness of Bacillus thuringiensis var. israelensis against Psorophora columbiae breeding in rice fields. *Mosq. News* **43**, 30–3.

McLaughlin, R. E. and Vidrine, M. F. (1984) Point source introduction system for control of Psorophora columbiae (Diptera: Culicidae) by Bacillus thuringiensis serotype H-14: results of a large scale operational test. *Environ. Entomol.* **13**, 366–70.

McLaughlin, R. E., Andrews, G. and Bell, M. R. (1971) Field tests for control of Heliothis spp. with a nuclear polyhedrosis virus included in a boll weevil bait. *J. Invertebr. Pathol.* **18**, 304–5.

McVay, J. A. R., Gudauskas, R. T. and Harper, J. D. (1977) Effects of Bacillus thuringiensis–nuclear polyhedrosis virus mixtures on Trichoplusia ni larvae. *J. Invertebr. Pathol.* **29**, 367–72.

Meneley, J. C. and Sluss, T. P. (1988) Development of 'Nolo Bait' (Nosema locustae) for the control of grasshoppers and locusts, in Proceedings of the Brighton Crop Protection Conference – Pest and Diseases, Vol. 3, British Crop Protection Council, Farnham, pp. 597–602.

Molloy, D. P. and Struble, R. H. (1989) Investigation of the feasibility of the microbial control of blackflies (Diptera: Simuliidae) with Bacillus thuringiensis var. israelensis in the Adirondack Mountains of New York. *Bull. Soc. Vector Ecol.* **14**, 266–76.

Molloy, D., Gaugler, R. and Jamback, H. (1981) Factors influencing efficacy of Bacillus thuringiensis var. israelensis as a biological control agent of black fly larvae. *J. Econ. Entomol.* **74**, 61–4.

Molloy, D., Wraight, S. P., Kaplan, B. *et al.* (1984) Laboratory evaluation of commercial formulations of Bacillus thuringiensis var. israelensis against mosquito and blackfly larvae. *J. Agric. Entomol.* **1**, 161–8.

Monnet, S., Gazon, M., Deramoudt, F.-X. *et al.* (1994) Production of polyhedral inclusion body in a high density insect cell bioreactor, in Proceedings of the VIth International Colloquium on Microbial Control, Montpellier, Society for Invertebrate Pathology, pp. 447–51.

Montoya, E. L., Ignoffo, C. M. and McGarr, R. L. (1966) A feeding stimulant to increase effectiveness of, and a field test with, a nuclear polyhedrosis virus of Heliothis. *J. Invertebr. Pathol.* **8**, 320–4.

Morales Ramos, L. H., McGuire, M. R. and Galán Wong, L. J. (1998) Utilization of several biopolymers for granular formulation of *Bacillus thuringiensis J. Econ. Entomol.* (in press).

Morris, O. N. (1969) Susceptibility of several forest insects of British Columbia to commercially produced Bacillus thuringiensis. *J. Invertebr. Pathol.* **13**, 134–46.

Morris, O. N. (1972) Inhibitory effects of foliage extracts of some forest trees on commercial Bacillus thuringiensis. *Can. Entomol.* **104**, 1357–61.

Morris, O. N. (1975) Effect of some chemical insecticides on the germination and replication of commercial Bacillus thuringiensis. *J. Invertebr. Pathol.* **26**, 199–204.

Morris, O. N. (1977a) Long term study of the effectiveness of aerial application of Bacillus thuringiensis–acephate combinations against the spruce budworm, Choristoneura fumiferana (Lepidoptera: Tortricidae). *Can. Entomol.* **109**, 1239–48.

Morris, O. N. (1977b). Relationship between microbial numbers and droplet size in aerial spray applications. *Can. Entomol.* **109**, 1319–23.

Morris, O. N. (1983) Protection of Bacillus thuringiensis from inactivation by sunlight. *Can. Entomol.* **115**, 1215–27.

Morris, O. N. (1985) Recent field experiences with Bacillus thuringiensis in Canada and research needs, in Microbial Control of Spruce Budworms and Gypsy Moths (eds D. G. Grimble and F. B. Lewis), CANUSA Forest Service, US Department of Agriculture, Broomall, pp. 39–46.

Morris, O. N. and Moore, A. (1975) Studies on the protection of insect pathogens from sunlight inactivation. II. Preliminary field trials. Canadian Forest Service, Report CC–X–113.

Morris, O. N., Trottier, M., McLaughlin, N. B. and Converse, V. (1994) Interaction of caffeine and related compounds with Bacillus thuringiensis ssp. kurstaki in Bertha armyworm (Lepidoptera: Noctuidae). *J. Econ. Entomol.* **87**, 610–7.

