11 The Spray Application of Mycopesticide Formulations

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Introduction

Biopesticides have been promoted for at least the last century, but their use remains limited to < 1% of the total pesticide market. Lisansky (1997) attributes this limited growth to lack of positive promotion by authorities and the agrochemical industry. Their perceived constraints include: narrow target spectra, poor performance relative to equivalent-cost chemical control and inconsistent product quality in comparison with chemicals. The *Biopesticide Manual* (Copping, 1998) lists six fungal agents that have been commercialized as mycoinsecticides, ten agents that have fungicidal or fungistatic properties, three mycoherbicides and one nematocidal product (Table 11.1). Together these constitute a tiny proportion (some 3%) of the already small worldwide biopesticide market (Georgis, 1997). The remainder consists largely of *Bacillus thuringiensis* subspp., other *Bacillus* products and viruses.

Poor performance can be attributed to:

- product inconsistency;
- a 'chemical paradigm', which creates the perception that users must see results immediately (rather than making cost-effective improvements in yield);
- difficulty or misunderstandings in use, which can result in poor efficacy.

In most Western field crops, 'spraying' usually means use of a tractor and boom whereas, in developing countries, this often involves hand-carried equipment (knapsack sprayers). In both cases the break-up (atomization) of the spray liquid is achieved using various types of hydraulic nozzle (see Matthews, 1992). Although this technology is more than a century old, many would argue that it has yet to be surpassed for simplicity and reliability. However, as we shall show in this chapter, hydraulic application is not necessarily the most efficient technique – especially when farmers are instructed by product labels to 'spray to runoff'. Most authorities agree that spray application is highly inefficient: Graham-Bryce (1977) has pointed out that, in foliar sprays against sucking insects, only 0.02–0.03% of the insecticide was utilized by the target. The most efficient dose transfer for insecticides quoted in the literature is 6% for aerial

(eds T.M. Butt, C. Jackson and N. Magan)

Table 11.1. Mycopesticide products and their application. (From Copping, 1998.)

Microbial agent	Pesticide type	Formulation/application
Ampelomyces quisqualis	**F**: powdery mildew hyperparasite	WG (with oil adjuvant): HV spray on to vines
Beauveria bassiana	**I**: Lepidoptera, Homoptera, Coleoptera	MG applied to axils/foliage WP, SC oil-based suspension: as HV sprays
Beauveria brongniartii	**I**: Scarabaeidae	MG, AL (HV sprays)
Candida oleophila	**F**: Postharvest fruit treatment	WG: spray or dip
Chondosterum purpureum	**H**: silver-leaf fungus prevents regrowth of unwanted deciduous trees	AL: sprayed or spread on to tree stumps
Colletotrichum gloeosporioides f. sp. *aeschynomene*	**H**: against *Aeschynomene virginica* (northern joint-vetch)	AL: sprayed on to weeds under high humidity
Coniothyrium minitans	**F**: prevention of *Sclerotinia*	AL: for spraying
Endothia parasitica	**F**: competes with more pathogenic *E. parasitica* strains	PA: applied to cuts or wounds of chestnut trees
Fusarium oxysporum	**F**: competes with more pathogenic *F. oxysporum* strains	SC, MG for glasshouse use
Gliocladium catenulatum	**F**: preventive esp. against *Pythium, Rhizoctonia, Botrytis, Didymella* and *Helminthosporium*	GR (WG?): granular, dip or foliar spray for seedlings and harvested produce
Gliocladium (=Trichoderma) virens	**F**: preventive esp. against *Pythium, Rhizoctonia, Fusarium, Theilaviopsis, Sclerotina* and *Sclerotium*	GR incorporated into soil
Metarhizium anisopliae	**I**: Coleoptera Isoptera Dictyoptera	GR (discontinued) DP: for termite galleries RB: ready-to-use baiting station
M. anisopliae var. *acridium (= Metarhizium flavoviride)*	**I**: Orthoptera: Acrididae	SU, OF: usually for ULV application
Myrothecium verrucaria	**N**: esp. against *Meloidogyne, Heterodera, Belonolaimus* and *Radopholus* spp.	Powder (DS?) applied as seed treatment or in soil drench
Paecilomyces fumosoroseus	**I**: Homoptera	WG for HV spraying
Phlebiopsis gigantean	**F**: prevention of *Heterobasidion annosum*	WP sprayed on to stumps
Phytophthora palmivora	**H**: against *Morrenia odorata* (strangler or milkweed vine)	AL sprayed on to weeds under high humidity
Pythium oligandrum	**F**: presentation of wide range of soil-borne pathogens	WP sprayed in glasshouses
Trichoderma harzianum	**F**: prevention of *Botrytis* and *Sclerotina*	MG soil treatment for vines and vegetables
T. harzianum and *Trichoderma viride*	**F**: preventive esp. against *Armillaria mellea, Pythium, Chondrosterium purpureum, Phytophthora, Fusarium, Rhizoctonia* and *Sclerotium*	MG, GR and others for soil application; also injected into woody crops and wound sealant
Verticillium lecanii	**I**: esp. Homoptera	WP sprayed at HV in glasshouses

F, Fungicide; **I**, Insecticide; **H**, herbicide; **N**, nematode. Formulation types conform to the Global Crop Protection Federation (GCPF, 1999) coding system (e.g. PA paste).
Solid formulations: MG, microgranules; GR, granules; DP, dusts; DS, powder for seed dressing. Solid formulations for mixing with water: WG, ~miscible granules; WP, wettable powders. Liquid formulations for mixing with water: SC, suspension concentrates. Ultra-low-volume (ULV) formulations: SU, ULV suspension; OF, oil-miscible flowable concentrate. AL is used for miscellaneous liquids applied undiluted: here mycopesticides as aqueous suspensios of spores, to be applied as soon as possible after receipt of product. HV, high-volume.

spraying of locust swarms. Higher efficiencies, with up to 30% of the tank mixture reaching the target, can sometimes be achieved with herbicides.

'Rational pesticide use' (Brent and Atkin, 1987) is an important concept in which treatment costs and the impact on non-target organisms are reduced by combining spatial and temporal precision of application with biologically specific products. Unfortunately, even with chemical pesticides, it can be difficult to define the true biological target, i.e. the site at which efficacy could be optimized (Hislop, 1987). With microbial agents, we cannot assume that simply delivering infectious propagules to the surfaces of target organisms will result in pest control. For example, complex pathogen-host attack and defence mechanisms exist against entomopathogenic fungi (Clarkson and Charnley, 1996; Blanford *et al.*, 1998) and 'chemical models' of biopesticide dose transfer may not be reflected in field results.

Table 11.1 includes several products that are formulated for specific application techniques (soil drenches, pastes for application to stumps, granules); these are applied according to manufacturers' specific instructions. However, it is the formulations for spray application that offer both the greatest challenge and the greatest opportunity for future development, and this will be the main theme addressed in this chapter.

