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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 dependant. 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*. 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

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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 support the development from the apical meristem (Peng *et al.* 2005b).

Bioherbicide propagules generally can not be translocated after landing on plant surfaces, and therefore it is important to ensure that the inoculum is deposited and retained at critical sites of action in adequate quantities to kill or suppress the target (Lawrie *et al.* 1997; Bateman 1999). In laboratory studies, a type of aerosol sprayer is commonly used and a bioherbicide agent is applied to the point of runoff for maximum dose and coverage. This method of application results in extremely high retention volumes on the plant, a level achievable only with unrealistic carrier volumes (>2,000 L/ha) for most field spray equipment (Peng *et al.* 2005b). As a result, many lab test results tend to overestimate the potential of bioherbicide candidates (Greaves *et al.* 2000) because under controlled conditions, extremely high inoculum doses may allow even low-virulent pathogens to cause sufficient damage to the target weed (Lawrie *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).

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 (Wisniewska 1991; Hart *et al.* 1992; Moerkerk and Combellack 1992; Gillespie 1994; Stamm Katovich *et al.* 1996). For example, Feng *et al.* (2003) observed only slightly increase of retention on corn (*Zea mays*) using fine- (Volume Median Diameter - VMD 175 µm) as opposed to coarse-droplet (VMD 491 µm) sprays, whereas Peng *et al.* (2005b) found about 40% greater retention on green foxtail (*Setaria viridis*) for fine droplets (VMD 207 µm) against coarse droplets (VMD 325 µm) when the same application volume was used. On different weed targets, Wolf *et al.* (2000) also reported that an increase in spray coarseness reduced the retention on giant foxtail (*Setaria faberi*) while exhibited little effect on smooth pigweed (*Amaranthus hybridus*). It was believed that larger droplets were more likely to rebound from giant foxtail leaves that are difficult to wet due to the surface roughness caused by wax crystals and a large number of trichomes (Wolf *et al.* 2000). Similar observations were reported with young leaves of wild oat (*Avena fatua*) on which only droplets about 100 µm could be readily retained (Lake 1977). These variations due to surface characteristics of weed targets and droplet spectra, coupled with different sizes of microbial propagules involved, can greatly complicate the effort aimed at optimizing the efficiency and effectiveness of bioherbicide delivery. This review will focus on application technologies deemed important to deposition, retention, and performance of fungal bioherbicide inoculum on weed targets.

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 volume distribution. 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 300-µm 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 routinely. Instead, applicators rely on assumptions or approximations 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 tend to oversimplify 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 waste by reducing the frequency of over- or under-dosing the target. High deposit variability has been associated with reduced control of insects (Uk and Courshee 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 insecticides and herbicides (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.* 2006).

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 high volume, low-velocity airflow approach (Furness *et al.* 2003). Concerns about spray drift have prompted studying coarser and air-induced sprays in tree crops, with improved on-target deposition observed under a wide window of application conditions. These technologies have been shown to maintain or even improve disease control in orchards and vineyards (Lesnik *et al.* 2005), due to increased timeliness and effectiveness of application.

For boom sprayers, the advantage of finer droplets has been placed in question. In one study, there was no improvement in deposition of fungicide on edible bean plants when hollow cone nozzles were used (Maze *et al.* 1992). In fact, uniformity and overall spray recovery on artificial collectors was even reduced when compared to conventional nozzles. In another study, no differences in sclerotinia stem rot incidence, severity, canola yield or seed quality were observed between hollow cone (fine) and flat-fan nozzles (Kutcher and Wolf 2006). These results from studies in Canada are in agreement with others that reported no advantage to hollow cone nozzles over flat-fan nozzles for fungicide applications in other crops (Kucharek *et al.* 1986; Egel and Harmon 2001). In most row crop conditions, very fine droplets generated by hollow-cone nozzles likely are of limited advantage for increasing spray deposition.

