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Pesticide Application

Delivery systems for Mycoinsecticides Using Oil-based Formulations

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Summary

The use of oils has been shown to enhance the efficacy of myco - insecticides, herbicides and hyperparasitic fungicides. Oils can also enable fungal pathogens to remain active under conditions of low humidity, and thus create opportunities for expanding the (presently limited) range of mycopesticide applications. Formulations of mycoinsecticides in oil present special opportunities when used in ultra-low volume (ULV) applications - and have been proven for acridid control (using aerial conidia of *Metarhizium anisopliae* var. *acridum*). However, for most of the world's agriculture, other nozzles such as hydraulic and air-shear atomisers are the main-stay of pesticide application. More research is required to evaluate the use of emulsified oils, and this study focuses on motorised sprayers, capable of achieving good coverage at acceptable (low-medium volume) rates of application. We present some data on the atomisation of oil-based formulations, comparing standard rotary sprayers with various motorised mistblowers. The principles of delivering microbial agents to their target sites are illustrated by estimating the numbers of spores "packed" into each droplet size class. From a theoretical point of view, reliance on large droplets may severely reduce the potential for environmentally benign biological agent activity. Moves to limit pesticide drift will impose further burdens on their development and fundamental questions must be answered about the future role of biopesticides in farming systems.

Key words: Mycoinsecticides, *Metarhizium anisopliae*, oil formulation, ultra-low volume, controlled droplet application (CDA), motorised knapsack mistblowers, spray drift

Introduction

The potential for development of mycopesticides has been boosted by the discovery that fungal conidia formulated in oils have shown greater infectivity than conventional water-based suspensions. Oils can substantially enhance the efficacy of entomopathogens against insects (Prior *et al.*, 1987), hyperparasitic fungi (Hofstein and Chapple, 1998) and mycoherbicides (Amsellem *et al.* 1990). Burges (1998) gives a comprehensive review of the formulation of biopesticides and further details on oil formulation. Being non-evaporative, their use is readily compatible with ultra-low volume (ULV) application techniques for spraying mycoinsecticides at low relative humidities (Bateman, 1997). This property has been exploited by the LUBILOSA[*] programme, which is dedicated to the replacement of chemical pesticides with environmentally friendly alternatives for locust and grasshopper control, and has developed the lipophilic conidia of the mitosporic (Deuteromycete) fungus *Metarhizium anisopliae* var. *acridum* into a product called 'Green Muscle®'.

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ULV spraying of oil-based formulations constitutes the normal delivery system for acridid pests. This approach has been field-tested and Green Muscle® has proved efficacious against a wide range of locusts and grasshoppers (Lomer *et al.*, 1999) at volume application rates (VARs) of as little as 0.5 l/ha (Langewald *et al.*, 1999). 'Green Muscle' had no effect on Carabidae, Tenebrionidae, Formicidae and Epydridae which were monitored during large scale field trials in Niger (Peveling *et al.*, 1999) and *M. anisopliae* isolate IMI 330189 can be classified as a "low risk" insecticide for all known classes of non-acridid, non-target organisms.

The precise role of oil in the infection process is incompletely understood; Ibrahim et al. (1999) noted that they appeared to extract fungistatic and stimulatory compounds from insect cuticles. After spraying mustard beetles in the laboratory, they also observed improved transport of conidia to areas of thinner cuticle (*e.g.* under the elytra). However in the field, direct impaction of spray droplets may constitute a less important mode of delivery than secondary acquisition of residues from treated plants (Bateman *et al.*, 1998). For either of these modes of action, controlled droplet application (CDA) techniques are crucial for successful spraying at such low VARs (practically never more than 2.5 l/ha for locust control).

CDA represents a very specialised mycopesticide delivery system, with limited opportunities for development of similar techniques against other pests since oil formulations can only be used with specialised application equipment (often rotary atomisers). An important challenge over the next few years will be the development of efficacious water-miscible formulations, which can be used reliably with conventional hydraulic sprayers on field crops. Very promising results have been obtained with oil-based adjuvants containing non ionic emulsifiers (Alves *et al.*, 1998), but the ultimate goal will be the preparation of ready-to-use suspo-emulsion (SE) formulations that are miscible with water, and have handling characteristics similar to chemical products.