Biological Considerations: Modes of Action

During the development of a mycoinsecticide for the biological control of locusts and grasshoppers, scientists of the international LUBILOSA[1] programme recognized that an effective delivery system requires a thorough understanding of the biological relationship with its target pest. For example, three distinct routes of fungal infection for locusts and grasshoppers have been identified:

1. Direct impaction with spray droplets. The infection or mortality resulting from 'direct contact' may vary from some 30% to > 99% 'direct hits' in the case of locusts and grasshoppers. However, this can be a very small component of the eventual dose transfer process (Bateman *et al.*, 1998).
2. Secondary pick-up of spray residues from vegetation or the soil. As with agrochemicals, secondary pick-up is often far more important than direct contact with spray droplets in most air-to-ground and ground-to-ground spray operations. Field observations suggest that spores may persist in the field for several days, depending on conditions (Langewald *et al.*, 1997).
3. Horizontal transmission (or 'secondary cycling') of the pathogen from individuals infected via the first two modes above, then death, sporulation and release of further inoculum into the environment, under suitable conditions (Thomas *et al.*, 1995).

At another level, it is extremely important to understand how the target and spray deposit interact at a biological level. For example, if the target is scale insects, the egg stage of a pest or a fungal pathogen, the mycopesticide must be brought to the pest. Application parameters are critical if the pest is sedentary and little horizontal transmission takes place; Bateman (1993) and Chapple *et al.* (2000) discuss the differences between biological and chemical active ingredients (a.i). However, if the pest is mobile, the interaction can be exploited (although here such factors as the repellency of many surfactants must be borne in mind). Although herbicide applications can be the most efficient forms of spraying (see above; Graham-Bryce, 1977), it can still be important to determine the optimal site on or near the plant for droplet deposition. The important practical question is 'Can application be optimized, within the limits of what the grower can be asked to do?' (Chapple *et al.*, 1996).

In theory, application technology is crucial when direct contact with target insects is needed; it may be less important if secondary cycling forms the principal method of mycopathogen infection in the field (i.e. classical biological control). Thomas *et al.* (1995) have suggested that high transfer of fungal propagules to target insects by direct impaction may be deleterious for the long-term management of acridids under certain circumstances. On the other hand, motorized mist-blower ('air-blast') sprayers were considered desirable for the efficient and speedy inoculation of a classical biological control agent in Australia; the rusts *Maravalia cryptostegiae* and *Phloeospora mimosae-pigrae* were effectively applied against rubber vine and *Mimosa*, respectively (A.J. Tomley, Queensland Department of Lands, personal communication).

Several laboratory and glasshouse studies have demonstrated that smaller droplets are more efficacious for arthropod pest control than larger ones. For example, the relative efficacy of different droplet size spectra, e.g. 30–60 μm droplets, were usually optimal with oil-based insecticide spray deposits, while 60–120 μm were most efficient with aqueous droplets (Adams *et al.*, 1990). Although this important effect has yet to be demonstrated with mycopesticides, it is not unreasonable to postulate that efficacy may be enhanced with an appropriate coverage of propagules in the target zone, obtained from suitable droplet size spectra.

Some Important Concepts in Spray Application

In order to better understand the cause of the spray inefficiency described above, it is useful to reflect on the implications of the large range of droplet sizes produced by most spray nozzles. Droplets are usually described by their perceived size (i.e. diameter), whereas the dose (or number of infective particles in the case of biopesticides) is a function of their volume. This increases by a cubic function relative to diameter ($\pi.d^3/6000$ to convert μm into picolitres); thus a 50 μm droplet represents a dose of 65 pl and a 500 μm drop represents a dose of 65 nanolitres (65,450 pl). This has long been recognized as one of the most important concepts in spray application (Himel, 1969), bringing about enormous variations in the properties of droplets (Table 11.2).

Different droplet sizes have dramatically different dispersal characteristics and are subject to complex macro- and microclimatic interactions (Bache and Johnstone, 1992). Greatly simplifying these interactions in terms of droplet size and wind speed, Craymer and Boyle (1973) concluded that there are essentially three sets of conditions under which droplets move from the nozzle to the target (see Fig. 11.1):

- Sedimentation dominates: typically larger (>100 μm) droplets applied at low wind speeds; droplets above this size are appropriate for minimizing drift contamination by herbicides.
- Turbulent eddies dominate: typically small droplets (< 50 μm), which are usually considered most appropriate for targeting flying insects, unless an electrostatic charge is also present, providing the necessary force to attract droplets to foliage. (NB: the latter effects only operate at very short distances, typically under 1 cm.)
- Intermediate conditions where both sedimentation and drift effects are important. Most agricultural insecticide and fungicide spraying is optimized by using relatively

Table 11.2. Ranges of droplet and drop sizes, with their volumes and properties.

Diameter range (μm)	Volume (maximum) in picolitres or l^{-12}	Properties/function
< 10	0.52	Potentially hazardous, very fine aerosols or particles, with a progressively increasing risk of inhalation by operators (greatest at approximately 1–3 μm)
< 50	65	Aerosols (appropriate for direct contact with small insects); most of the spray droplet spectrum in a Potter tower nozzle
50–100	524	Mists/fine sprays appropriate for oil-based ULV/mist-blower spraying
75–150	1,767	Maximizes coverage with water-based insecticide and fungicide sprays
150–300	14,137	Maximizes coverage with herbicide sprays, avoiding drift especially where wind (< 2 m s^{-1}) is present
300–500	65,450	Coarse spray: maximum avoidance of drift; at > 500 μm droplets become drops and progressively less efficient at covering foliage, leading to runoff, unless total volume applied is substantially increased
985	500,000	0.5 μl drops: lowest reliable volume that can be delivered by many microapplicators (for topical dosing in bioassays)

ULV, ultra-low-volume.

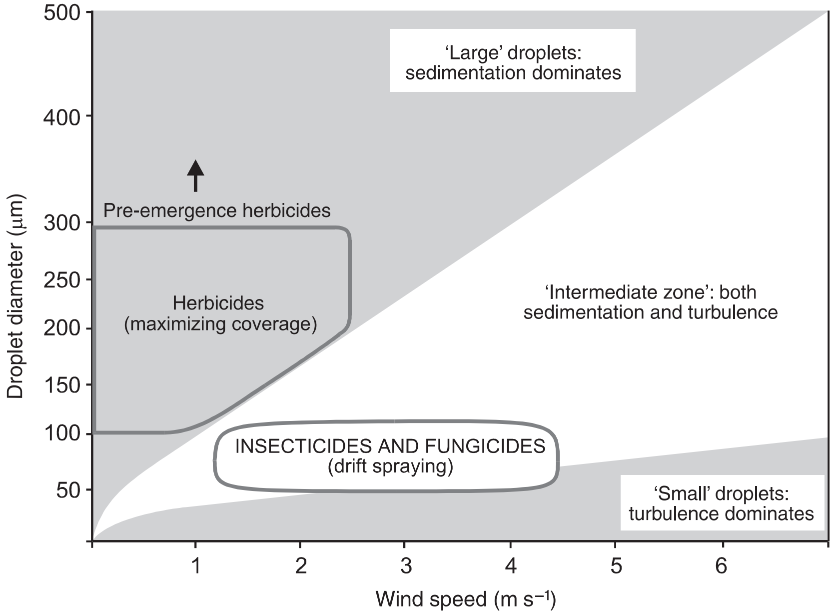

Fig. 11.1. Characteristics of 'large' and 'small' droplets. (Modified from Craymer and Boyle, 1973.)

small (say 50–150 μm) droplets in order to maximize 'coverage' (droplets per unit area), but these are also subject to drift.

