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Article

Biological Control of Spreading Dayflower (*Commelina diffusa*) with the Fungal Pathogen *Phoma commelinicola*

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Abstract: Greenhouse and field experiments showed that conidia of the fungal pathogen, *Phoma commelinicola,* exhibited bioherbicidal activity against spreading dayflower (*Commelina diffusa*) seedlings when applied at concentrations of 10^6 to 10^9 conidia·mL^{-1}. Greenhouse tests determined an optimal temperature for conidial germination of 25 °C–30 °C, and that sporulation occurred on several solid growth media. A dew period of ≥ 12 h was required to achieve 60% control of cotyledonary-first leaf growth stage seedlings when applications of 10^8 conidia·mL^{-1} were applied. Maximal control (80%) required longer dew periods (21 h) and 90% plant dry weight reduction occurred at this dew period duration. More efficacious control occurred on younger plants (cotyledonary-first leaf growth stage) than older, larger plants. Mortality and dry weight reduction values in field experiments were ~70% and >80%, respectively, when cotyledonary-third leaf growth stage seedlings were sprayed with 10^8 or 10^9 conidia·mL^{-1}. These results indicate that this fungus has potential as a biological control agent for controlling this problematic weed that is tolerant to the herbicide glyphosate.

Keywords: bioherbicide; biocontrol; dayflower; fungal pathogen; weed control

1. Introduction

Spreading dayflower (*Commelina diffusa* Burm. f.) is a perennial, monocotyledenous weed occurring worldwide in tropical and subtropical areas, and an annual weed in temperate climates. It spreads diffusely, creeping along the ground, branching heavily and rooting at the nodes, obtaining stem lengths up to 1 m [1]. *C. diffusa* can reproduce vegetatively and by seed, and cut stems root readily in moist ground. This weed prefers moist, fertile soil (e.g., gardens, cultivated fields), but will also grow on roadsides and in non-crop areas. It has a sprawling growth habit, and its long stems can create a tangled web in gardens and flower beds. It is related to several houseplant species e.g., wandering jew (*Tradescantia zebrine* (Schinz) D.R. Hunt) and perennial spiderwort (*Tradescantia virginiana* L.). *Commelina* spp. have been used as a ground cover to reduce soil erosion [2], which may have contributed to their spreading. Its potential as a fodder crop may be useful to provide protein to ruminants on smallholder farms [3].

When growing in rice and other lowland crops, this weed may act as a quasi-aquatic plant that can withstand flooding, and it readily infests cultivated lands, roadsides, pastures and wastelands [1]. *C. diffusa* is problematic, primarily in young crops (2–5 weeks old), but can also be a problem in mature crops due to its sprawling behavior [4]. It is a troublesome weed of cotton, rice and soybean in warm temperate areas of the U.S. and other countries [5–7]. It is also reported as a major weed of bananas in Mexico and Hawaii; beans, oranges, lemons, grapes, apricots, coffee and cotton in Mexico; papaya in Hawaii; sugarcane in Puerto Rico, and sorghum in Thailand [2]. It is also a weed in maize and vegetables in Mexico; bananas, papayas, and pineapples in the Philippines; rice in Colombia; sugarcane in Mexico and Trinidad; taro and pastures in Hawaii and coffee in Costa Rica [2].

C. diffusa is a host of the root-burrowing (*Radophilus similis*) [8], reniform (*Rotylenchulus reniformis*), banana lesion (*Pratylenchus goodeyi*) [9] and root-knot (*Meloidogyne exigua*) [10] nematodes. Severe outbreaks of cucumber mosaic virus have been correlated with high densities of *C. diffusa* serving as a reservoir of virus and aphid vectors [11].

Worldwide, there are about 170 species of *Commelina* and generally, most are difficult to control. Several *Commelinia* spp. exhibit resistance or tolerance to several chemical herbicides. *C. diffusa* was one of the first plants reported as being resistant to 2.4-dichlorophenoxy-acetic acid [12]. Herbicidal control of *C. diffusa* can be variable depending on the herbicide, growth stage, environmental parameters, *etc.*, and various herbicides and combinations of herbicides have exhibited a range of efficacy for control of *C. diffusa* and other related species as summarized [4,13–15]. Recent guidelines for control of *C. diffusa* in rice in Mississippi (USA) indicate that only ~50% of the 42 single herbicide or herbicide combination treatments provided good to excellent control, while 26% gave fair control and the remainder gave zero to poor control [16]. Some alternative herbicide options can be used to control this weed, alone or in combination with other modes of action in rice, during early post-emergence applications prior to flooding [17].

C. diffusa, Benghal dayflower (*C. benghalensis* L.), and Asiatic dayflower (*C. communis* L.) have been reported to be difficult to control with glyphosate in genetically-modified crops [18–24]. The ecological, biological and physiological factors related to glyphosate-tolerant *C. communis* in agronomic systems in Iowa have recently been studied [25]. Because of the increasing importance of *C. diffusa*, and its resistance or tolerance to many herbicides, alterative weed control measures may be

required. The use of bioherbicides has been recognized as a potential technological alternative to chemical herbicides in certain situations, and global interest exists in the bioherbicide concept, with active research and development projects established by commercial entities in the U.S., Canada, Europe, Australia, Japan, and other countries [26–30].

A leaf-spot disease (oblong lesions, *ca*. 1.3–1.8 cm) was observed on *C. diffusa* in a flooded rice field near Stuttgart, AR, USA (Figure 1). Infected leaf and stem tissues were collected and a fungal pathogen was isolated from this diseased tissue. This fungus was provisionally identified as *Phyllosticta commelinicola* E. Young, a synonym of *Phoma commelinicola* (E. Young) Gruyter [31]. The objectives of these studies were to isolate and examine this pathogen with respect to its growth and germination on various growth media, correlate inoculum concentration and bioherbicidal activity (inundative application) with plant growth stage, develop time courses for weed control and disease progression on *C. diffusa*, and evaluate weed control under field conditions. Knowledge of these basic parameters is essential for evaluating a plant pathogen as a bioherbicide for weed control [32].

