

Proceedings

The XIth International Bioherbicide Group Workshop

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Preface

The XIth International Bioherbicide Group Workshop will be held in Nanjing, China on August 23-24, 2013. We are here to share bioherbicide advances, experiences and technology worldwide. The specific topics on bioherbicide included in this workshop are: 1) Progress and prospect of bioherbicide, 2) Screening and biological study of bioherbicide, 3) Commercialization and registration of bioherbicide. More than 34 papers and abstracts, 17 oral presentations have been enthusiastically contributed to this workshop. The proceedings are compiled to reflect most of those academic contributions. We only edited the format according to authors' original presentation without involving in the contents and languages. We greatly regret any mistakes and imperfections in the proceedings.

I would like to express my sincere thanks to all of the speakers, administrators of local and central governments and the university, all participants, the organizing committee, and hard working staff members. I would greatly appreciate that Dr. Maurizio Vurro's communication and Dr. Karen Bailey's great work have contributed to the organization of this workshop.

Prof. Sheng QIANG

Host, the XIth International Bioherbicide Group Workshop
Weed Research Laboratory, Nanjing Agricultural University

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PART ONE Status and advancement of bioherbicide

1. The potential of the rust fungus *Puccinia punctiformis* for the biological control of *Cirsium arvense*.
Graeme Bourdot
2. Getting Beneficial Plant Pathogens into the Mainstream. **William L. Bruckart III**
3. Bioherbicide *Phoma macrostoma* for field crops. **Russell K. Hynes**
4. A Review of Bio-herbicide and Application on Weeds Controlling in Soybean Field. **Ai-qun Liu, Wei-ting Liu, et. al**
5. Investigating bitou bush Sudden Death Syndrome: A helping hand from metagenomic. **Louise Morin**
6. Potentially Integrate bioherbicide into organic rice farming. **Sheng Qiang**

PART TWO Screening and biological study of bioherbicide

1. Herbicidal activity of *Aureobasidium pullulans* PA-2 on weeds and its safety on crops.
Liang Cheng, Qing-yun Guo
2. Bioassay on the herbicidal activity of aqueous extract from *Conyza canadensis* L.
Xing-xiang Gao, Mei Li, et. al
3. Synthesis and herbicidal activity of novel 5-substituted derivatives of 3-(1-hydroxyethylidene)pyrrolidine-2,4-dione. **Bao-Feng Han, Chun-Long Yang**
4. The biological characteristics of biocontrol fungus *Curvularia* WYSJZ-B2 in tea garden of Fujian
Yong-cong Hong
5. Isolation, Purification and Structure Identification of Herbicidal Toxins from *Phoma herbarum* .
Ming-shan Ji, Si-jia Li, et. al
6. Growth Inhibition of Aqueous Extract from *Artemisia vulgaris* to Other Plant.
Mei Li, Xing-xiang Gao, et. al
7. Tenuazonic acid from *Alternaria augustivoide* induced oxidative stress and changes in some antioxidant enzyme activities in *Echinochloa crus-galli* leaves.
Zhao Liu, Shu-jun Jiang, et. al
8. Temperature and Dose influence *Phoma macrostoma* efficacy on seedling broadleaf weeds.
Joseph C. Neal
9. Isolation and Identification of four *Sclerotium rolsii* isolates, causing Southern blight on four different plants.
Wei Tang, Hua-qi He, et. al
10. Biological control of the invasive alien weed *Solidago canadensis*: combining an indigenous fungal isolate of *Sclerotium rolsii* SC64 with mechanical control.
Wei Tang, Jing Kuang, et. al
11. Invasive plant drives changes in soil enzyme activities, soil protein concentrations, and EE-BRSP under Cd stress .
Cong-yan Wang, Zhi-cong Dai, et. al
12. Differential gene expression for *Curvularia eragrostidis* pathogenic incidence in crabgrass (*Digitaria sanguinalis*) revealed by cDNA-AFLP analysis. **Jian-shu Wang, Xue-min Wang, et. al**
13. Overexpression of bacterial mercury membrane transport protein merT in *Arabidopsis* leads to enhanced mercury.
Ren Wang, Sheng Xu, et. al
14. Separation, Identification and herbicidal activity of Serratia Ha1. **Juan Yang, Li-hui Zhang, et. al**

15. Transcriptome analysis of a potential bioherbicide agent *Bipolaris eleusines*. **Jian-ping Zhang, Liu-qing Yu**
16. Effects of parasitism by *Cuscuta australis* on growth, reproduction and fluorescence characteristics of *Phytolacca Americana* **Bing Zhou, Xiao-hong Yan, et. al**

PART THREE Commercialization and registration of bioherbicide

1. Current situation of biopesticide registration in China. **Hua-rong Lin**
2. The compound and application of bioherbicide DISANCU and Organic acids. **Yu-xia Lu**
3. Sodium citrate in granular formulations of *Phoma macrostoma* for biocontrol of agricultural weeds. **Hubbard Michelle**
4. Optimal Condition of Conidial Production of *Nimbya alternantherae* and Identification of a Conidiation-deficient Mutant **Ya-feng Nie, Zhi-yi Chen, et. al**
5. Bio-control of *Sporobolus fertilis* with *Nigrospora oryzae*: effect of season and method of inoculation. **David Officer**
6. The Perspectives of Herbicide Registration and Management in China. **Hong-jun Zhang, Quan-le Bian**
7. Combined effect of herbicides ZJ0273 and haloxyfop on weed control, yield components and seed quality in transplanted *Brassica napus*. **Wei-jun Zhou, Hasitha K. Warusawitharana, et. al**
8. Field evaluation of “Junkeluo”, a biological control agent for broadleaf weeds in dry direct-seeded rice. **Chao Zhuang**

Status and advancement of bioherbicide

The potential of the rust fungus *Puccinia punctiformis* for the biological control of *Cirsium arvense*

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Biological control of *Cirsium arvense* (Californian thistle), one of the worst weeds in temperate areas of the world, remains an unrealized goal despite a long history of research. *Cirsium arvense* Neither the classical nor inundative approach has been successful. The host-specific rust fungus *Puccinia punctiformis*, associated with *C. arvense* throughout its global range, is one of many plant pathogens that have been considered. Our inadequate understanding of its complex life-cycle has precluded the rust's evaluation as a biological control agent. In particular, the spore type responsible and the susceptible infection court of the plant for establishing epiphytotics of the systemic disease were uncertain. The hypothesis tested here is that systemic infection is initiated by telia-bearing leaves of *C. arvense*, and that autumnal rosettes are the susceptible infection court. At 11 sites in four countries (3 in the USA, 3 in Russia, 1 in Greece and 4 in New Zealand), ca. 1.0 g of dried and ground telia-bearing *C. arvense* leaf tissue (using naturally-diseased leaves) was applied cumulatively at 3-day intervals to individual autumnal rosettes in populations with no evidence of the disease, giving inoculum loadings of 2, 4, 6, or 8 g per rosette. Averaged over sites and inoculum loading, the treatment effect was highly significant; systemically-diseased shoots emerged the following spring in close proximity to the inoculation points in 5.6 and 28.4% of control and rust-treated rosettes respectively. This suggests that telia-bearing leaf debris applied to autumnal rosettes in the field is a reliable method for inducing systemic disease that, in turn, may bring about the demise of the *C. arvense* population. While these results confirm that epiphytotics of systemic rust disease can be induced in field populations of *C. arvense*, more research is needed to determine how inoculations can be made more efficient and how the systemic disease affects the population dynamics of the weed.