Terms related to the effectiveness of application include the following:

- The efficiency of the spray application is determined by the complex phenomena that govern droplet transport from the nozzle to the target, which are described in texts such as Bache and Johnstone (1992). As a practical measure, Courshee (1959) coined the term deposit per unit emission (DUE) to describe the amount of pesticide recovered downwind in the target zone, relative to the volume emitted from the spray nozzle (originally in locust control operations). DUE is thus an overall measure of efficiency, permitting valid comparisons of spray recovery. It is usually expressed in units such as ml m^{-1} (mean quantity recovered in a unit area) per ml m^{-1} (liquid emitted over a unit of spray).

- Coverage is defined here as the extent to which a pesticidal spray has been distributed on a target surface. The biological implication is that good coverage increases the probability that a pest will encounter a pesticide. A rule-of-thumb guide to desirable levels of coverage is shown in Fig. 11.2 (but note that this is primarily based on what is known from the application of the chemicals). Because of the relationship between the diameter and volume of a sphere, there will theoretically be a cubic increase in numbers of droplets produced in relation to their average droplet diameter.
- Retention is the amount of spray liquid retained on (mostly the leaves of) crop plants. In other words, it is the remaining proportion of pesticide that has not 'run off' (usually high-volume spraying) or been eroded by weathering. There is an interaction between formulation effects on the tenacity of a deposit and the surface of the leaf to which it adheres. Droplets often bounce on leaves that are waxy (a property that is often influenced by age) and poor retention may occur with water-based formulations, especially those with high dynamic surface tensions. On the other hand, absorption of a.i. may occur with oil-based formulations. Leaf exudates (e.g. in apples and broad beans) may also contribute to the redistribution of a pesticide. Jefree (1986) has reviewed the ultrastructure and function of trichomes and epicuticular waxes. Chapple *et al.* (2000) discuss this important subject further.

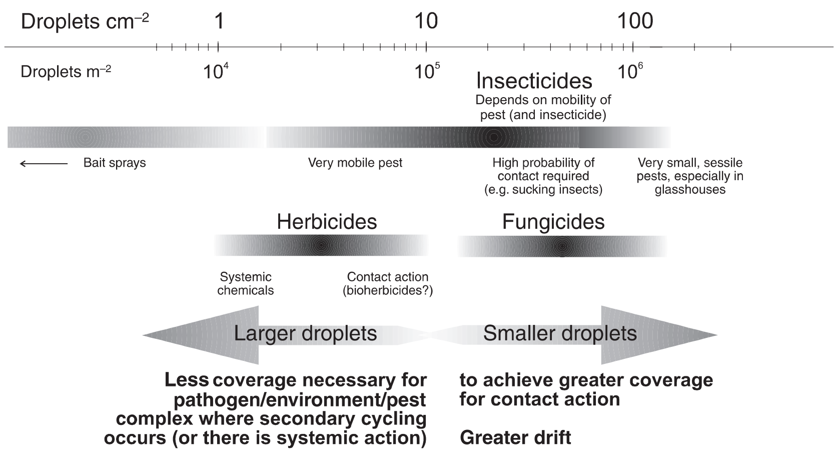

Fig. 11.2. A guide to spray droplet coverage. Horizontal bars indicate the desirable deposition in the target zone, based on chance encounter with the target. Note: (i) this diagram is for guidance only and no substitute for field evaluation; (ii) no reference has been made to the concentration, size or spreading of droplets.

- *The volume application rate (VAR)* is the amount of formulation applied per hectare. Table 11.3 gives a classification of VARs. Table 11.4 shows the theoretical coverage on plants if monodispersed droplets (i.e. all droplets have the same diameter) in these size classes were to be evenly applied at the lower limits of the ultra-low, very low, low, medium and high VAR ranges for field crops (from Matthews, 1992), assuming that all droplets were captured and retained. 'Per hectare' application often has very little relationship to the target area to be sprayed; leaf area indices of crops or weeds can range from fractions (pre-emergent weeds at the cotyledon stage) to > 5 (late-stage cereal crops). With bush and tree crops, VAR per hectare is even more inappropriate, and methods such as the unit canopy row (UCR) system have been developed where sprayer calibration is based on canopy size (Furness *et al.*, 1998).
- *Work rate* is the amount of ground (or crop) treated per hour/day and is linked to VAR. It is an important factor under certain circumstances, for example: (i) when the cost of labour is high; (ii) when a quick response is needed to a pest population that has exceeded an action threshold (especially if it is rapidly reproducing); or (iii) in migrant pest control.

Table 11.3. General classification of volume application rates (VAR in l ha^{-1}) for field and tree/bush crops. (From Matthews, 1992.)

	Field crops	Tree and bush crops
High volume (HV)	> 600	> 1000
Medium volume (MV)	200–600	500–1000
Low volume (LV)	50–200	200–500
Very low volume (VLV)	5–50	50–200
Ultra-low volume (ULV)	< 5[a]	< 50

[a] VARs of 0.25–2 l ha^{-1} are typical for aerial ULV application to forest or migratory pests.

Table 11.4. Coverage with specific droplet sizes (see Table 11.3) at different VARs if sprays consist of monodispersed drops (leaf area index taken as 1).

Mono-dispersed droplet size	Cross-sectional area of deposit (m^2)[a]	Droplets per m^2 (at 1 l ha^{-1})	% Cover (per ha) for lower limits of VAR classes				
			ULV (1)	VLV (5)	LV (50)	MV (200)	HV (600)
10	3.1×10^{-10}	190,985,932	6	30	NR	NR	NR
50	7.9×10^{-9}	1,527,887	1.2	6	60	NR	NR
75	1.8×10^{-8}	452,707	0.8	4	40	NR	NR
100	3.1×10^{-8}	190,986	0.6	3	30	120	NR
150	7.1×10^{-8}	56,588	0.4	2	20	80	NR
300	2.8×10^{-7}	7,074	0.2	1	10	40	120
500	7.9×10^{-7}	1,528	NR	0.6	6	24	72
985	3.0×10^{-6}	200	NR	0.3	3	12	37

[a] Single droplets (spread factor taken as 2).
NR, not realistic spraying scenario.
> 100% cover represents coalescence of droplets.

