Spray Retention and its Potential Impact on Bioherbicide Efficacy

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ABSTRACT

Hydraulic spray systems are widely used for application of agrochemicals due to ease of operation and consistent performance, despite relative inefficiency in delivering pest-control products to intended targets. Frequently, spray parameters are optimized for maximum product deposition and retention, although success of this strategy is case dependent. There is limited information on application improvements for microbial pesticides (biopesticides). Biopesticides, especially those that employ a fungus as the active ingredient, are generally applied with a liquid carrier but their deposition or retention has rarely been characterized. Depending on the size of microbe and plant morphology or architecture, interactions among spray parameters can be complex in terms of the impact on retention, distribution and performance of the biopesticide agent. Extrapolation of information from chemical pesticide applications may not always be appropriate. This review, based primarily on authors’ experience in spray retention involving three bioherbicide-weed systems, is aimed to highlight the impact of spray parameters and additives (adjuvants) on deposition, retention, and efficacy of bioherbicide agents. Information from additional bioherbicide agents is also considered for different sizes of fungal inoculum or characteristics of target plants. Although the focus is on potential bioherbicides, the information may also be useful to application of other microbe-based biopesticide agents. Strategies for maximizing biocontrol efficacy through optimization of spray parameters as well as other application technologies are discussed.

Keywords: adjuvant, canopy penetration, carrier volume, nozzle, spray quality, trajectory

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INTRODUCTION

In foliar application of either synthetic or microbial pesticides (biopesticides hereafter), the primary goal is to achieve maximum spray deposition, retention and target coverage. Most systems used to deliver biopesticides employ techniques and equipment developed originally for conventional pesticides (Smith and Bouse 1981; Bateman 1999), and this is unlikely to change significantly in the foreseeable future. Hydraulic nozzles, usually a tapered flat-fan design, are the primary means of applying herbicides because they provide a uniform spray pattern and a mix of droplet sizes that have historically been efficacious. Air-induced versions of these nozzles produce coarser droplets and are becoming more widely used for herbicide applications to minimize spray drift (Wolf et al. 2000) and for control of disease and insect pests in orchids (Knewitz et al. 2002). However, some aspects of spray targeting may be compromised if water volumes are not sufficiently high to maintain adequate droplet densities (Jensen et al. 2001; Howarth et al. 2004).

Biopesticide sprays involve delivery of microbial propagules usually suspended in a liquid carrier. Depending on the propagule size and target weed, inoculum deposition and retention can vary substantially (Jones 1998). Additionally, biopesticides often contain living microbial inoculum that may be subjected to shear-force impact of conventional spray equipment (Fife et al. 2005a, 2005b; Peng et al. 2005b; Byer et al. 2006a, 2006b), and special formulations or spray considerations may be required to maximize survival. Unfortunately, the effect of spray parameters on retention of biopesticide propagules is rarely documented. For some bioherbicides, targeting specific weed tissues may be of greater importance for efficacy. For example, BioMal®, a fungal bioherbicide against round-leaved mallow (Malva pusilla), caused more severe damage to the weed when infection occurred on stems and petioles than on leaves (Mortensen 1998), and therefore would be more efficacious if most fungal inoculum were deposited and retained on weed stems during application. Grasses can often be difficult to control with bioherbicide agents because their meristem tissues are protected by leaf sheaths (Greaves and MacQueen 1992) from direct infection. Severe damage to lower leaves of green foxtail by the fungal biocontrol agent Pyricularia setariae only retarded plant development temporarily and it
is the destruction of the young top leaf that interfered with the apical meristem most effectively and resulted in plant death (Peng et al. 2004). However, insufficient amounts of fungal inoculum may be deposited or retained on the vertically positioned top leaf when common spray devices and carrier volumes are used; frequently, only light disease damage occurs on the top leaf that continues to develop from the apical meristem (Peng et al. 2005b).

In other cases, excessive spray volumes and subsequent runoff have been considered counterproductive for microbial inoculum retention due to the possibility that the propagules may be washed off the leaf (Greaves et al. 1998). There have been few studies that examine the retention of microbial propagules in relation to various spray parameters. Applications using aerosol sprayers in the lab likely have little relevance to field scenarios in which spray volumes are generally below 200 L/ha and even 600 L/ha would be considered highly impractical (Matthews 1992). Aerosol sprayers may also generate a high proportion of fine droplets that can be either ‘empty’ (Jones 1998) or not reaching the target under field conditions due to the impact of atmospheric factors (Knoche 1994).

Although much can be learned from herbicide applications, studies specifically targeting the enhancement of biocontrol efficacy through optimization of spray parameters and other application technologies could also be useful (Boyetchko and Peng 2004). Spray retention is often used as an indicator for herbicide dose transfer that can be closely related to efficacy (Hart et al. 1992; Moerkerk and Combellack 1992). Retention characteristics, however, may vary with weed species or even biotypes and, can also be influenced by droplet size, travel speed, and spray adjuvants (Wolf et al. 2002a). In other cases, excessive spray volumes and subsequent runoff have been considered counterproductive for microbial inoculum retention due to the possibility that the propagules may be washed off the leaf (Greaves et al. 1998). There have been few studies that examine the retention of microbial propagules in relation to various spray parameters. Applications using aerosol sprayers in the lab likely have little relevance to field scenarios in which spray volumes are generally below 200 L/ha and even 600 L/ha would be considered highly impractical (Matthews 1992). Aerosol sprayers may also generate a high proportion of fine droplets that can be either ‘empty’ (Jones 1998) or not reaching the target under field conditions due to the impact of atmospheric factors (Knoche 1994).

Enhancing retention of bioherbicides by manipulation of spray parameters

Atomization of a spray liquid by a hydraulic nozzle produces a mix of droplet sizes ranging from 5 to over 1000 µm in diameter (Chapple et al. 1993). Droplet sizes contained in sprays are often described using the parameter VMD, which is the diameter that marks the 50th percentile of the spray's cumulative droplet size distribution not being retained. There is a substantial literature on the relationship between droplet size and product rates, carrier volumes, and canopy penetration by herbicides (Hislop 1987; Knoche 1994; Mathews 2000; Wolf et al. 2000). Several techniques are available for measuring droplet sizes, either in flight after leaving the nozzle or after deposition on artificial surfaces in the target zone (Bateman 1999; Wolf and Caldwell 2004). Laser particle-size analyzers provide a rapid, precise estimate of spray droplet size spectra (Bateman 1993).