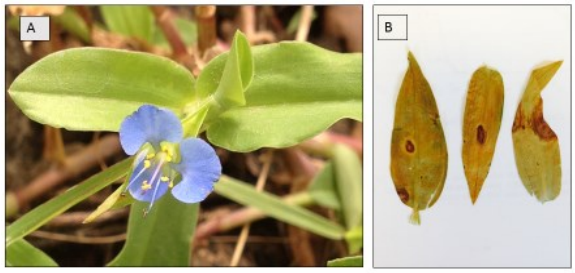

Figure 1. *Commelina diffusa* photographs. (**A**): flowering plant in the field; (**B**): pressed/dried specimen exhibiting leaf spotting incited by *P. commelinicola*.

2. Materials and Methods

2.1. Seed Sources, Test Plant Propagation

C. diffusa seeds were collected near Stuttgart AR, USA, planted in a 2:1 potting mix of Jiffy mix:sandy soil (Jiffy Mix, Jiffy Products of America, Inc., Batavia, IL, USA) contained in plastic trays (25 × 52 cm) and allowed to germinate. Germinated seedlings were transplanted into 10-cm^2 plastic pots (1 plant per pot) containing the soil mixture above, and grown under greenhouse conditions (28 °C to 32 °C, 40 to 60% relative humidity (RH), ~14 h day length, and 1650 $\mu E \cdot m^{-2} \cdot s^{-1}$ photosynthetically active radiation (PAR) measured at midday).

2.2. Isolation and Culture of Phoma commelinicola

Several isolates of the fungus were isolated from diseased *C. diffusa* tissue by surface sterilizing sections of diseased tissue in 0.05% NaOCl for 1 min, rinsing in sterile distilled water and then placing the sections on autoclaved (121 C, 15 min; at 103.42 kPa) potato-dextrose agar (PDA, Difco, Detroit, MI, USA) plates amended with the antibiotics chloramphenicol (0.75 $mg \cdot mL^{-1}$) and streptomycin sulfate (1.25 $mg \cdot mL^{-1}$). The plates were incubated for 48 h at 25 °C and then advancing edges of fungal colonies were transferred to PDA plates followed by incubation for 5 days at 25 °C under alternating 12-h light (cool, white fluorescent bulbs)/12-h dark regimens. Tests of these isolates indicated very similar

virulence on the host plant. We chose an isolate with the highest virulence (SFN-73) and used it in further studies. When re-inoculated onto healthy seedlings at 1×10^8, the fungus (SFN-73) was highly virulent and killed all inoculated plants within 5 days, while the controls remained healthy, thus fulfilling Koch's postulates (data not shown). The fungus was then sub-cultured on PDA without antibiotics, and preserved under refrigeration (4 °C to 5 °C) on sterilized sandy loam soil (25% water holding capacity), or on sterile silica gel containing skim milk [33].

Several media were examined for growth and conidial production of the fungus: water agar, 2.0% (WA), potato dextrose agar (PDA), yeast extract agar (YEA) and Czapek-Dox agar (CDA) from Difco (Detroit, MI, USA), V8 agar (V8A) [33] from Campbell Soup Co. (Camden, NJ, USA) and dayflower decoction agar (DFA). DFA was prepared by adding 100 g of finely ground, air-dried dayflower leaf and stem tissue to 2.0% WA to yield a 10% (w:v) product.

2.3. Effect of Temperature on Conidial Germination and Radial Growth Rate

Conidial germination was measured by spreading 100 µL of a suspension (1.0×10^6 conidia·mL^{-1}) prepared in sterile distilled water on PDA plates, and incubating them at 10 °C, 15 °C, 20 °C, 25 °C, 30 °C, or 35 °C on open-mesh wire shelves of an incubator (Precision Scientific Inc., Chicago, IL, USA). A 12-h photoperiod was provided by two 20 W, cool-white fluorescent lamps positioned in the incubator door. The light intensity at the plate level was 200 µE·m^{-2}·s^{-1} PAR as measured with a light meter (LI-COR Inc., Lincoln, NE, USA). Germinated conidia (500 plate^{-1}) were counted after 16 h using a haemocytomer.

For radial growth studies, 5-mm plugs were taken from the advancing margins of 7-day-old colonies of the fungus and placed in the centers of PDA plates. The plates were incubated at temperatures of 10 °C, 15 °C, 20 °C, 25 °C, 30 °C, or 35 °C as described above for the conidial germination studies. Colony diameters (fungal growth) were measured after 7 days of incubation. In each experiment, five replicate plates for each temperature were utilized. Both experiments were conducted twice, and the results of each experiment were pooled following testing for homogeneity.

2.4. Effect of Dew Period Duration on Weed Control and Dry Weight Reduction of C. diffusa Seedlings

C. diffusa seedlings (cotyledonary to first leaf growth stage) were sprayed (hand held sprayer; Spray-Tool, Aervoe Industries, Gardnerville, NV, USA) until runoff (*ca*. 100 L·ha^{-1}) occurred with a spray mixture containing 1.0×10^8 conidia·mL^{-1} in distilled water. Control plants were sprayed with distilled water. The inoculated plants were then placed in darkened dew chambers at 25 °C and 100% RH for periods of 3, 6, 9, 12, 15, 18, 21 or 24 h. Following this dew treatment, the plants were placed on sub-irrigated trays in the greenhouse as described above. Weed control and dry weight reductions were recorded 14 days after treatment (DAT). For dry weight determinations, the above ground biomass was harvested, oven-dried (48 h, 85 °C), weighed, and the percentage biomass reduction (compared with untreated control plants) was determined. The experiment was conducted twice with 3 sets of 10 plants for each experiment.

