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Relationship between the Spray Droplet Density of Two Protectant Fungicides and the Germination of *Mycosphaerella fijiensis* Ascospores on Banana Leaf Surfaces

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Abstract: The effect of fungicide spray droplet density (droplet cm^{-2}), droplet size, and proximity of the spray droplet deposit to fungal spores was investigated with *Mycosphaerella fijiensis* ascospores on the banana (*Musa* AAA) leaf surface for two contact fungicides: chlorothalonil and mancozeb. When droplet size was maintained at a volume median diameter (VMD) of 250 μm while total spray volume per hectare changed, *M. fijiensis* ascospore germination on the leaf surface fell below 1% for both fungicides at a droplet deposit density of 30 droplet cm^{-2}. At a droplet deposit density of 50 droplet cm^{-2}, no ascospores germinated in either fungicide treatment. When both droplet size and droplet cm^{-2} varied while spray volume was fixed at 20 litre ha^{-1}, ascospore germination reached 0% at 10 droplet cm^{-2} (VMD = 602 μm) for both fungicides. At lower droplet densities (2–5 droplet cm^{-2} VMD = 989 μm and 804 μm respectively), ascospore germination on the mancozeb-treated leaves was significantly lower than on the chlorothalonil-treated leaves. The zone of inhibition surrounding a fungicide droplet deposit (VMD = 250 μm) on the leaf surface was estimated to extend 1·02 mm beyond the visible edge of the spray droplet deposit for chlorothalonil and 1·29 mm for mancozeb. The efficacy of fungicide spray droplet deposit densities which are lower than currently recommended for low-volume, aerial applications of protectant fungicides was confirmed in an analysis of leaf samples recovered after commercial applications in a banana plantation. Calibrating agricultural spray aircraft to deliver fungicide spray droplets with a mean droplet deposit density of 30 droplet cm^{-2} and a VMD between 300 and 400 μm will probably reduce spray drift, increase deposition efficiency on crop foliage, and enhance disease control compared to aircraft calibrated to spray finer droplets.

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1 INTRODUCTION

Fungicides are applied in commercial banana (*Musa* AAA) plantations to manage Black Sigatoka disease (*Mycosphaerella fijiensis* Morelet), the major limiting factor in banana production where it occurs.[1] Fungicides are applied by aircraft at low spray volumes (15–25 litre ha^{-1}) to cover large areas efficiently. In many banana-producing regions, the protectant fungicides chlorothalonil ('Bravo'®), and mancozeb ('Dithane'®, 'Vondozeb'®, 'Manzate'®) comprise 25% to 50% of the total number of fungicide applications. The importance of these fungicides in the control of Black Sigatoka, and the high cost of fungicide applications, make it imperative that these applications be as efficient as possible.

One of the criteria used to judge the efficiency of aerial applications of fungicides is the density for spray droplet deposits per unit of leaf area, usually expressed as droplet cm^{-2}. It is a common perception by consultants and applicators in the chemical and agricultural industry that a high density of fungicide spray droplet deposits (>70 droplet cm^{-2}) is needed for low-volume, aerial applications to achieve adequate distribution of the fungicide on the leaf surface, and, consequently, effective disease control. High droplet deposit densities of protectant fungicides also are recommended in technical manuals used in the banana industry.[2,3]

However, there are no reports in the literature which serve as a basis for the current recommendations of the optimum droplet deposit density for low-volume, aerial applications of fungicides on crop foliage. Additionally, no observations have been published on the interaction of protectant fungicide droplet deposits and fungal pathogens on the plant leaf surface. Current recommendations on the optimal spray droplet deposit density for protectant fungicides appear to be based on studies examining the optimal size of spray droplets for aerially applied insecticides.[4–6] Since the mode of action and the target organisms of insecticides are different from those of fungicides, current recommendations for the optimal droplet deposit density of protectant fungicides on crop foliage may not be valid.

The objective of this study was to quantify the optimum spray droplet deposit density of two protectant fungicides on banana foliage by means of bioassays testing the inhibition of *M. fijiensis* ascospore germination on banana leaf surfaces.