The behaviour of droplets in the spray cloud begins with deceleration and evaporation. After exiting the nozzle at approximately 20 m/s, aerodynamic drag forces reduce droplet speed in relation to their mass. Smaller droplets reach terminal velocity first. For example, a 50-µm droplet will be at terminal velocity after travelling only 6.8 cm, whereas a 5-mm droplet will not reach terminal velocity for 1.45 m (Bache and Johnstone 1992). These characteristics help shed light on spray interception behaviour of droplets. Smaller droplets (< 200 µm) tend to move with predominant air flows, whereas the trajectory of larger droplets is more related to their initial velocity and inertia, and to gravity. As a result, the movement of smaller droplets is to a large degree dependent on prevailing meteorological conditions, plant canopies, and individual plant parts (Spillman 1984), and to a lesser degree on the atomizer pressure or orientation. For example, small droplets tend not to be intercepted by large targets such as mature leaves, due to the droplets’ inherently low kinetic energy, which allows them to move with airflows that go around such targets. On the other hand, the same small droplets are more efficiently collected on small targets such as stems or petioles. In order to better understand or predict spray movement into and through a canopy, spray quality may need to be described in terms of the proportion of the total volume (dose) available in specific size fractions that match the aerodynamic characteristics of the cropping or application situation in question.

Herbicide effectiveness is related to the quantity of active ingredient reaching the susceptible target site of weed to be controlled, but this quantity is impractical to measure directly. Instead, various indirect approximations are used to predict the relative effectiveness of various application or formulation methods. The most common approaches used for herbicides are to measure the amount of spray retained by target plants (Hislop et al. 1993) or to quantify uptake and translocation of the active ingredient (Tsuda et al. 2004). Although these measurements can go a long way toward explaining efficacy changes, both these approaches rely on the assumption that the processes involved and frequently do not fully account for efficacy changes observed in the field. Examples of application methods that increase deposition but have no positive impact on effectiveness abound (Cooke et al. 1986; Nicholls et al. 1995), indicating that other elements also affect efficacy. These factors need to be identified and incorporated into experimental methods. For example, more uniform spray deposition decreases water use by reducing the frequency of over- or under-dosing the target. High deposit variability has been associated with reduced control of insects (UK and Courshere 1982; Cooke et al. 1986). However useful the quantification of spatial/temporal variability structures of spray deposits and their impact on field-scale dose responses may be (Dorr and Pannell 1992), actual assessment of spray deposit variability is rare (Nordbo et al. 1993; Wolf et al. 1993). Further complications arise due to the heterogeneous nature of weed, and populations in different regions may have
unique anatomical and physiological features that can affect retention (Merritt 1982).

Studies of spray deposits on plant surfaces (Hess et al. 1974) (UK 1977) are required to identify application parameters critical to pesticide or biopesticide efficacy. It is clear that the form of deposit has relevance to activity, although no general statements are appropriate for all products or weeds. Small droplets are widely acknowledged to improve efficacy of many herbicides and microbial agents (Adams et al. 1990), due to increased spray coverage, under-leaf placement, and pest/droplet encounter frequency (Ford and Salt 1987). Deposit quality is important when considering the impact of droplet size on drying rates (Hall et al. 1994), which affect subsequent uptake by plants (Stevens et al. 1988). Mixture models can be used to determine the relationship of deposit structure (droplet size, number, and pesticide concentration) on pest mortality. Improved understanding of this relationship helped explain the differences in the efficacy of fine sprays between laboratory and field (Ebert et al. 1999). However, many current efforts suffer from their dependence on artificial targets (such as water-sensitive papers) for spray deposition analyses and a general lack of assessment of biological performance (Ozkan et al. 2000).

In recent years, spray nozzle design has undergone significant advances and many new options are available to applicators. In addition to traditional flat fan and hollow cone nozzles that have been used to apply high pressure, high volume, fine spray qualities in orchard and vine crops (Doll et al. 2005), applicators can now choose from an array of spray qualities, orientations, and air amendments. The principle that finer droplets allowed for higher droplet densities (Walklate 1992) was used to justify lower spray volumes (Cross et al. 2001). Sophisticated air-assist sprayers, which enclose the crop canopy and use multiple fans to direct the spray into the canopy from a number of directions simultaneously, may further enhance the value of this approach. Whether this type of approach translates to dose transfer of biopesticide propagules, which may be themselves larger than these fine droplets, is questionable. Electrostatic sprays have also been used to apply biopesticides (Law 2005), with significant improvements in total spray retention, particularly on difficult-to-reach plant parts such as flower parts, even with low carrier volumes (Scherm 2007). The authors applied bacterial suspensions in electrostatic sprays with a VMD of about 30 µm. Opportunities with larger propagules, however, were not discussed.

Improved deposit uniformity throughout the canopy can also be achieved without increasing water volume by utilizing a low-volume, high density, nozzles (Doll et al. 2003). Concerns about spray drift have prompted studying coarser and air-induced sprays in tree crops, with increased timeliness and effectiveness of application..

For boom sprayers, the advantage of finer droplets has been shown to maintain or even improve disease control in orchards and vineyards (Lesnik et al. 1994). In another study, no differences in sclerotinia stem infections (Bateman 1999; Wolf and Caldwell 2004). In addition, technologies have been adopted for measuring droplet sizes, either in flight or after deposition on artificial surfaces in a target zone (Bateman 1999; Wolf and Caldwell 2004). Laser particle size analyzers provide a rapid means to measure spray droplet size spectrum and the data can be processed electronically for extensive analyses. Estimates of the number of droplets in each size class are deduced from the data (Bateman 1993), which is useful for optimization of formulations or product dilution for the final tank mix. These protocols may be used to study retention of bioherbicidal inoculum provided the size of droplets and microbial propagules are appropriate. Bateman (1999) presented a theoretical distribution of microbial inoculum in the spray droplet size spectrum generated with different nozzles by converting droplet diameters to volumes and multiplying by the numbers of microbial propagules per unit volume in the spray tank. This provides an estimated drop size range in which there is a high chance for a droplet to contain at least one microbial propagule. Bioherbicidal concentrations used for field applications may range from 10⁶ to 10⁸ spores/L. (Masangkay et al. 1999; Zhang et al. 2002; Bailey et al. 2004; Rosskopf et al. 2005), and these concentrations will likely provide most droplets >150 µm diameter with more than one infective propagule (Bateman 1999). These theoretical models generally hold for microbial propagules up to 20 µm but may break down when propagules are much bigger or microbial concentrations are decreased dramatically (Bateman, pers. comm.).