2.5. Effect of Inoculum Concentration and Plant Growth Stage

C. diffusa plants (cotyledonary, 1 to 2 true-leaf, 3 to 4 true-leaf and 5 to 7 true-leaf growth stages) were sprayed with conidial suspensions of 1.0×10^6 to 1.0×10^9 conidia·mL^{-1} and held in a dew chamber for 16 h at 25 °C. Control plants were sprayed with distilled water only. Plants were moved to the greenhouse, and mortality and dry weight reductions were recorded 14 DAT. Experiments were conducted twice with 3 sets of 10 plants for each experiment. The experiment was conducted twice with 3 sets of 10 plants for each experiment.

2.6. Effects of Phoma commelinicola on Crop Seedlings

Greenhouse tests were conducted on seedlings of several crops to access the possible detrimental effects of this pathogen. Rice (*Oryza sativa* L.), soybean (*Glycine max* (L.) Merr.), cotton (*Gossypium hirsutum* L.) and corn (*Zea mays* L.) plants were grown from seeds in the greenhouse under the conditions described above. After 10 to 14 days (when seedlings were ~3–7 cm tall), plants were sprayed with *P. commelinicola* inoculum concentrations of 1.0×10^6 or 1.0×10^8 conidia·mL^{-1}. Visual disease symptomatology and dry weight analyses of fungal-inoculated plants were monitored 14 DAT. The experiment was conducted twice with 3 sets of 10 plants for each experiment.

2.7. Field Experiments

Field experiments were conducted in 1998 and 1999 at the University of Arkansas, Rice Research and Extension Center, Stuttgart, AR, USA, on Crowley silt loam (fine montmorillonitic, thermic Typic Albaqualfs), pH 6.2 to 6.5, with an organic matter content of ~1.0%. The experiments were established in an irrigated rice field, divided into 1.0 × 1.0 m micro-plots (1.0×10^{-5} ha). The field was naturally infested with dayflower seedlings (avg. 75 seedlings per plot) that were in the cotyledonary to first leaf growth stages. Within each plot, 18–20 test plants were randomly selected and marked using wooden stakes (7.6 cm long). Treatments consisted of *P. commelinicola* conidia applied at either 0.0 conidia·mL^{-1} (water control) or conidia in water applied at several concentrations from 1.0×10^6 to 1.0×10^9 conidia·mL^{-1}. The selected plants were monitored for disease development at 3-day intervals for 21 days. All treatments were replicated 4 times and the experiment was repeated.

2.8. Statistical Procedures

The greenhouse and field experiments were arranged as randomized complete block factorial designs with three and four replications, respectively. Data collected over the 2-year field testing period were examined for homogeneity of variance [34], combined, and analyzed using ANOVA. Field data from both years were pooled following subjection to Bartlett's test for homogeneity, and analyzed using analysis of variance. Because arcsine and square-root transformation of the data did not alter the interpretation, non-transformed data are presented. When significant differences were detected by the *F*-test, means were separated with Fisher's protected LSD test at the 0.05 probability level. Disease progression was based on a modified Horsfall and Barratt [35] rating scale of 0 to 5.0, assigning symptom expression as 0 represents unaffected, and 1.0, 2.0, 3.0, 4.0, and 5.0 represents 20, 40, 60, 80 and 100% leaf and stem injury (or dead plants), respectively. Percentage weed control was determined by dividing

the number of dead and severely injured plants (symptom expression ratings of 4.0–5.0) by the total number of plants treated × 100. Data were analyzed using standard mean errors and best-fit regression analysis. All data were analyzed using SAS (Version 9.1, SAS Institute, Inc., Cary, NC, USA) statistical software.

3. Results and Discussion

3.1. Isolation of Phoma commelinicola

The fungus was readily isolated from diseased tissue and observed to sporulate abundantly on PDA. Several isolates were collected and found to exhibit similar virulence. We chose an isolate with the highest virulence (SFN-73) and used it in these studies. When re-inoculated onto healthy seedlings at 1.0×10^8, the fungus (SFN-73) was highly virulent and killed all inoculated plants within 5 DAT, while the controls remained healthy, thus fulfilling Koch's postulates. The organism produced disease symptomatology typical of other diseases incited by other *Phoma* spp. (*i.e.*, lesions on leaves and stems) (Figure 1). The organism produced typical *Phoma* lesions on leaves and stems, with pycnidia scattered throughout the lesions. Under moist conditions, slimy masses of conidia accumulated on the upper surface of the leaf, breaking the epidermal layer and cuticle. Conidia were unicellular, hyaline (2.0 to 4.0 × 1.5 to 2.5 μm) extruded through ostioles contained in black pycnidia (60 to 165 × 45 to 140 μm), ostiolate, protruding into plant tissues or agar surfaces.

3.2. Germination and Growth of Phoma commelinicola

Germination of conidia on PDA occurred at 10 to 35 °C with optimal germination at 20 to 30 °C and maximum (85%) at 25 °C (Figure 2). The fungus grew at all temperatures tested (10 to 35 °C), but growth was significantly reduced at 10, 15 and 35°C (Figure 3). The fungus also grew and sporulated prolifically on several different solid substrate media. Dayflower decoction agar (DFA) and PDA produced the most abundant conidia (6.0×10^8 and 5.0×10^8, respectively) (Table 1). The lowest growth rate occurred on Czapek-Dox agar (4.1 mm·day^{-1}) and the highest rate of growth was found on DFA decoction agar (10.5 mm·day^{-1}). Due to the commercial availability of PDA, it was used in all other greenhouse and field experiments. This growth is comparable to conidial yields produced by other *Phoma* spp. that have been evaluated as bioherbicides [36,37].

