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Curvularia eragrostidis, a Promising Mycoherbicide Agent for Grass Weeds

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ABSTRACT

A fungal pathogen isolated from diseased leaves of large crabgrass (*Digitaria sanguinalis*) at three different geographic locations in China was identified as *Curvularia eragrostidis*. A series of biological assessments have been carried out to determine the potential of the fungus as a bioherbicide agent. The fungus is able to germinate and grow in a very wide range of temperature (10-40ºC) or pH (2-11) conditions, although 28°C and pH 6 were optimal. This implies a great versatility for infection of weeds under field conditions. Several phytotoxins have been identified from *C. eragrostidis* cultures. At least two of them, α,β-dehydrocurvularin and helminthosporin, are associated with the pathogenicity on crabgrass. The α,β-dehydrocurvularin impairs the PS-II reaction center and inhibits re-oxidation of the primary electron acceptor (*QA*) of photosynthesis. With slightly different modes of action, the helminthosporin affects the chloroplast function of large crabgrass leaves. Forty-one plant species belonging to 20 families were inoculated with *C. eragrostidis* to assess a potential host range. Many of these were important crop species commonly grown in China, including rice, corn, soybean, cotton, and peanut. The fungus caused no disease or any other negative impact on the crop species tested, while resulted in infection on several additional grass weeds including Chinese crabgrass and Chinese sprangletop. This reveals a potential broader spectrum of weed control. Formulation is urgently needed to make this bioherbicide agent perform consistently under field conditions.

Keywords: Microbial bioherbicide; fungi; weed biocontrol, phytotoxin, host range; chloroplast function

CONTENTS

INTRODUCTION 61
DISCOVERY AND BIOLOGICAL ASSESSMENT 61
MECHANISMS FOR WEED BIOCONTROL 62
Infection process 62
Toxins 62
Mechanisms of host infection 64
MASS PRODUCTION OF *C. eragrostidis* INOCULUM 64
WEED CONTROL EFFICACY UNDER FIELD CONDITIONS 64
CONCLUDING REMARKS 65
REFERENCES 65

INTRODUCTION

Curvularia eragrostidis J. A. Meyer has been reported as a pathogen of many plants including Indian lovegrass, barnyard grass, bermudagrass, corn, tea, yam, passion fruit, and striga (Michereff *et al*. 1995; Amusa 1997; Czerwenka Wenkstetten *et al*. 1997; Saha *et al*. 1999, 2000; Paula *et al*. 2000; Muniz *et al*. 2003; Zhang 2004; Dou *et al*. 2006). Most previous studies focused on biological and pathogenic characteristics of *C. eragrostidis* (Andrade *et al*. 1995, 1996; Michereff *et al*. 1995; Amusa 1997; Dasgupta *et al*. 2005; Saha *et al*. 2005). The fungus was first reported as a biocontrol agent for large crabgrass (*Digitaria sanguinalis* L.) by Zhu and Qiang (2003, 2004b), although several other fungal pathogens including *C. intermedia* and *Drechslera gigantean* (Walker and Tilley 1999; Chandramohan and Charudattan 2001, 2002; Tilley and Walker 2002) and rhizobacteria (Boyetchko 1997) had been evaluated earlier for biocontrol of large crabgrass and other weedy grasses. Evidente *et al*. (2006a, 2006b) later isolated the phytotoxin ophiobolins from *D. gigantean* and proposed its potential for control of grass weeds. In recent years, *C. eragrostidis* has been explored extensively for potential biocontrol of grass weeds, and this review will highlight some of the useful features revealed by these studies.