Although fine sprays may enhance retention on some target weeds, too fine droplets may contain few fungal spores or even be “empty” (Jones 1998). On green foxtail, retention of Pyricularia oryzae (spores) (30 × 10 µm, length × width) generally followed a pattern of liquid retention (Fig. 1), and therefore the liquid retention in this case serves as a valid indicator of spore retention (Peng et al. 2005b). However, when conidial suspensions of Alternaria alternata were applied to pigweed (Amaranthus retroflexus), spore retention did not follow that of the liquid and the majority of spores either failed to reach the target or was not retained on the plant (Lawrie et al. 2002b). Although the exact cause of the spore loss was not determined during this study, spore size/concentration and spray droplet size are likely the factors. Larger spores or higher spore concentrations tend to result in fewer than expected spores in spray drops. Based on liquid retention volume on pigweed, a significantly large portion of A. alternata spores were unaccounted for (Lawrie et al. 2002a). Depending on the nozzle type, often small droplets (<150 µm) make up more than 50% of the volume applied (Bateman 1999) and 20% or more of these droplets contain no microbial propagules, whereas more than 60% of larger droplets may each contain a wasteful amount of inoculum (Lawrie et al. 1997) which adds little to the severity of disease damage at the same infection site.

Despite the fact that protocols for liquid retention studies are readily available, it may often be necessary to verify the r0llow of a bioherbicidal agent depending on propagule size, concentration, and spray-droplet spectrum. The following case studies are used to show retention characteristics of selected bioherbicidal agents as affected by varying application methods, spray parameters, and weed targets.
Case study I – retention of *Pyricularia setariae* conidia (spores) on green foxtail

The host-specific fungal pathogen *P. setariae* (Ps) is a candidate for biocontrol of green foxtail (Peng et al. 2004). When spore suspensions were applied using an airbrush sprayer until runoff, the fungus caused a high level of weed mortality under controlled environment. This delivery method, however, tended to maximize spray retention volumes on plants (Peng et al. 2005b), consequently exaggerating the potential of biocontrol efficacy (Greaves et al. 2000). Lower efficacy occurred when the fungal inoculum was applied using conventional flat-fan hydraulic nozzles at 100-800 L/ha carrier volumes (Peng et al. 2001). A further study, based on liquid volumes retained on the plant, revealed that 2,000 L/ha sprays would be required for hydraulic nozzles to transfer a similar dose volume resulted from the airbrush spraying (Peng et al. 2005b). This indicates that the poorer efficacy is likely related to lower dose volumes delivered with hydraulic nozzles. Efficacy of the airbrush treatment could easily be matched by hydraulic-nozzle sprays as long as application volumes of the latter were increased to deposit an equivalent liquid volume on the plant (Peng et al. 2001). A carrier volume at 2,000 L/ha is obviously impractical for most field applications but a potential way of mitigating this is to increase the bioherbicide concentration. This strategy was successful on green foxtail, on which the carry volume of Ps was reduced from 2000 L/ha to 250 L/ha without compromising weed control (Peng et al. 2001). Higher inoculum concentrations may increase the number of fungal spores in spray drops as well as reduce the number of ‘empty’ droplets (Jones 1998). This also led us to believe that 250 L/ha provided sufficient coverage of green foxtail and that it was the spore dose, not the carrier volume that governed the ultimate efficacy of green foxtail control by *P. setariae* (Peng et al. 2001).

The size of spray droplets may be optimized to enhance retention efficacy on target weeds and many studies reported that finer droplets tended to result in higher retention efficiency on plant foliage (Knoche 1994; Wolf et al. 2000; Peng et al. 2003; Zhu 2004). The spectrum of spray droplets also affected liquid retention on green foxtail, with finer drops (VMD 207 µm) producing approximately 40% greater liquid volumes in comparison to coarse drops (VMD 325 µm) when application volumes were kept the same (Peng et al. 2005b). This increased spray retention may potentially cause higher weed-control efficacy or lower dose requirement for the biocontrol agent. Naturally, the question is whether this increased liquid retention has much bearing on bioherbicide loads, which has more direct impact on weed-control efficacy. The retention of Ps correlated strongly to that of liquid on green foxtail, but biological effects of these retention increases were harder to determine and the 40% higher retention with finer sprays did not consistently translate into more effective weed control (Peng et al. 2005b).

There may be several reasons for this: a) Disease responses to increased doses of the bioherbicide agent may be nonlinear and substantially higher inoculum doses can be required for noticeable efficacy improvements (Graham et al. 2004). Occasionally greater efficacy was seen with Ps applied in finer droplets, but the scale was generally less than that of spray retention increases. b) The greater liquid retention resulted from finer droplets may fail to increase the bioherbicide inoculum on plants because hydraulic flat-fan nozzles tend to generate a greater proportion of small drops (<150 µm) and most of them may not carry any fungal spores (Lawrie et al. 1997; Jones 1998). Other small drops with only a few spores may have low probability of causing successful infection (Jones 1998), especially for those fungal strains with low infection efficiency (Evans et al. 1996; Fujimoto et al. 2002). Fungal agents with large spores, such as *Drechslera gigantean* or *Exserohilum rostratum* used for biocontrol of green foxtail and other grassy weeds (Chandramohan et al. 2002; Peng and Boyetchko 2006), would likely suffer even greater inoculum losses if applied in fine drops. For example, in applications of *Myco-centospora acerina* for biocontrol of field violet (*Viola arvensis*), more than 78% of fine droplets generated by a hydraulic nozzle contained no fungal spores (Lawrie et al. 1997). On average, the size of *M. acerina* conidia is 100–250 × 14 µm. c) In a biological system involving pathogens and plants, host susceptibility and post-application conditions likely overweigh even impressive gains in spray retention and this was seen even with chemical pesticide applications in which increased retention sometimes failed to enhance efficacy in the field due to complex interactions among varying biological factors (Wolf and Caldwell 2004). It should also be pointed out that there are practical limitations for using too fine drops due to atmospheric interferences causing rapid evaporation and increased spray drift (Grover et al. 1997). Protective shields may help reduce off-target spray drift for fine drops, especially under high-wind conditions (Wolf et al. 1993) but this design has not been widely adopted in practice. One of the key messages from this study is that incremental increases of spray retention can be achieved with finer spray drops but this enhancement alone may not be sufficient to enhance Ps efficacy consistently against green foxtail.
Case study II – retention of Colletotrichum spp. spores