Table 1. Effect of various growth media [a] on radial growth and conidial production of *Phoma commelinicola* under various light and dark regimes [b].

Growth	Radial Growth (mm·day^{-1})			Conidia (10^8 plate^{-1})		
	Light	Dark	Light/Dark	Light	Dark	Light/Dark
WA	-	-	-	-	-	-
DFA	10.2 [a,c]	9.0 [a]	10.5 [a]	4.2 [a]	2.8 [a]	6.0 [a]
PDA	9.5 [b]	8.0 [b]	9.9 [b]	3.0 [b]	1.3 [b]	5.0 [b]
V8A	8.4 [c]	7.0 [c]	8.5 [c]	2.1 [c]	0.9 [c]	3.9 [c]
YEA	7.5 [d]	6.9 [c]	7.4 [d]	1.0 [d]	0.3 [d]	2.3 [d]
CDA	4.0 [e]	2.8 [d]	4.1 [e]	1.0 [d]	0.2 [d]	1.2 [e]

[a] WA, water agar; DFA, dayflower decoction agar; PDA, potato dextrose agar; V8A (V8 vegetable juice); agar; YEA, yeast-extract agar; CDA, Czapek-Dox agar; [b] Light conditions (24 h continuous light at 28 °C); Dark conditions (24 h continuous light at 28 °C); Light/dark conditions (12 h light/dark at 28 °C); [c] Means within the same column followed by the same letter do not differ at $p = 0.05$, according to FLSD.

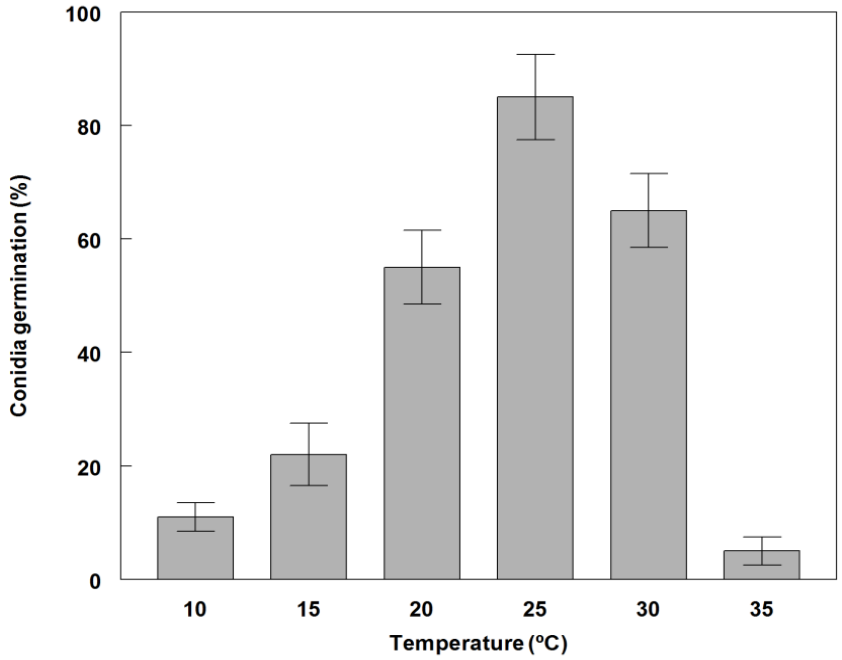

Figure 2. Effect of temperature on germination of conidia of *P. commelinicola* on PDA, 7 days after inoculation and growth under alternating light/dark conditions (12 h light/dark at 28 °C). Error bars represent Fisher's LSD ($p = 0.05$).

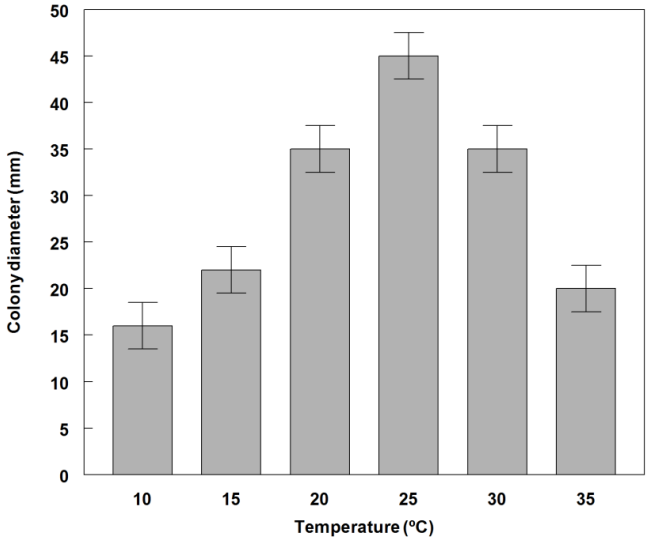

Figure 3. Effect of temperature on radial growth of *P. commelinicola* colonies on PDA, 7 days after inoculation and growth under alternating light/dark conditions (12 h light/dark at 28 °C). Error bars represent Fisher's LSD (p = 0.05).

3.3. Effects of Dew Period Duration on Weed Control and Dry Weight Reduction of C. diffusa Inoculated with P. commelinicola under Greenhouse Conditions

A minimum of 12 h dew at 25 °C was required to achieve ~65% weed control (Figure 4). Optimal weed control occurred at dew period durations of between 15 to 24 h. A similar trend was observed for the dry weight reduction data (~67% at 12 h) (Figure 5). Complete mortality (100%) was not achieved at any dew period, but many plants were severely stunted, which resulted in greatly reduced dry weight. Lengthy dew periods are commonly required for most bioherbicidal plant pathogens [32]. This factor has been a major constraint for commercial development of these biocontrol organisms [38].

