Weed Technology 2012 26:117–121

Weed Biology and Competition

Effects of Simulated Rainfall on Disease Development and Weed Control of the Bioherbicidal Fungi *Alternaria cassiae* and *Colletotrichum truncatum*

C. Douglas Boyette, Charles T. Bryson, Robert E. Hoagland, and Mark A. Weaver*

Alternaria cassiae and *Colletotrichum truncatum* are bioherbicidal pathogens of sicklepod, and hemp sesbania, respectively. The effects of simulated rainfall followed by 12 h simulated dew application, immediately or delayed by 1 to 4 h, on disease severity and weed control were studied for each pathogen on its weed host under greenhouse conditions. After each simulated rainfall event, treated plants were placed in a dew chamber for 12 h. Regardless of rainfall amount and/or timing, only slight differences occurred on *A. cassiae* disease severity and sicklepod control (85 to 100% for both parameters). However, when similar tests were imposed on *C. truncatum*, disease severity and hemp sesbania control were highly variable, ranging from 5 to 100%. Regardless of rainfall amount, disease development and control of hemp sesbania were greatly reduced (60%) when dew application was delayed by only 1 h following inoculation, regardless of rainfall treatment. Rainfall at 1.27 and 2.58 cm had little effect on disease development and control in hemp sesbania, but the effect of transfer time to dew application exhibited a greater role on these parameters. Thus the time between bioherbicide application and dew application was more important for *C. truncatum* than for *A. cassiae*. These results indicate that rainfall amounts and the timing of dew application caused differential effects on disease severity and weed control after application of these bioherbicides to their target weeds.

Nomenclature: Hemp sesbania [*Sesbania exaltata* (Rydb.)] ex A.W. Hill sicklepod [*Senna obtusifolia* (L.) Irwin & Barneby]; *Alternaria cassiae* Jurair & Khan; *Colletotrichum truncatum* (Schw.) Andrews and Moore.
Key words: Biocontrol, bioherbicide, simulated rainfall, rainfastness, wash off.

Alternaria cassiae y *Colletotrichum truncatum* son patógenos bioherbicidas de *Senna obtusifolia* y *Sesbania exaltata*, respectivamente. Se realizó un estudio bajo condiciones de invernadero para evaluar los efectos de lluvia simulada seguida inmediatamente o con retraso de 1a 4 h por 12 h de aplicación de rocío simulado, sobre la severidad de las enfermedades y el control de malezas para cada patógeno en su maleza hospedera. Después de cada lluvia simulada, las plantas tratadas se colocaron en una cámara de rocío por 12 h. Sin importar la cantidad de lluvia y/o el momento de aplicación, en *A. cassiae* solo ocurrieron leves diferencias en la severidad de las enfermedades y en el control de *S. obtusifolia* (85–100% para ambos parámetros). Sin embargo, cuando pruebas similares se impusieron a *C. truncatum,* la severidad de las enfermedades y el control de *S. exaltata* fueron altamente variables, con un rango de 5 a 100%. Sin importar la cantidad de lluvia, el desarrollo de la enfermedad y el control de la *S. exaltata* se redujeron dramáticamente (60%), cuando la aplicación de rocío se retrasó por solamente 1 h después de la inoculación, sin importar el tratamiento con lluvia. La lluvia a 1.27 y 2.58 cm tuvo poco efecto en el desarrollo de la enfermedad y en el control en *S. exaltata,* pero el efecto del tiempo entre transferencia y la aplicación de rocío jugó un papel más importante en estos parámetros. De tal manera, el tiempo entre la aplicación del bioherbicida y la del rocío fue más importante para *C. truncatum* que para *A. cassiae*. Estos resultados indican que las cantidades de lluvia y el momento de la aplicación del rocío causaron efectos diferenciales en la severidad de las enfermedades y el control de maleza después de la aplicación de estos bio-herbicidas a la maleza destino.

