# A review of work carried out relevant to the use of CDA for application of biological products

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Although there have been previous reviews and discussions of the application of biological products (Steinke and Giles 1995, Ignoffo and Falcon 1976, Matthews 1981) there have been no recent specific reviews on the use of Controlled Droplet Application (CDA) techniques for the application of biological products, although Ahmed and Leather (1994) concluded that "... all the micro-organisms can be applied by conventional equipment, but recent findings suggest benefits are obtained by ULV/CDA methods". This review attempts redress this by gathering together and summarising various literature which is relevant to the application of biological products using CDA and low or ultra-low volume techniques. It is not claimed to be exhaustive and it is recognised that there are many instances where commercial scale application has gone unreported in scientific literature.

## *Bacillus thuringiensis (Bt)*

Most commercial application of *Bt* using CDA has been via aerial application techniques in large scale treatment against forest pests. This is because a) the environmental constraints faced in the blanket treatment of large areas in such operations which mean that there is a greater tendency to use "softer" or biological sprays; and b) the logistical pressures of aerial application encourage the adoption of low volume application techniques.

Various workers report the large scale application of *Bt* through Micronair, spinning cage atomisers - a CDA aerial application method, e.g. Cadogan (1993), Cadogan *et al* (1995), Anon (1995), Montermini *et al.* (1993), Sundaram (1993), Payne et al (1996), Bernier et al (1990) and it is believed that many more instances go unreported as they are routine commercial applications. Typical application rates are 1.5-3.0 l/ha (total volume) using formulations of Foray, Dipel and Futura XLV with good control being reported. Laboratory studies indicate that oil-based formulations give better recovery (correlating with better control in bioassay) than aqueous formulations. It is suggested by Sundaram *et al.* (1993) that oil-based formulations are more efficient at impinging on foliage and show better rainfastness - possibly due to reduced reflection of droplets compared with aqueous formulations of high potency which can evaporate forming hard walls which make them more prone to bouncing off surfaces upon which they impact. In addition it is believed that the oil carrier helps drops to interact better with the waxy cuticle of the foliage than an aqueous carrier, thus enhancing drop retention and adhesion. The increased viscosity of oil-based formulations may reduce atomisation efficiency, however, and it has been shown that the performance of rotary screen atomisers may be adversely affected in their ability to produce smaller droplets.

Lewis (1983) comments that "microbials such as *Bt* and Baculoviruses achieve their maximum effect by thorough and stable coverage of the feeding zone with droplets of the proper size containing sufficient active material to cause lethal infections." A dose transfer model described by Hall *et al.* (1994) suggests that for *Bt*, application efficiency is inversely proportional to droplet size - i.e. "small is better". This is supported by various lab-based studies e.g. Smith *et al.* (1977a), Steinke and

Akesson (1993), Chapple *et al.* (1994), all of which indicate that effectiveness increases with reduced droplet size. Aston (1989) probably provides the most comprehensive study of the effects of droplet size and density on the activity of *Bt*. Using spinning disc rotary atomisers he found that there is a disproportionate partitioning of *Bt* infective crystals into smaller droplets with the number of crystals entering a droplet being proportional to the square of the droplet diameter rather than the diameter cubed as might be expected. Hence the concentration of *Bt* infective units increases as the droplet size decreases and this may partially explain why smaller droplets tend to improve performance. This effect may be related to another phenomenon observed which is an apparent loss of 40-70% of *Bt* infective crystal particles during the atomisation process - an effect also reported by Smith *et al* (1977b). Although Aston (1989) comments that this seems to be independent of the atomisation process, studies using hydraulic nozzles, (Smith *et al* 1977b), found that the biological effectiveness of a *Bt* spray in the field decreased when sprayed through hydraulic nozzles when spray pressure was increased even though the plant coverage increased with larger volumes and densities being deposited. It was suggested that shear forces caused by the higher pressures caused a greater loss of *Bt* crystals.

Deposition and feeding studies (Aston 1989, Bryant and Yendol 1988) have shown that the rate of uptake and ingestion of *Bt* is crucial to its success. If uptake is too slow this can lead to a feeding inhibition response being initiated before the insect has consumed a lethal dose of toxin. As *Bt* is relatively non-persistent, by the time feeding is resumed the *Bt* deposits on treated foliage may be inactivated. At the other extreme, ingestion of excessively high doses of toxin may cause a vomiting response on the part of the insect and also prevent mortality. Schmidt (*pers comm*) has indicated that the "dose per bite" is the crucial factor in ensuring high mortality. Another effect reported by Aston (1989) is that of feeding avoidance which increases with decreased droplet density. He comments that good coverage is necessary to minimise feeding avoidance effects. It is apparent, therefore, that there will be an optimum combination of droplet size, density and concentration for the most effective application of *Bt* for any given crop / pest situation.