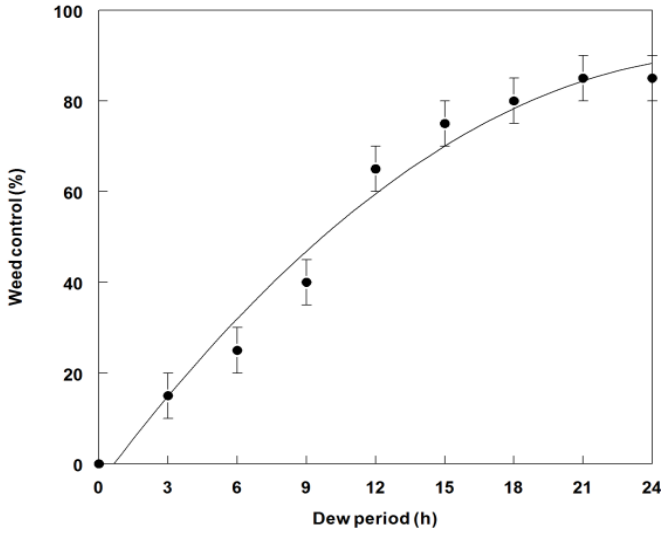

Figure 4. Effect of dew period duration at 25 °C on weed control of spreading dayflower inoculated with *P. commelinicola* at 1.0 × 10^8 under greenhouse conditions. Error bars represent Fisher's LSD (p = 0.05).

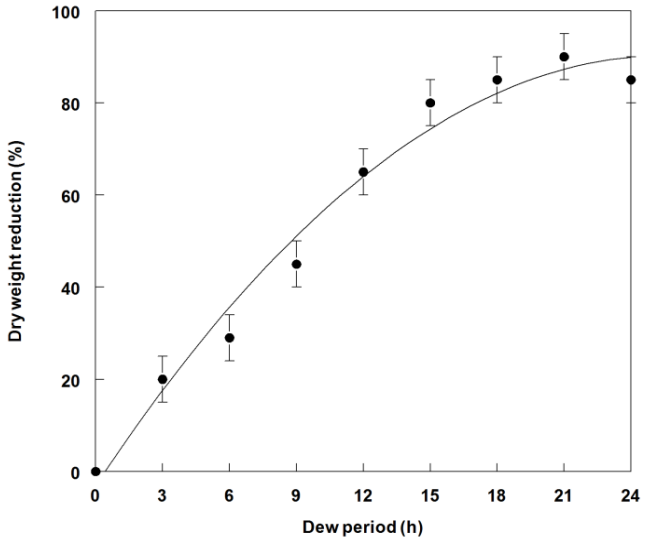

Figure 5. Effect of dew period duration on dry weight reduction of spreading dayflower inoculated with *P. commelinicola* at 1.0×10^8 under greenhouse conditions. Error bars represent Fisher's LSD ($p = 0.05$).

3.4. Effects of P. commelinicola Inoculum Concentration on Weed Control and Dry Weight Reduction of C. diffusa under Greenhouse Conditions

Generally, weed control on *C. diffusa* plants under greenhouse conditions was significantly increased at all growth stages as the fungal inoculum concentration increased (Figure 6). For example, at low inoculum concentration (0.001×10^9 conidia·mL^{-1}), the youngest plants were controlled about 5%, while the highest concentration (1.0×10^9 conidia·mL^{-1}) provided ~85% control. Plants in the 6- to 7- and 8- to 9-leaf stages were more resistant to infection than younger plants, *i.e.*, plants in the 8- to 9-leaf growth stages were controlled at the 0 and 15% levels by 0.001×10^9 and 1.0×10^9 conidia·mL^{-1}, respectively. Similar results were obtained for the dry weight reductions of plants at these growth stages and conidia concentrations (Figure 7). Since risk assessment of bioherbicides on non-target plants is necessary and important, we examined the effects of this bioherbicide on several crops under greenhouse conditions. Seedlings of several crops (rice, soybean, cotton and corn), sprayed with *P. commelinicola* inoculum concentrations at 1.0×10^6 and 1.0×10^8 conidia·mL^{-1}, exhibited no visual disease symptomatology or dry weight reduction when evaluated 14 DAT under greenhouse conditions (data not shown).