- *Controlled droplet application (CDA)* is a term probably coined by John Fryer of the Weed Research Organization in the UK (G.A. Matthews, personal communication). Bals (1969) stated that 'The efficiency of a spraying machine is inversely proportional to the range of droplets it emits, whilst the suitability for a specific problem depends on the actual size of the droplets emitted.' No atomizer is commercially available that can produce uniform (monodispersed) droplets, but rotary (spinning disc and cage) atomizers usually produce a narrower droplet size spectrum than conventional hydraulic nozzles. Therefore CDA can be considered in terms of optimizing technology to achieve a biological objective: delivering appropriately sized droplets (within practical engineering limits) for maximizing the control of a given pest target (where this is known). Unfortunately, the true biological target is often poorly defined and complex in nature, which, when combined with operational variables, makes most spraying inherently inefficient. However, there is often scope for improving existing practice (Hislop, 1987). Bals (1969) discussed the concept of producing small uniform pesticidal droplets to achieve adequate control with 'ultra-low dosage' combined with ultra-low volume (ULV) rates of application. Unfortunately, this is thought to have discouraged many chemical companies from promoting CDA techniques, since sales would be reduced (except when value could be re-added to an a.i. by proprietary delivery systems, such as the 'Electrodyn' (ED) (Coffee, 1981).
- *Droplet size* spectra generally refers to the measurement of – and statistics for describing – spray droplet spectra; they are described in the standard texts, including LeFebvre (1989) and Parkin (1992). At least two important measures of spray distributions are usually required, and the statistics used here are: (i) size: the volume median diameter (VMD), where half of the volume of spray contains droplets larger than the VMD (in μm) and the other half is in smaller droplets; and (ii) quality: the relative span, which is a dimensionless parameter calculated from the volume distribution only (see LeFebvre, 1989). A span that is substantially less than 1.0 is characteristic of a CDA spray (Bateman, 1993). With microbial control agents, particle distributions within droplet size spectra must be considered with respect to droplet volumes; this is discussed by Bateman (1993) and Chapple *et al.* (2000).
- *Spray drift*: with placement (localized) spraying of broad-spectrum or toxic chemicals, wind drift must be minimized, and considerable efforts have been made recently to quantify and control spray drift from hydraulic nozzles. On the other hand, wind drift is also an efficient mechanism for moving droplets of an appropriate size range to their targets over a wide area with ULV spraying. Himel (1974) made a distinction between exo-drift (the transfer of spray out of the target area) and endo-drift, where the a.i. in droplets falls into the target area, but does not reach the biological target. Endo-drift is volumetrically more significant and may therefore cause greater ecological contamination (e.g. where chemical pesticides pollute groundwater).

Formulations

A list of the formulation types with international standard abbreviations is given by the Global Crop Protection Federation (GCPF, 1999) and a comprehensive review on the formulation of biopesticides by Burges (1998). In brief, there are four major objectives when formulating biopesticides. Where possible the aim is to:

- stabilize agents during distribution and storage;
- aid handling and application of the product;
- protect agents from harmful environmental factors, thereby increasing persistence;
- enhance the activity of the agent at the target site.

With mycopesticides, the use of oils in formulations for spraying has shown great potential for the enhanced efficacy of insecticides (Prior *et al.*, 1988) and fungicides (Hofstein and Chapple, 1998), where the need for high humidity is also overcome. Amsellem *et al.* (1990) showed that invert emulsions (where oil constitutes the continuous phase) may eliminate the need for a minimum inoculum threshold with mycoherbicides. Oils can be pesticidal in their own right or may be phytotoxic (Wrigley, 1973), which may account for some biological activity.

The implementation of these developments for field crops is discussed by Chapple and Bateman (1997), but technical issues remain poorly understood; for example, they showed that the distribution of particles of the hyperparasitic mycofungicide *Ampelomyces quisqualis* and its emulsified oil adjuvant differs markedly before and after passage through a pump. There is a compound problem in that adjuvants will be wasted in the volume of spray droplets that are either physically too small to contain a particle or have a low probability of containing organisms. Droplet size in hydraulic nozzles can be substantially affected by the use of adjuvants (Hall *et al.*, 1993; Butler Ellis *et al.*, 1997).

Some of the interacting application parameters and how target pests might acquire the infective propagules are summarized in Fig. 11.3, emphasizing the role of formulations such as oil carriers.

Mycopesticide Application: Case-studies

Some of the technical implications of spraying mycopesticides can be illustrated with case-studies based on spray application scenarios for fungal insecticides, hyperparasitic fungicides and mycoherbicides. Measured droplet size spectra from typical sprayers are discussed with reference to product field concentrations and particle size spectra, where known. In all cases, the instrument used was a Malvern 2600 particle size analyser using model-independent analysis and the methodology described by Bateman and Alves (2000). For particle size measurements, the same instrument was fitted with a 63 mm lens and a PS1 sample cell that contained a small magnetic stirrer. Each reading consisted of a background measurement with either Shellsol T (for conidia with lipophilic cell walls) or distilled 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 data have been exported electronically and illustrated by line graphs indicating the percentage by volume in 32 size classes. X axes are accompanied by secondary scales indicating the equivalent droplet volume in picolitres (10^{-12} l) and the probable spore loading, with 'typical' tank mixture concentrations expressed as particles (conidia, etc.) per litre.

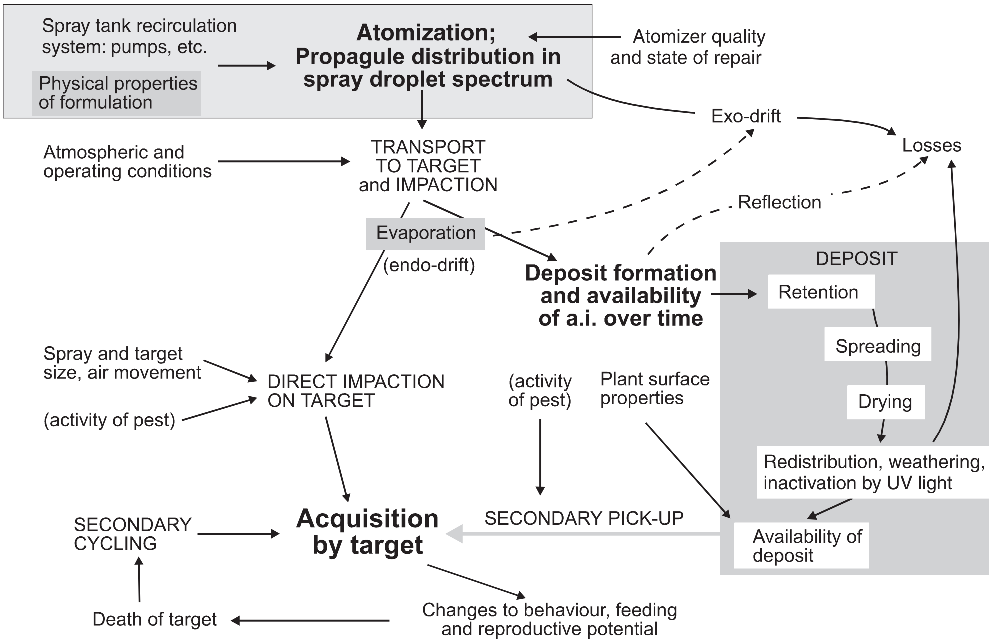

Fig. 11.3. Spray application processes and biopesticide formulations. Shaded zones represent effects that can be manipulated by formulation. This figure has been influenced by Young (1986), who describes the application of chemical pesticides. UV, ultraviolet.