Many studies demonstrate that the time between foliar pesticide application and rainfall is critical for rainfastness, absorption, and/or efficacy of many herbicides (Bryson 1988; Carrol et al. 1993; Feng et al. 2000; Koger et al. 2007; Gannon and Yelverton 2008), insecticides (Willis et al. 1992; McDowell et al. 1985; Nemec and Adkisson 1969), and fungicides (Troiano and Butterfield 1984; Vincent et al. 2007; Xu et al. 2008). In addition, it has been shown that various fungal pathogens of crop plants are potentially subject to splashing during simulated rainfall, which can serve as a mechanism for fungal dispersal, inciting epiphytotic conditions in some instances (Ntahimpera et al. 1997; Paul et al. 2004; Stensvand and Elkemo 2005; Ahimera et al. 2004). Conversely, research related to the effects of rainfall on bioherbicide efficacy is not well documented, and is generally based on conjecture and anecdotal evidence, with little empirical proof. Previously it has been shown that the bioherbicidal fungi *Alternaria cassiae* Jurair & Khan and *Colletotrichum truncatum* (Schw.) Andrus and Moore are effective bioherbicides of the problematic weeds sicklepod and hemp sesbania, respectively, when applied in an augmentative or inundative manner under favorable environmental conditions (Walker 1982; Walker and Boyette 1986; Boyette 1991; Boyette et al. 1993; Boyette 1994; Boyette et al. 2007). Heretofore, no studies have been conducted examining rainfall interactions on the efficacy of these pathogens following application to their weed hosts. Furthermore, few studies have addressed rainfall effects on other biological control agents following inundative application. The objective of this research was to evaluate the effects of simulated rainfall and interaction of a rainfall event at different lengths of time after foliar spray inoculations on disease progression and weed

---

DOI: 10.1614/WT-D-10-00127.1

* USDA-ARS, Southern Weed Science Research Unit, Stoneville, MS 38776. Corresponding author's email: doug.boyette@ars.usda.gov

control of sicklepod and hemp sesbania with their respective pathogens.

Materials and Methods

Isolation, Culture, and Inoculum Production. Single-spore strains of *Alternaria cassiae* [NRRL (Northern Regional Research Laboratory) 12553] and *C. truncatum* (NRRL 18434) were used in all experiments. Each isolate was preserved in screw-capped tubes containing sterilized soil (Bakerspigel 1953). Cultures of each isolate were then grown for 5 to 7 d on potato dextrose agar (PDA; Difco Laboratories, Detroit, MI) in 10-cm plastic Petri dishes that were incubated on open-mesh wire shelves of an incubator (Model I-35LLVL, Percival Scientific, Perry, IA) at 25 C under cool, white fluorescent lighting (12-h photoperiod). *Alternaria cassiae* sporulated prolifically, yielding approximately 10 g of spores at approximately 1.0×10^8 spores g^{-1} after 3 d. *Colletotrichum truncatum* also sporulated rapidly, and spores were rinsed from PDA plates routinely yielding 1.5 to 2.0×10^8 spores ml^{-1} after 7 d.

Inoculum Production. Spores of *A. cassiae* were produced by a dual-step method, as described by Walker (1982), and Walker and Riley (1982). Briefly, actively growing shaken cultures of *A. cassiae* were used to inoculate a liquid medium consisting of soy flour (15 g L^{-1}), corn meal (15 g L^{-1}), $CaCO_3$ (3 g L^{-1}), and distilled water. The fungus was grown for 24 h at 25 C in a 14-L fermenter (Model 10-E, New Brunswick Scientific Co., Inc., Edison, NJ), harvested, homogenized for 20 s in a blender (Vortex Genie, Scientific Industries, Inc., Bohemia, New York), and poured into 41 by 27 by 5.5-cm aluminum-foil–lined plastic trays in a chamber, and exposed for 10 min, at 24-h intervals, to ultraviolet light provided by 275-W sunlamps (Sylvania Manufacturing, Maple Grove, MN). Approximately 72 h after harvest of the mycelium, the resulting *A. cassiae* spores were harvested with a hand-held vacuum cleaner (Dust Buster, The Black & Decker Corporation, Towson, MD), transferred to glass vials, and stored at 4 C. Hemacytometer (Improved Nubauer Hemacytometer, Thermo Fisher Scientific, Waltham, MA) counts revealed that spore yields were approximately 1×10^8 spores g^{-1}.

Because *C. truncatum* sporulates sparingly in submerged liquid culture (Jackson and Schlisler 1992), a solid-substrate production technique was utilized to mass-produce this fungus. The cultures were grown for 5 to 7 d on PDA in 10-cm plastic Petri dishes that were incubated as described above. *Colletotrichum truncatum* spores were separated from the mycelium by filtering through double-layered cheesecloth (Softwipe Cheesecloth, American Fiber and Finishing, Inc., Albermarle, NC). The spore densities of each fungus were determined with the use of hemacytometers, and dilutions were made with distilled water to provide the desired inoculum concentrations (1.0×10^5 spores ml^{-1} for *A. cassiae*, and 1.0×10^7 spores ml^{-1} for *C. truncatum*).