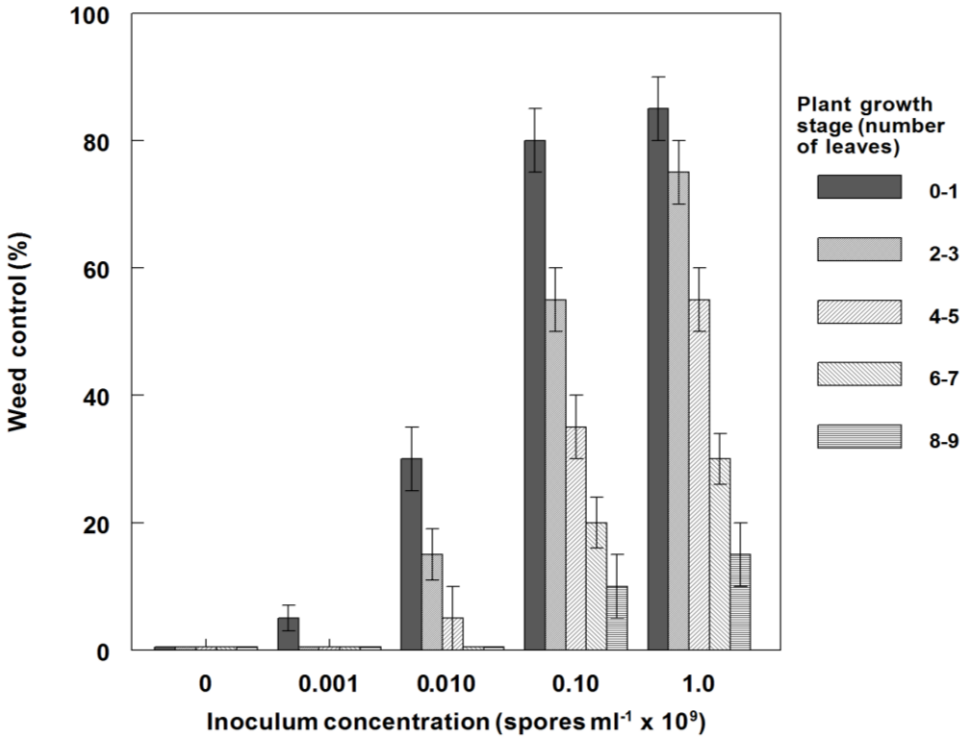

Figure 6. Effect of plant growth stage on weed control (mortality) of spreading dayflower inoculated with *P. commelinicola* at various inoculum concentrations under greenhouse conditions. Error bars represent Fisher's LSD (p = 0.05).

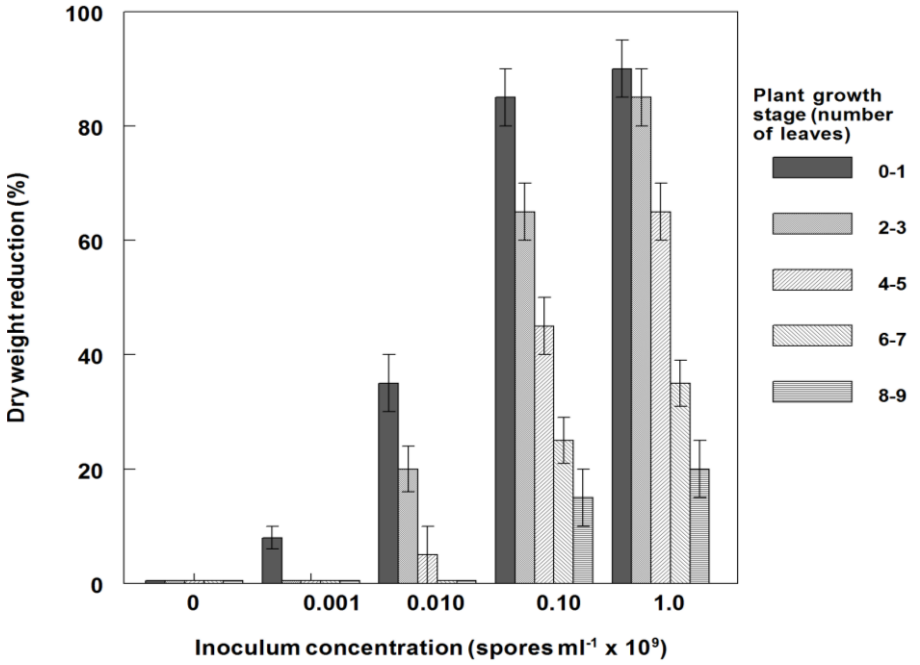

Figure 7. Effect of plant growth stage on dry weight reduction of spreading dayflower inoculated with *P. commelinicola* at various inoculum concentrations under greenhouse conditions. Error bars represent Fisher's LSD (p = 0.05).

3.5. Disease Progression of P. commelinicola on C. diffusa under Greenhouse Conditions

Disease on *C. diffusa* incited by *P. commelinicola* progressed in a linear fashion from 3 to 12 DAT under greenhouse conditions, with a disease rating of 3.5 occurring at 12 DAT (Figure 8). Disease progressed to 3.8 at 15 DAT and eventually increased to 4.5 at 21 DAT.

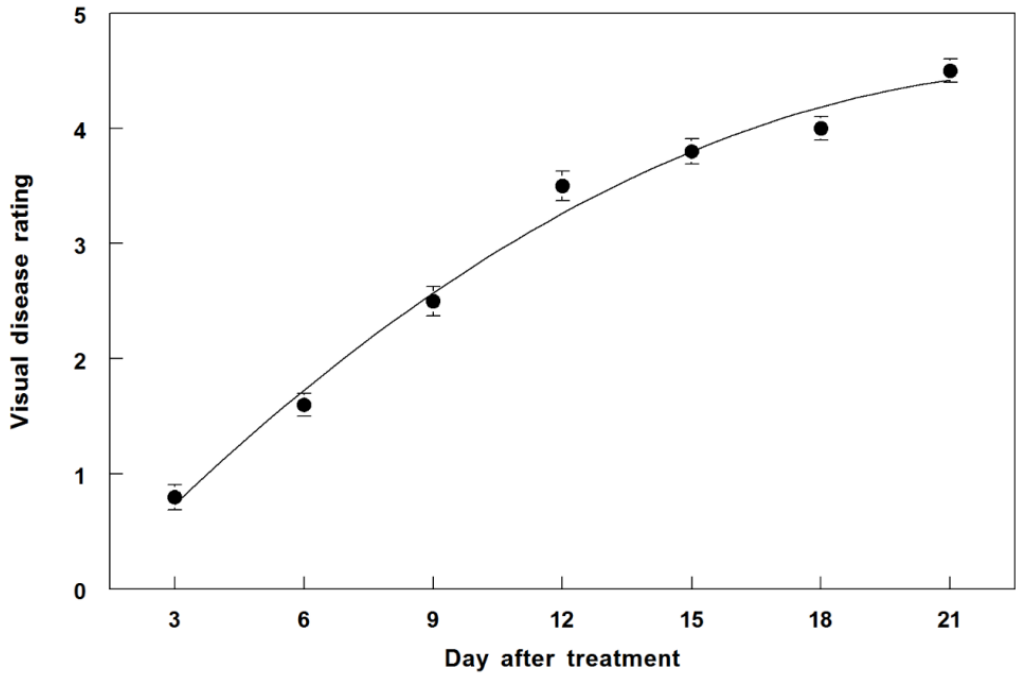

Figure 8. Disease progression of *P. commelinicola* on greenhouse-grown *C. diffusa* over a 21-day period after inoculation. The relationship for *P. commelinicola* disease progression is best described by the equation: $Y = -0.15 + 0.37X - 0.01X^2$, $R^2 = 0.98$. Error bars represent Fisher's LSD ($p = 0.05$).