Morris, O. N., Converse, V. and Kanagaratnam, P. (1995) Chemical additive effects on the efficacy of Bacillus thuringiensis Berliner subsp. kurstaki against Mamestra configurata (Lepidoptera: Noctuidae). *J. Econ. Entomol.* **88**, 815–24.

Moscardi, F. (1989) Use of viruses for pest control in Brazil: the case of the nuclear polyhedrosis

virus of the soybean caterpillar, Anticarsia gemmatalis. Mem. Inst. Oswaldo Cruz, Rio de Janeiro **84** (suppl. III), 51–6.

Most, B. H. and Quinlan, R. J. (1986) Formulation of biological pesticides, in Fundamental and Applied Aspects of Invertebrate Pathology, Proceedings of the IV International Colloquium on Invertebrate Pathology, Wageningen (eds R. A. Samson, J. M. Vlak and D. Peters), Society for Invertebrate Pathology, pp. 624–7.

Mulligan, F. S. III, Schaefer, C. H. and Wilder, W. H. (1980) Efficacy and persistence of Bacillus sphaericus and Bacillus thuringiensis H-14 against mosquitoes under laboratory and field conditions. *J. Econ. Entomol.* **73**, 684–8.

Nasci, R. S., Wright, G. B. and Willis, F. S. (1994) Control of Aedes albopictus larvae using time-release larvicide formulations in Louisiana. *J. Amer. Mosq. Control Assoc.* **10**, 1–6.

Navon, A. (1992) Interactions among herbivores, microbial insecticides and crop plants. Phytoparasitica **20**, 21–5.

Navon, A. (1993) Control of lepidopteran pests with Bacillus thuringiensis, in Bacillus thuringiensis, an Environmental Biopesticide: Theory and Practice (eds P. F. Entwistle, J. S. Cory, M. J. Bailey and S. Higgs), Wiley, Chichester, pp. 125–46.

Neisess, J. (1979) Evaluation of Sticking Agents mixed with Bacillus thuringiensis for Control of Douglas Fir Tussock Moth, Research Paper PNW-254, Pacific Northwest Forest Range Experimental Station, pp. 1–6.

Nichols, J. O. (1985) Pennsylvania's experiences with microbial control of the gypsy moth, in Microbial Control of Spruce Budworms and Gypsy Moths, CANUSA Symposium, USDA Forest Service, Broomall, pp. 29–30.

Nugud, A. D. and White, G. B. (1982) Bacillus thuringiensis serotype H-14; efficiency against Anopheles mosquito larvae and formulation studies, in Invertebrate Pathology and Microbial Control, Abstracts of the III International Colloquium on Invertebrate Pathology, University of Sussex, Brighton (eds C. C. Payne and H. D. Burges), Society for Invertebrate Pathology, p. 245.

Parnell, M., King, W., Ketunuti, V. and Wetchakit, D. (1996) The evaluation of very low volume application of water based formulations of nuclear polyhedrosis virus on cotton in Thailand, in Abstracts of the 29th Annual Meeting of the Society for Invertebrate Pathology, University of Córdoba, Spain, p. 60.

Patel, K. R., Wyman, J. A., Patel, K. A. and Burden, B. J. (1996) A mutant of Bacillus thuringiensis producing a dark brown pigment with increased UV resistance and insecticidal activity. *J. Invertebr. Pathol.* **67**, 120–4.

Pawar, V. M., Thombre, U. T. and Chaudhari, D. G. (1992) Effectiveness of baculoviruses as influenced by different additives, in Adjuvants for Agrochemicals (ed. C. L. Foy), CRC Press, Boca Raton, pp. 682–88.

Payne, N. J. and van Frankenhuyzen, K. (1995) Effect of spray droplet size and density on efficacy of Bacillus thuringiensis Berliner against the spruce budworm, Choristoneura fumiferana (Clem.) (Lepidoptera: Tortricidae). *Can. Entomol.* **127**, 15–23.

Pfrimmer, T. R. (1979) Heliothis spp: control on cotton with pyrethroids, carbamates, organophosphates, and biological insecticides. *J. Econ. Entomol.* **72**, 593–8.

Pinnock, D. E., Milstead, J. E., Kirby M. E. and Nelson, B. J. (1977) Stability of Entomopathogenic Bacteria, Miscellaneous Publication No. 10, Entomological Society of America, pp. 77–97.

Podgwaite, J. D. and Shapiro, M. (1986) Evaluation of sunlight protectants for gypsy moth, Lymantria dispar L., nucleopolyhedrosis virus, in Fundamental and Applied Aspects of Invertebrate Pathology, Proceedings of the IV International Colloquium on Invertebrate Pathology, Wageningen (eds R. A. Samson, J. M. Vlak and D. Peters), Society for Invertebrate Pathology, p. 154.