2 EXPERIMENTAL METHODS

2.1 Droplet deposit density tests

2.1.1 Treatments

In the test where total spray volume was fixed at (20 litre ha^{-1}), the treatments consisted of an untreated check and six different spray droplet deposit densities for each of two protectant fungicides applied to banana leaves: chlorothalonil (1·08 kg AI ha^{-1}) and mancozeb (2·24 kg AI ha^{-1}). The droplet deposit densities were 2, 5, 10, 20, 30 and 50 droplet cm^{-2}. The droplet sizes (volume median diameter, VMD) tested ranged from 305 μm to 989 μm.

For the test in which droplet size was maintained at VMD = 250 μm, fungicide rates (kg ha^{-1}) and total spray volume (litre ha^{-1}) varied according to the spray droplet deposit density. Droplet deposit densities tested were 5, 10, 20, 30 and 50 droplet cm^{-2}, which corresponded to total spray volumes of 2·0, 4·0, 8·0, 12·0 and 20·0 litre ha^{-1} respectively. Chlorothalonil (720 g $litre^{-1}$ SC; 'Bravo' 720) and mancozeb (448 g $litre^{-1}$ SC; 'Dithane' MB) formulations were always mixed into the spray on a fixed v/v basis (75 ml $litre^{-1}$ and 250 ml $litre^{-1}$ respectively). Consequently, as the total spray volume was decreased, the rates (kg AI ha^{-1}) of each fungicide decreased proportionately. For example, at the 20 litre ha^{-1} spray volume, the spray concentrations were equivalent to fungicide rates of 1·08 kg AI ha^{-1} and 2·24 kg AI ha^{-1} for chlorothalonil and mancozeb respectively, whereas at the 8·0 litre ha^{-1} spray volume, the fungicide rates were 0.43 kg AI ha^{-1} and 0·90 kg AI ha^{-1}.

2.1.2 Fungicide application

Fungicides were applied to the adaxial surface of banana leaves with an electrically powered 'Micronair' AU7000 rotary atomizer (Micronair Sales & Service, Inc., Miami FL) mounted on top of a stationary, outdoor spray tower located at ISK Biosciences Corporation's research facility in Santa Rita, Yoro, Honduras. The spray tower consisted a 4·5-m-high steel frame structure with a 4 × 4 m base, and was insulated on all four sides with polyethylene sheets (4·5 m high) to protect spray applications from exposure to crosswind. The rotary atomizer was mounted on top of the spray tower at a height of 4·2 m and positioned over the center of the square. Fungicides were mixed with water and the spray mixtures were placed in stainless steel beverage containers (11·3 litre capacity; R&D Sprayers, Inc., Opelousas, LA) for application. A steel cylinder containing compressed carbon dioxide gas was used to propel the fungicide mixture *via* plastic tubing (0·5 cm diameter) to the rotary atomizer. Fungicides were expelled onto individual, freshly cut banana leaves placed on the ground inside the spray tower. The fungicide mixtures were constantly agitated during application by placing magnetic stir bars inside the containers and mounting the containers on magnetic stir plates (Fisher Thermix® Stirrer, model 120MR, Fisher Scientific). The flow rate was maintained at 0·75 litre min^{-1} with a flow regulator valve and measured with a 'Tip Tester' (Spraying Systems, Wheaton, IL) inserted in

the spray line. Spray pressure was maintained at 207 kPa.