Motorised 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 standardised by FAO (1998). The most common design of nozzle is of the air-shear type, in which thin layers of liquid are introduced into the air stream and thus produce fine sprays. The combination of air assistance and production of relatively small droplets, enable motorised mistblowers to achieve good coverage at low VARs (*i.e.* without "spraying to run-off": G.A. Matthews, these proceedings). They are typically used to apply water based mixtures to trees at VLV rates (50-200 litres/ha), but low flow rate ULV adapters are available, achieving VARs of as little as 2.5 litres/hectare with oil-based formulations (*e.g.* for brown locust control in South Africa). Atomisation occurs either conventionally with an air-shear nozzle, or with a rotary atomiser: supplied separately or from the mistblower manufacturer. A more expensive option is to retro-fit nozzles such as the Micron 'Micronex' unit, which can be adapted to fit onto the air outlet of most mistblowers. Some manufacturers have also sought to improve atomisation of air shear nozzles, by altering the shape of the formulation feed tube in the air-stream.

The ramifications of spray application parameters, and how they might be influenced by formulation, are illustrated in Fig. 1. This has been influenced by Young's (1986) illustration describing the application of chemical pesticides, but emphasises the role of formulations, and that target pests can acquire pathogens by direct impaction, secondary pick-up or secondary cycling of infective propagules. In this paper, we describe systems for delivery of mycoinsecticides, especially focusing on oil-based formulations of *Metarhizium anisopliae* isolates and their atomisation. Two hand-held ULV sprayer nozzles (proven for locust control) are compared with a range of air assisted knapsack mistblowers that provide a suitable application technique for tropical perennial crops (which constitute a promising new market for biopesticides).

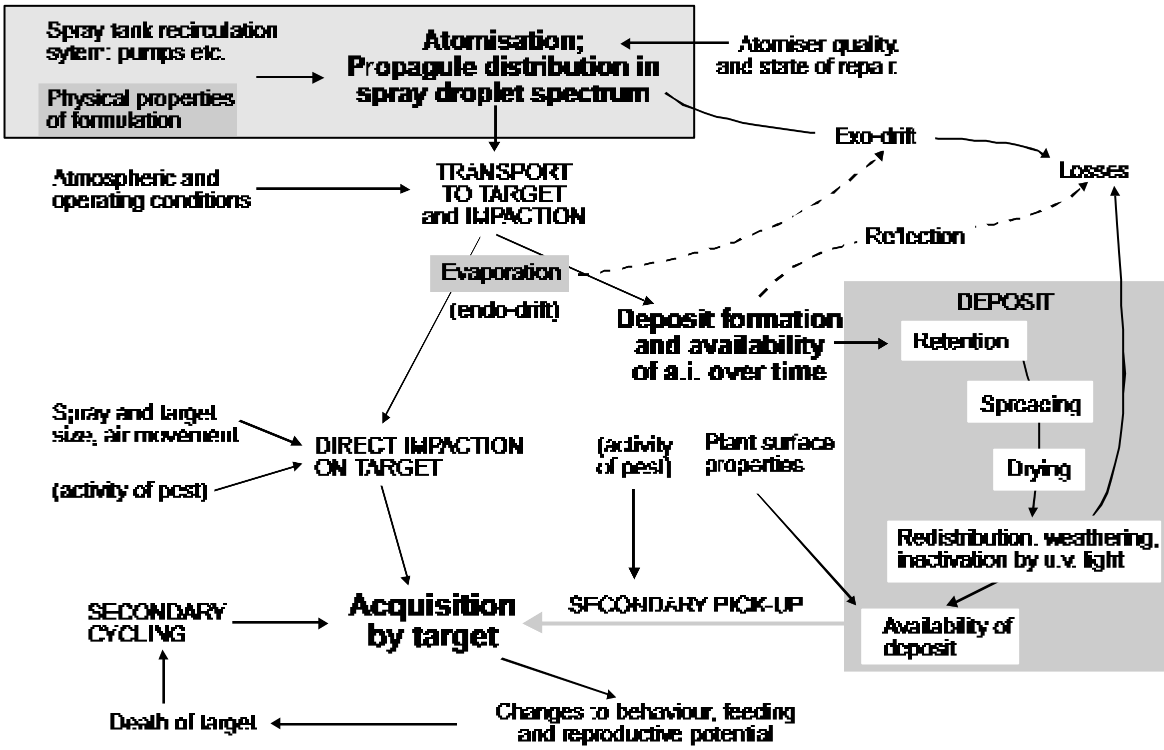

Fig. 1. Spray application processes and biopesticide formulations. Shaded zones represent effects which can be manipulated by formulation.