Podgwaite, J. D., Bruen, R. B. and Shapiro, M. (1983) Microorganisms associated with production lots of the nuclear polyhedrosis virus of the Gypsy moth Lymantria dispar. Entomophaga **28**, 9–16.

Pozgay, M., Fast, P., Kaplan, H. and Carey, P. R. (1987) The effect of sunlight on the protein crystals from Bacillus thuringiensis var. kurstaki HD1 and NRD12: a Rahman spectroscopic study. *J Invertebr. Pathol.* **50**, 246–53.

Pristavko, V. P. and Dovzhenok, N. V. (1974) Ascorbic acid influence on larval blood cell number and susceptibility to bacterial and fungal infection in the codling moth, Laspeyresia pomonella (Lepidoptera, Torticidae). *J. Invertebr. Pathol.* **24**, 165–8.

Pusztai, M., Fast, P., Gringorten, L. *et al.* (1991) The mechanism of sunlight inactivation of Bacillus thuringiensis crystals. *Biochem J.* **273**, 43–7.

Ragoonanansingh, R. N., Njunwa, K. J., Curtis, C. F. and Becker, N. (1992) A field study of Bacillus

sphaericus for the control of culicine and anopheline mosquito larvae in Tanzania. *Bull. Soc. Vector Ecol.* **17**, 45–50.

Raun, E. S. and Jackson, R. D. (1966) Encapsulation as a technique for formulating microbial and chemical insecticides. *J. Econ. Entomol.* **59**, 620–2.

Reardon, R. (1991) Aerial Spraying for Gypsy Moth Control: A Handbook of Technology, NA-TP-20, USDA Forest Service, Morgantown.

Reardon, R. C. and Podgwaite, J. D. (1994) Summary of efficacy evaluations using aerially applied Gypcheck® against gypsy moth in the USA. Environ. Sci. Health **B29**, 739–56.

Reardon, R., Podgwaite, J. D. and Cunningham J. C. (1992) Advances in application methods, formulation and use-strategies for gypsy moth virus, in Abstracts of the 25th Annual Meeting of the Society for Invertebrate Pathology, Heidelberg, p. 286.

Reardon, R. C., Podgwaite, J. D. and Zerillo, R. (1996) Gypcheck – the Nucleopolyhedrosis Virus Product, FHTET-96–16, USDA Forest Service, Northeastern Area, Morgantown.

Reiter, P. and McMullen, A. I. (1978). The action of lecithin monolayers on mosquitoes. I. General observations. *Ann. Trop. Med. Parasitol.* **72**, 163–8.

Renou, A. (1987) Achievements in the biological control of Heliothis armigera (Hbn.), a pest of the cotton crop in North Cameroon. Med. Facult. Landb. Rijk. Gent **52**, 311–8.

Richards, M. G. (1984) The use of a granulosis virus for control of codling moth, Cydia pomonella; application methods and field persistence, PhD thesis, University of London.

Richards, M. and Payne, C. C. (1982) Persistence of baculoviruses on leaf surfaces, in Invertebrate Pathology and Microbial Control, Proceedings of the III International Colloquium on Invertebrate Pathology, University of Sussex, Brighton (eds C. C. Payne and H. D. Burges), Society for Invertebrate Pathology, pp. 296–301.

Richter, A. R., Fuxa, J. R. and Abdel-Fattah, M. (1987) Effect of host plant on the susceptibility of Spodoptera frugiperda (Lepidoptera: Noctuidae) to a nuclear polyhedrosis virus. *Environ. Ent.* **16**, 1004–6.

Riethmüller, V. (1990) Labor-und Freilandutersuchungen über den Einsatz von Bacillus thuringiensis subspecies tenebrionis gegen Coleopteren unter besonderer Berücksichtigung der Larven des Kartoffelkäfers (Leptinotarsa decemlineata Say), Dissertation, Biological Division, College of Darmstadt.

Rigby, S. (1991) Bacillus thuringiensis in Crop Protection, PJB Publications, Richmond, Surrey.

Roberts, G. M. (1989a) The combination of Bacillus thuringiensis var. israelensis and surface active monolayers for mosquito control, PhD thesis, University of Southampton.

Roberts, G. M. (1989b). The combination of Bacillus thuringiensis var. israelensis with a monomolecular film. *Israel J. Entomol.* **23**, 95–7.

Roberts, G. M. (1991) Spreading of Bacillus thuringiensis var. israelensis over the water surface by a monomolecular carrier. *World J. Microbiol. Biotech.* **7**, 335–42.

