FORMULATION: IS IT AN EXCUSE FOR POOR APPLICATION?

K A JONES[1], A J CHERRY, D GRZYWACZ
Natural Resources Institute, University of Greenwich, Central Avenue, Chatham Maritime, Chatham, Kent ME4 4TB, UK

D H BURGES, 21 Withdean Avenue, Goring-by-Sea, Worthing, West Sussex, BN12 4XD, UK

ABSTRACT

Field results have shown that formulation of microbial insecticides is not essential to their success. However, large-scale use requires that a product is easily handled and has predictable activity. Formulation helps to achieve this by stabilising the product in storage, optimising application, enhancing the activity of the microbe and maximising environmental persistence. Application of any insecticide is an inefficient process. Formulation helps to negate this, as well as helping to make a product more acceptable to users. Whilst unformulated products will continue to have an important role to play, particularly in developing countries, effective formulation, in combination with efficient application, is essential key to wide-scale uptake of microbial insecticides.

INTRODUCTION

Formulation comprises aids to improving the effectiveness and user-acceptability of microbial insecticides. Several approaches are available to the formulator to achieve these functions, ranging from production of liquid suspensions to dry granules, and even incorporation of the agent in a living organism. The final product developed depends on a range of factors, such as behaviour and location of target, availability of formulation materials and application equipment, as well as customer preference. The range of possible formulation types used with microbial insecticides is listed by Rhodes (1993) and Jones and Burges (1997).

However, several field trials reported in the literature have indicated that the formulation of microbial insecticides has not drastically improved field activity (Bull, 1978, Couch and Ignoffo, 1981, Payne, 1986). There are also several examples, particularly from developing countries, that have demonstrated that unformulated products can be highly effective in the field, for example the use of home-produced nuclear polyhedrosis virus (NPV) to control *Spodoptera exigua* in Thailand (Jones *et al.*, 1993). Thus it would appear that formulation is not essential to successful control of insect pests with microbial pesticides. However, with experimental field trials a product is optimally handled and applied, but this normally will not be the case in commercial use. Moreover, such trials are subject to the normal extensive variation of field plots, which can mask desired effects. Formulation also may be necessary to improve characteristics, such as shelf-life, which are not a problem with experimental trials. Whilst unformulated products can give good control, they increase the likelihood of

[1] Seconded to CARE International, P.O. Box 1024, 134 Havelock Road, Colombo 5, Sri Lanka

failure and inconsistent results. This can be due to inactivation prior to use or at the target site, or as a result of variable amounts of the microbe reaching the target site. Large-scale uptake and commercial success of a microbial insecticide requires its activity to be predictable.

Jones and Burges (1997) list four main functions of formulation: 1) to stabilise the micro-organism during production, distribution and storage; 2) to improve handling and application of the product; 3) to protect the agent from harmful environmental factors at the target site; 4) to enhance activity of the agent at the target site by increasing its activity, reproduction, contact and interaction with the target pest. This paper deals with formulation from the point of application, an earlier paper in this book deals with improving storage characteristics of a product.

HANDLING

It is important that a product be easy to handle and apply. For example, wettable powders must easily mix with, and suspend evenly through the water carrier. If the micro-organism is not evenly suspended throughout a spray suspension, variability in the amount that reaches the target site will increase. Mixing is aided by the inclusion of a dispersant. A surfactant is also required to aid the powder to penetrate the surface tension at the liquid/solid interface. Alternatively, mixing problems can be reduced by adding a binder and forming water dispersible granules. These break surface tension easily, flow freely and do not cause a hazard with air-borne dust particles. However, they do require more agitation for dispersion. With products that are applied dry, formulation as granules reduces drift of fine dust and are easier to apply than powders.

Flowable suspensions or emulsions also must be able to mix evenly with any diluent, again this may require the inclusion of surfactants and, with the former, suspending agents to prevent rapid settling of the particulate micro-organism.

Dispersants and surfactants that have been used with microbial insecticides are listed by Burges (1997) and Burges & Jones (1997).

APPLICATION AND COVERAGE

Effective application requires appropriate equipment and suitable formulation. These two factors are inter-related. Equipment will not perform at an optimum without the appropriate formulation. In most cases microbial insecticides are applied using application equipment designed for chemical pesticides. Application technology in general has been reviewed by Matthews (1992) and Southcombe (1985), amongst others. Application of microbial insecticides has been discussed by Smith and Bouse (1981), Reardon (1991), Jones (1993), Bateman (1994) and Jones and Burges (1997). Even the best application method will normally result in only a small percentage of the product reaching the intended target site, for example with liquid sprays Himel *et al.* (1990) estimate that as little as 5% of the total active ingredient applied reaches the target site. Coverage is particularly important with bacteria, virus and protozoa, which need to be eaten by the insect pest, hence an even distribution is necessary in the pest feeding area. In contrast, fungi and nematodes, which act by contact, can be picked up as the target pest moves about, so distribution is less critical.