Mycoinsecticides

Although fungi such as *Metarhizium anisopliae* have been known as potentially useful biological control agents for over a century, little progress was made for several decades until *Verticillium lecanii* was developed for glasshouse crops (Hall and Papierok, 1982). Even though products such as 'Vertalec'® and 'Mycotal'® became available, their use was limited to this niche market because of the need to maintain high humidity (Helyer *et al.*, 1992). During the 1990s, the LUBILOSA programme developed a mycoinsecticide ('Green Muscle'®) using oil-based formulations of an isolate of *M. anisopliae* var. *acridum*, which has been field-tested against a number of acridid pest species (Lomer *et al.*, 1999) and is now recognized as an appropriate product for locust control in environmentally sensitive areas (FAO, 1997). The use of oil overcame the need for high humidity, enhanced the efficacy of the fungus and provided a suitable carrier for ULV application (Bateman, 1997). In a series of operational trials against *Oedaleus senegalensis*, it was shown that, although the organophosphorus chemical fenitrothion achieves an impressive 'knock-down', hopper populations recover within 2 weeks of application, whereas a more profound population reduction was achieved in the plots sprayed at ULV rates with *Metarhizium* conidia (Langewald *et al.*, 1999).

Figure 11.4 illustrates the atomization of an oil-based suspension of Green Muscle SU formulation with an 'Ulva+' rotary atomizer (commonly used for small-scale ULV spraying in Africa). Spraying ULV formulations for locust control usually requires a droplet size of approximately 40–120 µm diameter (FAO, 1992) and, like many other rotary atomizers, this sprayer achieves > 80% of the spray volume as 40–120 µm

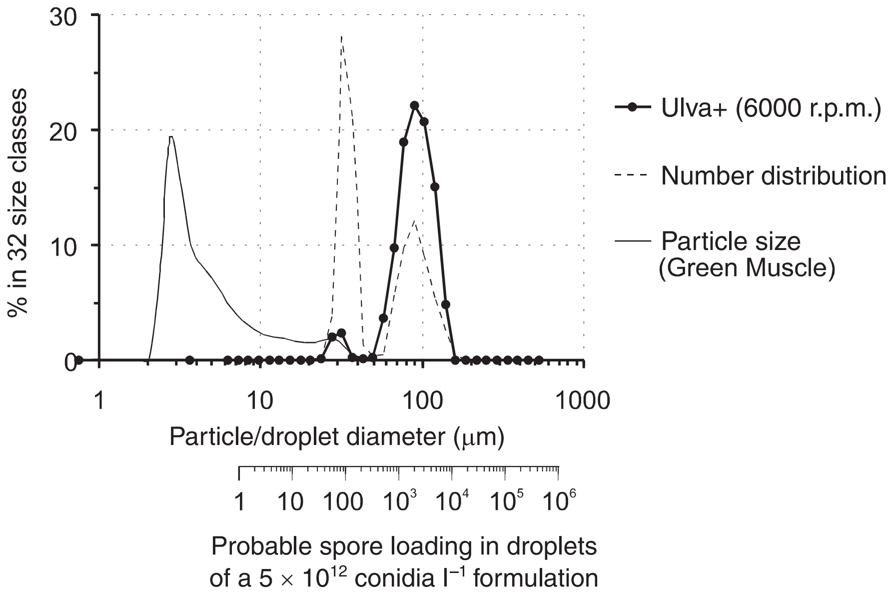

Fig. 11.4. Comparison of droplet size spectrum (volume and number distributions) of an 'Ulva+' rotary atomizer (60 ml min^{-1}) superimposed on the particle size spectrum of conidia in the 'Green Muscle' product. r.p.m., revolutions per minute.

droplets over a fairly wide range of rotational speeds (Bateman and Alves, 2000). Good-quality formulations consist of practically all single spores and most droplets contain in the region of 500–10,000 conidia with an operating concentration of 5×10^{12} conidia l^{-1} at a VAR of 1 l ha^{-1}. Only a very few droplets need to be encountered by target acridids in order to receive a dose that is lethal within 2–3 weeks.

Oil formulations of fungi such as *Paecilomyces farinosus* have been tested using other machinery, including cold foggers (Agudelo and Falcon, 1983), which are used for very small and flying insects. In Fig. 11.5 the droplet size spectra of a number of atomizers are shown juxtaposed to a particle scale of 10^{11} conidia l^{-1} (10^8 ml^{-1}). At this concentration, very few of the (aerosol-sized) droplets produced by the Microgen cold fogger would contain particles. This concentration might be more appropriate for the hydraulic nozzles illustrated, where in this scenario, a conidial application rate of 10^{13} spores might be dispersed in tank mixtures for a VAR of 100 l ha^{-1} (although with typical medium- to high-volume spraying the concentration would be up to ten times lower). The major problem here is that, at 'typical' operating pressures of around 300 kPa, much of the spray volumes of many flat-fan and commonly used hollow-cone nozzles are larger than the sizes most appropriate for covering foliage with insecticides and fungicides. For example, at 300 kPa, > 20% of the volume is in droplets of over 200 μm with the 'River Mountain' nozzle (fitted to many side-lever knapsack sprayers in China and South-east Asia). Only when high pressures (600 kPa) are applied to narrow-orifice hollow-cone nozzles (e.g. the HCX 2) are appropriate droplet spectra produced.