Roberts, G. M. and Burges, H. D. (1984) Combination of Bacillus thuringiensis var. israelensis and a surface active monolayer for mosquito control, in Proceedings of the Brighton Crop Protection Conference – Pests and Diseases, Vol. 1, British Crop Protection Council, Farnham, pp. 287–92.

Roome, R. E. (1975) Field trials with a nuclear polyhedrosis virus and Bacillus thuringiensis against larvae of Heliothis armigera (Hb.) (Lepidoptera, Noctuidae) on sorghum and cotton in Botswana. *Bull. Entomol. Res.* **65**, 507–14.

Rose, R. I. (1989) Enhancement of Bacillus thuringiensis and B. sphaericus persistence with slow-release formulations. *IOBC/WRPS Bull.* **12**(4), 77.

Salama, H. S., Foda, M. S. and Sharaby, A. (1985) Potential of some chemicals to increase the effectiveness of Bacillus thuringiensis Berl. against Spodoptera littoralis Boisd. *Angew. Entomol.* **100**, 425–33.

Salama, H. S., Foda, M. S. and Sharaby, A. (1986) Possible extension of the activity spectrum of Bacillus thuringiensis strains through chemical additives. *J. Appl. Entomol.* **101**, 304–13.

Salama, H. S., Foda, M. S. and Sharaby, A. (1989) Potentiation of Bacillus thuringiensis endotoxin against the greasy cutworm Agrotis ypsilon. *J. Appl. Entomol.* **108**, 372–80.

Salama, H. S., Saleh, M. R., Moawad, S. and Shams-El-Din, A. (1990a) Spray and dust applications of Bacillus thuringiensis Berliner and lannate against Spodoptera littoralis (Boisd.) (Lep., Noctuidae) on soybean plants in Egypt. *J. Appl. Entomol.* **109**, 194–9.

Salama, H. S., Salem, S., Zaki, F. N. and Matter, M. (1990b). Control of Agrotis ypsilon (Hufn) (Lep., Noctuidae) on some vegetable crops in Egypt using the microbial agent Bacillus thuringiensis. *Anz. Schad. Pflan. Umw.* **63**, 147–51.

Salama, H. S., Moawad, S., Saleh, R. and Ragaei, M. (1990c) Field tests on the efficacy of baits based

on Bacillus thuringiensis and chemical insecticides against the greasy cutworm Agrotis ipsilon in Egypt. *Anz. Schad. Pflan. Umw.* **63**, 33–6.

Santiago-Alvarez, C. and Ortiz-Garcia, R. (1992) The influence of host plant on the susceptibility of Spodoptera littoralis (Boisd.) (Lep., Noctuidae) larvae to Spodoptera littoralis NPV (Baculoviridae, Baculovirus). *J. Appl. Entomol.* **114**, 124–30.

Schnell, D. J., Pfannenstiel, M. A. and Nickerson, K. W. (1984) Bioassay of solubilized Bacillus thuringiensis var. israelensis crystals by attachment to latex beads. Science **223**, 1191–3.

Shapiro, M. (1982) In vivo mass production of insect viruses for use as pesticides, in Microbial and Viral Pesticides (ed. E. Kurstak), Dekker, New York, pp. 463–92.

Shapiro, M. (1984) Host tissues and metabolic products as ultraviolet screens for the gypsy moth (Lepidoptera: Lymantriidae) nucleopolyhedrosis virus. *Environ. Entomol.* **13**, 1131–4.

Shapiro, M. (1985) Effectiveness of B vitamins as UV screens for the gypsy moth (Lepidoptera: Lymantriidae) nucleopolyhedrosis virus. *Environ. Entomol.* **14**, 705–8.

Shapiro, M. (1986). In vivo production of baculoviruses, in Biology of Baculoviruses, Vol. II, Practical Application for Insect Control (eds R. R. Granados and B. A. Federici), CRC Press, Boca Raton, pp. 31–61.

Shapiro, M. (1989) Congo Red as ultraviolet protectant for the gypsy moth (Lepidoptera: Lymantriidae) nuclear polyhedrosis virus. *J. Econ. Entomol.* **82**, 548–50.

Shapiro, M. (1992) Use of optical brighteners as radiation protectants for gypsy moth (Lepidoptera: Lymantridae) nuclear polyhedrosis virus. *J. Econ. Entomol.* **85**, 1682–6.

Shapiro, M. and Argauer, R. (1995) Effects of pH, temperature, and ultraviolet radiation on the activity of an optical brightener as a viral enhancer for the gypsy moth (Lepidoptera: Lymantriidae) baculovirus. *J. Econ. Entomol.* **88**, 1602–6.