Most microbial pesticides are applied as liquid sprays, with either oil or water as the carrier. Low volumes are preferred in areas where water is scarce, because it reduces the volume and weight to be transported. Higher volumes are aimed at providing complete wetting of a target surface, although this also causes high run-off of spray from the target. The general trend, therefore, has been toward reduction of volume. With chemicals superior control has been obtained with lower volumes (Matthews, 1992). With microbial insecticides results have varied (e.g. Smith and Bouse, 1981; Topper *et al.*, 1984), but this is probably due to a lack of attention to efficient application. Laboratory studies have indicated that lower volume sprays are more effective (e.g. Barnett, 1982)

Reduction of volume increases need to optimise spray droplet size in order to maximise coverage of the target. Production of optimum sized droplets is influenced by viscosity, volatility and to a lesser extent surface tension of a liquid suspension (Sundaram, 1988) and therefore is greatly influenced by formulation. Coverage of a target surface is influenced by droplet viscosity, impaction and retention and depends on several factors, including formulation, as well as environmental conditions and the nature of the target surface (Johnstone *et al.*, 1977; Matthews, 1992; Rearden, 1991). A major feature of droplet retention is the ability of the formulation to 'wet' the surface of the target (Spillman, 1984).

The final size of a droplet reaching the target also depends on the amount of evaporation. Evaporation rate increases as droplet size decreases due to increasing surface to volume ratios. Oil evaporates much less than water as a result of different vapour pressure and viscosity and therefore oil carriers are most commonly used for ULV sprays. Both mineral and vegetable oils have been used with microbial insecticides. Vegetable oils appear to be more palatable to insects than mineral oils. Some mineral oils have also been found to rapidly inactivate some microbes (Cherry *et al.*, 1994; Stathers *et al.*, 1993). If water is used, an anti-evaporant or humectant is added. Anti-evaporants normally form a film at the droplet surface to reduce evaporation: often these are water-soluble polymers. Humectants, such as glycerol, glycols or molasses, increase hygroscopicity to reduce evaporation. Water is the carrier of choice with higher volumes where drop size is larger and the cost of oil would be prohibitive or environmentally damaging.

Particular sprayer types may also need spray liquid with physical properties within certain limits. For example, a ULV sprayer requires a solution/suspension within a predefined viscosity range. Many vegetable oils are too viscous and need to be mixed with a mineral oil. Some microbial products may contain large particles, e.g. insect debris in viral insecticides. Large particles can block nozzles causing incomplete or poor application, this is avoidable by grinding dry products, wet milling or filtration. With some sprayers droplets are formed by a spinning cage inside which formulation must prevent particles becoming 'caked,' which would otherwise block the orifices of the cage.

Micro-organisms can be hydrophobic or hydrophilic, which will affect both choice of carrier and wetter. Suspended particles, such as microbial agents, alter physical aspects of drops. Their influence, particularly on spray disintegration, is reduced, if they are 'wetted' (Smith and Bouse, 1981). Additives included for other function, such as environmental stability will also influence features such as viscosity and the wetting ability of the suspension.

Effectiveness of additives in a formulation will also be influenced by drop size. For example, the degree of filtration by a sunscreen will depend on both concentration and on droplet size (Killick, 1986). The influence of a feeding attractant may vary according to the number and size of drops, along with the concentration within a drop.

With application of liquid products to water for mosquito control drop sizes are less critical, and larger drops are preferable to avoid drift and penetrate overhanging foliage. With mosquitoes the same target species may occupy different habitats. Some species are surface feeders, others bottom feeders. Appropriate formulations must therefore be designed to deliver the correct dose of the agent to the zone harbouring target species, often over a period of time. Thus slow release formulations, such as solid briquettes, may be designed to disintegrate into some particles that remain at, or just below, the water surface and others that slowly sink to the bottom. Complete cover of a water body can be achieved through use of monomolecular films (Burges and Jones, 1997).

Additives may be incorporated into formulated products or added later to spray tank mixes. For ease of use, and to ensure that all necessary ingredients are present in the correct proportions, the former is preferable.

ENHANCED ACTIVITY

Additives can be included in a formulation to enhance the efficacy of the micro-organism. These can be split into two groups, ones that increase contact and uptake by the target host - attractants or feeding stimulants, or ones that synergise the activity of the micro-organism.

Use of an effective feeding attractant or bait is a more efficient method of dosage transfer than blanket application and random transfer (Cudwell, 1993). Attractants and stimulants are often recommended as tank mixes rather than being included in the formulation, alternatively they may be incorporated into a solid bait. They are mainly used with orally active micro-organisms and are normally feeding stimulants. They are particularly useful with products that maybe be slightly anti-feedant due to the presence of other formulation additives, or if the microbe itself has some anti-feedant properties, e.g. *B. thuringiensis*. The most effective appear to be plant extracts, and sugars (Burges and Jones, 1997). However, a stimulant must be selected with care as its effect may not be the same with all target pests (Burges and Jones, 1997).