Figure 11.6 illustrates some of the phenomena that may occur within the tank mixture and how this might affect the contents of the droplets themselves. Preparations containing well-separated conidia are extremely important for use with ULV sprayers

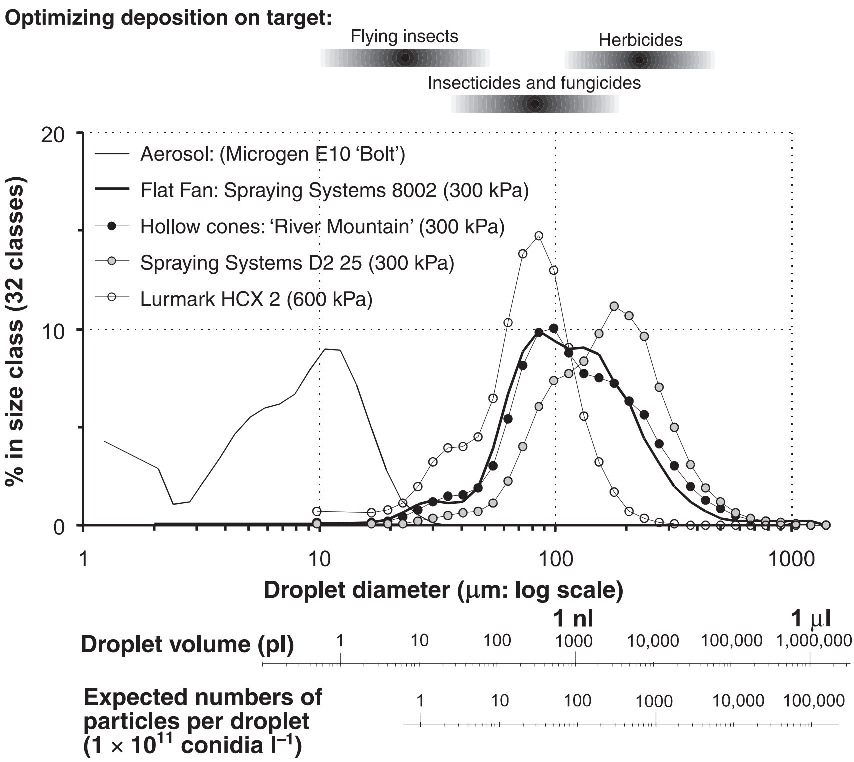

Fig. 11.5. Droplet size spectra of an aerosol generator (deodorized paraffin) and three hydraulic nozzles (water + 0.1% Agral, 300 kPa).

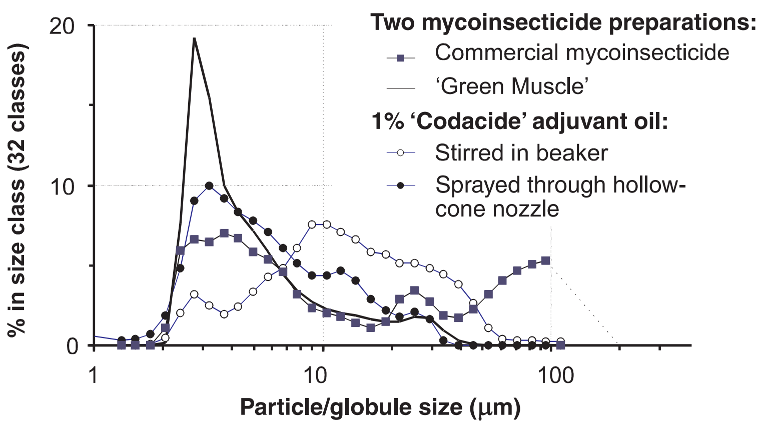

Fig. 11.6. Spectra of biopesticide formulations and emulsified oils.

and other equipment with narrow restrictions in the fluid line (Cherry *et al.*, 1999). However, problems could occur with certain commercial formulations in nozzles that produce fine sprays (quite apart from more practical filter and nozzle blockages). The action of pumping and spraying through a hollow-cone nozzle substantially broke up the globules of oil emulsions such as 'Codacide'. After passing through the sprayer, the

VMD of the globules in formulations is typically halved (and the presence or absence of conidia had little effect). This is due to the sheering force of the sprayer and pump systems, resulting in particles being more dispersed; there may therefore be an increased chance of infection with a sprayed liquid than with a formulation produced for a bioassay. Thus, although bioassays are an essential part of the development of any biological pesticide, pathogen delivery as sprayed droplets in trials is also essential.

Mycofungicides

The ultimate targets for any fungicide are the individual mycelium of the plant pathogen, but in practice this means maximum coverage on susceptible plant surfaces for protectant deposits (Mabbett, 1985). Much fungicide application research has been carried out using copper oxychloride, which being a particulate suspension may address some of the issues with microbial agents. The key practical issues are as follows:

1. Spray timing: a high work rate is especially important when protecting new foliage and young fruit, which are susceptible to infection (often by a complex of pathogens). Mabbett and Phelps (1983) found that use of low-volume and ULV applications of copper fungicides was the most practical way of achieving timely protection of seasonally induced new flushes of leaves against *Mycosphaerella citri*.
2. Spray placement: adequate coverage may be needed within the crop canopy and on the undersides of leaves (where pathogens may enter via the stomata – as in *M. citri*). Spray penetration into crops can be achieved by air assistance, provided by motorized mist-blowers and other motorized sprayers (e.g. orchard air-blast sprayers).
3. Longevity of deposit and redistribution: longevity (or persistence) is often dependent primarily on the nature of the pesticide and its formulation. The use of low-viscosity paraffinic oils (such as 'heavy alkylate' (Mabbett and Phelps, 1972)) is especially compatible with ULV application and has been shown to substantially improve the fungicide rain-fastness in comparison with water-based formulations (Mabbett and Phelps, 1983).

Fungicides perhaps require the greatest efficiency of coverage of all the pesticide types, and disease control in tree, bush and vine crops constitutes an important part of the market. Motorized knapsack mist-blowers (or air-blast sprayers) have many uses, although these sprayers were originally developed for obtaining good droplet coverage for mirid control in cocoa trees. Clayphon (1971) described the important criteria for evaluating machines, and technical requirements are now being standardized by the Food and Agriculture Organization (FAO, 1998). Because they produce relatively small droplets, mist-blowers are usually operated at lower VARs than the other types of hydraulic sprayers. They are typically used to apply water-based mixtures at 20–100 l ha^{-1} up to 250 l ha^{-1}, but low-flow-rate ULV adapters are available, achieving VARs of as little as 2 l ha^{-1} with oil-based formulations. Atomization occurs either conventionally with an air-shear nozzle or with a rotary atomizer supplied separately or (more economically) from the mist-blower manufacturers. The more expensive option is to retrofit nozzles such as the Micron 'Micronex', which can be adapted to fit on to the air outlet of most mist-blowers.