Materials and Methods

A series of droplet size measurements were carried out, using appropriate formulations and their blank equivalents, with a range of nozzles. Two mycopesticide delivery systems were considered:

1. ULV application using two standard ULV nozzles: the Micron Ulva+[2] and the Berthoud C5[3] (where the actual rotor is the same as the older C8). Green Muscle® SU consists of dry aerial conidia (available in sachets containing a measured quantity of product) are mixed with suitable oil carrier. For most field trials, spores have been suspended in a mixture containing equal volumes of two paraffinc oils: Ondina EL[4] (which is non evaporative and viscous) and Shellsol T (to adjust viscosity for ULV sprayers). Most recent trials have been carried out using 100 g (5×10^{12} conidia) in 1 litre formulation per hectare, but there has been interest in reducing this dosage to 50 g.a.i./ha in order to reduce costs.

2. The atomisation of four contrasting motorised mistblower nozzles was investigated:

Sprayer	*nozzle type*	*air velocity*
Solo 412 'JetPack'	air shear	86 m/s
Guarany 3.5 HP	air shear, shaped nozzle	83 m/s
Micron 'Micronex'	rotary nozzle fitted to the Solo 412 (as above)	
Jacto PL 50-BV	rotary (fitted as standard)	66 m/s

A spray mixture containing 1% 'Codacide'[5] oil was compared with a standard consisting of water +0.1% 'Agral'[6], which has been used frequently to evaluate spray nozzles (*e.g.* Arnold, 1983). 'Codacide' consists of rape-seed oil with 5% non-ionic emulsifier, which has been shown in the laboratory to be a non-toxic and potentially useful carrier for *Metarhizium* conidia (Alves, 1999). A 1% mixture would,

[2] Available from: Micron Sprayers Ltd., Three Mills, Bromyard, Herefordshire, HR7 4HU, UK
[3] Berthoud S A, B.P. 424 F 69653, Villefranche Cedex, Z.I. Nor Amas, F 69400, France
[4] Shell U.K. Ltd., Delta House, Wavell, Rd., Wythenshawe, Manchester M22 5SB, UK
[5] Microcide Ltd, Shepherds Grove, Stanton, Suffolk, IP31 2AR, UK
[6] Zeneca Agrochemicals, Fernhurst, Haslemere, Surrey, GU27 3JE, UK.

for example, be appropriate for a VAR of 200 l/ha (the boundary between VLV and LV spraying for tree crops) with an adjuvant application rate of 2 l/ha.

Droplet size spectra were measured with a Malvern[7] 2600 particle size analyser. The instrument was fitted with a 300 mm lens and each reading comprised of 1000 scans (equivalent to sub samples). Measurements were repeated at least once on a separate occasion to check for consistency and are presented here as means. Numbers were exported electronically into a data-base in the form of cumulative volume distributions over 32 size classes. Nozzles were directed horizontally and positioned approximately 250 mm in front of the beam. A 300 mm axial fan situated at the rear of the apparatus withdrew spray away from the sampling area (in order to minimise operator exposure to spray and prevent small droplets re-circulating in the beam). A background reading was taken with the mistblower engine operating, but not spraying.

In all experiments, formulations were delivered, from a pressurised (50 kPa) tank to the atomisers via a 'gap meter'[8] flow valve, in order to standardise flow rates. For low viscosity formulations, this method produced flow with a measured tolerance of <±10% at flow rates >30 ml/min.

