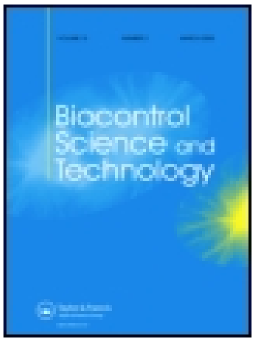

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Biocontrol Science and Technology

ISSN: 0958-3157 (Print) 1360-0478 (Online) Journal homepage: http://www.tandfonline.com/loi/cbst20

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To cite this article: G. D. INGLIS , D. L. JOHNSON & M. S. GOETTEL (1995) Effects of Simulated Rain on the Persistence of Beauveria bassiana Conidia on Leaves of Alfalfa and Wheat, Biocontrol Science and Technology, 5:3, 365-370, DOI: 10.1080/09583159550039819

To link to this article: http://dx.doi.org/10.1080/09583159550039819

Published online: 28 Jun 2010.

Biocontrol Science and Technology (1995) 5, 365–369

Effects of Simulated Rain on the Persistence of *Beauveria bassiana* Conidia on Leaves of Alfalfa and Wheat

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(*Received for publication 3 October 1994; revised manuscript accepted 30 April 1995*)

The effect of simulated rain at two intensities (26.7 or 112.7 mm h^{-1}*) and durations (30 or 60 min) on the persistence of* Beauveria bassiana *conidia applied in water on leaves of alfalfa leaves of alfalfa* (Medicago sativa*) or wheat (*Triticum aestivum*) was investigated. Initial populations of conidia on the leaves of both crops were similar, and ranged from* 1.7×10^5 *to* 3.5×10^5 *colony-forming units cm* $^{-2}$*. The simulated rain reduced the concentration of conidia on leaves by 28–61%. Although there was a slight effect due to rain intensity for alfalfa, there was no influence of either rain duration or crop type on the retention of* B. bassiana *conidia.*

Keywords: *precipitation, irrigation,* Beauveria bassiana*, entomopathogen, epigeal habitats, persistence*

INTRODUCTION

The control of a variety of insects by *Beauveria bassiana* can be obtained by the application of conidia in epigeal habitats (Feng *et al.*, 1994). However, the persistence of conidia in these habitats is usually poor, thereby reducing efficacy. In addition to the rapid deactivation of conidia by ultraviolet radiation (Inglis *et al.*, 1995), post-application precipitation is presumed to reduce conidial persistence (e.g. Gardner *et al.*, 1977; Daoust & Pereira, 1986; Johnson *et al.*, 1992). However, in a recent field experiment, rain appeared to have little impact on the loss of *B. bassiana* conidia from leaves (Inglis *et al.*, 1993). The objective of this study was to verify field observations by quantifying the effect of rain on the loss of *B. bassiana* conidia from leaves under controlled conditions.

MATERIALS AND METHODS

Inoculum Preparation and Application

Dry conidia of *B. bassiana* (GHA), supplied by Mycotech Corp., Butte, MT, USA, were suspended in sterile deionized water at a concentration of 1.7×10^9 viable conidia ml^{-1}. To facilitate the dispersion of conidia, the suspensions were vigorously agitated with a micropestle. The conidia were then applied to the leaves of wheat plants (*Triticum aestivum*, cv. AC Reed) and alfalfa (*Medicago sativa*, cv. AC Blue J), grown individually in 8.75-cm diameter pots

0958–3157/95/030365–05

containing Cornell mix. Immediately before treatment, the plants were trimmed to a height of 8–10 cm. The conidial suspension (100 μl) was applied to individual plants in a 10.8-cm diameter $\times$ 35.5-cm high Plexiglass cylinder using an airbrush (Artek, Rockford, IL, USA) at 103 kPa. The droplet size, density and distribution pattern were monitored using water-sensitive paper (Teejet Spraying Systems Co., Wheaton, IL, USA). Following inoculation, plants were maintained in the dark at ambient temperature for 15–20 min to allow the water carrier to evaporate, before exposing dry conidia on leaf surfaces to simulated rain.

Rain Simulation

A continuous spray, Guelph rainfall simulator (Tossell *et al.*, 1987) was used in a glasshouse with diffuse light and a temperature of 23–25°C. The boom of the simulator was situated 1 m above the bench, and plants were randomly arranged within the 0.5 $\times$ 0.5-m spray area; the rain coverage within this area had previously been determined to be uniform. Rain was applied (100 kPa) at medium (27 mm h^{-1}) and high (113 mm h^{-1}) intensities using the 1/8 4.3 W and 1/4 14 W nozzles respectively. Plants were exposed to rain for 30 or 60 min at each rain level.