The fungi C. truncatum (Ct) and C. gloeosporioides f. sp. malvae (Cgm) are bioherbicide candidates for scentless chamomile (Matricaria perforata) and round-leaved mallow (Malva pusilla), respectively (Makowski and Mortensen 1992; Mortensen 1988; Peng et al. 2005a). Although both weeds are considered the “broadleaf” type, they differ considerably in plant morphology and branch architecture. Scentless chamomile produces finely divided needle-like leaves, whereas round-leaved mallow has more typical broad leaves that are flat and present a greater surface area that intercepts vertically directed sprays efficiently (Byer et al. 2006a). Spores of Colletotrichum spp. are smaller than those of Ps, with Ct averaged 17±5 μm and Cgm 10±6 μm, respectively (Byer et al. 2006b). These relatively small spores are less likely to be affected by droplet size spectra, as reported in several previous studies (Egley et al. 1993; Lawrie et al. 2002a). Data repeatedly showed a similar trend for spores and liquid retention on both weeds, except that on round-leaved mallow both liquid and spore retentions peaked at about 1,000 L/ha and further increases of the application volume did not boost retention on the plant.

The retention saturation on round-leaved mallow at lower application volumes may be due to the plant morphology and architecture (Byer et al. 2006b). Although variable retention characteristics have been known with different plant species or even biotypes (Verity et al. 1981; Wisniewski et al. 1991; Gillespie 1994), there have rarely been reports specifically targeting bioherbicide applications. Coarse drops may be successfully captured by relatively large and horizontally positioned leaves of round-leaved mallow due to more vertical travel direction of spray drops (Matthews 2000) while smaller droplets may also be retained efficiently because of their low kinetic energy (Spillman 1984; Chapple et al. 1996) (Hartley and Brunskill 1958) suggesting the tendency of retention for large droplets would also depend on contact angle and droplets with smaller than 400 μm in diameter were less likely be reflected if the contact angle were not much greater than 90°. Efficient spray interception by round-leaved mallow plants might have resulted in earlier retention peak and possibly runoff at 1000 L/ha, due to hydrophobic waxy leaf surfaces (Matthews 1992). The potential for runoff may also be affected by leaf age, size and plant architecture at the time of application (Lawrie et al. 2002b). The relationship between observed and expected spores was linear on scentless chamomile but curvilinear on round-leaved mallow when application volumes increased from 500 to 2,000 L/ha (Fig. 2). This curvilinear relationship implied that at high application volumes, there is a greater loss of Cgm spores than the liquid carrier. Excessively high application volumes may therefore be counterproductive for retention of microbial inoculum in some cases (Greaves et al. 1998).

Despite different plant morphology/architecture as well as the retention efficiency, scentless chamomile and round-leaved mallow showed similar retention attributes in response to varying spray droplet spectra; finer drops generally resulted in higher retention than did coarse drops when the same spray volume was applied (Byer et al. 2006a). This retention trait was also similar to that observed on green fritail (Peng et al. 2005b). When biocontrol efficacy was examined in relation to droplet size and retention efficiency, however, different patterns were shown between scentless chamomile and round-leaved mallow; Ct applied in fine droplets (VMD 207 μm) caused greater weed control than did the fungus delivered in medium (VMD 267 μm) or coarse drops (VMD 325 μm). In contrast, Cgm efficacy was less responsive to the droplet size used and treatments in finer droplets, although generally giving higher liquid retention on round-leaved mallow, failed to achieved more effective weed control when compared to the treatments using coarse sprays (Byer et al. 2006a). Finer droplets frequently produce higher spray retention of chemical herbicides on weeds (Hartley and Brunskill 1958; Reichard 1988) and greater efficacy (Knoche 1994). The uncoupling of droplet size or spray retention with biocontrol efficacy of Cgm is probably due to inefficient delivery of fungal inoculum to lower weed stems, the critical infection site for bioherbicide efficacy, where severe diseases can girdle the main stem and cause the plant to collapse (Mortensen 1988; Mortensen and Makowski 1995). The increased spray retention on the whole plant of round-leaved mallow may have limited relevance to biocontrol because those large and horizontally positioned leaves might have intercepted a much greater proportion of the spray than does the lower stem. In this case, accurate delivery of the bioherbicide inoculum in droplets carrying optimal number of fungal propagules to the most vulnerable site of the weed would be of greater impact on efficacy (Amsellem et al. 1990; Doll et al. 2005) and ought to have been measured.