The size of the spray droplets applied to the banana leaves was controlled by regulating the revolutions per minute (rpm) of the rotary atomizer by means of a rheostat (0–140 A, 120 V Powerstat® Autotransformer, Superior Electric Co., Bristol, CN). The system was similar to that described by Dubs *et al.*[7] and allowed the droplet size and droplet deposit density to be changed while maintaining the total spray volume constant. At a fixed spray volume, as spray droplet deposit density was increased, droplet size decreased. In the test where droplet size was maintained fixed but droplet deposit density was changed, the total spray volume was controlled by adjusting the duration (seconds) of application. Droplet deposit density on the leaf samples was estimated by visual inspection under a stereo microscope. Droplet size (VMD) was estimated by analyzing droplet deposits on water-sensitive paper (Spraying Systems, Wheaton, IL) with a computerized optical image analyzer system ('Swath Kit', Droplet Technologies Inc., Crystal Lake, IL). The image analyzer system is integrated with computer software which calculates an estimate of the volume median diameter (VMD); this is the droplet diameter such that half of the spray volume comprises drops smaller than this size and half larger. In the determination of VMD, the software incorporates an estimation for the spread factor for spray droplet deposits on water-sensitive paper. The spread factor for each fungicide mixture was estimated by the silicone capture method,[8] which consists of capturing spray droplets in Petri plates containing a layer of 500-cS (low-viscosity) silicone fluid ('Boss'® DS Fluid, Accumetric, Inc., Elizabeth, KY) covering a layer of 300 000-cS (high-viscosity) silicone fluid. Spray droplets at the interface of the two silicone fluid layers are measured under a compound microscope (40 ×) and these measurements are compared to spray droplet deposits on water-sensitive paper. Linear regression analysis is performed on the data to relate the diameter of droplet deposits on water-sensitive paper with actual droplet sizes (measured in silicone fluid). During each application of the fungicide mixtures to banana leaves, the droplet spectrum emitted from the rotary atomizer was sampled by placing twelve 52 × 76 mm, water-sensitive spray cards on the ground inside the spray tower immediately adjacent to the banana leaves. These cards were then subjected to analysis using the swath kit as described above. This sample number ensured that the estimates of VMD were based on the measurements of at least 1000 spray droplet deposits.

2.1.3 Ascospore germination bioassay

Necrotic banana leaves containing parethecia of *M. fijiensis* were collected from field plots in Santa Rita, Honduras one to two days prior to use. The necrotic leaf tissue was dried on the laboratory bench overnight and cut into 2 × 2 cm pieces, which were stapled to 9-cm-diameter filter paper circles, eight leaf pieces per filter paper. Immediately prior to use, the filter paper circles containing the necrotic leaf tissue were soaked in sterile, distilled water for 5 min. The filter paper circles were then placed inside the lid of a 9-cm-diameter Petri plate. Leaf samples (9-cm-diameter circles) were cut from the fungicide-treated or untreated banana leaves and placed, adaxial surface facing up, on top of moist paper towels covering the bottom portion of the Petri plate. The Petri plate lids with necrotic leaf pieces were then placed over the leaf samples contained in the bottom plate and the Petri plates were left on the laboratory bench of 2 h to allow natural discharge of *M. fijiensis* ascospores onto the banana leaf samples. The filter paper and necrotic leaf pieces were then removed, and sterile, distilled water from a 1-litre, hand-held garden sprayer was misted onto the leaf samples to induce ascospore germination by simulating natural dew formation which occurs nightly on banana leaves in the field.[9] The covered Petri plates were stored on the laboratory bench for 48 h, after which time ascospore germination was quantified using the ascospore staining method described below. Germination tests were conducted with ascospores, since ascospores are the most important source of inoculum for *M. fijiensis*.[10]

The banana leaf samples were cut into strips (2·5 cm wide) and stained for 15 min in a 10 g litre^{-1} aqueous solution of Rose Bengal stain (Fisher Scientific), rinsed with distilled water to remove excess stain, and examined at 100 × under a compound microscope. Ascospore germination was quantified in ten microscopic fields of view chosen at random. An ascospore was considered germinated if it had a germ tube of at least 5 μm in length.

2.1.4 Data analysis

Five replicates were used per treatment. A replicate consisted of a 9-cm circular leaf sample. There were ten samples per replicate. A sample consisted of a microscopic field of view at 100 × magnification. The number of ascospores in a 100 × field of view ranged from 10 to 126 spores. For each replicate, the percentage germination of ascospores was calculated and data were averaged for each treatment. Data were combined from three separate tests.

2.2 Zone of inhibition tests

2.2.1 Methodology

The zones of inhibition surrounding fungicide spray droplet (VMD = 250 μm) deposits were estimated for chlorothalonil and mancozeb. The zone of inhibition was defined as the minimum linear distance from the edge of a spray droplet deposit on the banana leaf to

the point where *M. fijiensis* ascospores germinated in the vicinity of that droplet deposit. In order to avoid overlap of inhibition zones from different spray droplet deposits, a droplet deposit density of 2–5 droplet cm^{-2} was used, while maintaining the droplet size at VMD = 250 μm.