DISCOVERY AND BIOLOGICAL ASSESSMENT

Field surveys for diseases on crabgrass (*Digitaria* spp.) were conducted throughout China during 2000-2001. Plants with severe leaf-spot symptoms were obtained from Jiangsu and Yunnan Provinces. Sections of diseased leaf tissue were surface-sterilized for pathogen isolation and a total of six fungal isolates were identified as *C. eragrostidis* according to the taxonomic characteristics described by Ellis (1971). Conidial morphology for all isolates was similar with straight to slightly curved conidium marked by three transverse septa. In general, the size of the two central cells of each spore was equal or larger than the outer two polar cells, and the color of central cells was darker. The septa between the two central cells were usually distinct. Spores ranged in sizes; from 24 to 36 μm in length and 8 to 15 μm in width.

All isolates caused leaf lesions typical of *C. eragrostidis* on large crabgrass seedling (1- to 4-leaf stages), and similar

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Invited Review

to those observed under field conditions. Symptoms appeared on leaves 2-3 days after inoculation. Lesions were initially reddish brown, ring-shaped, and surrounded by chlorotic tissue, then rapidly enlarged to an oblong shape and gradually coalesced, killing the leaf within 4-6 days. Extensive leaves damage ultimately led to plant death, but large crabgrass plants not killed within 6 days of inoculation would usually survive although exhibiting severe stunting. The isolates QZ-2000 was most virulent among all six isolates obtained and yielded significantly more conidia than other isolates, thus was selected for further study (Zhu and Qiang 2003, 2004b).

The virulence of *C. eragrostidis* was influenced by dew duration, inoculum dose, host plant growth stage, temperature, photoperiod, and carrier (water) volume of spray. For infection and disease development, the optimal post-inoculation temperature range was 15-30°C. The virulence of the fungus on crabgrass was enhanced with increased spore concentration and dew duration. Both plant mortality and dry weight reduction of crabgrass seedlings increased as spore concentration increased. A spore concentration at 10^6 mL^{-1} caused over 85% mortality and 90% dry weight reduction on plants of 1- to 3-leaf stages when subjected to 24-h dew duration. At the same spore dose (about 6-7 × 10^7 spores m^{-2}), different carrier volumes used for sprays had an effect on weed control; higher carrier volumes resulted in greater efficacy of weed control but a minimum of 900 L ha^{-1} would be required to achieve over 90% of weed mortality (Zhu and Qiang 2004a).

Forty-one plant species in 20 families were inoculated with *C. eragrostidis* to assess the potential host range of the fungus. Most of these were economically important terrestrial plant species and cultivars grown in China, and others were species closely related to the crabgrass (**Table 1**). After inoculation, all plants were given 48-h dew at 20°C to provide a best-case scenario for infection. Responses of these plants to the *C. eragrostidis* isolate QZ-2000 (the most promising bioherbicide candidate) ranged from no visual symptoms to 98% mortality. On 6 of the 41 species inoculated, disease symptoms and/or a significant dry-weight reduction was observed; they were Chinese sprangletop, Adscendent crabgrass, large crabgrass, Chinese crabgrass, barnyardgrass, and green foxtail. At the seedling stage, large crabgrass and Adscendent crabgrass exhibited 96 and 98% mortality, respectively. Some grass species showed disease symptoms and a significant reduction in dry weight, but no plant mortality. For example, the dry weight of Chinese crabgrass and Chinese sprangletop was reduced by 63 to 68%, respectively, but plant mortality only reached 0 and 43%, respectively. Although disease symptoms were caused also on barnyard grass and green foxtail, the severity was generally low and the reduction on plant dry weight was only marginal.

None of the crop species tested was susceptible to the isolate QZ-2000, including Bermudagrass, tall fescue, perennial ryegrass, corn, soybean, rice, cotton, peanut, and water-melon. This is important because several crabgrass species and Chinese sprangletop are problematic weeds in many of these crop fields in China (Zhu and Qiang 2004b), and the non-pathogenicity of isolate QZ-2000 represents an opportunity to develop the fungus as a safe biocontrol agent against crab grasses and Chinese sprangletop for these crops.