Getting Beneficial Plant Pathogens into the Mainstream

William L. Bruckart, III

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Abstract: The use of plant pathogens for biological control of weeds has yet to become a main-stream component of weed management. There are two traditional approaches, i.e., inoculative (classical) and inundative (bioherbicial), which have resulted in safe and successful weed management on a number of occasions since the late 1970s. There are significant biological and environmental factors, in addition to the challenges of cost and profit, that have limited the general acceptance of this approach to weed management. Altogether, biological control of weeds by plant pathogens remains an untapped opportunity in weed control. Examination of successful programs provides insight into ways that might result in an improved outlook for weed pathogens and biological control. Characteristics of successful programs include: 1) selection of an important (intractable) problem, 2) building consilience and cooperation among stakeholders, scientists, and regulators at all levels, 3) communication and transparency – honest in all things (no false or utterly optimistic promises), particularly concerning the public, 4) a clear objective and intended outcome, agreeable to all, 5) consideration of all management options, i.e., biological control might not be the best approach or it might be promising only in combination with other pest control strategies, and 6) a plan to proceed in development and implementation. There are likely not-for-profit or non-commercial possibilities that should be considered. Examples of non-commercial approaches are given from other pest management programs. These are based on grower cooperatives or by state and federal cooperative ventures, are given. One major advantage is that a program will not be terminated if deemed unprofitable.

Bioherbicide *Phoma macrostoma* for field crops

Karen Bailey and Russell Hynes

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Field trials were conducted to determine the crop tolerance of alfalfa to the bioherbicide *Phoma macrostoma*, the efficacy of the bioherbicide on Canada thistle, *Cirsium arvense*, and wild mustard, *Sinapsis arvensis*, and the effect of bioherbicide application timing on weed control. *Phoma macrostoma* was harmful to alfalfa (cv. Long view) in the year of establishment significantly reducing the number of plants. The plant reductions were dependent on the bioherbicide rate applied. Alfalfa seeding rate did not compensate for the loss of plants when the bioherbicide was applied. Two year-old alfalfa was unaffected by the application of *P. macrostoma*. *Phoma macrostoma* effectively reduced *Canada thistle*, wild mustard, and other broadleaved weed seedlings under field conditions when applied prior to weed emergence. The bioherbicide was most effective on reducing plant number and biomass of wild mustard by 75-100%. The biomass of other broadleaved weed seedlings was reduced by 75%. The control of *Canada thistle* was more variable with reductions in weed cover ranging from 52-80% and the reductions in biomass from 35-69%. The effect of bioherbicide application time on weed efficacy was tested in a no-crop situation using tillage just prior to each application time thus creating a situation whereby the bioherbicide was applied prior to weed emergence. Early to mid-season applications of the bioherbicide reduced the number of plants of *Canada thistle* (48-78% reduction) and dandelion, *Taraxacum officinale*, (81-99% reduction). Other broadleaved weeds were reduced by 96% but only with the early application. Wild mustard was reduced by 96-100% at all application times. The bioherbicide was also able to reduce biomass of all weeds with the reductions exceeding 80% at the early and mid-season applications. Late applications were less effective in reducing weed numbers and biomass.

A Review of Bio-herbicide and Application on Weeds Controlling in Soybean Field

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Abstract: Weed is one of the major causes in crop yield reduction. The massive use of herbicide over the year has brought various environmental problems. With the implementation and in-depth of the strategy for sustainable development of agriculture, development and application of bio-herbicide has become a focus on weed control. Definition and classifications of bio-herbicide and its weeding principles of enzyme inhibitors were outlined in this paper, based on which the presence of bio-herbicide application, including natural plants abstracts, fungus, allelopathins and cover crops in soybean field in China and abroad were discussed and analyzed. The prospects for bio-herbicide application in soybean field under novel weeds control conception in no-till system were previewed.

Keywords: bio-herbicide, classification, no-till, application

Investigating bitou bush Sudden Death Syndrome: A helping hand from metagenomic

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Finding an effective biocontrol solution for bitou bush (*Chrysanthemoides monilifera* ssp. *rotundata*) has been on the research agenda for the last two decades in Australia. Bitou bush Sudden Death Syndrome (SDS), which refers to the rapid death of bitou bush patches observed in northern NSW, was investigated to assess if it could be exploited for biocontrol. Plant and soil samples were collected from sites affected or not with SDS. Some of the samples were processed in the laboratory to isolate potential pathogens. Concurrently, a metagenomic approach was undertaken to molecularly characterise and compare the fungal community present within bitou bush plants affected or not with SDS and in soil sampled from their root zone. The fungal community within native woody plant species growing in areas where bitou bush was affected by SDS was also characterised. The metagenomic approach unveiled that several *Diaporthe* (= *Phomopsis*) spp. were present in high abundance within bitou bush plants with unambiguous SDS symptoms. *Diaporthe* spp. were also isolated with the culturing method. In contrast, healthy bitou bush plants growing nearby diseased areas or at South Coast sites where SDS has never been reported were found to be primarily colonised internally by a *Penicillium* sp. These fungi were not found in any of the native plant species sampled. Experiments demonstrated that the *Diaporthe* spp. enter bitou bush plants via flowers and colonise stems as endophytes without causing visible symptoms. More research is required to determine if a certain level of internal colonisation of plants by *Diaporthe* spp. is required before SDS symptoms developed and/or if external environmental factors are necessary for symptoms to be triggered. We also hypothesise that the *Penicillium* sp. found in healthy bitou bush plants may provide some kind of protection to plants against invasion by potentially pathogenic fungi, but empirical evidence is required.

Potentially Integrate bioherbicide into organic rice farming

Sheng Qiang

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Abstract: Organic rice farming still relies on manual weed control costing much because of lack in effective weed control technique except duck-rice farming system in China. The development of a bioherbicide will become one of the most important technique options. The strain SC64 (*Sclerotium rolfsii*) has been studied for a potential bioherbicide in China. The strain SC64 can be mass-produced using rice and wheat straw as solid substrate. The culture with the fungus's mycelia was applied to paddy field at 1-3 weeks after direct-seeded or transplanted. Most of broad-leaved weeds and some monocotyledon weeds were infected and died after treatment for 3-5 days. The results showed that this application caused 60-85% mortality rate and 65-90% fresh weight reduction. Combined with *Helminthosporium gramineum* Rabenh. f.sp. *echinochloae*, the strain SC64 could effectively control all of main weeds occurred in paddy fields. In addition, the strain SC64 may be integrated into ecological control system and play a main role in weed control.