Shapiro, M. and Robertson, J. L. (1992) Enhancement of gypsy moth (Lepidoptera: Lymantriidae) baculovirus activity by optical brighteners. *J. Econ. Entomol.* **85**, 1120–4.

Shapiro, M., Agin, P. P. and Bell, R. A. (1983) Ultraviolet protectants of the Gypsy moth (Lepidoptera: Lymantriidae) nucleopolyhedrosis virus. *Environ. Entomol.* **12**, 982–5.

Shapiro, M., Dougherty, E. M. and Hamm, J. J. (1992) Compositions and methods for biocontrol using fluorescent brighteners. US Patent 5 124 149.

Shasha, B. S. and McGuire, M. R. (1992) Adherent starch granules. US Patent SN07/913 565.

Shasha, B. S., McGuire, M. R. and Behle, R. W. (1995) Lignin-based pest control formulations. US Patent Application SN08 568 159.

Sheppard, C. A. and Shapiro, M. (1994) Physiological and nutritional effects of a fluorescent brightener on nuclear polyhedrosis virus-infected Lymantria dispar (L.) larvae (Lepidoptera: Lymantriidae). Biol. Control **4**, 404–11.

Sheppard, C. A., Shapiro, M. and Vaughn, J. L. (1994) Reduction of mid-gut luminal pH in gypsy moth larvae (Lymantria dispar L.) following ingestion or nuclear or cytoplasmic polyhedrosis virus/fluorescent brightener on natural and artificial diets. *Biol. Control.* **4**, 412–20.

Shieh, T. R. (1989) Industrial production of viral pesticides. *Adv. Vir. Res.* **36**, 315–43.

Sivamani, E., Rajendran, N., Senrayan, R. *et al.* (1992) Influence of some plant phenolics on the activity of (-endotoxin of Bacillus thuringiensis var. galleriae on Heliothis armigera. *Entomol. Exp. Appl.* **63**, 243–8.

Sjogren, R. D. (1996) Insecticidal composite timed released particle. US Patent 5 484 600.

Skovmand, O. and Bauduin, S. (1998) Efficacy of a granular formulation of Bacillus sphaericus against Culex quinquefasciatus and Anopheles gambiae in West African countries. J. Vector Ecol. (in press).

Skovmand, O. and Eriksen, A. G. (1993) Field trials of a fizzy tablet with Bacillus thuringiensis var. israelensis in forest spring ponds in Denmark. *Bull. Soc. Vector Ecol.* **18**, 160–3.

Small, D. A. (1985) Aspects of the attachment of a nuclear polyhedrosis virus of the cabbage looper (Trichoplusia ni) to the leaf surface of cabbage (Brassica oleracea), PhD thesis, University of Oxford.

Small, D. A. and Moore, N. F. (1987) Measurement of surface charge of baculovirus polyhedra. *Appl. Environ. Microbiol.* **53**, 598–602.

Small, D. A., Moore, N. F. and Entwistle, P. F. (1986) Hydrophobic interaction involved in attachment of a baculovirus to hydrophobic surfaces. *Appl. Environ. Entomol.* **52**, 220–3.

Smirnoff, W. A. (1977) Experimental confirmation of the potential of a Bacillus thuringiensis and chitinase complex for repression of the Tortrix of spruce buds, Choristoneura fumiferana (Lepidoptera: Tortricidae). *Can. Entomol.* **109**, 351–8.

Smirnoff, W. A., Fettes, J. J. and Haliburton, W. (1962) A virus disease of Swain's Jack pine sawfly, Neodiprion swainei Midd. sprayed from an aircraft. *Can. Entomol.* **94**, 477–86.

Smith, D. B., Hostetter, D. L. and Ignoffo, C. M. (1977a) Laboratory performance specifications for a bacterial (Bacillus thuringiensis) and a viral (Baculovirus heliothis) insecticide. *J. Econ. Entomol.* **70**, 437–41.

Smith, D. B., Hostetter, D. L. and Ignoffo, C. M. (1977b) Ground spray equipment for applying Bacillus thuringiensis suspension on soybeans. *J. Econ. Entomol.* **70**, 633–7.

Smith, D. B., Hostetter, D. L. and Ignoffo, C. M. (1978a) Formulation effects on application of a viral insecticide (Baculovirus heliothis). *J. Econ. Entomol.* **71**, 814–7.

Smith, D. B, Hostetter, D. L. and Ignoffo, C. M. (1978b) Nozzle size–pressure and concentration combinations for Heliothis zea control with an aqueous suspension of polyvinyl alcohol and Baculovirus heliothis. *J. Econ. Entomol.* **72**, 920–3.

Smith, D. B., Hostetter, D. L. and Pinnell, R. E. (1980) Laboratory formulation comparisons for bacterial (Bacillus thuringiensis) and a viral (Baculovirus heliothis) insecticide. *J. Econ. Entomol.* **73**, 18–21.