Detailed studies have been carried out on biological characterization of the fungus and investigation of environmental factors that influences infection and disease development critically. A medium containing soyflour-cornmeal-sugar (SCS) favored the most rapid growth of fungal mycelium (Zhu and Qiang 2003). Fungal conidia are able to germinate in a wide range of temperature (10-40°C) or pH (2-11), although 28°C and pH 6 were optimal. This implies a great versatility for infection of weeds under field conditions. The conidial germination could be enhanced by adding a small amount of peptone, beef extract, Tween 80, crabgrass extract, or rapeseed oil in spore suspensions (Zhu and Qiang 2003).

The fungus preferred a growth medium containing glucose and $(NH_4)_2HPO_4$, respectively, as a carbon and nitrogen source for sporulation. This nutritional requirement is relatively easy to satisfy for fungal inoculum production. The optimal temperature for fungal sporulation is 28°C. Continuous lighting under near-UV spectrum yielded more spores than by 12-h alternating light and darkness (Zhu and Qiang 2003).

MECHANISMS FOR WEED BIOCONTROL

Infection process

Conidia of the fungus started to germinate on leaves of large crabgrass 1 hr after inoculation, often with appressorial formation and enzymatic cell-wall depredating activities around the infection site observed 3 hr later. Under optimal conditions, the fungus generally completed the penetration process 8 hrs after inoculation and was able to establish a parasitic relationship in host epidermal cells within 24 hrs. Leaf penetration occurred mainly at conjunction grooves of epidermal cell walls or directly through stomata. The enzymatic activity mediated by the appressoria and mechanical pressure exerted by a penetration peg played pivotal roles during the penetration of epidermal cells (Zhu 2004c).

Toxins

Bioassays showed that *C. eragrostidis* produced phytotoxins biologically active against many *Digitaria* species. In the absence of pathogen, crude toxins incited oblong brown leaf lesions and root inhibition similar to those caused by *C. eragrostidis* infection. Symptoms caused with culture filtrates were most severe 12-14 days after treatment. SCS liquid medium (2 g cornmeal soy flour and 15 g L^{-1}) was suitable for toxin production. The optimal cultural temperature and medium pH for toxin production were 30°C and 5.05, respectively (Zhu 2004c; Jiang 2005).

Two phytotoxic metabolites from the fungal culture filtrate were extracted with ethyl acetate, isolated with bioassay-guided column chromatography and thin-layer chromatography (TLC), characterized by UV, IR, MS, ^{1}H- and ^{13}C-NMR spectral data analyses, and eventually identified as α,β-dehydrocurvularin and helminthosporin (Jiang *et al*. 2006). Toxicity of these two toxins to large crabgrass was evaluated using bioassays based on the inhibition of seed germination, root elongation, and shoot growth. The results showed that the α,β-dehydrocurvularin significantly inhibited the germination with an IC_{50} at 44.68 μg mL^{-1}. α,β-Dehydrocurvularin also inhibited the elongation of root and shoot with IC_{50} at 29.72 and 316.97 μg mL^{-1}, respectively. The root and shoot of crabgrass responded to the α,β-dehydrocurvularin differently, with root being much more sensitive to this toxin. The helminthosporin exhibited a much weaker inhibitory effect on crabgrass than did the α,β-dehydrocurvularin, with IC_{50} ranging from 210 to 7,200 μg mL^{-1} for inhibition of germination, shoot and root elongation. However, the helminthosporin showed stronger toxicity on leaves of large crabgrass; causing bigger necrotic lesions (4.1 mm^2) at the 500 μg mL^{-1} concentration. In contrast, α,β-dehydrocurvularin at the same concentration caused smaller lesions (3.5 mm^2) on large crabgrass leaves. Adjuvants significantly promoted the bioactivity of α,β-dehydrocurvularin (but not helminthosporin) to large crabgrass (Jiang 2005; Jiang and Qiang 2005a, 2005b; Jiang *et al*. 2008).