Keywords: *Sclerotium rolfsii*, mycoherbicide, organic rice farming, *Helminthosporium gramineum* Rabenh. f.sp. *echinochloae*.

Screening and biological study of bioherbicide

Herbicidal activity of *Aureobasidium pullulans* PA-2 on weeds and its safety on crops

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Abstract: An isolate of indigenous fungus strain PA-2 was isolated from diseased poplar leaves in Ping'an, Qinghai Province and the metabolites of the strain showed high herbicidal activity to target weeds . According to its culture characters and 16S rDNA sequence, the strain was identified as *Aureobasidium pullulans*. The objectives were to determine mortality and fresh-weight reductions of various weeds and to evaluate the host range of the fungus as influenced by the fermentation filtrate. When *A. pullulans* was applied to *Galium aparine* L. var. *tenerum* (Gren. et Godr.) Reichb., *Chenopodium album* L., *Malva crispa* L., *Polygonum lapathifolium* L., *Avena fatua* L. in the post tests, 87.3%, 78.5%, 82.3%, 62.1%, 80.3% reductions in fresh weight occurred for weeds seedlings, respectively. Responses of inoculated weeds to *A. pullulans* ranged from light symptoms to 100% mortality. *Galium aparine* L. var. *tenerum* (Gren. et Godr.) Reichb. and *Malva crispa* L. exhibited significant reductions in fresh weight. No significant fresh-weight reductions were observed for *Triticum aestivum* L., *Vicia faba* L., *Hordeum vulgare* L. plants when compared to the control plants. In addition to slight spots on inoculated leaves, *Brassica napus* L. and *Pisum sativum* L. that exhibited these symptoms recovered within 7-15 days, often with no significant reductions in fresh weight. These results indicate that *A. pullulans* could be a potential microbial herbicide for control of target weeds in crops such as spring wheat, broad bean and highland barley.

Key words: *Aureobasidium pullulans*, weeds, biological control, crop safety

Bioassay on the herbicidal activity of aqueous extract from *Conyza canadensis* L.

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Abstract: *Conyza canadensis* L. is a major weed in China, often used as herbal medicine to inhibit bacterial growth and prevent diarrhoea in children. It also inhibits the growth of common mold. Bioassay on the herbicidal activity of aqueous extract from *Conyza Canadensis* were tested with petri dish and greenhouse cultivated method. The results showed that it strongly inhibited the germination and seedling growth of *Digitaria sanguinalis*, *Echinochloa crusgalli* and *Amaranthus retroflexus*, and the inhibitory effects increased with the increasing concentration of extracts. *Amaranthus retroflexus* was more sensitive to it than the others, and its seed germination and seed germination speed were completely inhibited at 0.05 and 0.1 g/mL concentration. At 0.05 g/mL concentration, the germination rate of *Digitaria sanguinalis*, *Echinochloa crusgalli* and *Amaranthus retroflexus* were 35.23%, 50.00%, and 0 respectively, and the germination speed were 23.52%, 50.25% and 0. The *Digitaria sanguinalis* and *Amaranthus retroflexus* roots growth were completely inhibited at 0.1 g/mL concentration of aqueous extracts, and the inhibition rate on *Echinochloa crusgalli* was 80.70%. At 0.0125 g/mL concentration, the root length inhibition of *Digitaria sanguinalis*, *Echinochloa crusgalli* and *Amaranthus retroflexus* were 54.05, 43.86 and 25.21%. The *Amaranthus retroflexus* hypocotyls were inhibited at 0.1, 0.05 g/mL concentration, but the inhibition rates were very lower, only 7.64% and 6.94% at 0.025, 0.0125 g/mL. At 0.025 g/mL concentration, the hypocotyls length inhibition of *Digitaria sanguinalis*, *Echinochloa crusgalli* and *Amaranthus retroflexus* were 57.14, 59.57 and 6.94%. To one seed, the germination speed was accord with the germination rate, but sensitively more, and the roots were inhibited more strongly than seedling height at same concentration.

Keywords: *Conyza Canadensis*, herbicidal activity, seed germination, seedling growth

Synthesis and herbicidal activity of novel 5-substituted derivatives of 3-(1-hydroxyethylidene) pyrrolidine-2, 4-dione

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Abstract: The natural product tenuazonic acid was used as leading compound, three series of twenty-five novel tetramic acid derivatives of 3-(1-hydroxyethylidene) pyrrolidine-2,4-diones were designed and synthesized. These derivatives included alkyl, substituted benzyl, cinnamyl, hypnone group, sulphide group, carboxylic acid ester moieties at 5-position. The structures of all title compounds were characterized by IR, ¹H NMR, MS, and Elemental analysis. The bioassay showed that most of title compounds exhibited noticeable herbicidal activities against *Brassica campestris* and *Echinochloa crusgalli* at the concentration of 100µg/mL. Compounds 6f and 6j exhibited better inhibitory activities than the leading compound tenuazonic acid against the stalk of *E. crusgalli* with inhibitory rates of 62.6% and 66.9%, respectively.

Key words: tenuazonic acid, pyrrolidine-2,4-dione, synthesis, herbicidal activity

Isolation, Purification and Structure Identification of Herbicidal

Toxins from *Phoma herbarum*

Ming-shan Ji, Li Si-jia Li, Zu-min Gu, He Zhu

(College of Plant Protection Shenyang Agriculture University, Shenyang)

Abstract: Based on existing research and guided by bioassay, *Phoma herbarum* toxins were isolated and purified by TLC, HSCCC, HPLC, etc. 1 pure active toxin 56d was gained from dayflower *Phoma herbarum* toxins. A comprehensive analysis of IR, MS and NMR shows that the chemical formula is $C_9H_8N_2O_6$, and the structural formula is 2-methyl-3, 5-dinitro benzoic acid methyl ester.

Key words: dayflower, *Phoma herbarum*, herbicidal activity, isolation and purification, structure identification

Growth Inhibition of Aqueous Extract from *Artemisia*

vulgaris to Other Plant

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Abstract: Interaction (growth inhibition or stimulation) among plants widely exists in nature and plays a vital role in crop production systems and pests (insects, nematodes, pathogene, weeds) management. *Artemisia vulgaris*, a herbaceous plant usually grow on the hillside or wasteland, was found could inhibit the germination, growth and development of other plants growing beside it. This study aimed to determine the effects of aqueous extract of *Artemisia vulgaris* on the seed germination and seedling growth of plants *Triticum aestivum* (wheat), *Sorghum vulgare* (sorghum), *Cucumis sativus* (cucumber), *Brassica campestris* (rape) and *Raphanus sativus* (radish) in Petri dishes in Laboratory. The results showed that the aqueous extract strongly inhibited the germination, germination speed and seedling growth of tested crops. Rape and radish were more sensitive than other species. The synthesis effects of rape, radish, cucumber, wheat, sorghum were 100%, 87.26%, 69.88%, 61.11% and 52.77% at the concentration of 0.0125 g/mL⁻¹. The MDA contents were also tested after treatment. The MDA contents of cucumber and radish increased largely after being treated. The results indicated that *A. vulgaris* could inhibit the growth of other plants and the inhibition effects, in some degree, were due to it could increase MDA and break cell membrane.

