Atomization and Sprays, 24 (4): 367–373 (2014)

A FLUORESCENT IMAGING TECHNIQUE FOR QUANTIFYING SPRAY DEPOSITS ON PLANT LEAVES

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Original Manuscript Submitted: 08/16/2013; Final Draft Received: 11/07/2013

Because of the unique characteristics of electrostatically-charged sprays, use of traditional methods has failed to accurately quantify deposition from these sprays. A new fluorescent imaging technique was developed that quantifies spray deposits from electrostatically-charged sprays on natural plant leaves. Results indicate that this technique can successfully detect and enumerate individual spray droplets on the underside of beet leaves. Further, this technique reveals the spatial distribution of the spray droplets. Researchers will be able to use this technique to quantify spray deposits on a multitude of artificial and natural deposition samplers.

KEY WORDS: *spray droplets, droplet count, spray application*

1. INTRODUCTION

In order for agricultural spray applications to be effective, the correct size of spray droplets must be applied to the intended target. The intended target may be a leaf, a fungal spore, or an insect. For many systemic herbicides, merely depositing several large droplets on the top side of a leaf is sufficient for adequate control. However, for control of many plant diseases or insects, the droplets must be smaller (less than 200 μm) to achieve good coverage and must deposit on the underside of the leaves where the insects or fungal spores often reside (Byrne and Bellows, 1991; O'Brien et al., 1993; Wilson, 1993; Ebert and Cartwright, 1997).

Artificial and natural samplers are both used to evaluate deposition from spray application treatments. Typical artificial samplers that have been used in laboratory and field studies include mylar sheets, water-sensitive papers, glass slides, strings, monofilament lines, mesh screens, and plastic soda straws (Fritz et al., 2007; Martin et al., 2010). Natural samplers include insects and plants, either whole or in part (e.g., leaves and stems). Fluorescent dyes often are added to the spray solution as tracers to confirm where the spray was applied and to quantify the amount deposited (MacIntyre-Allen et al., 2007). Mylar sheets are excellent for quantifying total spray deposition from conventional spray

1044–5110/14/$35.00

applications by solvent extraction of fluorescent dye that has been uniformly mixed into the spray solution. Water-sensitive papers do not require a fluorescent dye and can be processed with customized software to yield individual droplet data (Hoffmann and Hewitt, 2005; Thomson and Lyn, 2011), but spoil easily at high humidity and do not capture small droplets (less than $\sim$50 μm) very well. Plant leaves are natural samplers that can be washed top and bottom with solvents to extract and quantify total fluorescent tracer amounts (Carlton, 1992, 1996), but individual droplet data are difficult to extract from these samplers.

Conventional spray applications primarily rely on gravity to transport the spray to the intended target. Electrostatically charged sprays use gravity and an electrostatic field to direct the spray to the target (Carlton et al., 1995). While artificial samplers work well with conventional sprays, they can interfere with the deposition of electrostatically charged sprays. Sharp edges and corners on artificial samplers can induce a buildup of charge at those points. This buildup of charge can subsequently deter charged droplets from depositing at those locations. In addition, the lack of an electrical ground for these samplers reduces the affinity of charged droplets to these samplers. Although many plants have leaves with points and edges, the plants are grounded and provide excellent samplers because they are the actual intended target.

Since typical artificial samplers are not appropriate for sampling electrostatically charged sprays, natural plant materials must be used. However, current sampling techniques for plants only yield total spray deposition data. The study described herein details how to quantify the number of individual droplets on plant leaves, and visualize the spatial distribution of spray droplets on these leaves.

1.1 Objectives

The objectives of this study were to:

1. Detect fluorescent dye droplets using digital fluorescence imaging.
2. Quantify the number of individual spray droplets on plant leaves.
3. Visualize the spatial distribution of spray droplets.

2. MATERIALS AND METHODS

2.1 Spray Application

A solution of tap water and daylight visible dye (10% v/v DayGlo Rocket Red, ECO-13, DayGlo Color Corp., Cleveland, OH) was charged to +10 kV using a custom power supply, and applied at 483 kPa to mature beet plants at 45 cm above canopy using a ground-based electrostatic spraying system (3D Surface Sanitizer, Spectrum Electrostatic Sprayers, Houston, TX) with a TXVK-3 spray tip (Spraying Systems Co.,

Wheaton, IL). The spray was allowed to dry on the beet leaves for 10 min prior to collection. The leaves were placed in plastic zippered bags for transport to the laboratory.

