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SCIENTIFIC COMMUNICATION

Precision micro-spray tower for application of entomopathogens

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Micropulverizador de precisão para aplicação de entomopatógenos

RESUMO - Um micropulverizador portátil de baixo custo e fácil manuseio foi desenvolvido para experimentos envolvendo aplicações aquosas de diferentes entomopatógenos e inseticidas. O dispositivo foi calibrado usando diferentes combinações de pressão e tempo para aplicar uma ampla gama de doses numa superfície plana. Discos de papel-filtro foram pulverizados com corante azul para verificar a uniformidade das deposições (cobertura). A relação entre suspensão conidial de fungos entomopatogênicos e a deposição obtida desses propágulos numa superfície plana foi determinada. A combinação de 10 PSI e 3 s conferiu a melhor uniformidade de cobertura resultando num volume de deposição de 2,15 µL cm^{-2} (= 215 L ha^{-1}). A deposição de conídios foi positivamente linear às concentrações e esta relação foi similar para os fungos testados. Com base numa regressão linear comum a todos os fungos testados, foi possível estimar uma deposição de 150 conídios mm^{-2} (= $1{,}5\times10^{12}$ conídios ha^{-1}) à concentração de 1×10^{7} conídios mL^{-1}. Uma vez calibrado, este equipamento de pulverização pode ser utilizado numa variedade de bioensaios em pequenas arenas para teste de eficácia de entomopatógenos e pesticidas químicos.

PALAVRAS-CHAVE - controle biológico; sistema de pulverização; fungos entomopatogênicos; bioensaios.

ABSTRACT - An inexpensive, portable and easy handling spraying device was developed for experimental application of water suspensions of entomopathogens and insecticides. The micro-sprayer was calibrated using different settings of pressure and time to apply a wide range of doses on a plain surface. Filter paper cards sprayed with dye solutions were used to examine the deposition uniformity (coverage). The relationship between conidial suspensions of fungal entomopathogens and deposition rate of conidia was also investigated. As a result, the combination of 10 PSI and 3 s provided the most uniform coverage at a volume application rate of 2.15 µL cm^{-2} (= 215 L ha^{-1}). Conidial deposition rate was positively linear to fungal concentrations and regression lines were not significantly different among the fungal species tested. Hence, a single regression line was determined, which gave an estimate of 150 conidia mm^{-2} (= 1.5×10^{12} conidia ha^{-1}) based on a concentration of 1×10^{7}conidial mL^{-1}. Once calibrated, this spray device can be used in a variety of bioassays testing efficacy of entomopathogens and chemical pesticides in small confined experimental arena.

KEY WORDS - biological control; spray system; entomopathogenic fungi; bioassays.

Among entomopathogens, mitosporic fungi such as *Metarhizium anisopliae* senso latu (s.l.), *Beauveria bassiana* s.l., *Isaria fumosorosea* and *Lecanicillium* spp. have been widely used as eco-friendly biocontrol agents of mites and insects for many decades worldwide (Faria and Wraight 2007). A key factor governing the success of experimental testing of these microbial agents is the accurate application of the inoculum onto the target insect (direct contact) or surfaces of insect habitat (e.g. crop leaves) for residual contact. For that reason, it is critical to determine the known amount of a given active ingredient (e.g. conidia or blastospores) applied per unit area to determine dose-mortality responses of the target insect under specific incubation conditions. For instance, the virulence of fungal entomopathogens is generally

determined by lethal doses (LD) or lethal concentrations (LC) based on the known concentration deposited onto the insect or surface. Establishing the LD_{50} or LC_{50} of a fungal isolate allows comparisons with other isolates or different species and provides a starting point to establish appropriate concentrations for field testing. In addition, the estimation of median LD or LC for mycoinsecticides is valuable in the inundative application approach, in which a high number of infective propagules are required to kill the target under field conditions (Jaronski 2010). Many researchers establishing dose-responses of fungal suspensions under controlled conditions have used an air assisted spray tower (Potter 1952), which is now considered a standard device for this purpose. While effective, traditional spray towers are relatively non-portable and expensive for many workers in developing countries. Here, we described an inexpensive and portable precision spray tower that can be built using readily accessible materials and could be useful to microbial control researchers who do not currently have access to spray tower equipment.