Results and Discussion

Fig. 2 illustrates the atomisation of three versions of the oil-based Green Muscle SU formulation (blank oil, and two concentrations of spore suspension), with two rotary atomisers commonly used for small scale ULV spraying in Africa. Readings were taken over a range of voltages and the results are summarised using the VMD ($D_{[v,0.5]}$), together with the 10% ($D_{[v,0.1]}$) and 90% ($D_{[v,0.9]}$) volume percentiles (*i.e.* the values used to calculate the relative span). Spraying ULV formulations for locust control usually requires a droplet size of approximately 40-120 µm diameter (FAO, 1992) represented here by a shaded zone. The estimated numbers of conidia per droplet are illustrated by secondary Y axes for the two "live" formulations; droplets in the optimal range will typically contain between 150 - 3000 conidia. Although the Berthoud disc produced broader spectra at the slower speeds, both atomisers produced satisfactory droplet size spectra in the 5000 - 9000 rpm range; this is equivalent to approximately 6-10 v for the Ulva+ and 5-8 v for the Berthoud C5. In practice, disc speed is governed by adjusting the number of batteries used, but may also be substantially dependent on other factors including the flow rate and condition of both the 'D' cells used and the atomiser itself.

Fig. 3 shows the droplet size spectra produced by motorised mistblowers at a range flow rates with water + 1% 'Codacide'. At lower flow rates (≤ 500 ml/min) the air shear nozzles produced broad droplet size spectra that improved with increased flow rate with an accompanying increase in VMD. In contrast, the rotary 'Micronex' atomiser produces very narrow spectra at low flow rates, but when used at higher rates (>500 ml/min) there is no benefit to fitting this nozzle. With its powerful 3.5 HP engine and shaped formulation delivery opening, the Guarany machine produced the smallest droplets at all flow rates.

A more detailed comparison of droplet size spectra at one flow rate (200 ml/min) is shown in Fig. 4, with four atomisers and two formulations. The X axis is accompanied by two separate scales indicating: the equivalent droplet volume in picolitres and the probable spore loading with a 2.5 x 10^{10} conidia/l tank mixture. This nominal concentration would be appropriate for a VAR of 200 l/ha with a pathogen application rate of 5 x 10^{12} conidia/ha. Using these parameters, Table 1 shows estimates of the proportion (by volume) of spray droplets with a <50% chance of containing a single spore (those with a diameter of <34µm). Not only do these very small droplets have a greatly reduced chance of impaction on leaf surfaces (because of their aerodynamic properties: May & Clifford, 1967), but also the adjuvants contained in this spray volume will effectively be wasted (Chapple & Bateman, 1997). Droplets >91.5µm will probably contain >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.

[7] Malvern Instruments Ltd., Spring Lane South, Malvern, Worcs., WR14 1AT, UK
[8] CT Platon Ltd., Jays Close, Viables, Basingstoke, RG22 4BS, UK