There are a number of additives that have been shown to increase the efficacy of microbes. These include chemicals such as boric acid and sodium tetraborate which synergises NPVs (McKinely, 1985) and a range of chemicals, including CaO, CaSO4, Tweens, and caffeine, with *B. thuringiensis* (reviewed by Burges and Jones, 1997). Chemical insecticides have also been shown to increase efficacy of some microbial insecticides (e.g. Jones, 1994). However, caution is needed as some pesticides are antagonistic. Moreover, the formulator is adding toxic chemicals to a product, which may negate the environmental benefits of using beneficial micro-organisms.

Recently, Shapiro and Robertson (1992) reported that addition of an optical brightener resulted in dramatically increasing the efficacy of some NPVs. It appears that this is most dramatic with NPVs that are not highly pathogenic to their host. It is likely that synergists are most useful for use with less virulent strains or species of micro-organisms. It will be interesting, however, to determine whether a synergist will increase efficacy of a normally highly-virulent strain that has been partially inactivated, thereby improving persistence.

PERSISTENCE

Ideally a microbial insecticide should remain active throughout the period that a pest is present. Microbes can be inactivated by several environmental factors, including sun, high temperature, adverse humidity and leaf surface exudates. Also they may be physically removed from the target location by the action of wind, rain or leaching. The importance of each of these factors is dependent on where the target insect is located. On foliage sunlight is the most important factor, whereas in food or grain stores sun is not important, rather temperature and humidity are more likely to affect persistence.

Additives are included to protect the micro-organism from these adverse factors. Sunscreens are often added to a formulation to protect microbes from UV radiation in sunlight. These physically reflect the light, or selectively absorb damaging wavelengths. Reflectors include zinc oxide, titanium oxide, silicate and talcum. Absorbents include specialised dyes and chemicals, such as Congo red, indigo carmine and stilbene derivatives, which absorb specific wavelengths, as well as cheap and readily available additives that absorb over a wider range, such as molasses. Burges (1997) and Burges and Jones (1997) list a range of sunscreens that have been tested with microbial insecticides and rate their effectiveness.

Adverse effects of temperatures in the field cannot easily be countered through formulation. A more promising approach is selection of strains of micro-organisms active over a wider range of temperatures.

Humidity is particularly important for fungi. Fungal spores normally only germinate at humidities below 95+%. However, formulation of spores in oil can overcome this limitation and allow use of entomopathogenic fungal in environments with low humidities, such as deserts (Bateman *et* al., 1993).

Plant extracts can inactivate baculoviruses, or at least affected activity (e.g. Uchida *et al.*, 1984; Richter *et al.*, 1987). Similarly, chemicals on the insect cuticle may suppress the growth of fungal spores (St. Leger, 1993). Thus additives designed to overcome anti-microbial activity may be needed to improve the activity of products on these target substrates. High or low leaf surface pH on some plants may also be important (e.g. Young *et al.*, 1977), although buffering of the product has produced conflicting results (Falcon, 1969: Young and Yearian, 1976) and good control of cotton pests can be achieved with unbuffered formulations (Jones, 1994).

Micro-organisms can be lost from target surfaces through action of wind and rain, physical abrasion or flowing water. The inclusion of a sticker in a formulation can reduce the degree of loss. These include water-soluble materials, such as molasses, that can delay loss, and insoluble resins (Burges and Jones, 1997).

CONCLUSION

The title of this paper poses the question whether formulation is necessary or simply an excuse for poor application. Whilst it is undoubtedly true that application of any insecticides is normally grossly inefficient, and that formulation is a means to counter this, it is not the only reason to formulate a product. Formulation is desirable, not only to maximise application efficiency, but also to provide the best chance of the microbial insecticide to be effective over a range of environmental conditions and hence to provide a predictable level of control. It is also necessary to improve the general characteristics of a product, making it easier to handle and thereby more acceptable to users. Unformulated products will continue to be used by some farmers, particularly if they produce the microbe themselves. Perhaps a reflection of the potential of microbial insecticides is their ability to be highly effective without complicated processing and formulation, and this is an advantage to their continued use in resource-poor areas. However, unformulated products run the risk of failure and large-scale commercial use and farmer uptake will require appropriate and effective formulation.

A number of additives may act in more than one way, for example molasses can act as a sticker, a feeding stimulant, an anti-evaporant and a sunlight protectant. The ability of additives to affect several properties of a formulation means that complete formulations need to be field tested and assessed rather than individual additives, and that compromises may need to be made on the most appropriate formulation. Where possible formulation additives need to be non-toxic and environmentally benign in order to compliment these feature of the micro-organism.