Smith, D. B., Hostetter, D. L., Pinnell, R. E. and Ignoffo, C. M. (1982) Laboratory studies of viral adjuvants: formulation development. *J. Econ. Entomol.* **75**, 16–20.

Soares, G. G. Jr, Lewis, W. J., Strong-Gunderson, J. M. *et al.* (1993) Integrating the use of MVP® bioinsecticide, a unique Bt-based product, with natural enemies of noctuid pests: a novel concept in cotton IPM, in Proceedings of the 2nd Canberra Bacillus thuringiensis Meeting (ed. R. J. Akhurst), CSIRO, Canberra, pp. 133–45.

Stacey, A. L., Yearian, W. C. and Young, III, S. Y. (1977) Evaluation of Baculovirus heliothis with feeding stimulants for control of heliothis larvae on cotton. *J. Econ. Entomol.* **70**, 779–84.

Stacey, A. L., Luttell, R., Yearian, W. C. *et al.* (1980) Field evaluation of Baculovirus heliothis on cotton using selected application methods. *J. Ga. Entomol. Soc.* **15**, 365–72.

Stelzer, M. J., Neisess, J., Cunningham, J. C. and McPhee, J. R. (1977) Field evaluation of baculovirus stocks against Douglas-fir tussock moth in British Columbia. *J. Econ. Entomol.* **70**, 243–6.

Sundara-Babu, P. C. and Krishnan, A. (1970) Preliminary studies on the effect of Thuricide® for control of the semi-looper, Achaea janata, on the castor bean plant, Ricinus communis. *J. Invertebr. Pathol.* **15**, 129–30.

Sundaram, A., Leung, J. W. and Devisetty, B. N. (1993) Rain-fastness of Bacillus thuringiensis deposits on conifer foliage, in Pesticide Formulations and Application Systems, Vol. 13 (eds P. D. Berger, B. N. Devisetty and F. R. Hall), American Society for Testing and Materials, Philadelphia, pp. 227–41.

Sutherland, D. J. (1990) The future of bacterial control of mosquito and blackfly larvae, in Bacterial Control of Mosquitoes and Blackflies (eds H. de Barjac and D. J. Sutherland), Rutgers University Press, New Brunswick, pp. 335–42.

Tardif, R. and Secrest, J. P. (1970) Devices for cleaning and counting eggs of the gypsy moth. *J. Econ. Entomol.* **63**, 678–9.

Tamez-Guerra, P. McGuire, M. R., Medrano-Roldan, H. *et al.* (1996) Sprayable granule formulations for Bacillus thuringiensis. *J. Econ. Entomol.* **89**, 1424–30.

Tanada, Y. and Hukuhara, T. (1971) Enhanced infection of a nuclear polyhedrosis virus in the larvae of the armyworm Pseudoletia unipuncta by a factor in the capsule of a granulosis virus. *J. Invertebr. Pathol.* **17**, 116–26.

Tapp, H. and Stotzky, G. (1995) Insecticidal activity of the toxins from Bacillus thuringiensis subspecies kurstaki and tenebrionis adsorbed and bound on pure and clay soils. *Appl. Environ. Microbiol.* **61**, 1786–90.

Tatchell, G. M. (1981) The transmission of granulosis virus following contamination of Pieris brassicae adults. *J. Invertbr. Pathol.* **37**, 210–3.

Taylor, R. A. J., Chapple, A. C. and Hall, F. P. (1993) A model simulating the dose transfer of Bacillus thuringiensis (Bt) to third instar diamondback moth (Plutella xylostella) feeding on cabbage, in Pesticide Formulations and Applications Systems: Vol. 13, ASTM STP 1183 (eds P. D. Berger, B. N. Devisetty and F. R. Hall), American Society for for Testing and Materials, Philadelphia.

Tester, C. F. (1992) Influence of a genetically modified endophytic bacterium on composition and decomposition of corn residue – effect of Clavibacter xyli subsp. cynodontis biological control agents expressing the Bacillus thuringiensis crystal protein gene on maize residue degradation. *Soil Biol. Biochem.* **24**, 1107–12.

Tiong, R. H. C. and Munroe, D. D. (1976) Microbial control of an outbreak of Darna trima (Moore) on oil palm (Elaeis guineensis Jaq) in Sarawak (Malaysian Borneo), in Proceedings of the

Malaysian International Agricultural Oil Palm Conference, paper 41.

Timans, U. (1982) Zur wirkung von UV-strahlen auf das kernpolyhedervirus des schwammspiners, Lymantria dispar L. (Lep. Lymantriidae). *Zeit. Angewandte Entomol.* **94**, 382–401.