Fifty eight plant species including many notorious weeds and important crops were selected to study the spectrum of activity for the two phytotoxins, and the results showed that neither was highly selective, with 28 species sensitive to α,β-dehydrocurvularin and 27 to helminthosporin. *Chenopodium serotinum*, *Rumex japonicus* and *Oxalis corniculata* were most sensitive to α,β-dehydrocurvularin,

Table 1 Response of various plant species to inoculation with the *Curvularia eragrostidis* isolate QZ-2000 in controlled- environment conditions (48-h dew).

Family Species	Common name (cultivar)	Mortality (%)[a]	Dry-weight reduction (%)[b]
Amaranthaceae			
Amaranthus retroflexus L.	Redroot pigweed	0	0
Alternanthera philoxeroides (Mart.) Griseb.	Alligator weed	0	0
Cyperaceae			
Cyperus rotundus L.	Nutsedge	0	0
Rubiaceae		0	
Galium aparine var. *tenerum* (Gren. Et Godr.) Rcbb.	Tender catchweed	0	2
Scrophulariaceae			
Veronica persica Poir.	Iran speedwell	0	0
Caryophyllaceae			
Cerastium viscosum L.	Mouse-ear chickweed.	0	0
Stellaria media (L.) Cyrillus	Common chickweed	0	6
Leguminosae			
Glycine max (L.) Merr.	Soybean		
	(73-935); (78-82); (Suxie 1)	0	0
Arachis hypogaea L.	Peanut	0	3
Vicia sativa L.	Common vetch	0	10
Malvaceae			
Gossypium hirsutum L.	Cotton		
	(Sumian 12); (Nankang 3)	0	0
Commelinaceae		0	0
Commelina communis L.	Common dayflower	0	0
Asteraceae			
Eclipta prostrata (L.) L.	Eclipta	0	0
Xanthium sibiricum Patrin.	Siberian cocklebur	0	0
Eupatorium adenophorum Spreng..	Crofton weed	0	0
Erigeron annuus (L.) Pers.	Annual fleabane	0	1
Cannabinaceae			
Humulus scandens (Lour.) Merr.	Japanese hop	0	0
Boraginaceae			
Lamium amplexicaule L.	Henbit deadnettle	0	2
Vitaceae			
Cayratia japonica (Thunb.) Gagnep.	Japanese cayratia	0	4
Portulacaceae			
Portulaca oleracea L.	Purslane	0	7
Euphorbiaceae			
Euphorbia humifusa Willd.	Humifuse euphorbia	0	0
Euphorbia helioscopia L.	Sunn euphorbia	0	0
Convolvulaceae			
Calystegia hederacea Wall. Ex Roxb.	Ivy glorybind	0	0
Cruciferae			
Capsella bursa-pastoris (L.) Medic.	Shepherdspurse	0	0
Polygonaceae			
Polygonum lapathifolium L.	Dockleaved knotweed	0	6
Cucurbitaceae			
Citrullus lanatus (Thunb.) Mansfeld.	Water-melon (Su mi)	0	0
Chenopodiaceae			
Chenopodium album L.	Lambsquarters	0	0
Gramineae		0	
Echinochloa crusgalli (L.) Beauv	Barnyardgrass	0	36**
Alopecurus aequalis Sobol.	Equal alopecurus	0	7
Alopecurus japonicus Steud.	Japanese alopecurus	0	9
Avena fatua L.	Wild oat	0	14
Beckmannia syzigachne (Steud.) Fernald	American sloughgrass	0	12
Eleusine indica (L.) Gaertn	Goosegrass	0	0
Setaria viridis (L.) Beauv.	Green foxtail	0	26*
Digitaria ciliaris (Retz.) Koeler	Adscendent crabgrass	96**	92**
Digitaria sanguinalis (L.) Scop.	Large crabgrass	98**	94**
Digitaria radicosa (Presl) Miq.or D. Chinese Henr	Chinese crabgrass	0	63**
Oryza sativa L.	Rice		
	(Xie you 699); (9311); (Te you 63)	0	0
Zea mays L.	Corn		
	(Su yu nuo)	0	8
	(Chuan dan 14); (Ye dan 22)	0	0
Leptochloa chinensis (L.) Nees	Chinese sprangletop	46**	68**
Poa annua L.	Annual bluegrass	0	0
Cynodon dactylon (L.) Pers.	Bermudagrass	0	8
Festuca arundinaces Schreb.	Tall fescue	0	0
Lolium perenne L.	English perennial ryegrass	0	0