Key words: growth inhibition, aqueous extracts, *Artemisia vulgaris* L., seed germination, seedling growth

Tenuazonic acid from *Alternaria augustivoide* induced oxidative stress and changes in some antioxidant enzyme activities in *Echinochloa crus-galli* leaves

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Abstract: The aim of the study was to determine whether phytotoxicity of TeA against *Echinochloa crus-galli* leaves is correlative with oxidative stress caused by generation of reactive oxygen and the changes of antioxidant enzymes activity. The changes of malondialdehyde (MDA) content, hydrogen peroxide(H_2O_2), and activities of superoxide dimutase(SOD), glutathione reductase(GR) and catalase(CAT) were studied by leaf segment method *in vitro*. After the treatment of 500 $\mu\text{mol/L}$ TeA, the content of MDA and H_2O_2 increased 247.86% and 67%, respectively. The result indicated that the accumulation of MDA and H_2O_2 in *Echinochloa crus-galli* leaves was due to the reactive oxygen burst induced by TeA. TeA induced a significant increase in activities of SOD, GR and CAT. At 500 $\mu\text{mol/L}$ TeA, activities of SOD, GR and CAT increase more 1 fold than that of control. TeA induces oxidative stress in *Echinochloa crus-galli* leaves through generation reactive oxygen and upregulation of activities of SOD, GR and CAT.

Key words: *Echinochloa crus-galli*, Tenuazonic acid, Oxidative stress, Reactive oxygen, Antioxidant enzymes

Temperature and Dose influence *Phoma macrostoma* efficacy on seedling broadleaf weeds

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Abstract : *Phoma macrostoma*, a potential biocontrol agent for turfgrass weeds, was isolated from *Cirsium arvense* plants in Canada and is being tested in other regions of North America for control of broadleaf weeds in turf. This research was conducted to investigate the effects of varying temperature conditions on *Phoma macrostoma* control of seedling broadleaf weeds. Experiments were conducted in growth chambers to compare the efficacy of three doses of *Phoma macrostoma* on two species, *Senecio vulgaris* and *Lamium amplexicaule* grown in 4 temperature regimes – 15/20, 20/25, 25/30 and 30/35°C (dark / light period) temperatures. These data suggest that high temperatures common in the southeastern United States should not be an impediment to activity of *Phoma macrostoma* efficacy, and may actually improve the control of some broadleaf weed species.

Introduction

Turfgrass and landscape management are multi-billion dollar industries in the United States. Traditional pest management programs in these sites rely heavily upon synthetic chemistry for insect, disease and weed management. However, significant concerns exist over exposure to lawn care and landscape maintenance chemicals applied in urban watersheds. Interest in alternative and organic pest management options for urban lawns and landscapes continues to grow, with retail sales of organic lawn and garden products estimated to be \$460 million in 2008, a gain of 12% over 2007. Yet few bio-based alternatives for weed control are available.

Phoma macrostoma is a fungus isolated from Canada thistle (*Cirsium arvense*). Experiments conducted in Canada have demonstrated efficacy on common broadleaf weeds of turfgrass and landscape plantings (Bailey et al. 2009) with limited mobility and persistence in soil (Bailey and Derby 2005; Zhou et al. 2004). The organism is currently being tested in other regions of North America including North Carolina where the weather is much warmer compared to the region where this organism was collected.

Experiments were conducted to determine if higher temperature conditions common to the southeastern United States would affect *Phoma macrostoma* efficacy on common broadleaf weeds.

Materials and Methods

The experiment was conducted at the Southeastern Plant Environment Laboratory at North Carolina State University in Raleigh, NC. On July 6th, 2011 standard 4.5” pots were filled with a peat-lite + sand (1:1v/v) substrate. Pots were irrigated to settle the potting substrate. *Senecio vulgaris* (common groundsel) and *Lamium amplexicaule* (henbit) were then surface seeded to achieve approximately 10 to 20 seeds per pot. These species were selected for study based on preliminary data demonstrating they were susceptible, rapid and uniform germination, and upright growth habit necessary for the small spaces available in the growth chambers. Pots were placed in growth chamber at 20/15°C day/night for 19 days until first true leaves appeared. Treatments were applied to seedling weeds on July 25, 2011. The experiment was repeated on October 11, 2011. *Phoma macrostoma* treatments were applied using a hand-held shaker jar. *Phoma macrostoma* doses among this and other experiments were standardized based upon concentration of the fungal toxin, macrocadin, in the inoculum. In this experiment, *Phoma macrostoma* inoculum containing about 198 macrocadin units per gram was applied at dose of 42g/m², 84g/m² or 168g/m². Plots were arranged in a randomized complete block design with 6 replications (a single pot of each weed species per treatment per replicate). After treatment, pots were placed in chambers set at 20/15°C, 25/20°C, 30/25°C or 35/30°C day/night temperature and hand watered. Pots were hand watered daily and once a week with a complete nutrient solution at 100 ppm nitrogen. In the 2nd run of the experiment, plants were fertilized 3x per week with nutrient solution.

Table 1. Doses and temperature regimes

#	Treatment	Dose	temperature
1	Non-treated	na	20/15°C day/night
2	<i>Phoma macrostoma</i>	42g/m ²	20/15°C day/night
3	<i>Phoma macrostoma</i>	84g/m ²	20/15°C day/night
4	<i>Phoma macrostoma</i>	168g/m ²	20/15°C day/night
5	Non-treated	na	25/20°C day/night
6	<i>Phoma macrostoma</i>	42g/m ²	25/20°C day/night
7	<i>Phoma macrostoma</i>	84g/m ²	25/20°C day/night
8	<i>Phoma macrostoma</i>	168g/m ²	25/20°C day/night
9	Non-treated	na	30/25°C day/night
10	<i>Phoma macrostoma</i>	42g/m ²	30/25°C day/night
11	<i>Phoma macrostoma</i>	84g/m ²	30/25°C day/night

12	<i>Phoma macrostoma</i>	168g/m ²	30/25°C day/night
13	Non-treated	na	35/30°C day/night
14	<i>Phoma macrostoma</i>	42g/m ²	35/30°C day/night
15	<i>Phoma macrostoma</i>	84g/m ²	35/30°C day/night
16	<i>Phoma macrostoma</i>	168g/m ²	35/30°C day/night

Plant counts were recorded at treatment. Chlorosis was visually evaluated on a 0 to 3 scale where 0 = no chlorosis and 3 = severe chlorosis. Chlorosis evaluations were made 1, 2, 4 and 5 weeks after treatment (WAT). Phytotoxicity/control was visually evaluated on a 0 to 10 scale where 0 = no control (equal to the non-treated) and 10 = 100% control 4, 5 and 6 WAT. Fresh weights and plant counts were recorded 6 WAT.