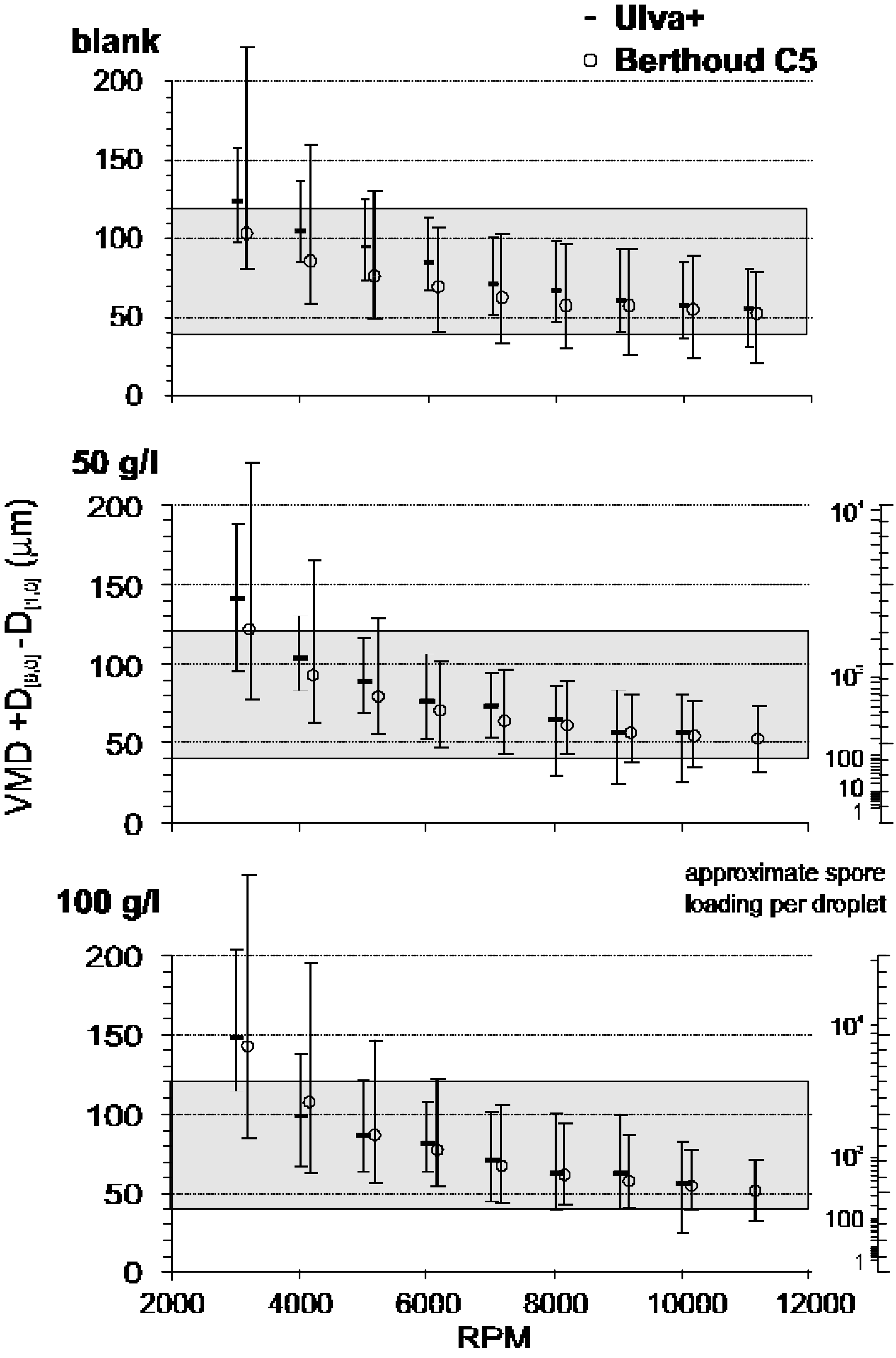

Fig. 2. Comparison of droplet size spectra for two rotary atomisers with three oil-based formulations of 'Green Muscle' SU at 60 ml/min. Readings were taken between 3000 and 11000 RPM at 1000 RPM intervals. Shaded zones indicate the 40 - 120 µm range (see text).

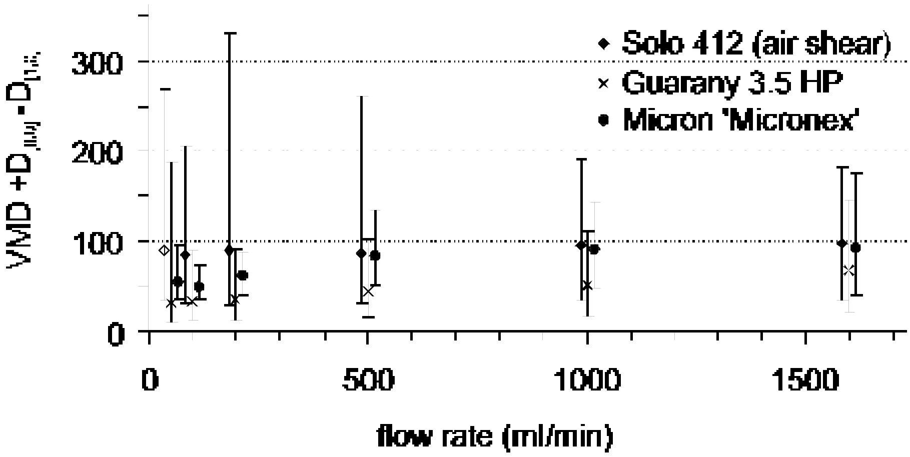

Fig. 3. Motorised mistblowers: machinery factors. The VMD + $D_{[9,0]}$ - $D_{[1,0]}$ is shown using water + 1% 'Codacide' from three motorised mistblowers at flow rates of 50, 100, 200, 500, 1000 and 1600 ml/min.