Chapple *et al.* (1994) comment that "it is clear that the advantages of increased efficiency from applying AI (of *Bt*) in smaller drops as shown in the laboratory and glasshouse have probably not been exploited to the fullest." Smith *et al.* (1977a) found that 90µm droplets gave consistently better results than 180 or 270µm droplets, though this report points out that increasing droplet densities and concentration also improve effectiveness and, outside controlled laboratory conditions, the three factors are generally interrelated and it is difficult to separate their effects. Morris (1980) indicates that the consensus of forest biologists is that a droplet size of 90µm and 25 drops/cm$^2$ is a guideline for successful application of *Bt* by aircraft to forests. Morris (1981) and Walton and Lewis (1982) reported that a droplet density of 20/cm$^2$ or better was necessary to achieve adequate control of forest pests with *Bt*, though Lewis (1983) makes the comment that in field tests effectiveness does not always correlate with droplet density.

Unreported studies (Clayton *pers comm*) have indicated that the one problem which may be faced in the field when applying *Bt* formulations through a hand-held, spinning disc atomiser arises with some of the more viscous, formulations which may have a tendency to clog nozzle orifices when applied as concentrated formulations at ultra low volume (ULV) rates of 1-3l/ha. These problems are significantly reduced, however, when applied in aqueous solutions at very low volume (VLV) rates of around 10l/ha. Although Vassal (1992) considers that *Bt* does not lend itself to ULV application, being relatively insoluble and therefore needing high volumes for application, Jacquemond (1982) found that water based ULV *Bt*/virus treatments resulted in better yields than either oil based ULV or conventional high volume water-based methods of application. Silvie *et al.* (1993) report the successful application of *Bt* (Foray) in cotton using hand held spinning disc applicators at very low volume (VLV) rates of 10l/ha. Spinning disc, CDA sprayers were chosen because they are the standard method of pesticide application for cotton in the area (West Africa).

### Fungal entomopathogens

Bateman (in press) is of the view that field success of biological insecticides is a function of virulent isolate, appropriate formulation and application, bemoaning the fact that the latter is often neglected by many insect pathologists. He draws an analogy with the respective disease triangle of susceptible host, climate and available pathogen often used to describe the infection process of plant pathogens.

Most work on the use of CDA for the application of fungal entomopathogens has been carried out in the context of locust and grasshopper control. This is largely for the same reasons as for *Bt* and forest pest control i.e. because "standard" existing application systems for the treatment in question are ULV, CDA systems and hence fungal compounds are developed to be compatible with these. Also there is considerable interest in the use of biological compounds for locust and grasshopper control because of the widescale nature of control operations against targets in a non-crop situation.

Much of the work carried out to date on the use of CDA for the application of fungal compounds for locust and grasshopper control is summarised by Bateman (in press). Current developments involve the formulation of fungal spores or conidia in oil for application through spinning disc sprayers. Locally available vegetable oils are commonly used, diluted with a light paraffin oil to reduce viscosity and hence improve liquid application characteristics. The oil formulation reduces spore desiccation and has the advantage of improving the efficiency and speed of kill in comparison to water based formulations, especially at low humidities and make the compounds ideal for application at ultra low volumes by CDA as evaporation is minimised. Applications of oil-based formulations of fungi including *Metarhizium flavoviride, Beauveria bassiana* and the microsporidium *Nosema locusta* through spinning disc sprayers are recorded by Bateman (1992, Bateman *et al.* (1994), Nasseh *et al.* (1992), Johnson *et al.* (1992) and Douro-Kpindou *et al.* (1995). Matthews (1992) considers some of the technical aspects of application of microbial insecticides for locust control and concludes that rotary atomisers are the most appropriate means of achieving the narrow droplet spectrum required and stresses the relevance of Controlled Droplet Application to maximise the effectiveness of very small volumes of mycoinsecticides by minimising waste. This is echoed by Bateman (in press) who considers that, unlike other application systems, the high concentration of conidia typically used in ULV suspension formulations (> $10^{12}$ conidia per litre) ensures that virtually all droplets contain viable spores - typically in the order of 100-10000 conidia per droplet in the size range of 40-120µm considered optimal by the FAO in its locust control guidelines. In this paper he also addresses some of the practical considerations of using SC formulations, including flow rate characteristics and the problems of blockage. Bateman (in press) considers the physical properties and atomisation of ULV formulations of myco-insecticides and comments that WULV (water based ULV spraying) may be a suitable technique for spraying certain pests in the humid tropics and cooler climatic zones as this would reduce the costs associated with relatively expensive, oil-based ULV formulations.

Other documented instances of the successful ULV application of oil-formulated fungal entomopathogens include Agudelo and Falcon (1983) who report high mortality to *Spodoptera exigua* larvae on sugar beet leaves, following ULV application of *Paecilomyces farinosus* hyphal bodies on cotton seed oil. Prior *et al.* (1988) found that *Beauveria bassiana* conidia formulated in oil for ULV mistblower application had higher rates of survival and infectivity against *Pantorhytes plutus* than aqueous formulations and speculated that this was due to their greater adhesiveness to the lipophillic insect cuticle.

Unpublished data from Colombia indicates that an experimental air-assisted CDA sprayer (Micron Motax) deposited significantly more volume of *B. bassiana* (against coffee berry borer) on coffee berries compared with the other high volume systems tested. However, in terms of biological efficacy, there was no significant difference between any of the machines tested - average levels of infection with *B bassiana* being 52%.