Greenhouse Experiments. Sicklepod plants and hemp sesbania were grown from seed (Azlin Seed Co., Leland, MS 38756) in a commercial potting mix (Jiffy-mix, Jiffy Products of America, Inc., Batavia, IL) contained in peat strips (Jiffy Products). Each strip contained 12 plants of either sicklepod or hemp sesbania. The potting mix was supplemented with a controlled-release (14:14:14, NPK) fertilizer (Osmocote, Grace Sierra Horticultural Products, Milpitas, CA). The plants were placed in subirrigated trays that were mounted on greenhouse benches. Greenhouse temperatures ranged from 25 to 30 C with 40 to 90% relative humidity (RH). The photoperiod was 12 h with 1,650 µmol m^2 s^{-1} photosynthetically active radiation as measured at midday with a light meter (LI-COR, Inc., Lincoln, NE).

When seedlings of each species were in the cotyledon to first true-leaf growth stage, they were inoculated with fully charged, hand-held aerosol sprayers (Spra-tool Power Pack, Aervoe Industries, Inc., Gardnersville, NV), containing aqueous preparations of their respective pathogens (*A. cassiae* or *C. truncatum*) each suspended in 0.02% (v/v) Polysorbate 80 nonoxynol surfactant (ICI Americas Inc., Enon, VA). Spray delivery rates were approximately 200 L ha^{-1} from a hand-held aerosol sprayer (Boyette et al. 2007). The plants were then subjected to rainfall with the use of a rainfall simulator, based on a design as described by Meyer and Harmon (1979), which reproduced water droplet size, velocity, and kinetic energy analogous to natural rainfall. The pH of the water was 7.8 and the water and air temperatures were 19 and 25 C, respectively. Following rainfall treatments of 10 min or 20 min to deliver 1.27 or 2.54 cm of rainfall, at intervals of either 0, 1, 2, 3, or 4 h between bioherbicide applications and rainfall events, groups of seedlings were placed in unlighted dew chambers [Dew Chamber, Model I 35-D, Percival Scientific Mfg., Boone, IA; 28 C, 100% relative humidity (RH)] for 12 h. Plants were then placed on greenhouse benches and monitored for disease development over an 8-d period and weed control. Disease severity was evaluated visually on a scale from 0 to 1 (where 0 = no disease, 0.2 = 20% disease, 0.4 = 40% disease, 0.6 = 60% disease, 0.8 = 80% disease, and 1.0 = complete plant death) to estimate disease progression (Sandrin et al. 2003). Weed control, plant height, and biomass reductions were determined after 21 days. Weeds were visually rated on a scale of 0 to 100, where 85 to 100 (severe injury or mortality) equaled control. Surviving plants were excised at the soil line, oven dried for 48 h at 85 C, and weighed, and the percent biomass reduction was calculated.

Experimental Design. The experiments were arranged in a split-plot design, with intervals between bioherbicide applications and rainfall events as the main plot, and rainfall amounts as the subplot. Each plant species and its corresponding pathogen were treated separately, but within hours of each other. Treatments were replicated four times, for a total of 48 individual plants. The experiments were repeated over time, and data were averaged following Bartlett's test for homogeneity of variance (Steel et al. 1997), and analyzed with the use of ANOVA. Some of the data did not follow a normal distribution pattern, and were converted by arc-sin transformation for analyses. When significant differences were detected by the *F* test, means were separated with Fisher's protected LSD test at the 0.05 level of probability. Disease- progression data were analyzed

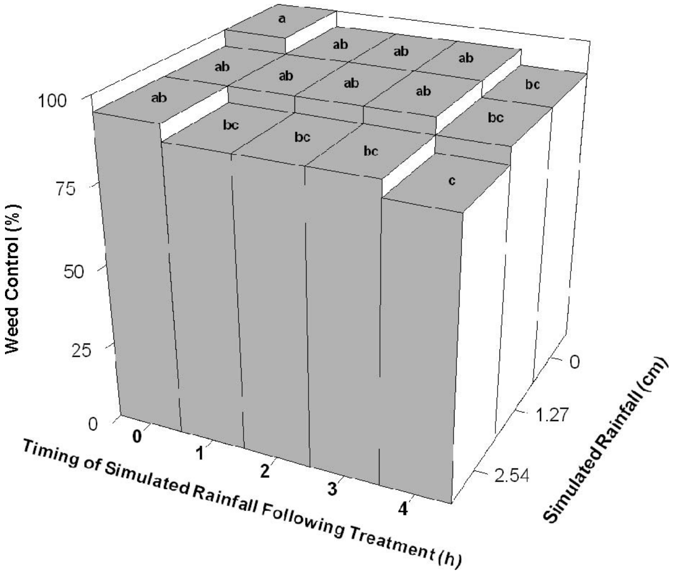

Figure 1. Effect of simulated rainfall amount and time following inoculation with *Alternaria cassiae* spores before rainfall treatments on sicklepod control. Weed control values in each column with the same letter are not significantly different according to $FLSD_{05}$.

with the use of standard error of the mean (SEM) and best-fit regression analysis. All data were analyzed with the use of SAS (Version 9.1, SAS Institute, Inc., Cary, NC) statistical software.