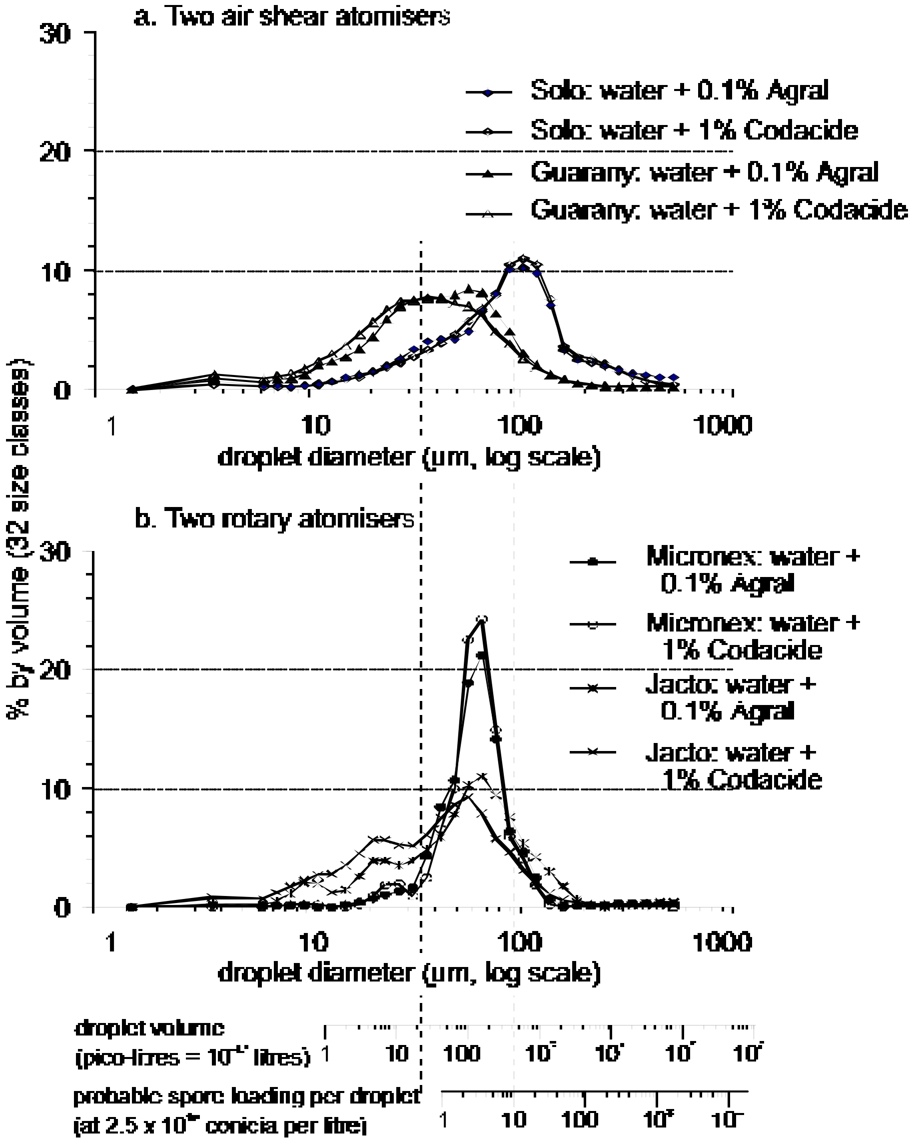

Fig. 4. Droplet size spectra of four motorised mistblower nozzles, operating at 200 ml/min, comparing 1% 'Codacide' with a water + 0.1% Agral standard. The vertical hatched lines demarcate the approximate droplet sizes at which there would be a <50% probability of droplets containing conidia and >10 conidia (see text).