Protocols for studying liquid spray retention (Wolf *et al.* 2000) have been used for studying microbial retention; a dye is dissolved in a spray carrier to estimate liquid volumes retained on the weed target (Wolf *et al.* 2000; Zhu 2004). After spraying, the dye solution is washed off from plants and the amount determined using a spectrophotometer. There is a substantial amount of information on spray drop sizes for herbicides to achieve optimal rates, carrier volume, and canopy penetration in a range of weed/production systems (Knoche 1994; Wolf *et al.* 2000; Feng *et al.* 2003; Zhu 2004; Zhu *et al.* 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 bioherbicide 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. Bioherbicide concentrations used for field applications may range from 10^6 to 10^8 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 setariae* 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 retention of a bioherbicide agent depending on propagule size, concentration, and spray-droplet spectrum. The following case studies are used to show retention characteristics of selected bioherbicide agents as affected by varying application methods, spray parameters, and weed targets.

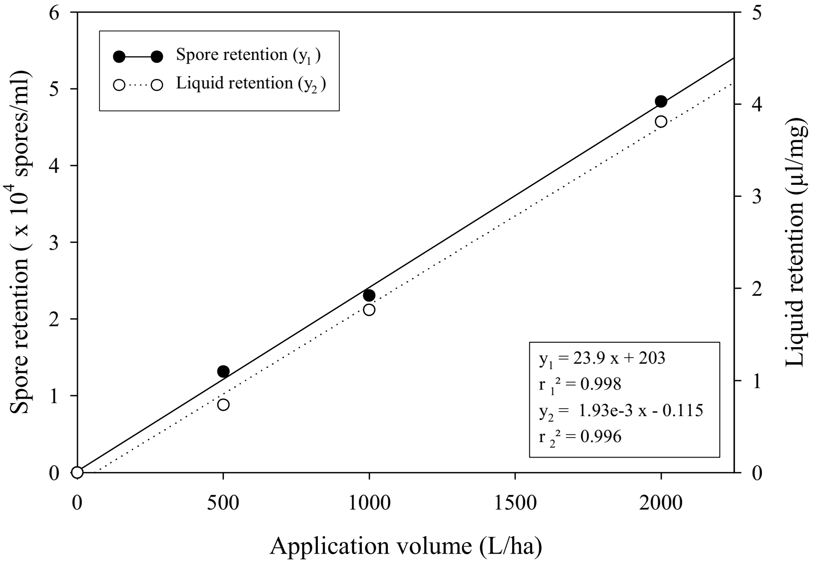

Fig. 1 Retention of Rhodamine WT dye and *P. setariae* spores on green foxtail applied using a broadcast sprayer at 500, 1,000, and 2,000 L/ha. Data were fitted with regression lines for which the correlation coefficients were calculated. From **Peng G, Wolf TM, Byer KN, Caldwell B** (2005) Spray retention on green foxtail (*Setaria viridis*) and its effect on weed control efficacy by *Pyricularia setariae*. *Weed Technology* **19**, 86-93.

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; Feng *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 *Mycocentospora 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; Wisniewska 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) suggested the tendency of reflection for large drops 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).