The portable micro-sprayer consists of a dual action gravity feed artist airbrush (Sagyma™ SW130K, Brazil; http://www.wkshop.com.br) with a 0.3-mm needle placed on the top of an cylindrical acrylic tower (inside dimensions 11.6 cm diameter by 23 cm height), which forms the spray tower (Fig. 1). The airbrush was connected to a gas pressure regulator (Record S.A., R9-CO_2, São Paulo, Brazil), and a 1.6 L pressurized air tank. Once mounted, the micro-sprayer can be disassembled for cleaning or replacement of parts. The area covered by the micro-sprayer is 105.68 cm^2, large enough for standard size Petri-dishes used in many laboratory bioassays. We calibrated this device by spraying deionized water onto 7-cm-diameter pieces of round filter paper (1 Qualitative, Qualy™- 14 µm) in order to evaluate the relationship between pressure (pounds per square inch, PSI) and spraying time. Each filter paper was weighed immediately before and after spraying to calculate microliters per square centimeter (µL cm^{-2}) at 5, 10, 15, 20, 25 and 30 PSI at 2, 3, 4 and 5 seconds (s). There were six replicates for each pressure and time combination (N = 144 observations). The study was repeated using a cotton blue stain solution (10% v/v) to evaluate the uniformity of the spray deposition. We examined the spray patterns on stained filter papers. Images were transformed into simple 8-bit grayscale color bands and then subjected to the image analyzer software ImageJ™ in order to determine the frequency distribution curves of color reflectance (in pixels) (Abramoff *et al.* 2004). According to this analysis, we selected 10 PSI to determine the relationship between the fungal concentrations and conidial deposition on a plain surface. Conidial suspensions of three fungal entomopathogens (*B. bassiana* CG1229 [spore size: 2.09±0.04 µm], *I. fumosorosea* CG1228 [1.39±0.03 × 3.58±0.08 µm], and *L. muscarium* ESALQ1408 [1.51±0.03 × 4.25±0.11 µm]) were prepared in an 0.01% v/v Tween 80 (Vetec®, RJ, Brazil) solution using nine concentrations ranging from 1×10^7 to 2.8×10^9 conidia mL^{-1} and sprayed for 3 s onto Petri plates (9 cm diameter) containing 6-8 glass coverslips (20 × 20 mm). Concentrations < 10^7 conidia mL^{-1} were not tested. We used 4 mL aliquots of test suspensions and tested each fungal concentration at least four times. The whole experiment was repeated twice. After spraying, coverslips were transferred into 45-mL Falcon® centrifuge tubes containing 5 mL of 0.1% Tween 80 solution and vortexed for 2 min, to dislodge conidia. Homogenized conidial suspensions for each spray replicate were enumerated by an improved Neubauer (hemocytometer) chamber (New Optik®, Brazil) with 0.1 mm depth. The average number of conidia recorded from coverslips was used to quantify deposition rates (i.e. conidia mm^{-2}).

A non-linear model was used to examine the effects of pressure and spraying time on the conidial deposition rates, while linear regression was used to describe the relationship between conidial deposition rate (conidia mm^{-2}) on a plain surface and fungal concentration (conidia mL^{-1}) (PROC REG, SAS Institute 2008). Regression lines of deposition rate against concentration of *I. fumosorosea*, *L. muscarium* and *B. bassiana* were compared to determine whether their slopes and intercepts were statistically different at 5% significance using the analysis of covariance (ANCOVA in PROC REG). Image analysis software was used to compare uniformity among different spray patterns according to pressure at a fixed spraying time of 3 s (ImageJ™, V1.47b, http://rsbweb.nih.gov/ij/notes.html). A highly significant relationship between water volume deposition and pressure and spraying time was indicated by the non-linear model (Fig. 1) (F= 713.2; df = 2, 141; P< 0.0001; adjusted R^2 = 0.91). We observed that the optimal coverage uniformity was achieved at 10 PSI and 3 s, which had the lowest frequency distribution curve for reflectance of color intensity (Fig. 1). Narrow reflectance curves (Fig. 1) correlated positively with spray uniformity, since wider bell-shaped curves represent a greater range and variability of droplet deposition. Higher and lower spray volume rates resulted in reduced deposition uniformity (Fig. 1). The selected pressure of our device fell

Table 1. Estimated of conidia deposited on a plain surface after spraying different conidial suspensions at 10 PSI for 3 seconds using the portable micro-spray tower.