Topper, C. P., Moawad, G. and McKinley, D. (1984) Field trials with a nuclear polyhedrosis virus against Spodoptera littoralis on cotton in Egypt. *Trop. Pest Manag.* **30**, 372–8.

Trought, K. (1989) Trends in Pesticide Formulation, PJB Publications, Richmond.

Turner, C. L., Bishop, A. H., Ryder, J. L. *et al.* (1994) Novel applications of Bacillus thuringiensis as a method for controlling blowfly (Calliphoridae sp.) and beetle (Dermestes sp.) infestations of post-harvest fisheries products, in Abstracts of the VI International Colloquium on Invertebrate Pathology and Microbial Control, Montpellier, Society for Invertebrate Pathology, p. 351.

Uchida, Y., Kawamoto, F., Himeno, M. and Hayashiya, K. (1984) A virus inactivating protein isolated from the digestive juice of the silkworm, Bombyx mori. *J. Invertbr. Pathol.* **43**, 182–9.

Vail, P. V., Henneberry, T. J. and Bell, M. R. (1977) Cotton leaf perforator: effect of a nuclear polyhedrosis virus on field populations. *J. Econ. Entomol.* **70**, 727–8.

Vail, P. V., Seay, R. E. and De Bolt, J. (1980) Microbial and chemical control of the cabbage looper on fall lettuce. *J. Econ. Entomol.* **73**, 72–5.

Vail, P. V., Hoffmann, D. F. and Tebbets, J. S. (1996) Effects of a fluorescent brightener on the activity of Anagrapha falcifera (Lepidoptera, Noctuidae) nuclear polyhedrosis virus to four noctuid pests. Biol Control **7**, 121–5.

Valero, J. R. (1989) Three Years of Experimental Aerial Application of Bacillus thuringiensis at 2. 01/ha against Choristoneura fumiferana, Information Report LAU-X-89B, Forestry Canada, Quebec.

Vlak, J. M. (1994) Novel strategies in the development of engineered baculovirus insecticides and concepts for biosafety, in Proceedings of the VI International Colloquium on Invertebrate Pathology and Microbial Control, Montpellier, Society for Invertebrate Pathology, pp. 496–501.

Vorgetts, L. J. and Buescher, M. D. (1985) in Proceedings of the 72nd Annual Meeting of the New Jersey Mosquito Control Association, Atlantic City, pp. 244–6.

Wassmer, D. (1995) Confusion in Margalitaville. *Wing Beats, Amer. Mosq. Contr. Assoc.* **6**(4, winter), 16–8.

Webb, R. E., Shapiro, M., Podgwaite, J. D. *et al.* (1993a) Field comparison of different strains of gypsy moth nuclear polyhedrosis virus against gypsy moth (Lepidoptera: Lymantriidae) in Western Maryland in 1990. *J. Econ. Entomol.* **86**, 1185–90.

Webb, R. E., Shapiro, M., Podgwaite, J. D. and Venables, L. (1993b) Field confirmation of potentiation of a baculovirus by the addition of a stilbene optical brightner. *Pest. Sci.* **37**, 218–9.

Webb, R. E., Dill, N. H., Podgwaite, J. D. *et al.* (1994a) Control of third and fourth instar gypsy moth (Lepidoptera: Lymantriidae) Gypcheck combined with a stilbene disulfonic acid additive on individual shade trees. *J. Entomol. Sci.* **29**, 82–91.

Webb, R. E., Shapiro, M., Podgwaite, J. D. *et al.* (1994b) Effect of optical brighteners on the efficacy of gypsy moth (Lepidoptera: Lymantriidae) nuclear polyhedrosis virus in forest plots with high or low levels of natural virus. *J. Econ. Entomol.* **87**, 134–43.

Webb, R. E., Dill, N. H., McLaughlin, J. M. *et al.* (1996) Blankophor as an enhancer of nuclear polyhedropsis virus in aborist treatments against the gypsy moth (Lepidoptera: Lymantriidae). *J. Econ. Entomol.* **89**, 957–62.

Weinberger, P. and Greenhalgh, R. (1984) Ecotoxicity of adjuvants used in aerial spraying, in Chemical and Biological Controls in Forestry (eds W. Y. Garner and J. Harvey Jr), American Chemical Society, Washington, DC, pp. 351–63.

Weiss, S. A., Dunlop, B. F., Georgis, R. *et al.* (1994) Production of baculovirus on industrial scale, in Proceedings of the VI Colloquium on Invertebrate Pathology and Microbial Control, Montpellier, Society for Invertebrate Pathology, pp. 440–6.