A comparison of droplet size spectra at one flow rate (200 ml min^{-1}) is shown in Fig. 11.7, with four atomizers and two formulations. The secondary *X* axis shows the probable spore loading with a 2.5×10^{10} conidia l^{-1} tank mixture concentration. This

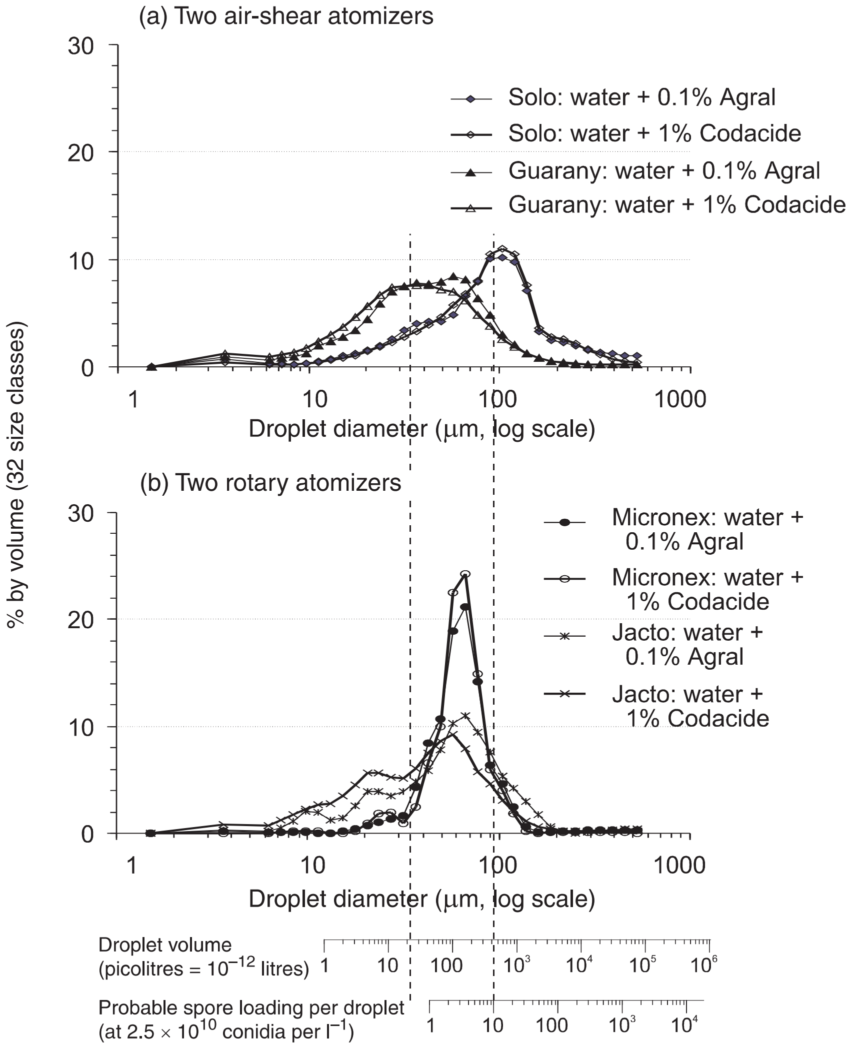

Fig. 11.7. Droplet size spectra of four motorized mist-blower nozzles, operating at 200 ml min^{-1}, comparing 1% 'Codacide' with a water + 0.1% Agral standard. The vertical hatched lines demarcate the approximate droplet sizes at which (on the left) there would be a < 50% probability of droplets containing conidia and (on the right) more than ten conidia per droplet. (From Bateman and Alves, 2000.) NB: equivalent axes.

nominal concentration would be appropriate for a VAR of 200 l ha^{-1} with a pathogen application rate of 5×10^{12} conidia ha^{-1}. Using these parameters, the proportion (by volume) of spray droplets with a < 50% chance of containing a single spore is high with sprayers, such as the 'Guarany 3.5HP', which produce a very fine spray spectrum. Not only do these very small droplets have a greatly reduced chance of impaction on leaf surfaces (because of their aerodynamic properties (May and Clifford, 1967)), but also the adjuvants contained in this spray volume will effectively be wasted (Chapple and Bateman, 1997). Droplets > 91.5 µm will probably contain more than 10 spores, and this has been used as an (arbitrary) upper limit for an 'optimal' droplet size range covering an order of magnitude of dose variation. By using a rotary atomizer, such as the 'Micronex', the volume of spray liquid contained in droplets that contain one to ten spores (effectively dispersing and impacting them on leaves) may be doubled in comparison with a simple air-shear nozzle.

Mycoherbicides

One of the top priorities in herbicide spraying is the accurate placement of larger (> 150 µm) droplets, with minimal drift to non-target plants. If the ground is the target (e.g. pre-emergence or systemic herbicides), control of droplet size may be relatively unimportant provided there are no small driftable (< 100 µm) droplets: a range of low-pressure hydraulic nozzles is available that produce drop spectra with the majority of the spray volume as larger droplets, and small droplet production can be eliminated with rotary or 'kinetic' nozzles (e.g. the dribble bar or watering-can). If weed leaves are the target, there is a need to balance the low-drift requirement with a droplet size that is small enough to be retained by the leaf surface.

Lake (1977) showed that small (100–200 µm) droplets are substantially more easily retained on water-repellent leaves (such as those of *Avena fatua*) than larger (300–400 µm) droplets, with water-based formulations of high surface tension. The interactions between droplet size and other application variables on the biological performance of foliage-applied chemical herbicides was examined by Merritt (1980). He also showed that only gross differences in droplet size (400 µm vs. 100 µm) affect performance: the need for small droplets can be reduced with suitable formulations containing surfactants to improve spray retention. In general, greater amounts of a.i. are retained as VAR is decreased; however, formulations applied at very high concentration may cause leaf scorch, reduced uptake of herbicide and thus a reduction in biological performance. In fact, the relationship is more complex. The literature has been reviewed recently by Knoche (1994).

For mycoherbicides, the production of excessively large droplets effectively 'wastes' a large proportion of particles. Since many mycoherbicidal fungi can only be produced economically for low rates of application, large droplets may be very inefficient, and a narrow-spectrum CDA sprayer, such as the 'Herbi', might be appropriate. This was recognised by Lawrie *et al.* (1997), who applied conidia of *Stagonospora* sp. and *Micocentrospora acerina* at 2×10^9 and 5×10^8 conidia l^{-1} using a Micron 'Micromax 84' (a tractor-mounted rotary atomizer).

A commonly adopted technique to maintain a moist environment around propagules is the use of humectants (such as guar gum) and other adjuvants (including oils). The spray analyses of the 'Herbi' shown in Fig. 11.8 indicate that a gum humectant is detrimental to both the size and the quality of the droplet spectrum; on the other hand, the use of an emulsified oil, such as 'Codacide', reduces the VMD, but maintains a narrow droplet spectrum. Using a hypothetical formulation containing 1×10^9 particles l^{-1}, the secondary *X* axis indicates that there is considerable potential for wasting inoculum with the production of excessively large drops of > 500 µm, each containing > 100 propagules.

There is currently much interest in minimizing spray drift, and low-drift nozzles, such as the 'Turbo Teejet', may be useful for certain biopesticides that are prone to cause nozzle blockages (Bateman *et al.*, 2000). However, as indicated in Fig. 11.8, such nozzles may also distribute inoculum inefficiently.