Spray deposition is normally highest when targets are perpendicular to the droplet trajectory (Elliott and Mann 1987; Richardson and Newton 2000). Nozzle angling and travel speed may be adjusted to enhance horizontal spray trajectory, hence reducing the contact angle to stems and improving deposition/retention on the vertical surfaces (Nordbo et al. 1993; Wolf and Caldwell 2004; Doll et al. 2005). This case study shows varying retention efficiency on weed targets influenced by plant morphology and architecture, as well as the opportunities to adjust drop size, carrier volume, and nozzle angling to optimize application efficiency and biocontrol efficacy.
Improving canopy penetration for better targeting lower stems

Although in many cases spray droplets of bioherbicide suspensions may behave similarly to those of chemical herbicides, efficacy of these droplets may vary substantially depending on whether a sufficient amount of inoculum can reach critical parts of the target. Bioherbicide agents generally cannot be relocated or translocated within the plant (Lawrie et al. 2002a) and it would be most efficient to direct most of the spray to vulnerable target sites for maximum impact. Aggressive weeds can often tolerate significant amounts of defoliation (McBrien and Harmsen 1987; Meyer 1998; Peng et al. 2001), and therefore many bioherbicide candidates have been directed towards lower stems of the weed (Daniel et al. 1973; Auld et al. 1988; Makowski and Mortensen 1989; Mortensen 1988; Boyette et al. 1993; Winder and Watson 1994; Peng et al. 2005a) where coalescing lesions may girdle the stem, causing the plant to collapse. From the weed control point of view, this is an efficient strategy. However, conventional spray systems, i.e. vertically positioned hydraulic nozzles, are generally not efficient in targeting lower stems, mainly due to interception of spray drops by the upper canopy, and possibly poor retention (Chapple et al. 1996). Although much research has been directed to novel spraying systems, it is clear that hydraulic nozzles will not be replaced any time soon due to their practicality and versatility in delivering all classes of agrichemicals. Spinning-disc sprayers may be a good example to the point. Such devices generate a narrow spectrum of droplet sizes with VMDs usually below 200 μm and often less than 100 μm to achieve coverage at relatively low application volumes (Bateman 1998). Such sprays can, however, have unpredictable trajectories, are generally poor for canopy penetration, and prone to spray drift (Schaef and Allsopp 1983). Although lowering the disc speed can enlarge droplet size (Hewitt 1992) and in-canopy applications enhance deposition on plant lower stems, the majority of the spray volume still falls in droplets under 125 μm in diameter (Bateman et al. 1998) and vertical distribution was generally uneven with insufficient amounts deposited at plant lower stems (Stonehouse 1993). In reality, spinning-disc sprayers have not been used commonly, especially for application of herbicides. It may be advisable that, for broadest adoption, common spray equipment should be considered for application of most biopesticide agents and dramatic modification of spray systems will more likely reduce rather than add to the likelihood of success (Chapple and Bateman 1999).

If hydraulic nozzles are the mainstay for agrichemical applications, what modifications may possibly be considered practical to enhance the deposition and/or retention of bioherbicide inoculum targeting weed stems? Chapple et al. (1996) proposed a “double nozzle” design to improve biopesticide applications over conventional nozzle systems. This device requires only a minor change over current spray equipment by adding a set of fine nozzles at an angle in the travel direction of the boom to apply biopesticide inoculum into the clouds of water droplets produced with medium or coarse nozzles mounted in a vertical position. Spray drops larger that 150 μm are considered more efficient carriers for many biopesticide propagules (Chapple et al. 1996; Bateman 1999) and too large drops may contain a wasteful amount of inoculum. The advantage of this double-nozzle system is to minimize the number of “inefficient” large drops for biopesticide delivery, which may account for over 80% of the carrier volume in a coarse spray. This system also reduces drift of “biologically efficient” fine droplets that contain bioherbicide inoculum because these fine droplets will be entrained into coarse water drop clouds and together they produce the overall deposition and retention characteristics of coarse sprays (Chapple et al. 1997). Target loading can be increased by this system, especially on vertical surfaces and at the base of a canopy (Taylor and Andersen 1997). This modification is considered relatively simple and flexible; all the main features of a conventional sprayer are retained and where using “double nozzles” is not required, the fine nozzles can be shut off and the single-nozzle system restored quickly. In a study where glyphosate was applied with the “double-nozzles” onto soybeans, the efficacy was higher than that of coarse sprays alone and the herbicide rate could be reduced substantially (Hall et al. 1996). Nozzle spacing and angling are important to provide coverage of the drops when targeting low plant organs. For this reason, the double-nozzle system (Chapple et al. 1996). The drawback of this system is potential requirements for a larger volume of water as well as the generation of droplets in a broad size spectrum. The latter may result in unpredictable behaviors of spray drops during field applications (Matthews 2000).