Results and Discussion

Greenhouse Experiments. Disease severity and weed control between the two pathogens varied greatly in response to timing between bioherbicide application and simulated rainfall events prior to placement in the dew chamber. Overall, little differences were associated with rainfall amount or timing of the rainfall event for sicklepod control with *A. cassia* (Figure 1). The best treatment (100% control of sicklepod) was achieved with no rainfall followed by an immediate dew application. However, even in the worst treatment (2.54 cm rainfall followed by a 4-h delay before a dew application), substantial control (85%) was attained (Figure 1). Disease progression was slower when sicklepod plants received either 1.27 or 2.54 cm rainfall, regardless of timing relative to bioherbicide application (Figures 2A–C). Disease progression was also delayed when there was a 2- or 4-h delay between the bioherbicide application and rainfall event, requiring 8 days to achieve maximum sicklepod disease progression (Figures 2B and 2C).

The results with *C. truncatum* upon disease progression and control of hemp sesbania were strikingly different. With *C. truncatum*, hemp sesbania mortality was not significantly different when plants received 0, 1.27, or 2.54 cm of rainfall, followed by an immediate dew application (Figure 3). Regardless of rainfall amount, mortality of hemp sesbania was greatly reduced (60%) when the dew application was delayed even at 1 h following inoculation (Figure 3). Weed mortality resulting after a 4-h delay in dew treatment, regardless of rainfall, was not significantly different than that