3.6. Effects of P. commelinicola Inoculum Concentration and C. diffusa Growth Stage on Weed Control and Dry Weight Reduction under Field Conditions

In field experiments, the highest weed control (55 to 70%) occurred on cotyledonary to third-leaf stage plants at 1.0×10^7 to 1.0×10^9 conidia·mL^{-1}, 21 DAT (Figure 9). A similar trend occurred in dry weight reduction with ~80% reduction after 21 DAT at 1.0×10^8 or 10^9 conidia·mL^{-1} (Figure 10). The LD_{50} and GR_{50} values for weed control (Figure 9) and dry weight reduction (Figure 10) were 2.0×10^7 conidia·mL^{-1} and 4.0×10^6 conidia·mL^{-1}, respectively.

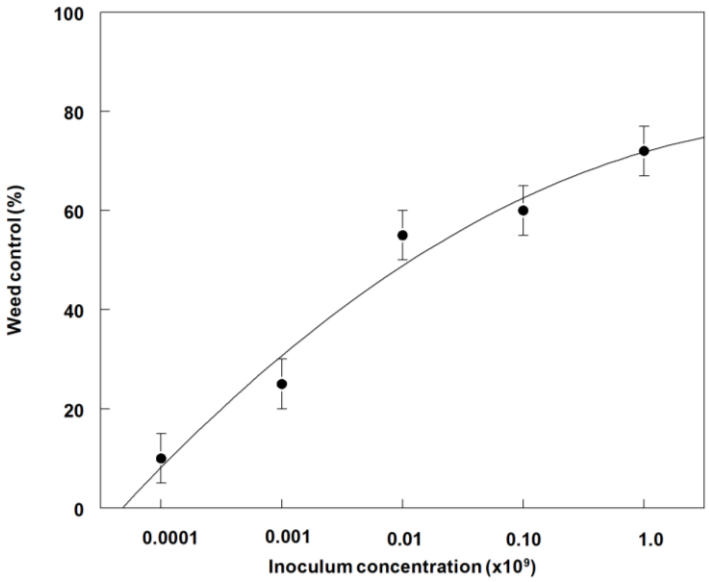

Figure 9. Effect of *P. commelinicola* inoculum concentration on the control of *C. diffusa* in the cotyledonary to third leaf growth stage under field conditions. $Y = -18.8 + 29.2X - 2.2X^2$, $R^2 = 0.96$.

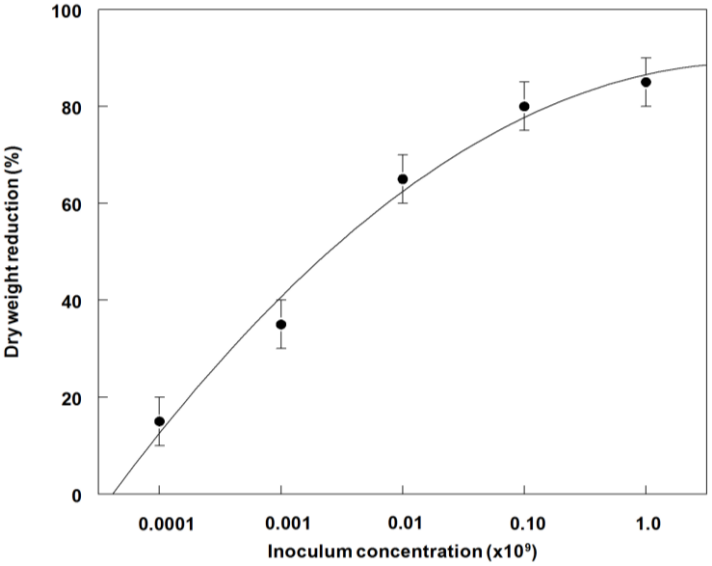

Figure 10. Effect of *P. commelinicola* inoculum concentration on the dry weight reduction of *C. diffusa* in the cotyledonary to third leaf growth stage under field conditions. $Y = -22.0 + 37.8X - 3.2X^2$, $R^2 = 0.98$.

3.7. Disease Progression of P. commelinicola on C. diffusa under Field Conditions

The disease progression of this fungus on *C. diffusa* under field conditions (Figure 11) was similar to that found under greenhouse conditions (Figure 8). However, 15 days were required to achieve a rating value of 3.5 as compared to 12 days under greenhouse conditions (Figure 8). The maximal disease rating of 3.8 occurred at 21 DAT in the field (Figure 11). No visual infectivity or injury was observed on rice plants using this formulation under field conditions (data not shown).