while corn, soybean and the species from *Leguminosae*, *Caryophyllaceae*, *Cannabinaceae*, *Euphorbiaceae*, *Labiatae* and *Scrophulariaceae* were resistant to the toxin at the 500 μg mL^{-1}. *Chenopodium serotinum* was also the most sensitive species to helminthosporin. *Beckmannia syzigachne*, *Chenopodium album*, and *Alopecurus japonicu* were also highly sensitive, while soybean, cotton, tomato, and the weeds *Amaranthus retroflexus* and *Echinochloa crus-galli* were resistant at 500 μg mL^{-1}. These results indicated that the two phytotoxins may have a broad spectrum of activity against a range of weeds in certain crop systems. For example, α,β-dehydrocurvularin may be used to control *D. sanguinalis* and several other problematic weeds in corn and soybean fields and helminthosporin may be used for weed control in cotton and soybean fields (Jiang 2005; Jiang *et al*. 2008).

The effect of α,β-dehydrocurvularin on cell mitosis was studied using adventitious root tips of *Allium sativum*, and the results showed that the toxin interfered with cell division due to a multinucleolus abnormality and inhibition of the spindle formation. This impact on the cell mitosis process consequently suppressed root elongation (Jiang 2005; Jiang *et al*. 2008).

Effects of the toxins on photochemical activities of large crabgrass were studied to understand the pathogenic mechanism. It was found that α,β-dehydrocurvularin strongly inhibited the activities of the Hill reaction, non-cyclic photophosphorylation (PSP), and the activities of Mg^{2+}-ATPase and Ca^{2+}-ATPase. However, the toxin did not affect the activity of cyclic PSP and had little impact on photosynthetic pigment content. The Mg^{2+}-ATPase is more sensitive to α,β-dehydrocurvularin than Ca^{2+}-ATPase; at 200 μg mL^{-1}, the toxin reduced the electron transport rate of PS-II system by 19.4% but showed no impact on the PS-I. Chlorophyll fluorescence data showed that the toxin caused a decrease in Fv/Fo, F_v/F_m, $\Phi PS\,II$, qP, qN, Fm values of large crabgrass and an increase in Fo values (Jiang 2005). These changes in chlorophyll fluorescence parameters indicate that the toxin damages the PS-II reaction center and blocks the reoxidation of the primary electron acceptor (QA). Based on these observations, α,β-dehydrocurvularin is suspected to damage PS-II reaction center and inhibit the reoxidation of the primary electron acceptor (QA). Consequently, the electron transport between PS-II reaction center and QA or between QA and the second electron acceptor (QB) are blocked, and PSP and carbon assimilation were inhibited. Eventually, metabolism of large crabgrass was disrupted. The decrease of qN value implied that the toxin causes the accumulation of reductive-electron acceptor and increases the production of free radicals, resulting in the damage to PS-II reaction center.

Helminthosporin also strongly inhibited the Hill reaction, non-cyclic PSP, and activities of Mg^{2+}-ATPase and Ca^{2+}-ATPase in large crabgrass; detached leaves treated with helminthosporin at 150 μg mL^{-1} showed significant declines in Fv/Fo, F_v/F_m, $\Phi PS\,II$, and Fm values when compared to those in control leaves 24 hours after treatment, while little change was observed in qP, qN, or photosynthetic pigment content. In contrast, the Fo increased considerably (Jiang and Qiang 2005a, 2005b; Jiang *et al*. 2006). These results indicate that although both toxins cause damage to chloroplast functions of large crabgrass leaves, their action targets vary slightly. Likely, chloroplast is not the only site of action for these toxins due to additional bioactive impact observed, although the impact on the photosynthetic process is considered the primary factor of pathogenesis for *C. eragrostidis* on large crabgrass (Jiang 2005).