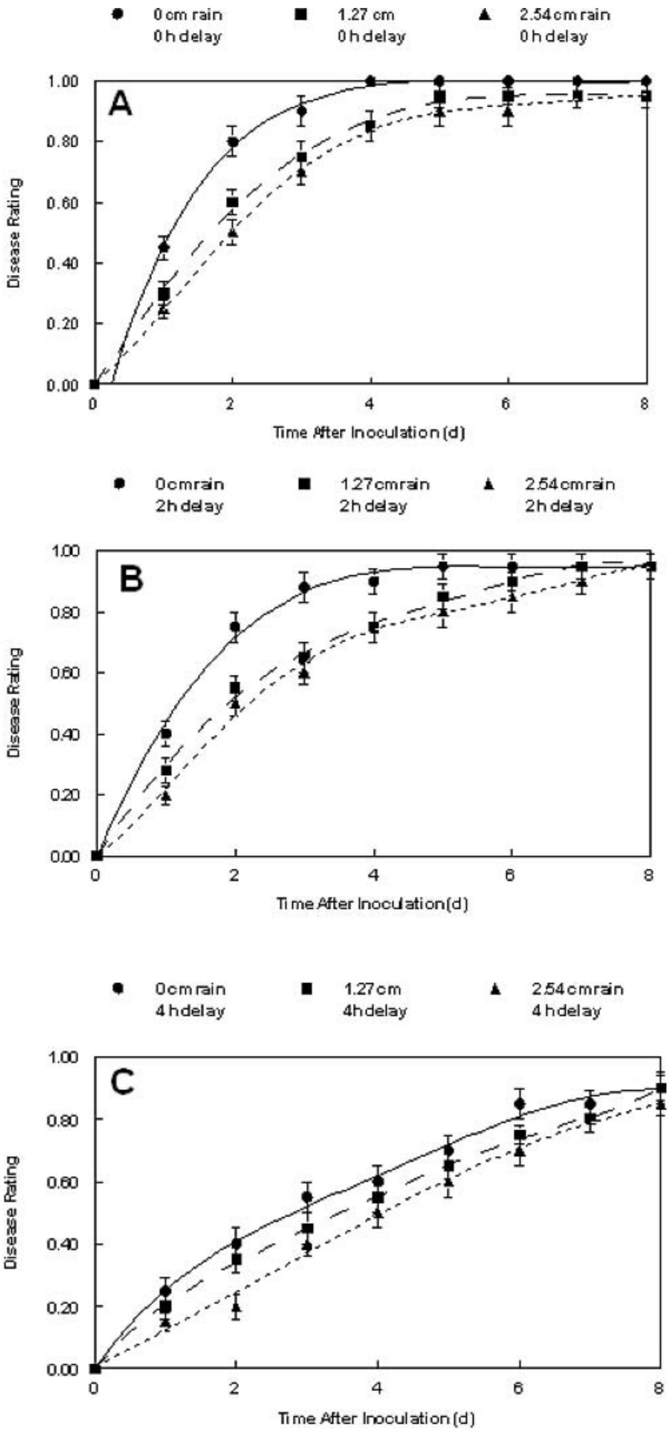

Figure 2. Disease progression of *Alternaria cassiae* infecting sicklepod based upon disease rating (0–1, where 1.0 = plant mortality) over time. Spray application rates were approximately 200 L ha^{-1}. Equations describing relationships of the treatments are: (0 rain/0 delay): $Y = -2.13 = 50.98X + 1.82X^2 - 6.33X^3$; $R^2 = 0.99$; (1.27 cm rain/0 delay) $Y = -0.36 + 34.30X - 1.63X^2 - 0.75X^3$; $R^2 = 0.99$; (2.54 cm rain/0 delay) $Y = 0.19 + 18.74X + 8.25X^2 - 3.24X^3$; $R^2 = 0.99$; (0 cm rain/2-h delay) $Y = 1.17 + 55.82X - 11.67X^2 + 1.03X^3$; $R^2 = 0.99$; (1.27 cm rain/2-h delay) $Y = 0.380 + 33.64X - 2.88X^2 - 0.77X^3$; $R^2 = 0.98$; (2.54 cm rain/2-h delay) $Y = -0.41 + 17.20X^2 + 8.46X^3$; $R^2 = 0.9$; (0 cm rain/4-h delay) $Y = -0.15 + 33.15X - 9.32X^2 + 1.80X^3$; $R^2 = 0.99$; (1.27 cm rain/4-h delay): $Y = 0.17 + 27.71X - 8.76X^2 + 2.23X^3$; $R^2 = 0.99$; (2.54 cm rain/4-h delay) $Y = 0.55 + 11.68X + 0.04X^2 + 0.12X^3$; $R^2 = 0.99$. Error bars represent ± 1 SEM.

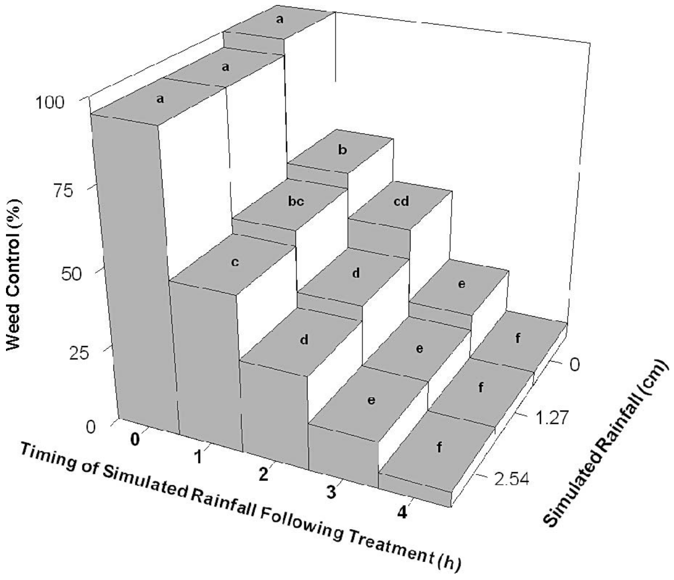

Figure 3. Effect of simulated rainfall amount and time following inoculation with *Colletotrichum truncatum* spores before rainfall treatments on hemp sesbania control. Weed control values in each column with the same letter are not significantly different according to FLSD$_{05}$.

in the untreated controls (Figure 3; control data not plotted). Only 3 d were required to achieve 100% hemp sesbania mortality with no rainfall and an immediate dew treatment. However, 5 d were required to achieve maximum mortality (95%) and disease when plants received either 1.27 or 2.54 cm rainfall followed by an immediate dew treatment (Figure 4A). With no rainfall, a delay of 2 h in dew treatment, maximum mortality (40%) of hemp sesbania required 5 d, with only slight differences between either 1.27 or 2.54 cm of rainfall (Figure 4B). A 4-h delay in dew application (Figure 4C) at all rainfall levels did not result in mortality or disease significantly different than untreated controls (control data not plotted). Reduction in dry weight of both sicklepod and hemp sesbania were almost identical to mortality (data not presented).

Sicklepod exhibits a thigmotrophic response (folding of leaves), even after slight disturbance, such as that from the spray inoculation process (Walker 1982). Because the *A. cassiae* spore germination and infection process begins within a few minutes following inoculation (van Dyke and Trigiano 1987), it is possible that the fungal spores were trapped by the folded leaves, with the folded leaves serving as a protectant from rainfall wash off. Although this response also occurred with hemp sesbania leaves, the folding was not as extreme as with sicklepod, and it could have had less of an effect in protecting the *C. truncatum* spores.