Table 1 *Percent by volume of droplets unlikely to contain spores (diameter of <34 µm) and those in "optimal" 34-91.5 µm size band (see text)*

Sprayer	spray volume with <34µm droplets		volume in 34-91.5µm size band	
adjuvant:	1% 'Codacide'	0.1% Agral	1% 'Codacide'	0.1% Agral
Solo 412 'JetPack'	13	15	39	34
Guarany 3.5 HP	47	40	44	50
Micron 'Micronex'	7	6	85	85
Jacto PL 50-BV	42	27	49	54

Using these criteria, there is clearly a benefit to using rotary atomisers, with the "Micronex" giving the narrowest droplet size spectra and maximising the volume of droplets (85%) in the optimum range. In general, the adjuvant had little effect on droplet spectrum, although the incorporation of 'Codacide' oil tended to reduce droplet sizes with the Jacto nozzle over a range of flow rates (Alves, 1999). In contrast, droplet size is often increased by the addition of emulsified oils (and other adjuvants) with hydraulic nozzles. Butler Ellis *et al*, (1997) showed that this resulted from a general increase in droplet size spectra with flat fan tips.

Bateman, Luke & Alves (1999) confirmed that the same effect applies to hollow cone nozzles, and that the presence or absence of conidia in the formulation made negligible differences to droplet spectra. This study indicated that the absence or presence of spores, at concentrations of up to 100 g/l, likewise made little difference to the rotary atomisation of oil-based formulations. This is useful, since sprayer evaluation using 'blank' equivalents rather than live formulations may overcome certain practical difficulties, including quarantine restrictions and limited supplies of experimental material.

This paper has described the production of small droplets, carried air currents to their site of action: either in the form of wind drift or blown with a fan. Small droplets are known to be more efficacious than larger ones (Munthali & Wyatt, 1986) and this probably applies to biopesticides (Chapple *et al.*, 1994). Several papers in these proceedings reflect recent emphasis in application research, which has focused on the reduction of spray drift. The most commonly implemented solution to the drift "problem" has been the use of hydraulic nozzles that increase droplet size spectra (without necessarily improving spray quality). Large droplets (>200 µm) have long been known to have a increased risk being wasted, by bouncing off foliage and falling to the ground (Brunskill, 1956). In the example illustrated in Fig. 4, enlarged droplets in the 200-900 µm diameter range would each expend in the orders 100 - 10,000 infective particles. This increase in inefficiency of spray application could be especially disastrous if such nozzles were used to apply to relatively expensive, slow acting biopesticides. Because of their specificity, the environmental risks associated with biopesticides can be considered to be substantially less than conventional chemicals and they should therefore enjoy the benefits of efficient application methods using small droplets.

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References

Alves R T 1999. Development of mycoinsecticide formulations and application techniques appropriate for pest control. PhD thesis, University of London, 225 pp.

Alves R T, Bateman R P, Prior C. 1998. Performance of *Metarhizium anisopliae* formulations with oil adjuvants on *Tenebrio molitor*. *Proceedings 5th International Symposium on Adjuvants for Agrochemicals*. Held at Memphis, Tennessee, USA; Aug. 1998.

Amsellem Z, Sharon A, Gressel J, Quimby P C. 1990. Complete abolition of high inoculum threshold of two mycoherbicides (*Alternaria cassiae* and *A. crassa*) when applied in invert emulsion. *Phytopathology* **80**: 925-929.

Arnold A C. 1983. An evaluation of fourteen hand-held battery-operated spinning disc sprayers, *Tropical Pest Management*, **29**: 105-121

Bateman R P, Douro-Kpindu O K, Kooyman C, Lomer C, Oambama Z. 1998. Some observations on the dose transfer of mycoinsecticide sprays to desert locusts. *Crop Protection* **17**: 151-158.

Bateman R P. 1997. Methods of application of microbial pesticide formulations for the control of grasshoppers and locusts. *Memoirs of the Entomological Society of Canada* **171**: 67-79.

Bateman R, Luke B, Alves R. 1999. Some observations on the application of oil-based formulations of mycoinsecticides. Proceedings: "*Spray oils beyond 2000: Sustainable pest and disease management*" Eds. G.A.C. Beattie & D.M Watson, University of W. Sydney, Australia.