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APPLICATION SYSTEMS FOR MICROBIAL PESTICIDES: NECESSITY NOT NOVELTY

A C CHAPPLE
KOUNTOURIOTOU 13, TRIANDRIA TK55337, Thessaloniki, Greece
e-mail: <chapple@lino.openet.gr>

R P BATEMAN
International Institute of Biological Control, Silwood Park, Buckhurst Rd., Sunninghill, Ascot, Berkshire SL5 7TA, UK. *e-mail:* <r.bateman@cabi.org>

ABSTRACT

Biopesticides face a challenge in moving from protected crops - e.g. glasshouses, nurseries - to broad acre crops. For those biopesticides that are to be distributed into canopies using sprays, any attempt to bypass existing application practices for chemicals (the use of "novel" application systems) is likely to prove a serious obstacle to the adoption of a biopesticide by growers or farmers. Although the ubiquitous hydraulic nozzle - flat fan or hollow cone - has many shortcomings, principally the high inefficiency of use of active ingredient, there are no widely used, acceptable alternatives. Other application systems may offer advantages over the hydraulic nozzle, but if a "novel application system" is a prerequisite for the use of a biopesticide, then the likelihood of that biopesticide being used is very low. The most conspicuous exceptions to the use of hydraulic sprayers have been in the deployment of rotary nozzles appropriate for ultra-low volume (ULV) application of biopesticides for the treatment of natural and semi-natural ecosystems (*e.g.* locust control and forestry).

Because choice of application system for broad acre crops is more or less fixed, it is important to ensure that preliminary glasshouse and field trials represent as closely as possible the eventual application scenario. Data is presented which shows the difference between the particulate distributions in the spray volume before and after passage through a pump. The consequences of not considering application systems are discussed.

INTRODUCTION

In the real sense of the word, biopesticides are not "novel": they have been in use in glasshouses for decades and in protected crops, nurseries, and in turf pest control for almost as long. Their current "novelty" value is mainly due to the increased attention that broad acre crops are receiving, with growers, regulators, researchers, and the public alike, all keen to replace conventional broad-acre agricultural chemical control with safer, more acceptable methods of crop protection. However, true biopesticides have largely failed to penetrate the broad acre market and most progress is being made with biorational pesticides such as *Bacillus thuringiensis* (*Bt*), botanicals and the synthetic avermectins. The principal exceptions are forestry pest control with viruses (Entwistle *et al.*, 1990, Evans, 1994) and the operational scale tests against locusts and grasshoppers with *Metarhizium* spp. (*e.g.* Kooyman *et al.*, 1997). One rare (and non-entomological) example that is in current use with broad acre crops is the hyper-parasite *Ampelomyces quisqualis* (commercialised as AQ10® by Ecogen Inc., Langhorne, PA, USA), for the control of powdery mildew in grape vines.

There are many obstacles to transferring a biological control agent from the laboratory or glasshouse to the field. These obstacles include broadening target specificity, ease of use (i.e. commercially useable formulations with long shelf lives), obtaining comparable costs to the existing agrochemical alternatives, and reliable, cheap mass production, to name but four. In general, the agrochemical paradigm offers limited assistance, and novel approaches are required, exploiting other fields such as food technology, fermentation, or the pharmaceutical industry, for example. These proceedings offer readily available examples of innovative thinking and novel approaches.

The processes involved in pesticide application - often referred to as dose transfer - start with the formulation and its properties in the spray tank, through atomisation, transport to and impaction on target surfaces, distribution of the deposit and subsequent environmental degradation, to biological effects. The authors would argue that for one important facet of the crop protection process - *i.e.* the <u>method</u> of application of the pest control agent - "novelty" is a risky and problematical approach and, on past experience, unlikely to succeed. However, in comparison with chemical pesticides, application issues with biologicals are under-researched (D. Dent, these proceedings). This paper outlines the temptations and limitations of using application technology to solve the problems posed by implementing biological control in the place of conventional systems, as well as the hazards of using non-representative systems in the original research.

APPLICATION TO BROAD ACRE CROPS

Almost without exception, the step from the laboratory to the glasshouse results in a loss of efficacy of the active ingredient (a.i.*): more a.i. per unit area is required to obtain the same result, often to the point of making the product commercially non-viable. This problem also applies to conventional agrochemicals: a continuing series of symposia at five year intervals addresses this exact problem (BCPC, 1994). Biologicals are not exempt, and, it can be argued, are possibly more prone to loss of efficacy under true field conditions as they are more sensitive to errors such as poor tank mixing.