Results

In the midst of the first run of the experiment, irrigation in one of the growth chambers failed; therefore data are not available for the 30/25°C day/night treatment for that study. Other growth chambers were unaffected. Both species expressed symptoms of chlorosis and bleached foliage. Severity of chlorosis increased for four weeks on both species and declined there after. Chlorosis on henbit was greater at 20/15 temperature compared to higher temperatures (Figure 1). However, despite the presence of significant chlorosis, there was essentially no reduction in henbit growth or above-ground biomass in the first run of the study (Figure 2). However, in the second run of the experiment, henbit fresh weight was reduced in all temperature regimes by increasing doses of *Phoma macrostoma* (Figure 3).

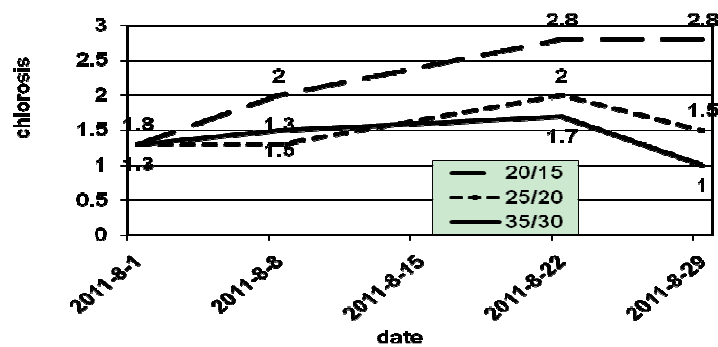


Figure 1. Chlorosis ratings (0 = green tissues; 3 = most foliage chlorotic or bleached) on *Lamium amplexicaule* treated with 168 g/m² *Phoma macrostoma*. First run of the experiment.

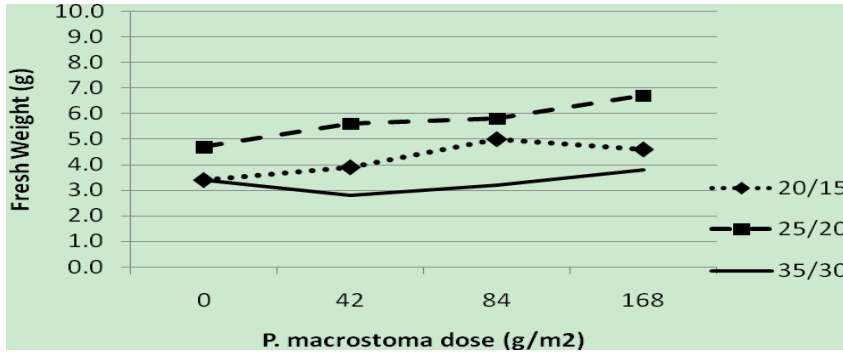


Figure 2. *Phoma macrostoma* dose and temperatures effects on *Lamium amplexicaule* fresh weight (g); 6 weeks after treatment. First run of the experiment.

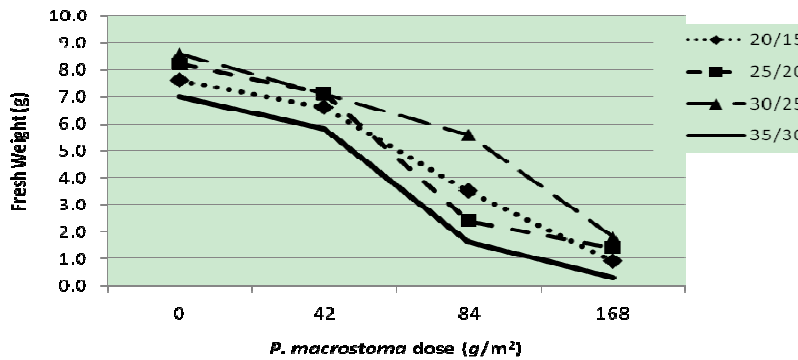


Figure 3. *Phoma macrostoma* dose and temperatures effects on *Lamium amplexicaule* fresh weight (g); 6 weeks after treatment. Second run of the experiment.

For common groundsel, significant differences were observed between runs of the experiment. In the first run of the experiment, common groundsel was controlled greater than 90% by four weeks after treatment with 168g/m² at 30/35 C with complete (100%) control by five weeks (Figure 4). Higher temperature regimes resulted in greater control. In this run of the experiment, only the highest application dose of 168 g/m² controlled common groundsel.

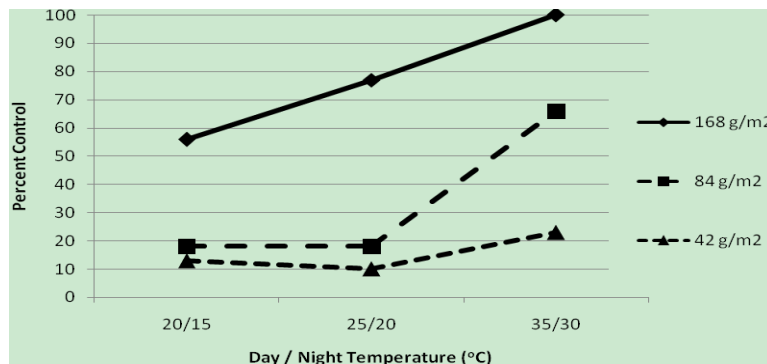


Figure 4. Percent control of common groundsel 5 weeks after treatment; 1st run of the experiment

Common groundsel control was greater in the 2nd run of the experiment. Essentially complete control was observed with all three doses in the two highest temperature regimes

but at lower temperatures, only the highest dose provided greater than 90% control (Figure 5). Data for above-ground fresh weight and plant counts 6 WAT supported the results of visual evaluations.