Fungicide application to banana leaves, ascospore discharge, incubation and staining were conducted in a manner similar to that described for the droplet deposit density study. Forty-eight hours after inoculation with ascospores, leaf samples were stained and examined at 100 × under the compound microscope. Fungicide droplet deposits on the leaf surface were clearly visible; both chlorothalonil and mancozeb spray droplet deposits stained reddish brown with Rose Bengal. When a group of *M. fijiensis* ascospores was found in the vicinity of a fungicide droplet deposit, measurements were taken of the distance between the edge of the spray droplet deposit and the closest point where ascospores germinated in the vicinity of the droplet deposit. Up to three measurements were taken per droplet deposit, depending on the location of ascospores around the droplet deposit. Care was taken to ensure that inhibition zones of other fungicide droplet deposits did not overlap with the observed inhibition zone by choosing droplet deposits that were separated from other droplet deposits by at least 5 mm in all directions.

2.2.2 Data analysis

For each fungicide, the inhibition zones surrounding 20 spray droplet deposits were measured. If more than one measurement of the inhibition zone was taken for a spray droplet deposit, measurements were averaged for that droplet deposit. A *t*-test assuming unequal sample variance was conducted to compare the widths of the inhibition zones surrounding chlorothalonil and mancozeb spray droplet deposits. The experiment was conducted three times with similar results. Data were combined from all three tests.

2.3 Field applications

Leaf samples were collected from three different banana plantations immediately after a commercial application of chlorothalonil in order to quantify the spray droplet density on collected leaf samples and the percentage germination of *M. fijiensis* ascospores inoculated on the leaf samples in the laboratory. The applications were made with an Ag Cat, piston engine, fixed-wing aircraft fitted with ten Micronair AU5000 rotary atomizers and covering a swath width of 24 m. The spray volume applied was 20 litre ha^{-1}. Prior to conducting this evaluation, it was known that the airplane was calibrated to deliver coarser spray droplets (VMD = 360 μm) than are normally used (VMD = 250 μm) in fungicide sprays in banana. This estimation was confirmed by placing water-sensitive card samples at 2-m intervals across the entire spray swath during the commercial applications and estimating the VMD with the image analyzer system previously described. Leaf samples were collected from the most recently emerged, fully expanded leaf that had not previously received a fungicide application. In each test, two leaf samples were collected from each of ten plants (20 samples total) spanning one spray swath. Leaf samples were immediately taken to the laboratory where the spray droplets density was quantified for each sample. The ascospore bioassay was then conducted as previously described. Percentage ascospore germination was averaged for each leaf sample. The field trial was conducted three times. Only data from the first trial are summarized, since results from the subsequent two trials revealed a slightly narrower range of droplet deposit densities on foliage, and ascospore germination data were identical to results from the first trial (0% germination).

3 RESULTS

3.1 Droplet deposit density tests

3.1.1 Observations

Fungicide spray droplet deposits and ascospores of *M. fijiensis* were clearly visible on the banana leaf surface after the staining procedure with Rose Bengal (Plates 1 and 2), permitting quantification of spore germination. The ascospores and germ tubes stained dark red and the spray droplet deposits stained reddish brown.

3.1.2 Maintaining droplet size fixed while varying droplet deposit density and total spray volume

The relationship between the density of spray droplet (VMD = 250 μm) deposits and the percentage germination of ascospores was similar for both fungicides, with the percentage germination of ascospores declining sharply as the spray droplet density increased (Fig. 1). With both fungicides, percentage germination of asco-

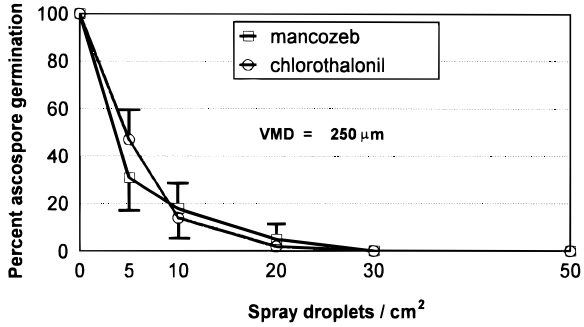

Fig. 1. Relationship between the spray droplet deposit density of (□) mancozeb and (○) chlorothalonil and *Mycosphaerella fijiensis* ascospore germination on banana leaves. Data represent the mean of three experiments.