Brunskill R T, 1956, Physical factors affecting the retention of spray droplets on leaf surfaces, *Proceedings of the 3rd. British Weed Control Conference (vol. 2)*: 593-603.

Burges H D. (ed.) 1998. *Formulation of Microbial Biopesticides, beneficial microorganisms, nematodes and seed treatments*. Publ. Kluwer Academic, Dordrecht, 412 pp.

Butler Ellis M C, Tuck C R, Miller P C H. 1997. The effect of some adjuvants on sprays produced by agricultural flat fan nozzles. *Crop Protection*, **16**: 41-50.

Chapple A C, Bateman R P. 1997 Application systems for microbial pesticides: necessity not novelty. In "*Microbial Insecticides: Novelty or Necessity?*" *British Crop Protection Council Proceedings/Monograph Series* **No. 68**: 181-190.

Chapple AC, Taylor R A J., Hall F R, Downer R A. 1994. Understanding the dose transfer process as a prerequisite to a more predictable lab-to-field performance correlation. In: *Comparing Laboratory and Field Pesticide Performance II. BCPC Monograph No. 59*. H G Hewitt. J Casely, L G Copping, B T Grayson and D Tyson (eds.): 267-272.

Cherry A, Jenkins N, Heviefo G, Bateman R, Lomer C. 1999. A West African pilot scale production plant for aerial conidia of *Metarhizium* sp. for use as a mycoinsecticide against locusts and grasshoppers. *Biocontrol Science and Technology*, **9**: 35-51

Food and Agriculture Organisation of the United Nations (FAO) 1992. The desert locust guidelines, IV: Control. Food and Agriculture Organisation Booklet, Ed. P. Symmons, 64 pp.

Food and Agriculture Organisation of the United Nations (FAO) 1997. Evaluation of field trial data on the efficacy and selectivity of insecticides on locusts and grasshoppers. *Report to FAO by the Pesticide Referee Group*, 6th meeting, Rome, 10-12 December 1996. 21 pp.

Hofstein R, Chapple A. 1998. Commercial development of biofungicides. In *Biopesticides: Use and Delivery* Eds. F.R. Hall & J.J. Menn. Humana Press, Totowa, NJ, USA, **Ch. 6**: 77-102.

Ibrahim, L., Butt, T.M., Beckett, A., Clark, S.J. 1999. The germination of oil-formulated conidia of the insect pathogen *Metarhizium anisopliae*. *Mycological Research*, **103**: 901-907.

Langewald J, Ouambama Z, Mamadou A, Peveling R, Stolz I, Bateman R, Attignon S, Blanford S, Arthurs S, Lomer C. 1999. Comparison of an organophosphate insecticide with a mycoinsecticide for the control of *Oedaleus senegalensis* (Orthoptera: Acrididae) and other Sahelian grasshoppers at an operational scale. *Biocontrol Science and Technology*, **9**: 199-214.

Lomer C J, Bateman R P, Dent D, De Groote H, Douro-Kpindou O-K, Kooyman C, Langewald J, Ouambama Z, Peveling R, Thomas M. 1999. Development of strategies for the incorporation of biological pesticides into the integrated management of locusts and grasshoppers. *Agricultural and Forest Entomology* **1**: 71-88.

May K R, Clifford R. 1967. The impaction of aerosol particles on cylinders, spheres, ribbons and discs. *Annals of Occupational Hygiene*, **10**: 83-95

Munthali D C, Wyatt I J 1986. Factors affecting the biological efficiency of small pesticide droplets against *Tetranychus urticae* eggs. *Pesticide Science*, **17**: 155-164.

Peveling R, Attignon S, Langewald J, Ouambama Z. 1999. An assessment of the impact of biological and chemical grasshopper control agents on ground-dwelling arthropods in Niger, based on presence/absence sampling. *Crop Protection*, **18**: 323-339

Prior C, Jollands P, Le Patourel G. 1988. Infectivity of oil and water formulations of *Beauveria bassiana* (Deuteromycotina; Hyphomycetes) to the cocoa weevil pest *Pantorhytes plutus* (Coleoptera: Curculionidae). *Journal of Invertebrate Pathology* **52**: 66-72.