These toxins help mediate the bioherbicidal effect of the fungus on large crabgrass by decreasing the efficiency of light energy conversion due to the damage to the chloroplast function, which inhibits ATP, NADPH and metabolism of the plant (Jiang and Qiang 2005a). The suppression of mitosis limits new growth from the meristem tissue, resulting in further decrease in photosynthesis area. These phytotoxic effects are considered being of importance for effective biocontrol of large crabgrass.

Mechanisms of host infection

Several extra-cellular esterase isoenzymes were detected in saprophytic and parasitic phases during the germination of *C. eragrostidis* conidia. Epicuticular wax is normally the first line of defense for plants to fend off various invaders. The waxes produced by *D. sanguinalis* (host) significantly stimulated the growth of *C. eragrostidis* conidial germ tubes but showed no effect on appressorial formation. In contrast, the epicuticular wax of *Festuca arundinacea* (a non-host turfgrass species) inhibited both the extension of germ tubes and differentiation of appressoria. Analysis using gas chromatography–mass spectrometry found that the constituents of epicuticular waxes of *D. sanguinalis* and *F. arundinacea* were remarkably different; with only 4 common compounds shared among 24 and 21 compounds identified, respectively, in the waxes of the two species (Wang 2006). Epicuticular waxes of *D. sanguinalis* induced secretion of esterases from the fungus (Wang 2006). An esterase preparation from germinating *C. eragrostidis* conidia could completely degrade the epicuticular wax of *D. sanguinalis* after 4 h incubation at 25°C, but only digested the wax of *F. arundinacea* partially (Wang *et al*. 2008). These results suggest that the components of the wax and their chemical characteristics are important to successful infection of *D. sanguinalis* by *C. eragrostidis*.

MASS PRODUCTION OF *C. eragrostidis* INOCULUM

Conidia of *C. eragrostidis* are more efficacious than mycelia for infection of crabgrass, and therefore the method for mass production of fungal conidia was explored using a combination of submerged and solid-substrate fermentation in a two-staged process described by Walker (1983). The highest yields of conidia were achieved by producing mycelia in a submerged culture, then transferring them to a solid substrate to promote conidiation. Wheat bran, sawdust, or rice bran were the most suitable and economical solid substrates tested (Xu 2004).

When these solid substrates were mixed in an optimal ratio, better conidiation was obtained. The type and proportion of the materials, water content, incubation temperature, and light condition are important for the conidial production. The sawdust from Dawn Redwood (*Metasequoia glyptostroboides* Hu & W.C. Cheng) was superior to those from other tree species. The optimal ratios for rice bran-sawdust and wheat bran-sawdust mixtures used for solid substrates were 3: 1 and 2.5: 1, respectively. The optimal amount of water added to the substrate is 1.6: 1 (v/v) prior to autoclaving. The most favorable temperature was 28°C for mycelial growth, but 25°C for sporulation. Within a range, the fungal sporulation was stimulated by a prolonged lighting period and continuous lighting was more favorable to *C. eragrostidis* sporulation than 12-hr alternate lighting and darkness (Xu 2004; Gao 2005; Qiang *et al*. 2005). However, when the duration of lighting exceeded three consecutive days, germination of resulting conidia could be reduced or even inhibited, and consequently the inoculum was less efficacious.

WEED CONTROL EFFICACY UNDER FIELD CONDITIONS

A large number of field trials were conducted between 2002 and 2006 at two research field sites near Nanjing to determine efficacy of the fungus in varying applications for control of large crabgrass and other weeds under different field conditions. Treatments included different rates of fungal conidia, use of adjuvants or humectants, and mixtures of the fungus with selected herbicides at reduced rates.