Conidial Enumeration

Prior to rain exposure, 10 alfalfa leaflets (ca. 1 $\times$ 0.5 cm) and 10 $\times$ 1-cm wheat leaf segments were randomly collected from plants in each pot. The wheat leaf segments were removed 1–2 cm from the tip of the lamina. After exposure to rain, 10 leaflets or leaf segments were removed from the same leaves sampled prior to rain exposure. Control treatments were alfalfa and wheat plants inoculated with conidia but not exposed, although positioned adjacent to the rainfall simulator in the glasshouse for 60 min. No *B. bassiana* conidia were recovered from leaves of the alfalfa and wheat plants sampled prior to inoculation.

Conidia were recovered by placing the leaf samples in 20 ml-vials and washing them in 5 ml of 0.01-M phosphate buffer amended with 0.05% Tween-80 (v/v) (buffer–Tween) for 2 h on a rotary shaker (300 rpm). The wash solutions were diluted three times, and a 100-μl aliquot from each dilution was spread on a semi-selective oatmeal–dodine medium (Chase *et al.*, 1986). The number of colony-forming units (CFU) at the dilution yielding 30–300 CFU/dish was recorded after 5–6 days at 25 $\pm$ 1°C. The total area of the leaf pieces was determined after washing with a leaf area meter (Model 3100, Li-Cor Inc., Lincoln, NE, USA). Leaf areas ranged from 4.0 to 11.9 and 4.2 to 10.3 cm^2 for alfalfa and wheat respectively. The reductions in conidial number due to rain exposure at time x were calculated as: ([CFU cm^{-2} at time $T_0 -$ CFU cm^{-2} at T_x]/CFU cm^{-2} at T_0) $\times$ 100. If the number of conidia recovered at T_x was larger than at T_0, the percentage increase in number was calculated as: ([CFU cm^{-2} at time $T_0 -$ CFU cm^{-2} at T_x]/CFU cm^{-2} at T_x) $\times$ 100.

To assess the effectiveness of the wash procedure, conidia sprayed on alfalfa and wheat leaves were recovered and populations cm^{-2} of leaf were calculated as above. After washing, the leaf segments were rinsed in sterile buffer–Tween. Then, 4.5-mm diameter disks were cut from each, macerated, diluted in buffer–Tween and the homogenate spread on oatmeal–dodine agar. The number of CFU cm^{-2} from the washes and homogenates were compared.

Statistical Analyses

Each of the three trials conducted was arranged as a completely randomized design with two levels of crop, rain intensity and duration, each replicated three times. The normality of the conidial population data was tested using the Shapiro-Wilk's W test. Log_{10} transformations were required to normalize the conidial population data, but untransformed means and standard errors of the means ($\pm$) are presented throughout the text. Prior to pooling the data, the homogeneity of variance between trials was examined using Bartlett's test. The combined data was analyzed with weighted analysis of covariance, using the general linear model routine of SAS (SAS Institute, 1988). Conidial populations on leaves before rain exposure were used as the covariate, and, in all instances a trial effect was included in the models. In the absence of interactions between crop, rain intensity, rain duration and the covariate, individual treatments within each crop were compared with the control treatment using weighted analysis of covariance and least-square means.

RESULTS AND DISCUSSION

The wash technique employed recovered 95.6–99.7% of the conidia from alfalfa and wheat leaves. Within 10 min of application the water carrier had evaporated, and initial populations of dry conidia on leaves of the plants ranged from $1.6 \times 10^5 \pm 0.39 \times 10^5$ to $3.5 \times 10^5 \pm 0.64 \times 10^5$CFU cm^{-2} (Table 1). There was no difference ($P = 0.28$) in the deposition of conidia on the two crops. Conidia of *B. bassiana* are rapidly killed by solar radiation under field conditions (Inglis *et al.*, 1993). However, under the diffuse light conditions of the greenhouse, the conidia were not affected by the 60-min exposure (Table 1, control treatment).

The role of precipitation on the persistence of fungal taxa possessing hydrophobic propagules (i.e. *Beauveria*, *Metarhizium* and *Nomuraea*) in epigeal habitats has not previously been studied. In this study, it was observed that simulated rain caused the removal of *B. bassiana* conidia from leaves of alfalfa ($P = 0.011$) and wheat ($P = 0.007$), with the decrease in population density ($P \leqslant 0.05$) ranging from 28.1 ± 12.5 to 60.6 ± 9.0% (Table 1). The duration of rain exposure had no effect ($P = 0.42$), and conidia were removed equally from leaves of both crops ($P = 0.65$). There was a weak effect ($P = 0.031$) due to the difference in rain intensity. Although the interaction between crop and rain intensity was non-significant ($P = 0.094$), the rain intensity effect occurred only for alfalfa (Table 1). Rain has been shown to decrease the foliar persistence of *Bacillus thuringiensis* (Frankenhuyzen & Nystrom, 1989), and to facilitate the dispersal of some fungal propagules (Fitt *et al.*, 1989). Droplet velocity and size affect the dispersal of fungal propagules (Fitt *et al.*, 1989). Although the nozzles and pressure used produced a relatively wide range of drop sizes, the 1-m boom height selected produced rain with relatively low droplet velocities.