As suggested earlier, larger drops may be more effective for canopy penetration but often poor for retention on vertical surfaces due to droplet’s kinetic energy and large contact angle. Improved weed control was achieved from “double nozzles” onto soybeans. This may also increase the chance of loading “biologically efficient” small droplets at lower stems (Nordbo et al. 1993; Wolf and Caldwell 2004; Doll et al. 2005). In a study using a double-nozzle boom to optimize spray retention on simulated wheat spikes for fungicide treatment against head blight, Wolf and Caldwell (2004) found that this nozzle configuration, arranged in front and back with a 60° angle from the vertical, had maximal to increase spray collection and retention (Elliott and Mann 1997; Richardson and Newton 2000). Travel speed may also be increased to enhance horizontal trajectory for improved retention on weed stems (Nordbo et al. 1993; Wolf and Caldwell 2004; Doll et al. 2005). In a study using a double-nozzle boom to optimize spray retention on simulated wheat spikes for fungicide treatment against head blight, Wolf and Caldwell (2004) found that this nozzle configuration, arranged in front and back with a 60° angle from the vertical, had maximal to increase spray collection and retention (Elliott and Mann 1997; Richardson and Newton 2000). Travel speed may also be increased to enhance horizontal trajectory for improved retention on weed stems (Nordbo et al. 1993; Wolf and Caldwell 2004; Doll et al. 2005). In a study using a double-nozzle boom to optimize spray retention on simulated wheat spikes for fungicide treatment against head blight, Wolf and Caldwell (2004) found that this nozzle configuration, arranged in front and back with a 60° angle from the vertical, had maximal to increase spray collection and retention (Elliott and Mann 1997; Richardson and Newton 2000). Travel speed may also be increased to enhance horizontal trajectory for improved retention on weed stems (Nordbo et al. 1993; Wolf and Caldwell 2004; Doll et al. 2005). In a study using a double-nozzle boom to optimize spray retention on simulated wheat spikes for fungicide treatment against head blight, Wolf and Caldwell (2004) found that this nozzle configuration, arranged in front and back with a 60° angle from the vertical, had maximal to increase spray collection and retention (Elliott and Mann 1997; Richardson and Newton 2000). Travel speed may also be increased to enhance horizontal trajectory for improved retention on weed stems (Nordbo et al. 1993; Wolf and Caldwell 2004; Doll et al. 2005). In a study using a double-nozzle boom to optimize spray retention on simulated wheat spikes for fungicide treatment against head blight, Wolf and Caldwell (2004) found that this nozzle configuration, arranged in front and back with a 60° angle from the vertical, had maximal to increase spray collection and retention (Elliott and Mann 1997; Richardson and Newton 2000). Travel speed may also be increased to enhance horizontal trajectory for improved retention on weed stems (Nordbo et al. 1993; Wolf and Caldwell 2004; Doll et al. 2005). In a study using a double-nozzle boom to optimize spray retention on simulated wheat spikes for fungicide treatment against head blight, Wolf and Caldwell (2004) found that this nozzle configuration, arranged in front and back with a 60° angle from the vertical, had maximal to increase spray collection and retention (Elliott and Mann 1997; Richardson and Newton 2000). Travel speed may also be increased to enhance horizontal trajectory for improved retention on weed stems (Nordbo et al. 1993; Wolf and Caldwell 2004; Doll et al. 2005). In a study using a double-nozzle boom to optimize spray retention on simulated wheat spikes for fungicide treatment against head blight, Wolf and Caldwell (2004) found that this nozzle configuration, arranged in front and back with a 60° angle from the vertical, had maximal to increase spray collection and retention (Elliott and Mann 1997; Richardson and Newton 2000). Travel speed may also be increased to enhance horizontal trajectory for improved retention on weed stems (Nordbo et al. 1993; Wolf and Caldwell 2004; Doll et al. 2005).
raulic nozzles. They attributed this partially to much finer droplets generated by the airbrush sprayer that would not bounce off the target easily. At the same time, they also pointed out that a more horizontally directed angle with the airbrush spraying would likely contribute to the high deposition on the stem. Similar to previous examples, angling hydraulic nozzles also increased deposition or retention on vertical surfaces of the pigweed, including stems and apices. This would be useful for the bioherbicide agent *Alternaria alternate* because, as in the case of round-leaved mallow (Byer et al. 2006b), stem girdling by coalesced lesions is also critical to effective biocontrol of the pigweed (Lawrie et al. 2002a). In general, vertical surfaces of weeds may be best targeted with spray droplets projected as horizontally as possible (Wolf and Caldwell 2004).

**Enhancing spray retention and bioherbicide efficacy with adjuvants**

Spray adjuvants are frequently used to alter the physiochemical properties of the spray liquid to improve deposition/retention during application of agrichemicals. These adjuvants can change spray drop-size spectrum and velocity, in-flight and/or impact behavior, and deposit-target interactions, consequently influencing retention of agrichemicals and final biological effects on the target (Miller et al. 2001). Adjuvants can be classified as stickers, spreaders, wetters, drift retardants, anti-oxidants, anti-evaporants, etc. (Hall et al. 1993) and these names are fairly descriptive of their functions in aiding pesticide applications. Despite the fact that more adjuvants have become available in recent years, it is still unclear which should be used in what conditions because there are multiple steps from atomization to final biological effect during the application process and adjuvants may potentially impact on many of the steps (Hall et al. 1993). Screening trials are generally necessary for a specific target weed to evaluate the effect of droplet traits and behavior on deposition/retention efficiency as affected by different adjuvants. Besides spray quality, swath pattern can also be affected dramatically with use of adjuvants (Chapple et al. 1993) and this impact is often concentration-dependent, due largely to changes in viscosity of the spray material used in a liquid carrier (de Ruiter et al. 2001). Adjuvants can be classified as stickers, spreaders, wetters, drift retardants, anti-oxidants, anti-evaporants, etc. (Hall et al. 1993) and these names are fairly descriptive of their functions in aiding pesticide applications. Despite the fact that more adjuvants have become available in recent years, it is still unclear which should be used in what conditions because there are multiple steps from atomization to final biological effect during the application process and adjuvants may potentially impact on many of the steps (Hall et al. 1993). Screening trials are generally necessary for a specific target weed to evaluate the effect of droplet traits and behavior on deposition/retention efficiency as affected by different adjuvants. Beside spray quality, swath pattern can also be affected dramatically with use of adjuvants (Chapple et al. 1993) and this impact is often concentration-dependent, due largely to changes in viscosity of the spray solution (Wolf et al. 1997). There is also potential phytotoxicity of adjuvants depending on the amount of material deposited per unit area on plant surfaces, penetration of the material into the leaf, and cellular toxicity. Often this direct phytotoxicity is related to the adjuvant chemistry and concentration used in a liquid carrier (de Ruiter et al. 2001).

**Desirable adjuvants for bioherbicide delivery**

In addition to maximizing deposition or retention as in the case of agrichemical application, adjuvant selection for bioherbicides may also need to consider some unique requirements by the living organism. Bio-agents will have to survive the process of application as well as the duration from landing on the plant surface to the occurrence of environment favorable conditions for the microbial inoculum to germinate and subsequently infect the weed. Ideally, adjuvants for bioherbicides should not only optimize deposition/retention efficiency, but also maintain the survival of microbial inoculum during the period post application (Zidack and Quimby 1998; Bateman and Chapple 2001). To meet this criterion, adjuvants selected should, first of all, be compatible with the bioherbicide agent. In a study evaluating commercial surfactants/adjuvants for potential formulation of the bioherbicide agents *Colletotrichum spp.* and *Phoma spp.* Zhang et al. (2003) found that the surfactant Tween® 20 reduced conidial germination of several fungal strains, whereas Tween® 40 or Tween® 80 stimulated the germination. It was observed that the latter two surfactants helped release *Colletotrichum* spp. conidia from self-inhibition of germination at high inoculum concentrations. Caution should be exercised when inferring the compatibility of an adjuvant because results can vary substantially depending on the agent or specific biological event in question. For example, Bailey et al. (2004) examined several adjuvants to aid application of the bioherbicide agent *Pleospora papaveracea* against opium poppy (*Papaver somniferum*) and found that the surfactant Tween® 80 inhibited appressorium formation but not conidial germination of the fungus. Most commercially available adjuvants are designed to facilitate application of agrichemicals and not compatible with the bioherbicide agent. Many of these products are somewhat too harsh to microbial inoculum, hence unsuitable for use with bioherbicide agents.