Concentration (conidia mL^{-1})	Deposition (conidia mm^{-2}) [a]		Equivalent field deposition (conidia ha^{-1}) [b]
	Mean±SE	95% Fiducial Limits	
1×10^7	150±34	34 – 658	1.5×10^{12}
5×10^7	811±120	193 – 3401	5.0×10^{12}
1×10^8	1674±261	397 – 7060	1.7×10^{13}

[a] Deposition rates estimated by the single regression lines with common slope and intercept for all fungal entomopathogens tested (Y = -5.15 + 1.05x, where y = log-deposition and x = log-concentration). Standard error (SE) is given to the mean.
[b] Calculated based on 1 hectare of plain area (= 10^{10} mm^2).

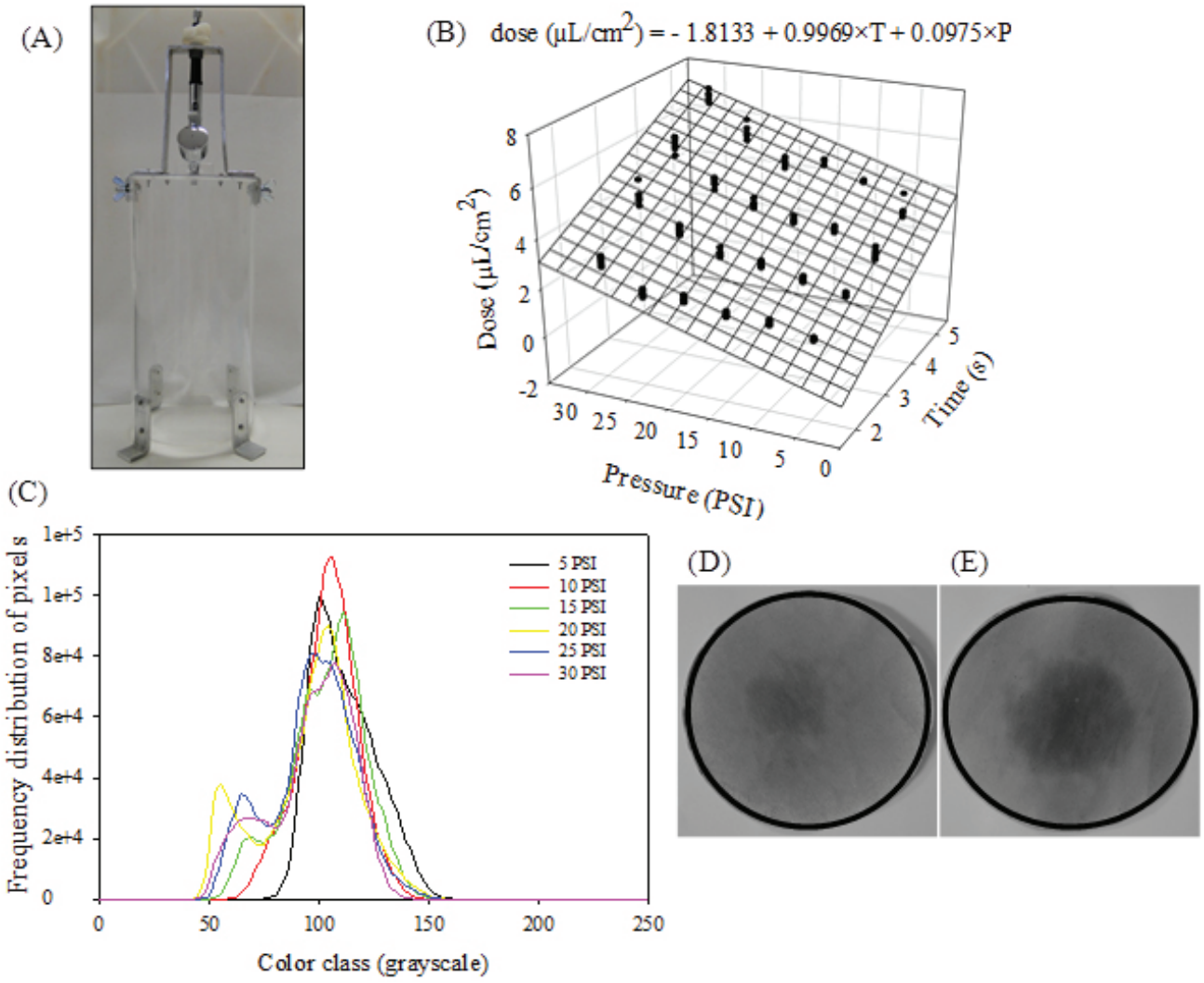

Figure 1. Calibration of the micro-spray tower (A). Non-linear regression plot for dose (µL of water cm^{-2}) in function of spraying time (seconds) and pressure (PSI) as explanatory variables (B). Spray patterns at different pressures at 3 s (fixed) expressed in frequency distribution of reflectance from sprayed cards in grayscale (8-bit picture) (C). Spray patterns of water application on filter paper cards in grayscale at 10 PSI/3 s (D) and 30 PSI/3 s (E).