In trying to understand why this loss of efficacy occurs, any researcher is going to investigate the differences between the glasshouse and the field. Amongst obvious candidates - e.g. micro- and macro-environmental effects, antagonistic organisms, the often large differences between glasshouse and field-grown crops - the application system soon comes under scrutiny. Unlike the above list of differences, this is one of the few that can be manipulated in the field. The main system of application used in broad acre crops - the hydraulic flat fan or hollow cone nozzle - has changed little since Victorian times, and boom suspension systems

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* Throughout this text, active ingredient (a.i.) refers to any active component of a crop pest control agent, whether of biological or chemical origin.

and four-wheel drive tractors notwithstanding, would have been completely comprehensible to engineers of the time. Although effective, the hydraulic application system is also highly inefficient. Estimates of inefficiency are typically 99% (Graham-Bryce, 1983) or higher (Hall & Adams, 1990), and one is immediately tempted to ask if there are better ways of delivering a.i. into crop canopies.

The work attempting to understand the inefficiency of the hydraulic application system has lead to the development of alternative application methods, ranging from spinning discs and electrohydrodynamic sprayers (Coffee, 1981; Downer *et al.*, 1992) (also known as controlled droplet application: CDA), to electrostatically charged sprays, foggers, rotary cages, etc. All offer advantages over the hydraulic nozzle for specific circumstances. The most thoroughly researched alternative sprayers are based on rotary atomisers, but despite other advantages (*e.g.* reduced labour inputs, low volume application rates, greater timeliness of application) the resultant control has often been disappointing or no better than the simpler, conventional approach (Arnold *et al.*, 1984abc; Cooke, *et al.*, 1985; McKinlay, 1985), despite sometimes depositing greater quantities of a.i. into the canopy (Cayley, *et al.*, 1985).

Rotary atomisers should not be considered novel application systems (Lodeman, 1896, Bals, 1969) and have stood the test of time - especially in certain niche markets where ultra-low volume (ULV) application rates are of paramount importance. A good example is locust control where ULV spraying is the standard application technique (Symmons, 1992). The production of very small droplets in arid environments requires the use of formulations that evaporate slowly and usually are based on oil; such formulations also enhance the infectivity of *Metarhizium* and overcome the need for high humidity for germination of fungal conidia (Bateman *et al.*, 1993; Bateman, 1997).

Much of the research into improved application systems has been based round the work on conventional insecticides which has shown that for a given amount of a.i., efficiency of utilisation of insecticides is inversely proportional to drop size (i.e. small drops work better for the same amount of a.i.: see Adams *et al.*, 1990 for a review). The same is true for biorationals such as *Bt* (Bryant & Yendol, 1988; Maczuga & Mierzejewski, 1995). This work in turn was driven by theoretical considerations concerning the "optimum drop size" for pesticides (Potts, 1946, but more usually referenced to Himel, 1969). However, it must be stressed that despite their theoretical pedigree, elegance of design, and their successful use in reducing pesticide inputs under certain conditions, not one "novel" application system has replaced the hydraulic nozzle as the mainstay of pesticide application, world-wide. No novel application system has delivered improved application efficiency relative to the hydraulic nozzle over the wide range of circumstances commonly met by the farmer or grower when spraying broad-acre crops. Recent research and advances notwithstanding [i.e. air-assisted sprayers (Hislop *et al.*, 1993), electrostatic charging of hydraulic nozzles (Western & Hislop, 1997; Wolf *et al.*, 1995), and "double nozzle" injection systems (Chapple *et al.*, 1997b)], it is extremely unlikely that any biological pesticides will be applied into broad acre crops other than through hydraulic nozzles in the near future.

There is therefore a general rule concerning application when considering the introduction of biological pesticides into broad acre crops: field trials and the preliminary glasshouse work must reflect the application system predominant in the market at which the biological is aimed. Normally, this will be one of two systems: the hydraulic nozzle (flat fan or hollow cone) found on boom mounted sprayers or knapsack sprayers; and the airblast sprayers used in orchards and certain row crops (e.g. vines). It should be noted however, that in other circumstances, other application systems may predominate: e.g. the use of granular formulations for targets such as corn borers, or spinning disc sprayers for insecticide application in African cotton (Matthews, 1992).

Modification of the spray cloud to alter deposit delivery - beyond the scope of this paper - is possible within the constraints of a standard application system (Hall *et al.*, 1993). It is also possible to ask growers or farmers to alter some aspects of their application practice: these include changing nozzle, altering volume application rate, and adding adjuvants to the spray tank (Chapple *et al.*, 1997a).

THE TRANSITION FROM GLASSHOUSE TO FIELD

In the development of any crop protection agent, the step from glasshouse to field is fraught with difficulty. Taking a biological pesticide as an example, when applying entomopathogenic nematodes to protected crops (glasshouses, nurseries) it is acceptable to ask the grower to apply the nematodes in large volumes of water for drench application. However, one litre of water added post application per 50 cm diameter pot is the equivalent of applying the contents of approximately one olympic-sized swimming pool per 44 ha, or 5.1 l/m^2. By contrast, applying 200 l/ha, the volume application rate per unit ground area is 0.02 l/m^2 (*i.e.* ignoring leaf area indices). With exceptions (e.g. turf and irrigated crops), it is unreasonable to expect the broad-acre grower to apply such vast quantities of water. Another example is mycoherbicide glasshouse trials, where as much as 2 or 3 ml of liquid are applied per target plant, which can translate into field application rates as high as 5,000 l/ha: a completely untenable requirement for a broad acre grower.