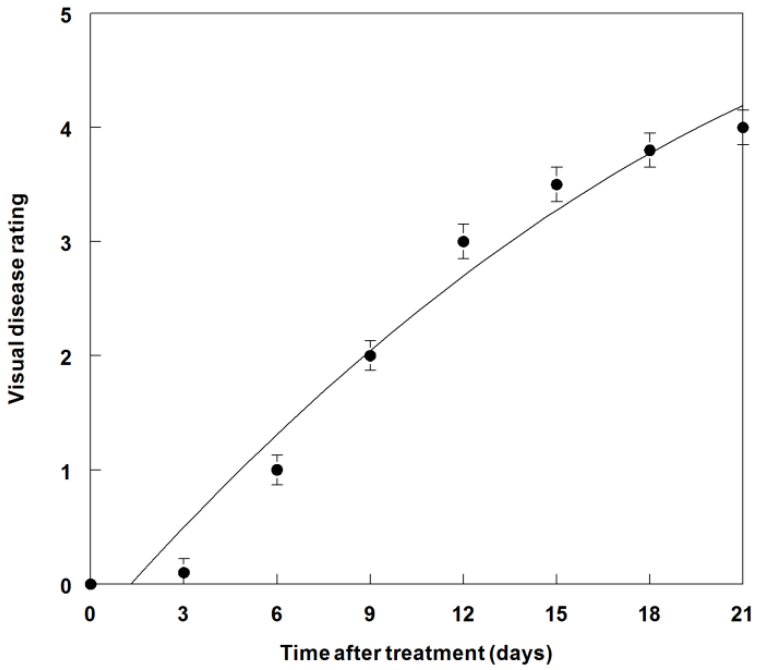

Figure 11. Disease progression of *P. commelinicola* on field-grown *C. diffusa* over a 21-day period after inoculation. The relationship for *P. commelinicola* disease progression is best described by the equation: $Y = -0.39 + 0.31X - 0.04X^2$, $R^2 = 0.96$. Error bars represent Fisher's LSD ($p = 0.05$).

Despite the high phytopathogenic potential shown by various *Phoma* spp. and pathovars, the bioherbicidal potential of these microbes has been largely ignored. Some attempts to evaluate this diverse genus as bioherbicides have been reported. For example, Heiny [39,40] isolated a highly host specific strain of *P. proboscis* from diseased field bindweed (*Convolvulus arvensis* L.). Heiny and Templeton [37] reported significant bioherbicidal effects when conidia of this fungus were applied to weed seedlings, under temperatures from 16–28 °C, and ≥9 h dew period and the compatibility with synthetic herbicides was investigated [40]. Studies of environmental factors on the effectiveness of a *Phoma herbarum* strain against *Commelina communis* showed that temperatures of 28–32 °C and a 48-h dew period were required for optimal control [41]. A strain of *P. herbarum* from diseased leaves of *Parthenium hysterophorus* L. was found in central India [42]. The fungus caused >90% inhibition of seed germination, seedling mortality and leaf damage, followed by a reduction in the height of this weed [42]. Three strains of *Phoma herbarum* were isolated from the diseased leaves and stem of *Lantana camara* L. [43] and all strains incited severe infection of weed seedlings [44]. Other *Phoma* spp. have also been reported from various regions of India: *P. campanulata* on *Cassia fistula* L.; *P. exigua* on *Sesamum indicum* L.; *P. eupyrena* on *Achyrenthus aspera* L.; *P. glomerata* on *Crotalaria juncea* L. and *Parthenium hysterophrous* L.; *P. lantanae* on *Lantana camara*; *P. palmarum* on *Calotropis procera* L.; *P. tridocis* on *Tridex procumbens* L.; and *P. herbarum* var. *ipomoeae* and *P. euphorbiae* on *Euphorbia hirta* [45,46]. Recently, *Phoma macrotoma* has been developed as a commercial bioherbicide (Phoma^{TM}) for controlling various broadleaf weeds in turf [47].

Fungal conidia are the predominant propagules used in bioherbicidal research [26,29,30]. However, mycelial formulations of various fungal bioherbicides, including *Phoma* spp., have shown weed control

potential against some important weeds. For example, mycelial suspensions of *P. herbarum* incited significantly more disease severity than conidia on dandelion (*Taraxacum officinale* Weber) [48]. Similarly, mycelial fragments of *P. exigua* and *P. herbarum* incited significantly more disease severity of *T. officinale* than fungal conidia [49]. Zhao and Shamoun [36] found that culture growth media and age of mycelial cultures could affect the disease severity of *P. exigua* on the perennial evergreen weedy shrub, salal (*Gaultheria shallon* Pursh).

The Clearfield™ system has become the predominant rice production system in southern rice producing states [50]. The rice cultivars utilized in this system are natural mutants with tolerance to the herbicide imazethapyr (2-(4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1H-imidazol-2-yl)-5-ethyl-3-pyridine-carboxylic acid) (Newpath™). Although this herbicide controls many grassy and broadleaf weeds, it fails to control some weeds, including *C. diffusa,* which can result in tremendous weed infestations if other weed control measures are not utilized [51]. Whether this isolate of *P. commelinicola* could be incorporated in this system remains to be determined.

4. Conclusions

P. commelinicola may be an effective bioherbicide for controlling *C. diffusa*, a problematic weed in rice production in the southern U.S. Fungal conidia can be readily produced on several solid substrate growth media. The fungus grows and germinates over a wide range of temperatures. Although a rather lengthy dew period is required (15 to 18 h) to achieve levels of control of 75 to 80%, respectively, this free moisture condition is met in flooded rice fields. No visual disease symptomology was observed on rice (cv, Starbonnet). However, further research is in progress to define the host range of the fungus using various rice cultivars and other economically important rice weeds. Special consideration will be given to the effects of this pathogen on other principal *Commelina* spp., especially *C. benghalensis*, an exotic, invasive weed that is resistant to herbicides in many areas of the world, including the southern U.S. [52]. We also wish to examine the effects of surfactants and other adjuvants to improve the efficacy and use of mycelial formulations of this organism for control of *Commelina* spp. Possible interactions (synergistic, additive or antagonistic) of this pathogen with herbicides will be examined since important synergistic interactions of other plant pathogens with herbicides have been discovered [32,53,54]. Furthermore, since another pathogen (*C. gloeosporioides* f. sp. *aeschynomene*, LockDown^{TM}) [55] is compatible with the Clearfield^{TM} rice production system, additional research will also examine the compatibility of *P. commelinicola* in this rice production protocol.

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Conflicts of Interest

The authors declare no conflict of interest.

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