When fungus was applied alone at various doses, the weed control was attained. At the same application volume,

increasing conidial concentration up to 5×10^5 mL^{-1} often enhanced weed-control efficacy noticeably but beyond that point, increasing fungal inoculum dose had little effect. Weed-control efficacy and consistency were improved by adding adjuvants or humectants such as Tween-80, oil, lecithin, glycerol, ascorbic acid in spray formulations (Gao 2005). Synthetic herbicides including nicosulfuron, fenoxaprop-P, imazethapyr at substantially reduced rates (0.1-0.2X of recommended rates) could also be used with the fungus as a synergizer to enhance the efficacy and consistency of weed control. This greatly reduced herbicide rates should help the efforts aimed to decrease the chemical load in the environment. For example, fungal conidial suspensions at 5×10^5 spores mL^{-1} amended with nicosulfuron at 0.1-0.2X recommended rates plus adjuvants in a carrier volume greater than 600 L/ha generally achieved significant weed suppression (> 50% fresh weight reduction) under a range of field conditions. Under favorable weather conditions with frequent rain, this fungal-chemical mixture controlled large crabgrass at the 2- to 3-leaf stage by up to 90% when compared to untreated controls (Xu 2004; Zhu 2004; Gao 2005). The herbicides at the drastically reduced rates were generally ineffective to control the weed,, but may weaken the resistance of weed to the fungus infection and inhibit the growth of weed at early stages (Charudattan 1986). Herbicides at low rates, although insufficient for weed control, may change permeability of cell membrane and thus promote fungus growth and infection (Greaves and Sargent 1986). The high humidity during frequent rains may promote the germination of conidia and growth of mycelia, eventually enhancing the overall infection on weeds (Templeton *et al*. 1979).

Results of field experiments indicate that low infestation by large crabgrass may be controlled effectively by this mycoherbicide agent. Under heavy infestation, high weed mortality often was not achieved although the growth of large crabgrass was markedly suppressed. Due to this selective suppression, crops were able to compete with the weed vigorously and form the dominant plant community. Adding herbicides at drastically reduced rates synergizes the control of large crabgrass by the bioherbicide agent under field conditions while minimize impact of the chemical to the environment.

CONCLUDING REMARKS

Years of research has revealed that *C. eragrostidis* has great potential to be developed as a mycoherbicide for large crabgrass; the fungus is highly virulent and kills the weed rapidly. It causes no negative impact on many important crops grown in China, including rice, corn, cotton, soybean, peanut, water-melon and turf grasses, therefore making it a safe option in these crops where crabgrass is often a major weed problem. Additionally, conidia of the fungus can be readily mass produced using a two-stage fermentation process, with the potential to meet current industry technological standards. The synergy with selected herbicides at much reduced rates may aid in efficacy, consistency, and flexibility of weed control under field conditions. In an integrated weed control system, this strategy may decrease use of chemical herbicides and alleviate concerns about herbicide residual in the environment.

Going forward, a commercially acceptable *C. eragrostidis* formulation is required to further enhance the weed-control performance under field conditions. Proper formulations may help improve moisture retention around fungal inoculum and alleviate the stress caused by dry conditions (Walker and Connick 1983), affecting the practicality of microbial bioherbicides (Ghorbani *et al*. 2005). Invert emulsions (Amsellen *et al*. 1991; Auld 1993; Egley and Boyette 1995; Womack *et al*. 1996; Bourdot *et al*. 2006; Boyette *et al*. 2007), water-in-oil-in-water emulsions (Auld 2003), wetting agents (Auld *et al*. 1990; Jahromi *et al*. 2006), and biopolimers (Shabana *et al*. 1997; Chittick and Auld 2001) have been demonstrated to improve environmental adaptability of mycoherbicides. The effectiveness of these formulation options may be examined for *C. eragrostidis* on large crabgrass to determine applicability. From the commercial stand point, at least a shelf life of 8-10 months will be required for this product because of the nature of production cycle and distribution system. Finally, a mammalian toxicology study should be conducted as soon as possible to ensure human and animal safety for regulatory considerations, which will require a partnership with industry.

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