Colletotrichum truncatum spores are highly susceptible to dehydration (Jackson and Schisler 1992; Egley and Boyette 1995); thus delaying the rainfall and dew application could have resulted in the greatly reduced weed control efficacy. Because *C. truncatum* spores are hydrophilic whereas *A. cassiae* spores are hydrophobic (Weaver et al. 2007), it is possible that the *C. truncatum* spores also are more prone to wash off than *A. cassiae* spores. Although no attempts were made to

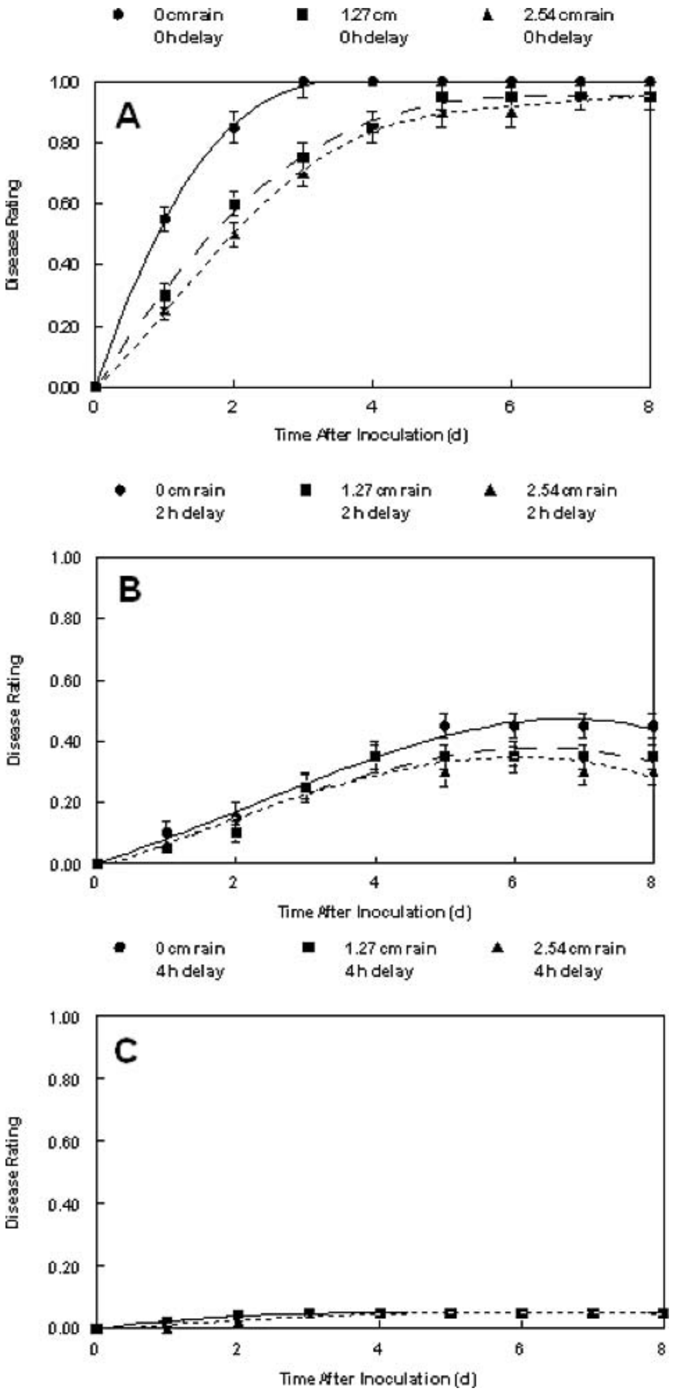

Figure 4. Disease progression of *Colletotrichum truncatum* infecting hemp sesbania based upon disease rating (0–1, where 1.0 = plant mortality) over time. Spray application rates were approximately 200 L ha^{-1}. Equations describing relationships of the treatments are: (0 rain/0 delay) $Y = 0.59 + 65.32 - 9.15X - 2.32X^2 + 0.89X^3$; $R^2 = 0.99$; (1.27 cm rain/0 delay) $Y = -0.36 + 34.29X - 1.\,63X^2 - 0.76X^3$; $R^2 = 0.99$; (2.54 cm rain/0 delay) $Y = 0.19 + 18.74X + 8.25X^2 - 3.24X^3$; $R^2 = 0.99$; (0 cm rain/2-h delay) $Y = 0.40 + 6.72X + 1.08X^2 - 0.16X^3$; $R^2 = 0.99$; (1.27 cm rain/2-h delay) $Y = -1.82 + 7.82X + 0.47X^2 - 0.11X^3$: $R^2 = 0.97$; (2.54 cm rain/2-h delay) $Y = 0.71 + 3.68X - 0.85X^2 + 0.06X^3$; $R^2 = 0.92$; (0 cm rain/4-h delay) $Y = -0.12 + 2.94X - 0.5X^2 + 0.03X^3$; $R^2 = 0.99$; (1.27 cm rain/4-h delay) $Y = -0.64 + 1.87X - 0.12X^2$; $R^2 = 0.99$; (2.54 cm rain/4-h delay) $Y = 0.46 + 2.94X - 0.53X^2 + 0.03X^3$; $R^2 = 0.95$. Error bars represent ± 1 SEM.