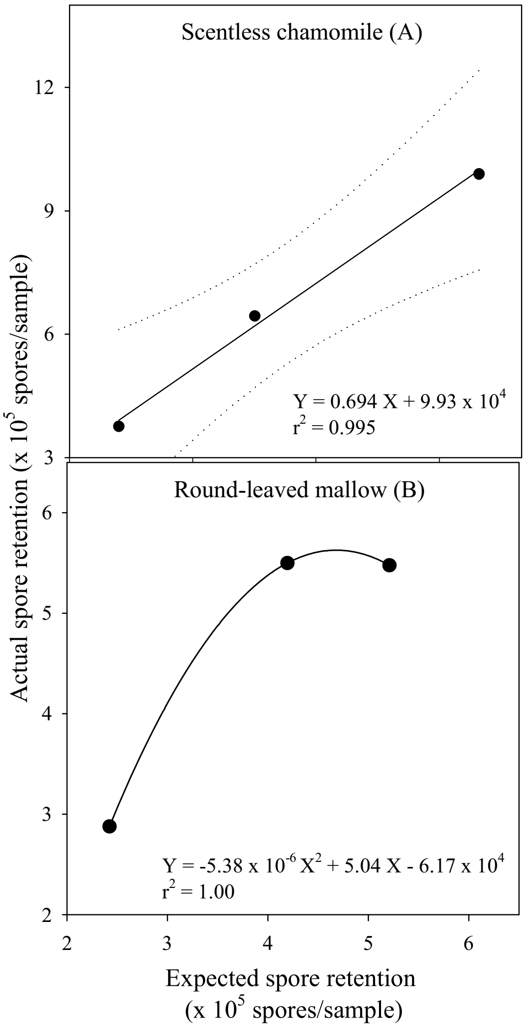

Fig. 2 Mean spore retention regressed against expected values based on liquid retention measurements on scentless chamomile (A) and round-leaved mallow (B). From **Byer KN, Peng G, Wolf TM, Caldwell BC** (2006) Spray retention for liquid and mycoherbicide inoculum in three weed-biocontrol systems. *Biocontrol Science and Technology* **16**, 815-823.

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 foxtail (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 1997; 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 can not be translocated after landing on the target (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 1999). Such sprays can, however, have unpredictable trajectories, are generally poor for canopy penetration, and prone to spray drift (Schaefer 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 1997).

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 uniform distribution of droplets when using 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 (Lake 1977; Chapple *et al.* 1996). In applying the bioherbicide fungus *Microsphaeropsis amaranthi* to waterhemp (*Amaranthus rudis*), (Doll *et al.* 2005) examined various types of hydraulic nozzles and found that a hollow cone nozzle, often used for directed applications of fungicides and insecticides in air blast sprays in orchards, can generate a finely atomized spray pattern that provides excellent coverage of the target plant due to large numbers of fine droplets swirling around within the plant canopy. As a result, maximum spray retention and disease severity was obtained, particularly on stems. Although hollow cone nozzles are not commonly used for herbicide application, the better spray retention on stems and more efficacious weed control underscore the efficiency of these small droplets in carrying a sufficient number of fungal propagules and getting them onto vertical surfaces. To reduce spray drift, the boom may be lowered into the canopy. This may also increase the chance of loading "biologically efficient" small droplets at the stem base (Hislop 1987; Stonehouse 1993; Hall *et al.* 1996).

Nozzle angling may be used to increase spray deposition on vertical surfaces because the target in a perpendicular position to droplet trajectory is most efficient for 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 potential to increase spray deposition as well as uniformity over the conventional single-nozzle system on the vertical target. Faster travel speeds also increased overall spray deposits, mostly from the contribution of the front nozzle. The authors concluded that a combined use of double nozzles, coarser sprays, and faster travel speeds could increase spray retention on vertically positioned wheat spikes by more than 100% when compared to a conventional sprayer (Wolf and Caldwell 2004). They also believed that the collection efficiency might be further enhanced on wheat spikes by increasing spray droplet velocity and this view is shared by others for better targeting vertical surfaces (Zhu *et al.* 1996). This strategy may also be applicable to the delivery of chemical herbicides, especially for weeds with more vertically positioned leaves (Wolf and Caldwell 2004). A comparable double-nozzle configuration was also evaluated for application of *P. setariae* to increase retention of the bioherbicide agent on the vertical top leaf of green foxtail, which was considered critical to regrowth from the apical meristem tissue (Peng *et al.* 2004). Improved weed control was achieved with this device over conventional vertically positioned nozzles (Peng *et al.* 2001).