2.2 Digital Imaging

The spray deposits on the underside of stemless beet leaves were imaged with an 8 megapixel 4-CCD digital still camera (model F828, Sony Corporation, Tokyo, Japan) fitted with a 58 mm infrared filter (B&W Infracolor #099, Bad Kreuznach, Germany) and a fixed aperture setting of f5.6 and 1.0 s exposure time. Two 1.2 m blacklight bulbs (model F40BLB, General Electric, Fairfield, CT) placed 36 cm overhead illuminated the plant leaves. The camera was secured to a tripod with the lens positioned 48 cm above the target leaves. The matte black platform was cleaned after imaging each leaf to remove residual traces of fluorescent dye.

2.3 Image Processing

Each of the digital images was processed using Image J, an open source, Java-written image processing program. After starting Image J, the digital image file in .TIFF format [Fig. 1(a)] was opened by choosing FILE-OPEN. Various image settings were adjusted by selecting IMAGE –ADJUST - COLOR THRESHOLD. The Brightness parameter was set via slider bar to a minimum of 46 and a maximum of 255. This minimum level insured that the droplets being detected by the software were indeed real and not just stray background fluorescence. The Saturation parameter was left at the default setting of 0 to 255. The Hue parameter was set to 0 to 6 for the Rocket Red color of fluorescent dye used in this study. The Thresholding Method was set to ”default” and Threshold Color was set to ”Red.” Color Space was set to ”HSB” and a dark background was indicated. The resulting filtered image was saved by choosing PLUGINS –UTILITIES - CAPTURE IMAGE [Fig. 1(b)]. By choosing ”Select,” the software identified and outlined the individual spray droplets. This image also was saved by following the above procedure [Fig. 1(c)].

The next step in the process was to analyze these spray droplets. From the top menu, ANALYZE - ANALYZE PARTICLES was chosen. The Size of particles to be evaluated was set from 0 to infinity, and the Circularity was set from 0.60 to 1.00. The other parameters are mostly visual in nature, but were as follows: Show: Outlines, Display Results, Clear Results, Summarize. When these parameters were accepted by selecting ”OK,” three new windows appeared: a drawing of the droplets analyzed [Fig. 1(d)], a Results table (Fig. 2), and a Summary table (Fig. 3).

3. RESULTS

Following the above outlined procedure, the software produced an excellent drawing of the identified droplets [Fig. 1(d)] and a table of results (Fig. 2). The drawing indicates

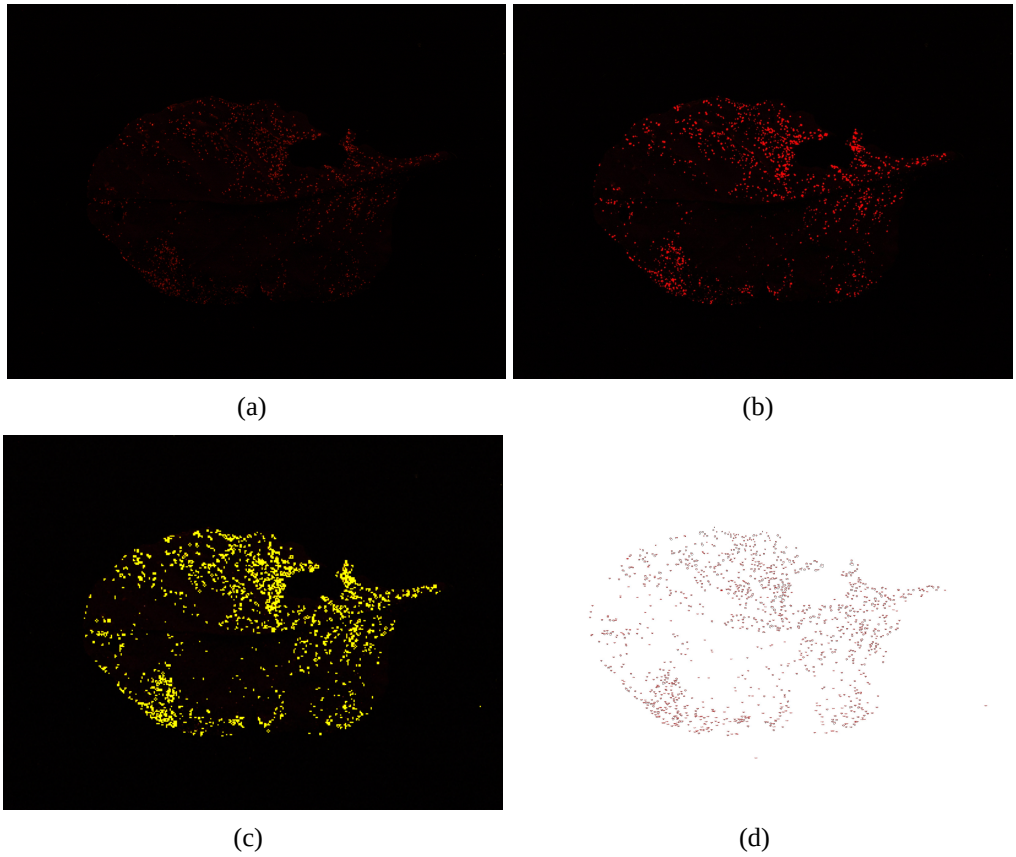

FIG. 1: Droplets containing fluorescent dye applied to the underside of a beet leaf by an electrostatic sprayer: (a) original digital image, (b) digitally thresholded image, (c) image with droplets outlined, and (d) computer drawing of selected droplets with background removed.