within the recommended range for Potter spray towers (Potter 1952). The preferred setting (10 PSI for 3 sec) provided a volume application rate of 2.15 µL cm^{-2} (= 215 L ha^{-1}), which is close to the standard recommended by the IOBC in many insecticide trials under controlled laboratory conditions (i.e. 200 L ha^{-1}) (Candolfi *et al.* 2000). In practice, optimal spray volumes under field conditions may vary widely according to crop type, product formulation, spray application technique, weather conditions and so on.

Slopes and intercepts for deposition rate and concentration regression lines were not significantly different among fungi tested (Interaction fungus×concentration: t = 0.03; df = 1, 53; P = 0.98; Fungus: t = -0.21; df = 1, 53; P = 0.84), which suggests that the increase in deposition rate of conidia depends on the conidial concentration (Fig. 2). A single regression line for all fungal species was fitted to explain this relationship (Fig 2). There was also a clear relationship between conidial deposition (conidia mm^{-2}) and concentration (conidia mL^{-1}) (F = 117.32; df = 1, 19; P < 0.0001, adjusted R^2 = 0.85). Estimated deposition rates for concentrations at 1×10^7, 5×10^7 and 1×10^8 conidial mL^{-1} were equivalent to 150, 811 and 1674 conidia mm^{-2}, respectively (Table 1). The portable micro-spray tower can apply a range of concentrations of entomopathogenic fungi which correspond to field doses for commercial aerial and terrestrial applications of entomopathogenic fungi, where application rates fall within 1×10^{12} to 1×10^{14} conidia ha^{-1} (Jaronski 2010).

In conclusion, we described an inexpensive spraying device for application of fungal entomopathogens as well as other microbial agents to portions of crop foliage or onto target arthropods. This device can be also used with various chemical pesticides. The portability of the system (e.g. requiring no electrical source) allows it to be used in remote locations where other types of spray towers cannot be used.

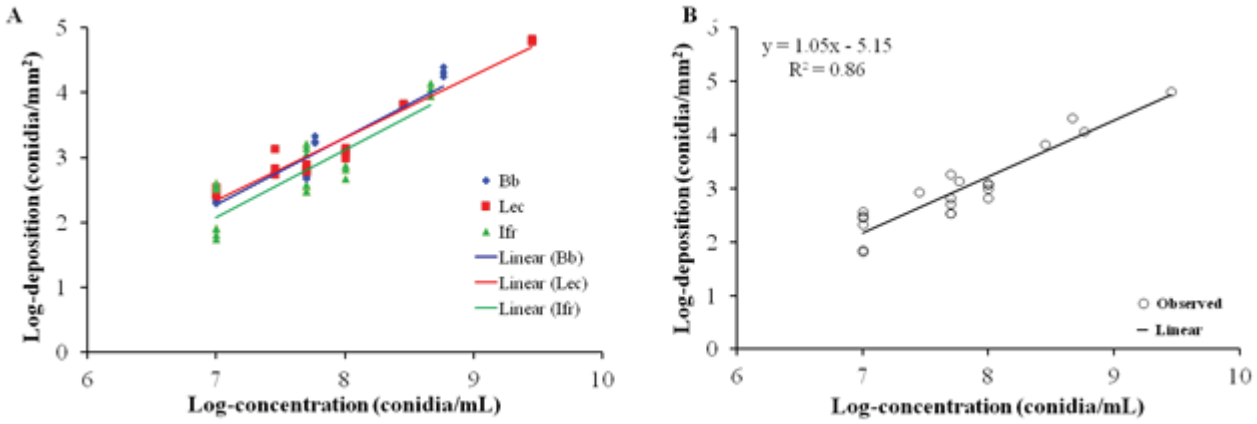

Figure 2. Regression lines of conidial deposition rates (conidia mm^{-2}) against conidial concentrations (conidia mL^{-1}) of *I. fumosorosea*, *L. muscarium*, and *B. bassiana* (A) and a common regression line for all fungal entomopathogens (B). Conidial suspensions were sprayed for 3 s at 10 PSI.

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