Lawrie *et al.* (2002) reported that airbrush sprays, even at low volumes, consistently resulted in greater retention and better coverage on stems of redroot pigweed (*Amaranthus retroflexus*) when compared to applications using hydraulic 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 was deemed 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 additives can change spray drop-size spectrum and velocity, in-flight and/or impaction 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. 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 environmental conditions that are conducive 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 it would not be surprising that 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 spores of *Colletotrichum truncatum* for biocontrol of hemp sesbania (*Sesbania exaltata*) and this formulation noticeably enhanced the efficacy of weed control over the fungal inoculum suspended in water. This emulsion stimulated fungal germination and appressorial formation, consequently lessening the dew requirement under field conditions. It was believed that the oil protected the spores from desiccation during the dew-free period and the surfactant Silwet L-77 promoted rapid germination and appressorium formation once the dew materializes (Boyette *et al.* 2007). A single application of this oil-based fungal formulation with a tank mix of 0.2% (v/v) Silwet L-77 controlled hemp sesbania by 95% in soybean fields when compared to the adjuvant alone, which had no visible effect. This formulation is also beneficial to a similar bioherbicide agent, *C. gloeosporioides*, used for control of sicklepod (*Senna obtusifolia*); the adjuvant Silwet L-77 reduced the requirement of wetness duration for successful fungal infection from 16 h to 8 h (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.

Product name	Functional ingredient	Property	Source
Alginate	Sodium alginate	Thickener	Fisher Scientifics Canada, Inc.
Amigo	Polyoxyalkylated alkyl phosphate ester	Surfactant	Bayer CropScience
Assist	Paraffin oil	Surfactant	BASF Canada Inc.
Bond	Synthetic latex primary aliphatic oxyalkylated alcohol	Spreader, sticker	Loveland Industries Inc. UK
Canplus 411	Crop oil concentrate	Surfactant	Syngenta Crop Protection Canada, Inc.
DR2000	Complex carbohydrate polymer	Thickener	Bayer CropScience
Dura-Gel®	Gelatinized starch	Sticker	Ingredient Warehouse, USA
Ekol	Vegetable oil	Surfactant	JIZA a spol. v.o.s., Czech Republic
Fenugreek gum	A plant-based biopolymer	Humectant	Agriculture and Agri-Food Canada, Saskatoon, SK
Gelatin	Collagen protein	Sticker	Lipton Inc. Canada
Glycerin	Glycerol	Humectant	Fish Scientifics Canada, Inc
Intac	Polyacrylamide polymer	Thickener	Loveland Industries Inc. UK
LI 700	Surfactant blend	Thickener	United Agri Products, USA
Merge	Proprietary surfactant blend	Surfactant	BASF Canada Inc.
Metamucil	Psyllium hydrophilic mucilloid	Humectant	Procter & Gamble Canada Inc.
Prevail C	Mineral oil	Surfactant	Dow AgroScience Canada Inc.
Score	Petroleum hydrocarbons	Surfactant	Syngenta Crop Protection Canada, Inc
Soydex	Proprietary	Surfactant	Helena Chemical Co. USA
Turbocharge	Mineral oil plus surfactant bland	Surfactant	Syngenta Crop Protection Canada, Inc
Tween 80	Sorbitan monooleate	Surfactant	Fisher Scientific Canada, Inc.
Watersorb	Acrylic acid /acrylamide polymer	Humectant	Polymers Inc. USA
Water Lock G400	Biopolymers	Humectant	Grain Processing Co. USA
Xanthan	Bacterial polysaccharides	Thickener	Sigma Chemical Co. USA

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

Adjuvant	Retention (µl/mg)	Compatibility (% germination)	Efficacy (% disease)
Control (spores in water)	2.6	75	12
Bond (1.0%)	4.1 ** [a]	69	16
Ekol (1.0%)	6.6 **	73	18
Intac (1.0%)	7.4 **	61 ** [a]	5 ** [a]
Tween 80 (1.0%)	6.5 **	74	27 **
Xanthan (1.0%)	4.8 **	83 **	13

[a] Treatment means are significantly different from the control (LSD, $P < 0.05$).

Fig. 3 Effect of fenugreek gum on biocontrol efficacy of *Pyricularia setariae* 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%).

factants, thickeners, binders, stickers, spreaders, or humectants were selected and evaluated using a medium spray quality (VMD 267 µm) at 200 L/ha spray volume. Each adjuvant was tested at 0.1 and 1% (v/v) concentrations. Products or concentrations that substantially increased spray retention over water controls were assessed further for compatibility with the fungal agents and potential to improve biocontrol efficacy.

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 quality (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 dependant 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 (Egley *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 infection 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, atomizers, and use of 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 not 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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