Williams, T., Doyle, C. and Cory, J. (1992) Host–virus relationships for iridescent viruses and blackflies, in Abstracts of the 25th Annual Meeting of the Society for Invertebrate Pathology, Heidelberg, p. 314.

Wilmot, T. R., Allen, D. W. and Harkanson, B. A. (1993) Field trial of two Bacillus thuringiensis var. israelensis formulations for control of Aedes species mosquitoes in Michigan woods. *J. Amer. Mosq. Control Assoc.* **9**, 344–5.

Wilson, G. R. and Benoit, T. G. (1993) Alkaline pH activates Bacillus thuringiensis spores. *J. Invertebr. Pathol.* **62**, 87–9.

Winstanley, D. and Rovesti, L. (1993) Insect viruses as biocontrol agents, in Exploitation of Microorganisms (ed. D. G. Jones), Chapman & Hall, London, pp. 105–36.

Wood, H. A. (1994) Recombinant baculovirus pesticides: protecting crops and our environment, in Proceedings of the VI Colloquium on Invertebrate Pathology and Microbial Control, Montpellier, Society for Invertebrate Pathology, pp. 428–9.

Wright, D. J., Iqbal, M. and Verkerk, R. H. J. (1995) Resistance to Bacillus thuringiensis and abamectin in the diamondback moth, Plutella xylostella: a major problem for integrated pest management? Mededelingen – Faculteit Landbouwkundige en Togepaste Biologische Wetenschappen, Univ. Ghent **60**(3b), 927–33.

Yamamoto, T. and Tanada, Y. (1978a) Phospholipid, an enhancing component in the synergistic factor of a granulosis virus of the armyworm, Pseudaletia unipuncta. *J. Invertebr. Pathol.* **31**, 48–56.

Yamamato, T. and Tanada, Y. (1978b) Biochemical properties of viral envelopes of insect baculoviruses and their role in infectivity. *J. Invertbr. Pathol.* **32**, 202–11.

Yamamoto, T. and Tanada, Y. (1980) Acylamines enhance the infection of a baculovirus of the armyworm, Pseudaletia unipuncta (Noctuidae, Lepidoptera). *J. Invertbr. Pathol.* **35**, 265–8.

Yap, W. H., Thanabalu, T. and Porter, A. G. (1994) Expression of mosquitocidal toxin genes in a gas-vacuolated strain of Ancylobacter aquaticus. *Appl. Environ. Microbiol.* **60**, 199–202.

Yearian, W. C. and Young, S. Y. (1978) Application of Microbial Insecticides on Field Crops, Miscellaneous Publication No. 10, Entomological Society of America, pp. 21–6.

Yendol, W. G., Hamlen, R. A. and Rosario, S. B. (1975) Feeding behaviour of gypsy moth larvae on Bacillus thuringiensis (Bacteria)-treated foliage (Lymantria dispar: Lep. Lymantriidae). *J. Econ. Entomol.* **68**, 25–7.

Yendol, W. G., Heldung, R. C. and Lewis, F. B. (1977) Field investigation of a baculovirus of the gypsy moth. *J. Econ. Entomol.* **70**, 598–602.

Young, S. Y. (1989) Problems associated with the production and use of viral pesticides. Mem. Inst. Oswaldo Cruz., Rio de Janeiro, **84** (suppl. III), 67–73.

Young, S. Y. and Yearian, W. C. (1976) Influence of buffers on pH of cotton leaf activity of a Heliothis nuclear polyhedrosis virus. *J. Ga. Entomol. Soc.* **11**, 277–82.

Young, S. Y. and Yearian, W. C. (1986) Formulation and application of baculoviruses, in The Biology of Baculoviruses, Vol. II, Practical Application for Insect Control (eds R. R. Granados and B. A. Federici), CRC Press, Boca Raton, pp. 157–79.

Zaritsky, A., Zalkinder, V., Ben-Dov, E. and Barak, Z. (1991) Bioencapsulation and delivery to mosquito larvae of Bacillus thuringiensis H14 toxicity by Tetrahymena pyriformis. *J. Invertebr, Pathol.* **58**, 455–7.

Ziniu, Y., Songya, L. Jingyuan *et al.* (1996) Cloning and expression of Bacillus thuringiensis Cry IIA gene in Anabeana, in Abstracts of the 2nd En Gedi Conference on Bacterial Control of Agricultural Insect Pests and Vectors of Human Disease, Ben-Gurion University, Negev, Shoresh, Abstract No. 29.

Zou, Y. and Young, S. (1996) Use of fluorescent brightener to improve Pseudoplusia includens (Lepidoptera: Noctuidae) nuclear polyhedrosis virus activity in the laboratory and field. *J. Econ. Entomol.* **89**, 92–6.