Field trials tend to reflect actual field spraying practices more closely than the glasshouse trials that precede them. Glasshouse trials are, more often than not, aimed at obtaining the maximum efficacy from an a.i., even where this means using commercially non-viable formulations (e.g. simple suspensions of the micro-organism under study) or excessively high volume application rates to "simulate" dew periods. However, even the traditional application techniques for field trials - small, compressed-air driven precision sprayers - do not reflect the application system likely to be used in the field. The principal differences are the absence of a pump and re-circulation system.

Recent, preliminary work by the authors has shown that the frequency distribution of *A. quisqualis* spores in the spray volume differs before and after passage through a pump. Samples were measured with a 'Malvern 2400' particle size analyser fitted with a 63 mm lens using model independent analysis. The instrument was fitted with a PS1 sample cell that contained a small magnetic stirrer. Each reading consisted of a background measurement with tap-water, followed by the gradual introduction of concentrated suspensions using a pipette. A reading was taken when the obscuration of the laser was optimal in the "illustrate live" command. The whole procedure was repeated to check for consistency; each reading consisted of 1000 scans (equivalent to sub samples).

Figure 1 shows the distribution of spores: in a glass beaker "B" (e.g. the distribution present in a small hand-held mist-blower used in a laboratory test); in a larger volume "T" (50 l.: e.g. the volumes used in larger scale glasshouse trials with air-pressurised containers); after recycling through a pump "R" (e.g. in a field sprayer tank, either airblast or conventional hydraulic nozzle); and after recycling and spraying through a flat fan nozzle "S" at 40 psi [276 kPa] - i.e. adding the shear stresses of atomisation to those of the pump. It should be noted that this work was done under a "worst case" scenario: i.e., the formulation was not allowed time to hydrate. Other data (unpublished) indicate that the formulation breaks up into near-monodispersed particles after hydrating for a few minutes.