Viruses

Jones (1988) comments that "the importance of application to the successful use of viruses cannot be overemphasised." Rabinda *et al.* (1988) compared the application of an NPV for *Heliothis* control in chickpea using a spinning disc sprayer at 12.5 l/ha (described as ULV but more accurately VLV) and knapsack sprayer at 500 l/ha. The former had the addition of 10 or 20% crude sugar, presumably as an anti-evaporant. Details are not given either of the droplet size or the swath widths used and it is not known whether the "ULV" application was a placement or drift spray. The "ULV" application was more effective, though not significantly so, than the high volume treatment. Smith *et al.* (1984) found drift deposit aerosol virus application to be effective against *Heliothis* in soyabean causing 85% mortality, while Mulock *et al.* (1990) report the use of a spinning disc sprayer for ULV drift application (using a droplet size of 50µm and a swath width of 2m) of an NPV virus, although comparison was not made with high volume techniques.

An ongoing programme being carried out by the Natural Resources Institute (NRI) in the UK on NPV viruses for control of *Heliothis armigera* in cotton, chickpea and pigeonpea and *Spodoptera littoralis* in cotton has indicated that VLV (c 10l/ha) application of aqueous formulations of virus using spinning disc sprayers gives control equal to or better than conventional high volume techniques. (Cherry *pers comm*). In trials at ICRISAT Cowgill and Bhagwat (1996) report the use of a battery operated spinning disc sprayer for successful application of *Heliocoverpa* NPV in chickpea, though few details are given about application parameters. Comparisons were not made with conventional techniques, although a knapsack sprayer was used to apply the insecticide comparison in trial. In this context the work in West Africa (Silvie *et al.* 1993) referred to above using both *Bt* and virus for cotton pest control is also relevant.

In forestry Killick (1990) found that small droplets led to higher infection levels than large droplets in experimental ground applications of NPV sprayed against the Pine Beauty Moth (*Panolis flammea*). This may be partially explained by unpublished work, cited in the same paper, in which droplets were collected on spiders' webs and it was found that smaller droplets had a disproportionally greater presence of virus. Smith *et al.* (1977) found that the use of smaller droplets increased the effectiveness of *Baculovirus heliothis*. In aerial applications to lodgepole pine Entwistle *et al.* (1990) found that poor results were obtained against *Panolis flammea* using a spinning cage Micronair and attributed this to an overwide droplet spectrum. Results improved markedly when using a stacked spinning disc applicator (two Micron X15 atomisers used in conjunction) which produced a much narrower droplet spectrum. Best results were obtained with the highest droplet numbers and lowest PIB (polyhedral inclusion bodies) dosage per droplet. It was concluded that control can be maximised by selection of an optimal droplet density with a narrow droplet spectrum being essential for uniform deposition of the pathogen. Payne *et al.* (1996) using Micronair spinning cage atomisers to apply NPV for gypsy moth control in mature oak canopy concluded that control of droplet size to produce a "finer spray" could reduce wastage to the ground.

## Mycoherbicides

There is relatively little reported on the use of CDA for application of mycoherbicides, possibly because this is an area of biological control which has received less attention than for example insect control. Quimby and Boyett (1987), discussing application technology of biological herbicides, comment that a number of questions need to be answered including diluent systems, optimum volume of diluent and droplet size. For experimental purposes during the initial evaluation of a weed pathogen the spray volume and inoculum concentration is very high, usually about 900 to 1,000 l/ha with $10^5$ or $10^6$ spores per ml, permitting coverage to the point of run off, although these spray volumes can generally be reduced to more practical levels by increasing the amount of inoculum per unit of spray volume.

Ellison (1993 and *pers comm*) used a spinning disc Herbaflex applicator to apply potential mycoherbicides of the *Colletotrichum* genus for control of the weed *Rottboellia cochinchinensis*. Application was almost to the point of run off, at high volume rates with CDA being chosen to minimise inoculum waste. Application of more viscous emulsion formulations by a hydraulic pressure nozzle sprayer resulted in an increase in droplet size and a considerably increased loss of inoculum to the soil. Perry and Williams (1984) evaluated the Micron ULVA spinning disc sprayer as a tool for inoculating plant pathogens in the field and greenhouse. Good leaf cover and subsequent levels of infection were obtained with droplet sizes in the range 60-70µm. Pathogens with larger propagules showed a lower rate of infection when the droplet size was reduced to 30-40µm.

## Nematodes

The only reported instance of the use of CDA for application of entomopathogenic nematodes is by Lello *et al.* (1996). It was calculated that the minimum droplet diameter needed to accommodate an infective juvenile (IJ) of the organism *Steinernema carpocapsae* was 178µm and as coverage and control over droplet size were deemed to be important application parameters conventional nozzles were compared with a spinning disc system. The spinning disc system (Micro Ulva adapted to produce droplets in the desired size range) gave 50% mortality while applying less than 9% of (IJs) compared with the most effective hydraulic nozzle. It is understood (Wright *pers comm*) that work is continuing on optimising the performance of the spinning disc treatment by selection of a sprayer better suited to the higher feed rates and slower disc speeds required.

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