the droplets selected for analysis and allows the user to assess the spatial distribution of those droplets. The table displays droplet data for each individual droplet identified and measured. Results outputted include a unique droplet number, the projected area of the droplets (in user-selected units), the average droplet dimension, and the minimum and maximum droplet dimensions. In addition, the software also produced a summary data table (Fig. 3). This table summarizes the data for each image analyzed. It reports the image name, the total number of droplets analyzed, the total projected area and the average projected area of those droplets, the percent area of the image that the droplets occupy, and the average droplet size for all droplets measured. The user has the option of saving these tables for further data or statistical analysis.

For the particular study at hand, the software detected 1201 droplets on the underside of this particular leaf. By running the leaf through a leaf area meter, the droplet density, in

Results

File Edit Font Results

	Area	Mean	Min	Max
1	21	30	17	66
2	71	32.507	16	75
3	130	36.738	16	112
4	39	27.359	17	51
5	176	36.841	16	99
6	36	26.389	15	55
7	18	28.444	16	55
8	4	24.750	19	29
9	97	33.825	15	78
10	6	25.667	22	29

FIG. 2: Abridged results table output from analysis of droplets. Table shows sequential droplet number as assigned by Image J, area of droplet (in user-selected units), mean, minimum, and maximum droplet dimensions.

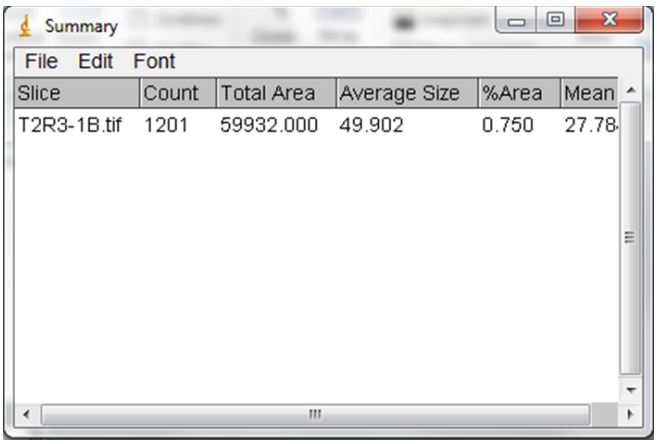

FIG. 3: Summary table output from analysis of droplets. Table shows the image that was analyzed, the total number of droplets, the total area those droplets occupied, the average size of the droplets (in user-selected units), the percent area of the image that the droplets occupied, and the mean droplet size.

drops/cm^2, could be calculated and compared to the droplet density of the other spray application treatments. In addition, it could be seen from Fig. 1(c) that the charged droplets covered more of the interior and perimeter of the underside of the leaves, whereas the droplets from the uncharged treatments tended to reside around just the perimeter of the underside of the leaf.

4. CONCLUSIONS

The imaging technique described herein provided excellent quantification of spray droplets on plant leaves and visualizing their spatial distribution. Other spray deposition analysis techniques, using both natural and artificial samplers, have been proven for conventional spray applications, but do not provide individual droplet data for natural plant samples and may skew sampling of electrostatically charged sprays because of the buildup of electrostatic fields at sharp points on the samplers. This technique fills that void for electrostatic aerial spray application but has its limitations also. Namely, for this technique to be successful, the spray droplets must be unique and distinct from one another. Since relatively few droplets typically deposit on the underside of plant leaves, this technique is ideal for this situation. The leaf surface or target of interest also needs to be as flat or two-dimensional as possible. This makes it easier for all of the droplets in the image to be in focus at one time. In addition, this technique requires relatively controlled conditions for imaging. Field imaging would be possible, but proper lighting and focus would be critical. Although the objective of this work focused on electrostatic agricultural spray applications, the technique presented may be broadly applicable to other areas such as spray coatings, printing, and food sanitation. Future work would include quantification of the spatial distribution of the deposited droplets and methods of fluorescent imaging three-dimensional targets.

ACKNOWLEDGMENTS

The author thanks Spectrum Electrostatics for use of a handheld electrostatic sprayer, and Mr. Chris Parker for his valuable assistance in collecting and compiling the data.

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