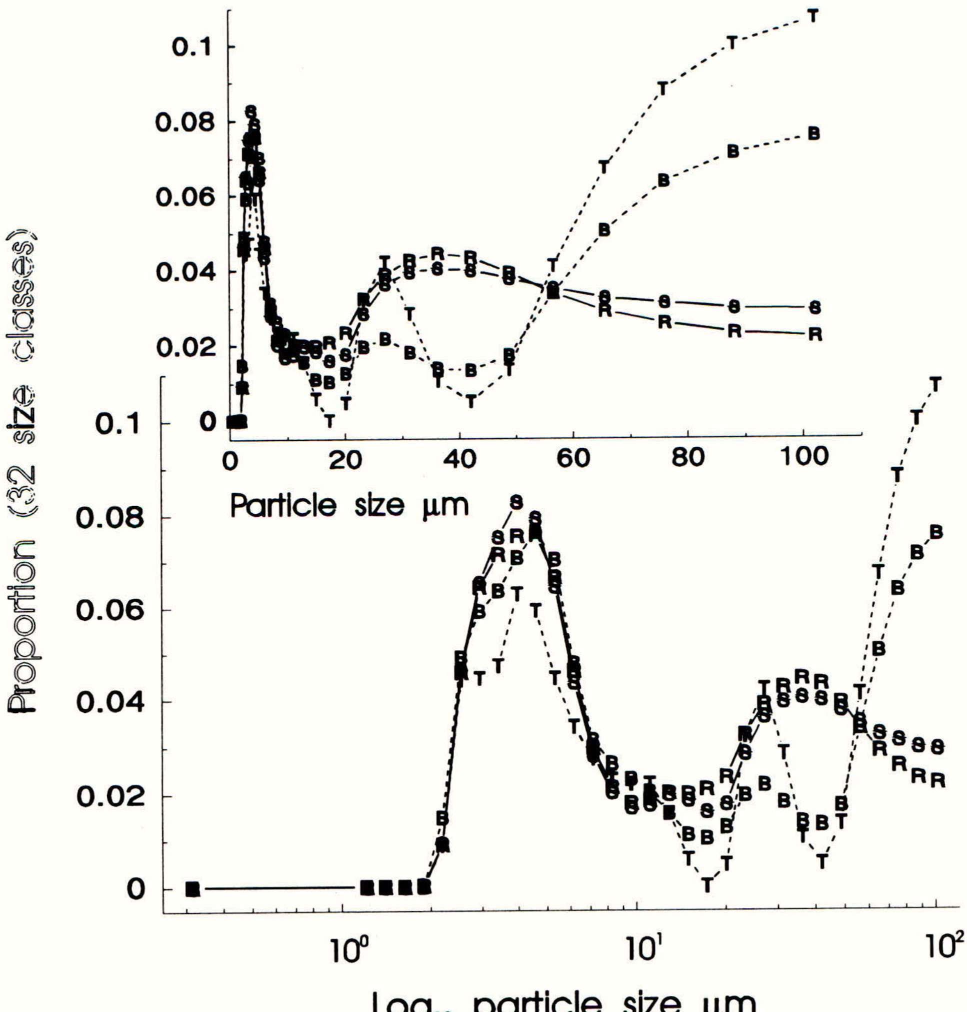

Figure 1. Frequency distribution of particle sizes (linear and $\log_{10}$ axes) of *Ampelomyces quisqualis* (formulation) when: stirred in a beaker, **B**; stirred in 50 l. of water in a spray tank, T; after 5 minutes recycling though a diaphragm pump, **R**; and after recycling and spraying through a flat fan hydraulic nozzle, **S**.

As with *Metarhizium* for locust control, the use of oil has been found substantially to improve the efficacy of *A. quisqualis*. Figure 2 shows the effect of pump recycling on the adjuvant oil, ADDQ®, (an Ecogen Inc. proprietary oil adjuvant). The distribution of the oil in the beaker was visibly so poor as to negate any need to see the effects in 50 l.: most of the oil remained on the surface, despite vigorous stirring. Passage through a pump completely altered the distribution of the particles of oil, such that the mean particle size of oil drops was much closer to that of *A. quisqualis*, which has dimensions of approx. 5μm by 3μm.

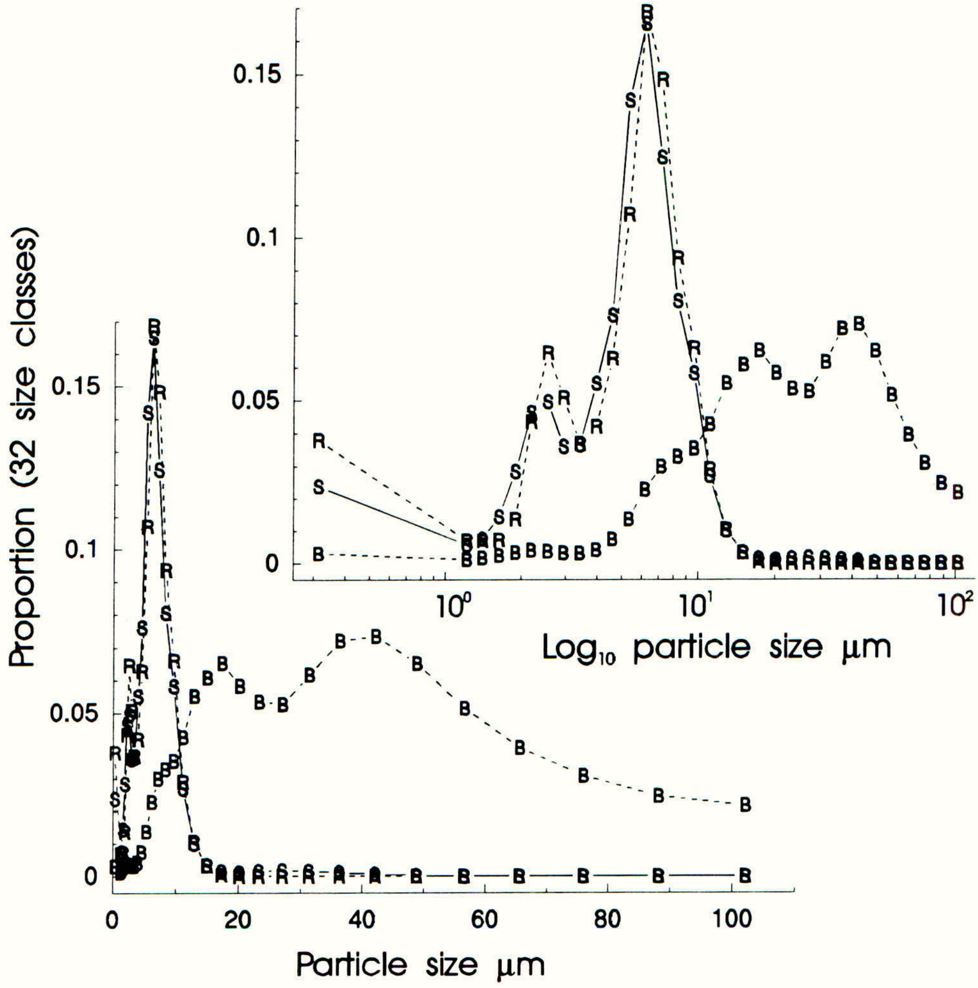

Figure 2. Frequency distribution of oil particles of ADDQ, an oil enhancement agent for *A.quisqualis*, when: stirred in a beaker **B**; after 5 minutes recycling though a diaphragm pump, **R**; and after recycling and spraying through a flat fan hydraulic nozzle **S**.