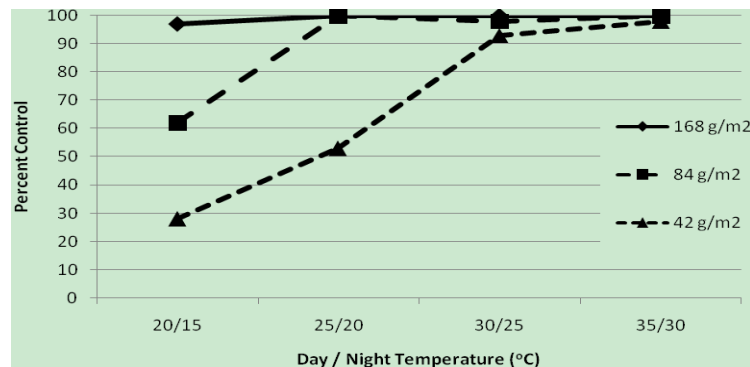


Figure 5. Percent control of common groundsel 5 weeks after treatment; 2nd run of the experiment

Conclusions

These data demonstrate that the efficacy of *Phoma macrostoma* as a biological control agent may be influenced by temperature conditions. The results support the conclusion that higher temperature conditions should not be detrimental to its activity on susceptible weeds. In fact, these data suggest that plant stress induced by higher temperatures may actually improve control of some species such as common groundsel that exhibit greater growth rates at lower temperatures. These data also suggest that under lower temperature regimes, application dose may need to be increased to maintain commercially acceptable control. Differences in results between experiments may be associated with different fertility levels. Plants were fertilized with balanced water soluble nutrient solution once a week in the first run of the experiment, but due to miscommunication with staff, in the 2nd run of the experiment plants received fertilization three times per week. Greater fertilization increased plant vigor, with non-treated *Lamium* plants producing about twice the biomass in the second run compared to the first run of the study (Figures 2 and 3). It is plausible that greater fertilization and plant vigor enhanced control with *Phoma. macrostoma*; a hypothesis supported by recent research (Bailey et al. 2013). Growth chamber experiments may not be representative of results in field conditions. In outdoor, field conditions, higher temperature climates are often associated with other environmental conditions such as high light, water deficits, and soil microflora that may alter plant and pathogen responses. These results should be confirmed by further testing.

Isolation and Identification of four *Sclerotium rolsii* isolates, causing Southern blight on four different plants

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Abstract: *Sclerotium rolsii* Sacc., a versatile soil borne pathogen, commonly occurs in the tropics, subtropics and other warm temperature regions, especially at high humidity and warm temperatures. It may cause a variety of diseases, named as Southern blight, crown rot and white mold. The isolate SC64 of *Sclerotium rolsii*, isolated from diseased *S. canadensis* plants with destructive stem rot symptoms, has been studied as a potential bioherbicide for this weed and other broad leaf weeds and some sedges. In order to screen for more candidate isolates, four pathogen isolates CSC, DSC, HSC and QSC, estimated as *Sclerotium rolsii* based on morphological characters, was isolated from damaged plants with a destructive stem rot disease symptom sporadically occurring on Bryophyta; *Chlorophytum comosum*; *Arachis hypogaea* and *Begonia evansiana* in Nanjing and Jiangdu city, Jiangsu province of China. ITS sequence (687, 681, 685 and 665 bp) analysis through initial amplifying from the pathogen with the universal primers ITS1 and ITS4, and comparing with those of related species acquired from GenBank database confirmed the morphological identification of these strains. Isolates CSC, DSC, HSC and QSC had similar biological characteristics as *S. rolsii* SC64, except isolate HSC had relatively bigger sclerotia and colony diameter under same temperature conditions.

Key words: *Sclerotium rolsii*, rDNA-ITS sequence, phylogenetic tree, bioherbicide

Biological control of the invasive alien weed *Solidago canadensis*: combining an indigenous fungal isolate of *Sclerotium rolfsii* SC64 with mechanical control

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Abstract: *Solidago canadensis* L. is a major invasive weed that is highly tolerant to disturbances and difficult to control in China. In order to develop a rapid non-chemical control strategy for this weed in heterogeneous environments, we investigated different treatments including mechanical control (cutting and hoeing) and inoculation with an indigenous pathogen, *Sclerotium rolfsii* SC64, which was isolated from *S. canadensis* and applied by means of a solid formulation. Greenhouse and field trials were conducted to test how the control regimes (i.e. individual treatment methods, combination of these methods and different treatment timing) influences the control efficiency. The fungal isolate *S. rolfsii* SC64 caused 70% plant mortality and fresh weight reduction of *S. canadensis* under 150 cm growth stage, and efficacy increased to 80% when the above-ground material was removed. However, the use of cutting, hoeing or treating with *S. rolfsii* SC64, on its own, did not provide sufficient control of *S. canadensis*. Cutting treatments performed in July and September only eliminated sexual reproduction of *S. canadensis*. Combinations of cutting, hoeing and treating with isolate SC64 during the growing season in May, July and September was able to kill more than 90% of the ramets. This combination of methods not only eliminated sexual reproduction of *S. canadensis*, but also destroyed its underground stems and prevented its regrowth. Therefore, this integrated approach may provide an optima control strategy for *S. canadensis*.

Key words: Canadian goldenrod, mycoherbicides, integrated weed management

Invasive plant drives changes in soil enzyme activities, soil protein concentrations, and EE-BRSP under Cd stress

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Abstract: Anthropogenic activities have triggered the unprecedented environmental changes in recent decades, such as biologic invasion and heavy metal pollution. At present, numerous studies founded the main reason for the successful invasion of invasive plants was that they can accelerate the succession of soil microbial communities in rhizosphere and then strengthen microbial functions to facilitate their further invasion process. Thus, there is considerable interest in understanding the relative competitive ability of invasive plant and its effects on the activities of soil microbial community under heavy metal pollution (HMP) in order to insight into the mechanism of the successful invasion of invasive plants. In this study, the invasive plant (*Wedelia trilobata*), one of the most worst invasive species listed by International Union for Conservation of Nature and Natural Resources (IUCN), and its allied native plant (*W. prostrate*) were chosen and treated with simulated Cd (0, 25, 50, 100, and 200 mg kg⁻¹ of soil) and without or with benomyl (B, 100 mg kg⁻¹ of soil). Results showed that Cd stress significant reduces leaf biomass and stem biomass of *W. trilobata*, while Cd stress did not pose significant effects on root biomass of *W. trilobata*. *W. prostrate* had all died off after three-month growth with Cd stress unfortunately. This means that the relative competitive ability of *W. trilobata* were obviously higher than that of its allied native plant under the stress condition mediated by HMP. Cd can significant affect the activities of urease and alkaline phosphatase. *W. trilobata* increased soil pH values and protein concentrations, while it decreased easily extractable Bradford-reactive soil protein (EE-BRSP) and alkaline phosphatase activities. Thus, this study may be found two possible ways for invasion mechanism of invasive plant under HMP through affect the physicochemical property of soil and/or the activities of soil microbial community: (1) invasive plant can decrease the solubility of heavy metals and then accelerate its invasiveness under HMP through increased soil pH values, and (2) invasive plant can increase its invasiveness through increase the utilized amount of nutrient, such as nitrogen and phosphor. According to the results of this study, HMP may enhance the invasiveness of invasive plants and then facilitate their invasion process.