determine and compare spore numbers present on plants receiving rainfall as compared to plants receiving no rainfall, microscopic examination did reveal the presence of *A. cassiae* and *C. truncatum* spores on their respective weed hosts after rainfall treatments. Previous research here and elsewhere has examined the effect of different crop oils (Auld 1993; Boyette 1994; Egley and Boyette 1995; Mintz et al. 1992; Sandrin et al. 2003) and other humectants, such as psyllium (Charudattan et al. 1995), upon free-moisture requirements of bioherbicides as they relate to weed control efficacy. It is possible that these adjuvants may also aid in adhesion of the fungal spores to weed tissues (Egley and Boyette 1995), thus reducing potential wash off.

Our results demonstrate that rainfall and the timing of a dew period can affect disease progression and overall weed control efficacy of these bioherbicides. However, interactions of rain and dew with biological agents are very complex and are dependent on the hydrophobic/hydrophilic character of the spores or propagules, the weed surface topography, thigmotrophic/nastic movements of target plants, the physical/chemical nature of formulation ingredients, and other factors. Therefore, the potential effects of rainfall on a given biological agent cannot be accurately predicted, and specific studies are needed to assess a given agent and target combination. Future research will be conducted to determine if certain adjuvants will prevent desiccation and/or wash-off of *C. truncatum* as well as other *Colletotrichum* spp. that we have previously evaluated as bioherbicides.

Literature Cited

Ahimera, N., S. Gisler, D. P. Morgan, and T. J. Micailides. 2004. Effects of single-drop impactions of the dispersal of *Botryosphaeria dothidea* conidia. Phytopathology 94:1189–1197.

Auld, B. A. 1993. Vegetable oil suspension emulsions reduce dew dependence of a mycoherbicide. Crop Protect. 12:477–479.

Bakerspigel, A. 1953. Soils as a storage medium for fungi. Mycologia 45:596–604.

Boyette, C. D. 1991. Host range and virulence of *Colletotrichum truncatum*, a potential mycoherbicide for hemp sesbania (*Sesbania exaltata*). Plant Dis. 75:62–64.

Boyette, C. D. 1994. Unrefined corn oil improves the mycoherbicidal activity of *Colletotrichum truncatum* for hemp sesbania (*Sesbania exaltata*) control. Weed Technol. 8:526–529.

Boyette, C. D., R. E. Hoagland, and M. A. Weaver. 2007. Biocontrol efficacy of *Colletotrichum truncatum* for hemp sesbania (*Sesbania exaltata*) is enhanced with unrefined corn oil and surfactant. Weed Biol. Mgt. 7:70–76.

Boyette, C. D., P. C. Quimby, Jr., C. T. Bryson, G. H. Egley, and F. E. Fulgham. 1993. Biological control of hemp sesbania (*Sesbania exaltata*) under field conditions with *Colletotrichum truncatum* formulated in an invert emulsion. Weed Sci. 41:497–500.

Bryson, C. T. 1988. Effects of rainfall on foliar herbicides applied to seedling johnsongrass (*Sorghum halepense*). Weed Technol. 2:153–158.

Carrol, M. J., R. L. Hill, E. Pfeil, and A. E. Herner. 1993. Washoff of dicamba and 3,6-dichlorosalicylic acid from turfgrass foliage. Weed Technol. 7:437–442.

Charudattan, R., Y. A. Shabana, J. T. DeValerio, and E. N. Rosskopf. 1995. Broad-spectrum bioherbicide to control several species of pigweeds and methods of use. U.S. patent 5393728.

Egley, G. H. and C. D. Boyette. 1995. Water–corn oil emulsion enhances conidia germination and mycoherbicidal activity of *Colletotrichum truncatum*. Weed Sci. 43:312–317.

Feng, P.C.C., J. J. Sandbrink, and R. D. Sammons. 2000. Retention, uptake, and translocation of ^{14}C-glyphosate from track-spray applications and correlation to rainfastness in velvetleaf (*Abutilon theophrasti*). Weed Technol. 14:127–132.