TABLE 1
Ascospore Germination at Different Spray Droplet Deposit Densities of Chlorothalonil and Mancozeb on the Banana Leaf Surface, Total Spray Volume 20 litre ha^{-1}

Spray droplet deposit density (droplet cm^{-2})	*VMD (μm)*	*Ascopore germination (%)*	
		Chlorothalonil	*Mancozeb*
0	na	100	100
2	989	22·8	5·0[a]
5	804	17·3	0·1[a]
10	602	0·0	0·0
20	447	0·0	0·0
30	399	0·0	0·0
50	305	0·0	0·0

[a] Difference between chlorothalonil and mancozeb significant ($P = 0·05$) according to *t*-test. No significant difference in other cases.

spores fell below 1% at the droplet deposit density of 30 droplet cm^{-2}.

3.1.3 Varying droplet size while changing droplet deposit density (fixed spray volume)

Using a total spray volume of 20 litre ha^{-1}, the spray droplet density was varied from 2 to 50 droplet cm^{-2}, with a corresponding change in droplet size as shown in Table 1. Percentage ascospore germination reached 0% for both fungicides at a deposit density of 10 droplet cm^{-2} (VMD = 602 μm). At the deposit densities of 2 droplet cm^{-2} (VMD = 989 μm) and 5 droplet cm^{-2} (VMD = 804 μm), the percentage of ascospores that germinated in the mancozeb treatment was significantly less ($P = 0·05$) than in the chlorothalonil treatment.

3.2 Zone of inhibition tests

Since both the ascospores and the fungicide spray droplet deposits were made visible under the microscope with the staining technique, the inhibition zones of fungicide spray droplet deposits could be measured. The mean distances between the edge of a spray droplet deposit and the first germinated spore in the vicinity of that spray droplet deposit were 1·02 and 1·29 mm for chlorothalonil and mancozeb respectively (Table 2).

TABLE 2
Zone of Inhibition surrounding Fungicide Spray Droplet (VMD = 250 μm) Deposits on the Banana Leaf Surface, data Combined from Three Separate Tests

Fungicide	*Mean distance from edge of spray droplet (mm)*	*Variance*	*Observations*
Chlorothalonil	1·02	0·04	60
Mancozeb	1·29[a]	0·10	60

[a] Difference between treatments significant ($P = 0·05$) according to two-sample *t*-test with unequal variances.

3.3 Field applications

Ascospore germination was not detected in any of the banana leaf samples collected in the field immediately following commercial applications with an airplane calibrated to spray large spray droplets (VMD = 360 μm). Spray droplet deposit densities ranged from 14·2 to 56·8 droplet cm^{-2} with a mean 33·1 ($\pm$19·6, $s = 9·4$) droplet cm^{-2}.

4 DISCUSSION

This study quantified the relationship between the droplet deposit density of protectant fungicides on crop foliage and the inhibition of fungal spore germination. A droplet deposit density of 30 droplet cm^{-2} (VMD = 250 μm) of the fungicides chlorothalonil and mancozeb resulted in over 99% inhibition of *M. fijiensis* ascospore germination on the banana leaf surface. With larger spray droplets (VMD = 602 μm), a droplet deposit density of 10 droplet cm^{-2} resulted in 100% inhibition of ascospore germination. The results suggest that the current recommendation of a minimum of 70 spray droplets cm^{-2} to give effective control of Black Sigatoka in banana is incorrect. Based on the results of this study, where a protectant fungicide was applied both in a controlled setting and in a commercial application, the recommended droplet deposit density in low-volume applications (20 litre ha^{-1}) is 30 droplet cm^{-2}.