Key words: Cd, Heavy metal pollution, Plant invasion, Soil enzyme

Differential gene expression for *Curvularia eragrostidis* pathogenic incidence in crabgrass (*Digitaria sanguinalis*) revealed by cDNA-AFLP analysis

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Abstract: Gene expression profiles of *Digitaria sanguinalis* infected by *Curvularia eragrostidis* strain QZ-2000 at two concentrations of conidia and two dew durations were analyzed by cDNA amplified fragment length polymorphisms (cDNA-AFLP). Inoculum strengths was more determinant of gene expression than dew duration. A total of 256 primer combinations were used for selective amplification and 1214 transcript-derived fragments (TDFs) were selected for their differential expression. Of these, 518 up-regulated differentially expressed TDFs were identified. A total of 35 TDFs were successfully cloned and sequenced of which 25 were homologous to genes of known function according to the GenBank database. Only 6 genes were up-regulated in *Curvularia eragrostidis*-inoculated *D. sanguinalis*, with functions involved in signal transduction, energy metabolism, cell growth and development, stress responses, abscisic acid biosynthesis and response. The appears that a few pathways that may be important parts of the pathogenic strategy of *C. eragrostidis* strain QZ-2000 on *D. sanguinalis*. Our study provides the fundamentals to further study the pathogenic mechanism and and screen for optimal *C. eragrostidis* strains as potential mycoherbicide to control *D. sanguinalis*.

Key words: *Digitaria sanguinalis*, *Curvularia eragrostidis*, Gene expression, cDNA-AFLP

Over expression of bacterial mercury membrane transport protein merT in Arabidopsis leads to enhanced mercury tolerance

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Abstract: This study investigated molecular mechanism of over expressing mercury transporter (merT) from *Pseudomonas alcaligenes* in *Arabidopsis*. The ability of the transgenic *Arabidopsis*'s ability to remediate mercury was also investigated. *merT* was ectopically expressed in *Arabidopsis thaliana*. Semi-quantitative RT-PCR was used to analyze expression of antioxidant enzyme genes. Hg concentration was analyzed by an inductively coupled plasma optical emission spectrometer. Histochemical detection of H₂O₂ and O₂^{•-} were conducted by 3,3-diaminobenzidine and nitroblue tetrazolium-staining methods. Compared with wild-type plants, overexpressing *merT* in *Arabidopsis* enhanced the tolerance to Hg and increased the Hg concentration in the shoots of the transgenic plants. Further results showed that enhanced total activities or corresponding transcripts of antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX) were observed in transgenic *Arabidopsis* under Hg stress. These results were confirmed by the alleviation of oxidative damage, as indicated by the decrease of thiobarbituric acid reactive substances (TBARS) contents and reactive oxygen species (ROS) accumulation. *merT* increased Hg tolerance in transgenic *Arabidopsis*, and decreased production of Hg-induced ROS, thereby protecting plants from oxidative damage.

Key words: *Arabidopsis*, Mercury membrane transport protein, Mercury tolerance, Oxidative damage, Reactive oxygen species

Separation, Identification and herbicidal activity of *Serratia* Ha1

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Long-term and large dosage application of chemical herbicides has led to weed resistance, pollution and residue, and so to develop safety, pollution-free and environment-friendly herbicide is becoming imperative. Micro-herbicide has the advantages as novel structures, broad-spectrum, multi-target site of action and easier to save and will provide the researchers a bright prospect.

In recent years, marine microorganisms have become a new direction for novel drug research in domestic and overseas. Marine environment of high pressure, high salt, oligotrophic, hypoxia, less light and cold results in significant different metabolic pathways of microorganisms and probably provides a large quantity of agricultural compounds with novel structures and high bioactivities.

A bacterial strain named Ha1 with high herbicidal activity was isolated from marine microorganisms in this study. Light microscopy showed strain Ha1 was gram-negative, straight, obtuse, rod shaped, growth outer flagellaum, diameter 0.5~0.8 μm and long 0.9~2.0 μm . The physiological and biochemical tests showed Ha1 was indole test-positive, catalase enzyme reaction strongly positive contacts, could produce red pigment at 28 $^{\circ}\text{C}$, but couldn't produce red pigment at 37 $^{\circ}\text{C}$, and regained the ability of producing red pigment at 28 $^{\circ}\text{C}$. 16S rDNA gene sequences of strain Ha1 were obtained (comprising 1598 nucleotides) and submitted to GenBank. On the basis of the traditional Identification System and 16S rDNA gene sequencing methods, Ha1 was identified as *Serratia*. The gene sequence was deposited in the GenBank database under the accession number KC935341.

In this study, to assess the herbicidal potential of metabolites produced by Ha1 strain, we selected two kinds of methods: inhibition of *Digitaria sanguinalis* and seed germination test. The results showed that the IC₅₀ of the crude extracts to *D. sanguinalis* roots and shoots were 3.332 mg·mL⁻¹ and 2.828mg·mL⁻¹, respectively. And the IC₅₀ of inhibition of *D. sanguinalis* was 1.158 mg·mL⁻¹. It can be concluded that the metabolites of Ha1 strain had the potential to be developed as a microbe-based herbicide.

Transcriptome analysis of a potential bioherbicide agent *Bipolaris eleusines*

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Abstract: *Bipolaris eleusines* is a pathogen isolated from a naturally infected barnyardgrass plant and has been considered as a potential bioherbicide candidate for control of barnyardgrass (*Echinochloa crus-galli*). A crude extract of *B. eleusines* containing ophiobolin A as the principal phytotoxin also showed high efficacy against several weeds including barnyardgrass, monochoria (*Monochoria vaginalis*), small-flower umbrella sedge (*Cyperus difformis*), false loosestrife (*Ludwigia prostrate*), and Indian rotala (*Rotala indica*) in paddy rice fields. However, there are many challenges in commercialization of this fungus and its phytotoxin, such as the lower active phytotoxins. To learn the genetic background of this fungus and phytotoxin biosynthesis will be helpful to directly and definitely improve their yields of these active phytotoxins. Therefore, we studied the transcriptome of *B. eleusines* through generating expressed sequence tags (ESTs) on a 454 sequencing platform and cloned several phytotoxin biosynthesis genes. A total of 26,555,560 high-quality ESTs with an average read length of 559 bp were generated. These ESTs were assembled into 32,100 unigenes. 97.9% of these unigenes (31432) were annotated and showed different levels of homology with other organisms using BLAST searches (E-value $\leq 1e-5$) against the SwissProt, KEGG, Nr, COG and GO databases. Seventy-six unigenes were also analyzed be similar to that of transcription factor genes. Sixty unigenes (encoding 17 enzymes) were found to be involved in terpenoid biosynthesis. Following these ESTs involved in terpenoid biosynthesis, *BeHMGR*, *BeIPPI*, *BeFPPS* and *BeGGPPS* of four key enzymes involved in ophiobolin A biosynthesis pathway were isolated from ophiobolin A-producing fungus stratin *B. eleusines*. These information might be helpful not only for theoretical research of further defining the mechanism of ophiobolin A biosynthesis, but also supply more clues of target enzymes to modulate the production of ophiobolin A for practical application.