Prasad (1994) evaluated nine commercial adjuvants for potential formulation of *Chondrostereum purpureum*, a bioherbicide agent for control of deciduous shrubs in forest vegetation management in Canada (Harper et al. 1999; Pitt et al. 1999), and found that seven of the products were toxic to the fungus. These adjuvants included some of the common surfactants used in agrichemical applications, including Silwet L-77 and Triton X-100. If these surfactants were diluted from 0.1 to 0.01% in the formulation, however, the toxic effect would be significantly alleviated although the functionality of the surfactant may also be reduced. On the other hand, Silwet L-77 used in an oil-in-water emulsion made of unrefined corn oil showed little negative impact on mycelium of *Colletotrichum coccodes* (Boyette 2006). Direct effects of this adjuvant on spray retention were not reported. However, Silwet L-77 is considered an excellent wetting/spreading agent that can reduce the VMD of spray droplets when high-flow nozzles and low pressure are used (Stevens 1993). Finer droplets may have greater retention efficiency on certain weeds (Peng et al. 2005; Byer et al. 2006b). By reducing surface tension of the carrier, Silwet L-77 may also improve adherence of spray droplets to highly water repellent leaf surfaces (Stevens 1993). These retention features may provide the above *Colletotrichum* spp. bioherbicide agents with double benefits; increasing the spray or retention efficiency as well as promoting rapid germination and infection on the host. Available information clearly indicates a possibility of using adjuvants to improve spray retention efficiency. The key question is whether the improvement will be substantial enough to make a material difference in biocontrol efficacy. Of course, adjuvants to be considered for this type of application will have to be compatible with the bioherbicide inoculum. With these objectives, we carried out the following study to identify promising additives for tank-mix applications with *P. setariae* and *C. truncatum* for enhancement of spray deposition/retention efficiency and biocontrol efficacy against green foxtail and scentless chamomile.

**Case study III – Evaluation of spray adjuvants for bioherbicide delivery**

*P. setariae* and *C. truncatum* were selected as a model system in this study because retention patterns of their fungal propagules were similar to that of liquid carrier on respective weed targets (Byer et al. 2006b). More than 20 commercial adjuvants (Table 1), with advertised features as sur-
Table 1 Adjuvants evaluated for potential enhancement of spray retention on green foxtail and scentless chamomile.

<table>
<thead>
<tr>
<th>Product name</th>
<th>Functional ingredient</th>
<th>Property</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alginate</td>
<td>Sodium alginate</td>
<td>Thickener</td>
<td>Fisher Scientific Canada, Inc.</td>
</tr>
<tr>
<td>Amigo</td>
<td>Polyoxyalkylated alkyl phosphate ester</td>
<td>Surfactant</td>
<td>Bayer CropScience</td>
</tr>
<tr>
<td>Assist</td>
<td>Paraffin oil</td>
<td>Surfactant</td>
<td>BASF Canada Inc.</td>
</tr>
<tr>
<td>Bond</td>
<td>Synthetic latex primary aliphatic oxyalkylated alcohol</td>
<td>Spreader, sticker</td>
<td>Loveland Industries Inc. UK</td>
</tr>
<tr>
<td>Canplus 411</td>
<td>Crop oil concentrate</td>
<td>Surfactant</td>
<td>Syngenta Crop Protection Canada, Inc.</td>
</tr>
<tr>
<td>DR2000</td>
<td>Complex carbohydrate polymer</td>
<td>Thickener</td>
<td>Bayer CropScience</td>
</tr>
<tr>
<td>Dura-Gel®</td>
<td>Gelatinized starch</td>
<td>Sticker</td>
<td>Ingredient Warehouse, USA</td>
</tr>
<tr>
<td>Ekol</td>
<td>Vegetable oil</td>
<td>Surfactant</td>
<td>JIJA a spol. v.o.s., Czech Republic</td>
</tr>
<tr>
<td>Fenugreek gum</td>
<td>A plant-based biopolymer</td>
<td>Humectant</td>
<td>Agriculture and Agri-Food Canada, Saskatoon, SK</td>
</tr>
<tr>
<td>Gelatin</td>
<td>Collagen protein</td>
<td>Sticker</td>
<td>Lipton Inc. Canada</td>
</tr>
<tr>
<td>Glycerin</td>
<td>Glycerol</td>
<td>Humectant</td>
<td>Fish Sciences Canada, Inc</td>
</tr>
<tr>
<td>Intac</td>
<td>Polyacrylamide polymer</td>
<td>Thickener</td>
<td>Loveland Industries Inc. UK</td>
</tr>
<tr>
<td>LI 700</td>
<td>Surfactant blend</td>
<td>Thickener</td>
<td>United Agri Products, USA</td>
</tr>
<tr>
<td>Metamucil</td>
<td>Proprietary surfactant blend</td>
<td>Surfactant</td>
<td>BASF Canada Inc.</td>
</tr>
<tr>
<td>Previa C</td>
<td>Petroleum hydrocarbons</td>
<td>Humectant</td>
<td>Procter &amp; Gamble Canada Inc.</td>
</tr>
<tr>
<td>Score</td>
<td>Synthetic latex primary aliphatic oxyalkylated alcohol</td>
<td>Surfactant</td>
<td>Dow AgroScience Canada Inc.</td>
</tr>
<tr>
<td>Soydex</td>
<td>Proprietary</td>
<td>Surfactant</td>
<td>Helena Chemical Co. USA</td>
</tr>
<tr>
<td>Turbocharge</td>
<td>Mineral oil plus surfactant bland</td>
<td>Surfactant</td>
<td>Syngenta Crop Protection Canada, Inc</td>
</tr>
<tr>
<td>Water Lock G400</td>
<td>Biopolymers</td>
<td>Humectant</td>
<td>Polymers Inc. USA</td>
</tr>
<tr>
<td>Xanthan</td>
<td>Bacterial polysaccharides</td>
<td>Thickener</td>
<td>Sigma Chemical Co. USA</td>
</tr>
</tbody>
</table>

Table 2 Effect of selected adjuvants on retention, spore germination and efficacy of *Pyricularia oryzae* against green foxtail.