The significance of calibrating airplanes to deliver a droplet deposit density on banana foliage that is lower than that previously recommended is that it allows the applicator to use spray droplets with a VMD between 300 μm and 400 μm while continuing to use the standard spray volume of 20 litre ha^{-1}. In order to obtain a droplet deposit density of 70 droplet cm^{-2} with a spray volume of 20 litre ha^{-1}, a droplet size (VMD) in the range of 250–280 μm must be used. Smaller spray droplets have significant disadvantages with aqueous fungicide spray mixtures; smaller droplets have a lower terminal velocity and will be suspended in the air for a longer time period than larger droplets. This is likely to increase evaporation losses from the spray droplets, thereby reducing the efficiency of recovery on crop foliage, especially with high temperatures and low relative humidity levels. Additionally, smaller droplets will be more susceptible to drift onto non-target sites. This is particularly true for droplets smaller than 100 μm.[11] The use of anti-evaporants in pesticide formulations has been reported to reduce losses of spray volume during aerial applications.[12] Increasing droplet size may have a

similar effect, since the surface-area-to-volume ratio of the spray droplets would decrease, and spray volume losses due to evaporation would probably be less.

In addition to droplet deposit density on crop foliage, another important factor to consider when determining optimal droplet size for pesticide applications is penetration of the spray droplets into the plant canopy. Although canopy penetration was not addressed in this study, the recommended droplet size based on the data from this study (VMD = 300–400 μm) falls within the recommended range of droplet sizes (VMD = 200–400 μm) needed to obtain effective penetration in the plant canopy by aerially applied pesticides.[13] Additionally, evaluation of fungicide coverage on new foliage resulting from a commercial application in this study indicated that there was complete inhibition of *M. fijiensis* ascospore germination on leaf surfaces with a mean droplet deposit density of 33 droplet cm^{-2} (VMD = 360 μm).

Further work is needed to analyze the recovery efficiency of fungicide applications in banana using spray aircraft calibrated to deliver different droplet sizes ranging from VMD = 250 μm to VMD = 400 μm. It is probable that calibrating aircraft to deliver larger droplets will reduce spray drift, increase fungicide recovery efficiency, and enhance disease control. The results of this study also suggest that refinements can be made to the recommendations for spray volume and fungicide rates for the control of Black Sigatoka.

This study confirmed that a zone of inhibition surrounds a fungicide spray droplet deposit on the leaf surface, which could be recognized by the presence of ungerminated spores at a distance from the edge of the visible spray droplet deposit. One explanation for this zone of inhibition may be that small fungicide particles, which are not visible with the compound microscope, are dispersed beyond the edge of the visible spray droplet deposit. A more plausible explanation is that the fungicide is partially dissolved into the free water on the leaf surface and it is in this aqueous medium that uptake into and inhibition of fungal metabolism occurs. With *M. fijiensis*, a film of water on the leaf surface is required for ascospore germination.[14]

The relationship between the size of a fungicide spray droplet and the diameter of the inhibition zone surrounding the droplet deposit on the leaf surface was not addressed in this study. However, it appears likely that, as the size of the deposited spray droplet increases, the zone of inhibition will be correspondingly, larger since the concentration of fungicide in the free water surrounding the spray droplet deposit will be greater. This would explain the observation that the droplet deposit density required for 0% ascospore germination in this study was 10 droplet cm^{-2} when the VMD was 602 μm, whereas when the VMD was 250 μm at the same droplet deposit density, 14% germination was observed.

The observation that mancozeb resulted in a significantly higher level of inhibition of ascospore germination than chlorothalonil at droplet deposit densities of 2 and 5 droplet cm^{-2} (VMD = 989, 804 μm respectively) is consistent with the observation of a larger inhibition zone surrounding a mancozeb droplet deposit compared to chlorothalonil. A probable explanation for this is that mancozeb fungicides are more soluble in water (20 mg $litre^{-1}$) than chlorothalonil (0·9 mg $litre^{-1}$),[15] and they may be more easily taken up into the free water solution on the leaf surface, redistributing over a larger area and increasing their chances of coming into contact with the fungal spores. This explanation is consistent with higher rain washoff rates of the mancozeb fungicides compared to chlorothalonil.[16]

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