Table 1 summarises other, theoretical, work (from Chapple, 1995). *A. quisqualis* is typically applied at approximately 1.25 x 10^{11} spores/ha (25 g/ha @ 5 x 10^{9} spores/g formulation). For typical application parameters (tractor speed, hydraulic pressure, air velocity, etc.), the numbers of drops per hectare applied can be calculated for a given application system. If the atomisation system is considered as a sampling system, removing spores from the spray tank in different sized samples (the drops), then it is possible to calculate the probability that any drop size will contain a spore. This in turn, can be transformed to the l/ha and percent of drops or applied volume with either no spores present (wasted delivery) or more than one spore present (over-dosing: see Table 1).

"Efficient distribution" of *A. quisqualis* can be defined as distributing single spores evenly over all leaf surfaces. With many microbial insecticides, more than one spore is required per target, so Table 1 must be considered in light of the delivery requirements for a given biological control agent. However Table 1 also shows that a large proportion of the droplets contain no spores, although the percent volume of liquid atomised with no spores is lower. Any water-soluble adjuvants or enhancement agents will be delivered evenly dispersed through the spray cloud, and although many drops will be delivered with adjuvant but without a spore, the volume of adjuvant wasted is relatively low. However, for any adjuvant oils, even when passed through a pump such that the mean oil particle size is approximately that of the spore it is intended to accompany (Figure 2), one is relying on the probability that a drop will contain both spore and oil. This goes some of the way to explaining why, for example, several litres/ha of oil are required to be added to approx. 70 g/ha of formulated A. *quisqualis* enhancement to take effect.

Table 1. Effects of changing application method (hydraulic flat fan, air blast sprayer, and spinning disc), when applying an a.i. as single discrete particles of uniform size (here, the biofungicide *Ampelomyces quisqualis* is applied at approximately 1.25 x 10^{11} spores/ha).

Nozzle[1]	l/ha[2]	% Drops with:		% Volume[3]	l/ha[4] with	% Drops	% Drops
		0 spores	1 spore[3]	no spores	no spores	>1 spore	>300µm
2080-14	159	63.8	15.3	5.8	9.2	20.9	30.0
2080-16	242	66.2	14.6	5.4	13.1	19.2	50.3
2080-30	560	71.8	11.7	4.7	26.2	16.5	76.2
Airblast	250	95.6	3.9	57.1	142.7	0.5	0.0
Spinning	1.5	6.5	12.0	0.4	0.01	81.5	0.0
disc[7]	11.2	39.9	23.6	7.4	0.8	36.5	0.0

1 Three hydraulic flat fan nozzles (Hardi International A/S, DK-2630, Denmark), under similar operating conditions, an orchard airblast sprayer, and a spinning disc sprayer (Ulva+, Micron Ltd, Three Mills, Bromyard, Herefordshire, HR7 4HU, UK).
2 l/ha: Volume application rate under field operating conditions.
3 % of the applied drops containing no spores, by number and by volume.
4 Actual l/ha applied containing no spores.
5 % of the applied drops containing more than one spore per drop.
6 % of the applied number of drops >300µm.
7 1.5 l/ha for oil and 11.2 l/ha for water based formulation

The above assumes that the oil particles are very small - of the order of size of the attendant spores - and are uniformly sized, which they are not. However, from the limited data presented in Figure 2, it can be seen that it is unreasonable to expect an adjuvant oil to be as effective based on the distribution of particles produced in a beaker / spray bottle as the same oil after shearing and mixing by a pump. Again, this data goes some of the way to explaining why successful results were obtained in the field with adjuvant oils with *A. quisqualis* (using motorised knapsack sprayers) relative to the conflicting results in the glasshouse - e.g. little or no enhancement under "ideal" conditions, using pressurised-air sprayers.

CONCLUSIONS

The authors would argue two points, based on:

- general past experience of field application systems;
- the available literature documenting the success and failure of novel application systems;
- theoretical considerations of the distribution of uniform sized particles (here, microbial-based pesticides) in spray volumes and spray clouds;
- preliminary data investigating the effects of field spraying systems on particulate distributions, pre and post-sheer stress.

First, problems arise when transferring a microbial pest control agent from the laboratory and glasshouse to the field. Attempting to circumvent or solve these problems by using spray application systems that are non-standard for the target crop is more likely to reduce rather than add to the likelihood of success. The scope for altering a currently used field application system such as the hydraulic nozzle is quite limited, and has been discussed in detail elsewhere (Chapple, 1996).

Second, there are substantial differences between the end result - in-flight drops and final deposits containing a.i. - from application systems typically used in the lab, glasshouse, and field trials (e.g. pressurised canisters, high volume applications) when compared with the systems typically used in the field (large spray tanks with pumps and recycling systems). These differences may have significant effects on the eventual distribution of the a.i. through a canopy and substantially change the efficacy of the biopesticide under study.

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