<table>
<thead>
<tr>
<th>Adjuvant</th>
<th>Retention (µl/mg)</th>
<th>Compatibility (% germination)</th>
<th>Efficacy (% disease)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2.6</td>
<td>75</td>
<td>12</td>
</tr>
<tr>
<td>Bond (1.0%)</td>
<td>4.1 **</td>
<td>69</td>
<td>16</td>
</tr>
<tr>
<td>Ekol (1.0%)</td>
<td>6.6 **</td>
<td>73</td>
<td>18</td>
</tr>
<tr>
<td>Intac (1.0%)</td>
<td>7.4 **</td>
<td>61 **</td>
<td>5 **</td>
</tr>
<tr>
<td>Tween 80 (1.0%)</td>
<td>6.5 **</td>
<td>74</td>
<td>27 **</td>
</tr>
<tr>
<td>Xanthan (1.0%)</td>
<td>4.8 **</td>
<td>83 **</td>
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*P* < 0.05.

Most adjuvants did not change spray retention volumes substantially when compared to water controls. Some increased the volume on green foxtail by 58 to 185% (Table 2), but none did so on scentless chamomile. Although mechanisms for the higher retention were not determined, these adjuvants may possibly have had an impact on spray characteristics (Chapple et al. 1993; Stevens 1993), which may in turn affect retention efficiency on green foxtail (Peng et al. 2005b) (Wolf et al. 1997) pointed out that the influence of an adjuvant on retention can be concentration dependent if the change in concentration affects dynamic surface tension of the spray mixture. In this study, adjuvant at the higher concentration often resulted in greater retention on green foxtail when compared to the lower concentration, but there were also practical limits due to changes in the carriers’ physical properties. For example, a gum made of the legume crop fenugreek (at 1.0% concentration) noticeably increased the viscosity of Ps suspensions as well as retention of spray on green foxtail plants when the treatment was applied with an airbrush sprayer. Consequently, this adjuvant mix increased the weed control when compared to the fungus delivered in the Tween® 80 surfactant (Fig. 3). However, this benefit could not be demonstrated with hydraulic flat-fan nozzles. Although viscous forces within the drop can act to absorb kinetic energy during the process of flattening and recoil on target surfaces and reduce rebound (Hall et al. 1993), the fenugreek gum at this concentration completely collapsed the spray pattern of flat-fan nozzles, resulting in uneven distribution. Novel atomizers such as twin-fluid nozzles have been suggested to overcome limiting physicochemical properties (Égley et al. 1993), but their lack of general availability has limited broad adoption.

Most of the adjuvants that had significant retention improvement appeared compatible with Ps, with no major impact on spore germination (Table 2). This indicated that these products were suitable for tank mixing with the fungus for the purpose of spore retention improvement on green foxtail. However, application of the fungal inoculum at a sub-lethal dose with most of these adjuvants did not achieve more effective weed control when compared to the fungus applied in water. Tween® 80 was the only exception, doubling disease severity (Table 2). The use of “Intac” caused even less disease than the fungus in water, a circumstance that may be associated with the slight reduction in spore germination by this adjuvant. This is another example which demonstrates that gains in spray retention may not necessarily be translated into substantial increases in weed biocontrol. It is not clear if any of the adjuvants interfered with other biological events of the fungus during in-

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*P* < 0.05.

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Fig. 3 Effect of fenugreek gum on biocontrol efficacy of *Pyricularia oryzae* against green foxtail under greenhouse conditions. From left to right: control (blank), fungus in Tween® 80 (1.0%), and fungus in fenugreek gum (1.0%).

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fication process, including appressorium formation and penetration. However, experience reminds us that spray retention alone often influences the efficacy of bioherbicide agents incrementally (Peng et al. 2005b). The outcome of weed control may be influenced more profoundly by formulation additives that facilitate the process of plant infection by the bioherbicide agent (Auld et al. 2003; Boyetchko and Peng 2004; Hynes and Boyetchko 2006).

CONCLUDING REMARKS

The traditional approach to herbicide application, “the dose makes the poison”, is reflected in the magnitude of research dedicated to understanding and increasing the amount of product on the target plant. Some of the successful approaches include changes in spray quality, spray trajectory, atomization and droplet adjuvants to achieve this goal. Due to the heterogeneous nature of crop canopies, weed morphologies, and modes of action of active ingredients, a certain degree of customization in spray parameters/additives is often required for the full benefit to be realized. In the case of bioherbicides, a significant number of complicating factors conspire to make the task significantly more challenging and less well understood. The first is that the dose response of many bioherbicides cannot be compared to traditional herbicides. In the former, often much greater gains have to be made for an efficacy benefit to be appreciable, largely due to the mechanism of biocontrol agents. Second, bioherbicide dose is uniformly distributed within the atomized droplets in a spray cloud. In fact, unlike soluble synthetic herbicides, the larger the agent propagules, the less likely they will be delivered to the target in small droplets. This poses a fundamental difficulty when combined with the third challenge, namely that there are specific sites of infection on the target plant that may be favoured over other sites for biological control. For example, the delivery of a large propagule to a plant stem is made more difficult given that the finer, not coarser droplets are better at reaching and being retained by stems. A fourth complicating factor is the fate of the bioherbicide propagule after it has been delivered to the plant. Its ability to infect the host will depend not only on a range of environmental factors, but also on the physical compatibility of the carrier with the propagule and the host tissue. It is important to ensure that adjuvants that enhance retention efficiency will not decrease germination or appressorial formation of the biocontrol agent. Otherwise, gains in spray retention can easily be negated.

Indeed, improvements in the performance of bioherbicides through enhancement of spray retention can be elusive. Substantial gains will come with considerable additional investment in research on all fronts including strain selection and formulation. Breakthroughs will more likely be case-specific, depending on technological, economical, and market successes. It is incumbent on the biocontrol research community to continue investing in fundamental aspects of the delivery technology to improve the effectiveness and efficiency of biopesticides.

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