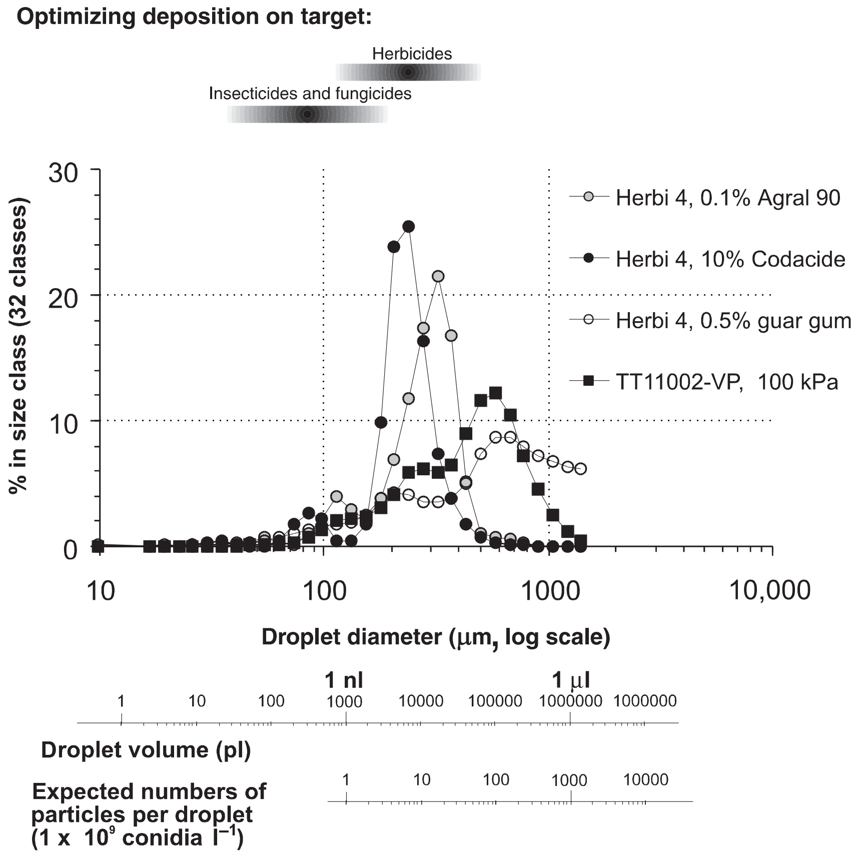

Fig. 11.8. Possible examples of herbicide application, including three formulations atomized by the Micron 'Herbi 4' and water + 0.1% Agral atomized by a Spraying Systems 'Turbo Teejet' with a 10^6 conidia ml^{-1} formulation.

It is interesting that similar techniques and problems have been encountered with studies on entomopathogenic nematodes. Mason *et al.* (1998) examined the numbers of infective juveniles (IJs) in the output of spinning-disc atomizers and found that, at lower concentrations (the highest being 1.2×10^7 IJs l^{-1}), many droplets contained no nematodes. Chapple *et al.* (2000) point out that large particles (such as nematodes, and thus presumably macroconidia or hyphae) can influence droplet production.

Conclusions

Conventional hydraulic nozzles are the mainstay of pesticide application techniques. However, from a theoretical point of view, reliance on them may severely reduce the potential for environmentally benign biological agent activity – hence the references to certain 'novel' techniques. However, changes in policy and practice to limit pesticide drift (especially in Europe and North America) effectively encourage the use of larger droplet sizes in ordinary spray nozzles. Not only has 'exo-drift' been reduced at the expense of 'endo-drift', but this also imposes substantial burdens on the development of these environmentally advantageous microbial agents. Clearly, fundamental questions must be answered about the future role of biopesticides in farming systems, including 'Is drift an issue at all with biopesticides?'

In general, the only available spray application system for mycopesticides is that system in widespread use in the target crop in the geographical area of interest or local market. This also applies where a mycopesticide is being included with other pesticides (e.g. as part of an integrated pest management (IPM) system). Alternative application systems (e.g. CDA, mist-blower, etc.) often have substantial advantages over the hydraulic nozzle so must not be ignored, especially for niche markets where either no application system exists or where other application systems are ineffective. It has long been recognized that there is considerable grower resistance to investing in specialized equipment (Parish, 1970); although it not impossible to change application practice, the benefits have to be clearly demonstrated. A grower can be asked to change the nozzle, the VAR and other parameters, such as nozzle pressure and forward speed. With biological control agents such as mycopesticides, it may be possible to ask more of growers than would normally be asked when applying conventional agrochemicals. However, there are limits, and these must be explored properly before making label recommendations.

The use of oil formulations and adjuvants has been shown to enhance the activity of several mycopesticides. Since it would be impractical and uneconomical to use oils in conventional medium- to high-volume application equipment, the use of emulsified oils may provide a technical solution. However, trials with *Beauveria bassiana* products in North America have produced indifferent results (Wraight and Carruthers, 1999) and further research is needed.

Substantial work has been carried out to increase spray coverage with air-assisted sprayers and has been reviewed in a symposium by Lavers *et al.* (1991). Motorized knapsack mist-blowers produce relatively small droplets and are usually operated at lower VARs than the other types of hydraulic sprayer. They can achieve very good coverage, but, when used for medium- rather than low- or very-low-volume applications, they may use (expensive) adjuvants inefficiently in tank mixtures containing microbial pesticides (Chapple and Bateman, 1997).

On a larger scale, various tractor-boom-mounted air-assist nozzles have been developed, often with the aim of reducing drift with chemical pesticides, but also achieving increased canopy penetration and under-leaf coverage by air entrainment into field-crop canopies. Taylor and Andersen (1997) describe the benefits of spray booms where a perforated sleeve provided a curtain of air alongside the hydraulic nozzles and projected the spray into the crop. Less draconian modifications to spray booms include twin fluid nozzles, such as Cleanacres 'Airtec' deflector nozzles (which also substantially reduce drift), or vehicle-mounted booms with drop legs that pass in between crop rows (e.g. as produced by Benest Engineering). These nozzles also require a compressor and their use with biopesticides is discussed further in Bateman *et al.* (2000). Instead of using air to entrain fine droplets into crop canopies, the use of coarse sprays from conventional hydraulic tips in a 'double nozzle' system has been proposed by Chapple *et al.* (1996); this might also avoid the need for surfactants in oil-based mycopesticide formulations, but this device is only just becoming available commercially.

The development of other techniques for enhancing biological pesticides has been advocated, but requires further research and awaits the availability of commercial equipment. Electrostatic charging of sprays may improve under-leaf coverage and ED nozzles offered benefits of low maintenance and power requirements in comparison with other CDA systems (Coffee, 1981). However, the ED is no longer available commercially and, in any case, the polar solvents used in ED formulations are toxic to biological agents.

Failure to consider the numerical aspects of dose transfer of particles during application may be catastrophic during the testing of new microbial control agents. Although there is potential for substantially improving the efficacy of biopesticides with better delivery systems, this is limited (probably to less than ten times) and must be constrained by practical considerations, such as acceptability to growers.

Note

[1]Lutte Biologique contre les Locustes et les Sauteriaux: a collaborative, multidisciplinary research and development programme funded by the governments of Canada (CIDA), the Netherlands (DGIS), Switzerland (SDC) and the UK (DfID).

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