Rain, particularly that falling immediately after the application of inoculum, has been implicated with decreased efficacy of *B. bassiana* by many researchers (e.g. Gardner *et al.*, 1977; Johnson *et al.*, 1992). Results from the present study indicate that rain could reduce the efficacy of this entomopathogen in epigeal habitats. However, the authors had previously observed that rain accompanied by wind in excess of 80 km h^{-1} within 30 min of application had no apparent effect on persistence of *B. bassiana* conidia on leaves (Inglis *et al.*, 1993). It now appears that the rapid deactivation of conidia by solar radiation that occurs under field conditions obscures the effect of conidial removal by rain, as determined by changes in populations over time.

Although simulated rain removes *B. bassiana* conidia from leaves, a substantial proportion of the conidia applied in water remain after exposure to relatively high-intensity rain. Furthermore, the effects of rain are not all detrimental, because infection can be enhanced under conditions of high ambient humidity (Schaerffenberg, 1964) provided by precipitation or irrigation (Campbell *et al.*, 1985). Whether precipitation will enhance or jeopardize the development of an epizootic will depend on whether conidial populations are decreased below the inoculum threshold required to incite mycosis in the target insect. Formulation adjuvants such as sticking agents might also reduce the impact of rain on conidial removal and prolong the time that inoculum densities remain above the threshold level.

ACKNOWLEDGEMENTS

The authors wish to thank the following people at Agriculture and Agri-Food Canada, Lethbridge: Ron Feniuk for his technical assistance, Dr J. J. Miller for the use of the rain simulator, Toby Entz for his statistical advice and Drs J. R. Byers, D. A. Gaudet and L. M. Kawchuk for their critical reviews. The research was funded in part by a grant from Farming for the Future, Alberta Agricultural Research Institute and a Pest Management Alternatives Office graduate student scholarship to G. D. Inglis. This manuscript is Lethbridge Research Centre contribution #3879461.

TABLE 1. Effect of simulated rain on retention of *B. bassiana* conidia on leaves of alfalfa and wheat

	Alfalfa			Wheat		
Rain treatment	Before (CFU cm^{-2} × 10^5)	After (CFU cm^{-2} × 10^5)	Reduction[a] (%)	Before (CFU cm^{-2} × 10^5)	After (CFU cm^{-2} × 10^5)	Reduction[a] (%)
Medium intensity[b]						
30 min	1.96 ± 0.35	1.13 ± 0.12ab[c]	28.1 ± 12.5	1.90 ± 0.28	1.10 ± 0.20bc[c]	35.4 ± 11.80
60 min	2.50 ± 0.43	2.09 ± 0.52bc	24.6 ± 14.0	1 83 ± 0.20	0.85 ± 0.21ab	56.0 ± 6.70
High intensity[d]						
30 min	2.19 ± 0.45	0.74 ± 0.24a	60.6 ± 9.00	1.60 ± 0.39	1.07 ± 0.50ab	49.7 ± 14.0
60 min	2.58 ± 0.81	1.21 ± 0.41ab	46.8 ± 12.9	1.72 ± 0.48	1.16 ± 0.55ab	50.7 ± 9.70
Control[e]	3.51 ± 0.64	4.24 ± 0.96c	−2.2 ± 16.8	3.07 ± 0.46	3.28 ± 0.52c	−4.0 ± 7.80

[a]Percentage reductions in conidial populations were calculated as: ([CFU cm^{-2} at time T_0 − CFU cm^{-2} at T_x]/CFU cm^{-2} at T_0) × 100. Percentage increases were calculated as: ([CFU cm^{-2} at time T_0 − CFU cm^{-2} at T_x]/CFU cm^{-2} at T_x) × 100.

[b]Medium-intensity rain = 26.7 mm h^{-1}.

[c]Post-rain exposure treatment means (± standard error) within each crop not followed by the same letter are significantly different ($P \leqslant 0.05$) according to least-square means. Conidial populations on leaves after rain exposure were adjusted for the covariate (conidial populations before rain exposure) but unadjusted means are presented.

[d]High-intensity rain = 112.7 mm h^{-1}.

[e]No rain treatment and exposure to the same light conditions for 60 min.

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