Gannon, T. W. and F. H. Yelverton. 2008. Effect of simulated rainfall on tall fescue (*Lolium arundinaceum*) control with glyphosate. Weed Technol. 22:553–557.

Jackson, M. A. and D. A. Schisler. 1992. The composition and attributes of *Colletotrichum truncatum* spores are altered by the nutritional environment. Appl. Environ. Microbiol. 58:2260–2265.

Koger, C. H., D. M. Dodds, and D. B. Reynolds. 2007. Effect of adjuvants and urea ammonium nitrate on bispyribac efficacy, absorption, and translocation in barnyardgrass (*Echinochloa crus-galli*). I. Efficacy, rainfastness, and soil moisture. Weed Sci. 55:399–405.

McDowell, L. L., Willis, G. H., Smith, S., and Southwick, L. M. 1985. Insecticide washoff from cotton plants as a function of time between application and rainfall. Trans. Am. Soc. Agric. Eng. 28:1896–1900.

Meyer, L. D. and Harmon, W. C. 1979. Multiple intensity rainfall simulator for erosion research on row sideslopes. Trans. Am. Soc. Agric. Eng. 22:100–103.

Mintz, A. S., D. K. Heiny, and G. J. Weidemann. 1992. Factors influencing the biocontrol of tumble pigweed (*Amaranthus albus*) with *Aposphaeria amaranthi*. Plant Dis. 76:267–279.

Nemec, S. J. and Adkisson, P. L. 1969. Effects of simulated rain and dew on the toxicity of certain ultra-low volume (ULV) insecticidal formulations. J. Econ. Entomol. 62:71–73.

Ntahimpera, N., L. V. Madden, and L. L. Wilson. 1997. Effect of rain distribution alteration on splash dispersal of *Colletotrichum acuatum*. Phytopathology 87:649–655.

Paul, P. A., S. M. El-Allaf, P. E. Lipps, and L. V. Madden. 2004. Rain splash dispersal of *Gibberella zeae* within wheat canopies in Ohio. Phytopathology 94:1342–1349.

Sandrin, T. R., D. O. TeBeest, and G. J. Weidemann. 2003. Soybean and sunflower oils increase the infectivity of *Colletotrichum gloeosporioides* f. sp. *aeschynomene* to northern jointvetch. Biol. Control 26:244–252.

Steel, R.G.D., J. H. Torrie, and D. A. Dickey. 1997. Principles and Procedures of Statistics—A Biometrical Approach. 3rd ed. New York, NY: McGraw-Hill.

Stensvand, A. and H. Elkemo. 2005. Use of a rainfall frequency threshold to adjust a degree-day model of ascospore maturity of *Venturia inaequalis*. Plant Dis. 89:198–202.

Troiano, J. and E. J. Butterfield. 1984. Effects of simulated acidic rain on retention of pesticides on leaf surfaces. Phytopathology 74:1377–1380.

Van Dyke, C. G. and R. N. Trigiano. 1987. Light and scanning electron microscopy of the interaction of the biocontrol fungus *Alternaria cassiae* with sicklepod (*Cassia obtusifolia*). Can. J. Plant Pathol. 9:230–235.

Vincent, A., J. Armengol, and J. Garcia-Jimenez. 2007. Rainfastness and persistence of fungicides for control of *Alternaria* brown spot of citrus. Plant Dis. 91:393–399.

Walker, H. L. 1982. A seedling blight of sicklepod caused by *Alternaria cassiae*. Plant Dis. 66:426–428.

Walker, H. L. and C. D. Boyette. 1986. Influence of sequential dew periods on biocontrol of sicklepod (*Cassia obtusifolia*) by *Alternaria cassiae*. Plant Dis. 70:962–963.

Walker, H. L. and J. A. Riley. 1982. Evaluation of *Alternaria cassiae* for the biological control of sicklepod (*Cassia obtusifolia*). Weed Sci. 30:651–654.

Weaver, M. A., M. E. Lyn, C. D. Boyette, and R. E. Hoagland. 2007. Bioherbicides for weed control. Pages 93–110. *in* M. K. Upadhyaya and R. E. Blackshaw, eds. Non-Chemical Weed Management. Oxon, United Kingdom: CAB International.

Willis, G. H., L. L. McDowell, S. Smith, and L. M. Southwick. 1992. Foliar washoff of oil-applied malathion and permethrin as a function of time after application. J. Agric. Food Chem. 40:1086–1089.

Xu, X. M., R. A. Murray, J. D. Salazar, and K. Hyder. 2008. The effects of temperature, humidity, and rainfall on captan decline on apple leaves and fruit in controlled environment conditions. Pest Mgt. Sci. 64:296–307.

Received September 24, 2010, and approved August 24, 2011.