Key words: Expressed sequence tags (ESTs), Phytotoxin, Ophiobolin, Biological control, Barnyardgrass

Effects of parasitism by *Cuscuta australis* on growth, reproduction and fluorescence characteristics of *Phytolacca americana*

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Abstract: *Phytolacca americana*, an invasive plant widely distributed in China, has caused serious threaten to the biodiversity of local systems. Parasitic plant *Cuscuta australis* often parasites *P. americana* in the field, to investigate its potential for controlling *P. americana*, effects of parasitism by *C. australis* on growth, reproduction and fluorescence characteristics of *P. americana* were studied by greenhouse pot bioassay and fluorimetry. The results showed that, leaf number, plant height, root diameter, leaf biomass, stem biomass and root biomass of *P. americana* parasited by *C. australis* were significantly inhibited after 30 days, with the values of 15.53, 25.80 cm, 0.64 cm, 1.13 g, 0.64 g and 0.24 g, only 43.38%, 70.15%, 43.54%, 49.13%, 53.78% and 9.72% as the control respectively. It was the same with inflorescence number, length, mean flowers, maturing rate and flavor biomass, with the values of 0.73, 1.31 cm, 7.21, 0 and 0.01 g, only 17.38%, 18.17%, 26.72%, 0% and 0.66%. Compared with the control, parameters as Fv/Fm, ETR, Φ PSII, qL, NPQ in parasitic treatment were significantly lower, it was the same with Chla and Chlb contents, but there was no significant effects on Chla/Chlb ratio. It could be concluded that parasitism by *C. australis* significantly inhibited the growth, reproduction and photosynthesis of *P. americana*, which means the potential of *C. australis* developed as the agent for controlling *P. americana*.

Keywords: *Cuscuta australis*, *Phytolacca Americana*, parasitism, growth and reproduction, fluorescence characteristics

Commercialization and registration of bioherbicide

Current Situation of Biopesticide Registration in China

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Abstract: Without effective crop protection, losses in agricultural production owing to pests, weeds and diseases have been estimated to vary from 10 to 50%. Since pesticides are intentionally released into the environment to protect crops from harmful insects, weeds and pathogenic microorganisms, it is common practice to evaluate their product chemistry, toxicity, environment, residue, efficacy and other necessary information during the registration procedure before placing pesticides on the market. Biopesticides, directly making use of living organisms or biological active substances as a pesticide, as well as synthetic compounds with the same structure as natural pesticides, have different data requirement from chemical pesticides. Since 2008, more attention was paid by government on the management and application of biopesticides. Here, compared to the chemical pesticides, five categories of biopesticides, including microbial pesticides, biochemical pesticides, botanical pesticide, and natural enemy, were shown for their registration requirements in China. Some difficulties in detecting biopesticides were mentioned in this paper. And in future, some requirements are discussed to reduce in order to support the development of biopesticides.

Key words: Biopesticide, registration, data requirement, microbial pesticide, biochemical pesticide, botanical pesticide

A mixture of organic acid with Mycoherbicide DISANCU enhances weed control effect

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ABSTRACT : The previous studies showed DISANCU (*Curvularia* sp., QZ2000) had a good weed control effect only when combining with low-rate herbicide. Organic acids could replace herbicide for a synergistic agent so that, in that case, it could make DISANCU application be entirely organic. A series of the compatibility and cup, pot, plot and field trial experiments were conducted combining of oxalic acid and acetic acid with DISANCU. The results showed that as the concentration of oxalic acid increased from 1000ppm to 8000ppm in the mixture with spores, the stem control effect reached more than 90% and 70% from less than 80% and 70% respectively in the cup and pot experiments. The mixture of acetic acid ranging from 1000ppm to 8000ppm with spores demonstrated increasing stem control effect from less than 72% and 60% to more than 90% and 60% respectively. The plot and field experimental results showed that the stem control effects were less than 60% and 71% respectively when the concentration of oxalic acid ranged from 1000ppm to 4000ppm after 4 weeks. But the mixture of 8000ppm oxalic acid with spores demonstrated more than 70% and 80% the stem control effect respectively. As the concentration of acetic acid increased from 1000ppm to 8000ppm, DISANCU demonstrated more than 65% and 70% the stem control effect respectively. In conclusion, the addition of organic acid to DISANCU can enhance weed control efficacy of the mycoherbicide.

Key words: DISANCU, organic acid, *C. eragrostidis*

Sodium citrate in granular formulations of *Phoma macrostoma* for biocontrol of agricultural weeds

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Phoma macrostoma Montagne has been registered for biological control of broadleaf weeds in turfgrass. However, the formulation employed in turfgrass is unsuitable for agricultural applications because it is lightweight and resides on the soil surface without breaking down. Thus, it is of interest to develop formulation(s) by which *P. macrostoma* can be safely, cost-efficiently and effectively used to control agricultural weeds such as Canada thistle (*Cirsium arvense* (L.) Scop.) and dandelion (*Taraxacum officinale* G.H. Weber ex Wiggers). Important traits of granular formulations include stable viability of the active ingredient and rapid and complete dispersion in the target environment. Formulations can also enhance biological activity of the active organism. We hypothesised that the addition of sodium citrate as an adjuvant would promote the dispersion of a bioherbicide granular formulation in the soil and weed control capacity of *P. macrostoma* while not reducing fungal survival. Preliminary results demonstrate that 0.1% sodium citrate in potato dextrose agar (PDA) increased *P. macrostoma* colony growth rate. Furthermore, 0.1% and 2.5% sodium citrate increased granules disintegration in water, while not changing fungal survival in granules or pre-emergent dandelion control in well-watered greenhouse trials. We plan to examine the impact of sodium citrate on granule disintegration in soil and on pre-emergent weed control in lightly misted greenhouse trials. Sodium citrate is not detrimental to *P. macrostoma* granule formations, but its benefits remain to be fully explored.

Optimal Condition of Conidial Production of *Nimbya alternantherae* and Identification of a Conidiation-deficient Mutant

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Abstract: *Alternanthera philoxeroides* Griseb is an invasive plant and is currently spreading in many area of China. *Nimbya alternantherae* is considered as a beneficial plant pathogen causing foliar and stem necrosis of *A. philoxeroides*. However, conidia are the most common propagule used in biocontrol programs and must be produced in large quantities, quickly, inexpensively, and efficiently. In this study, factors that are important in establishing a conidia production system for *N. alternantherae* strain SF-193 were examined. After the strain was exposed to black light lamp, we investigated the effects of light conditions, time of the exposure, temperature and time in darkness on its conidial production, and then explored how to get mass conidia of the pathogen with dilution method. The results showed that the optimal conditions of conidial production were that the strain was kept in darkness after the exposure to black light lamp, the time exposing to black light lamp was 24 hours, the temperature and the time kept in darkness were 20°C and 16 hours respectively, and conidial yield was 1.46×10^5 conidia·mL⁻¹. Based on these conidial conditions, we found that conidial yield was 1.84×10^6 conidia·mL⁻¹ from the plate which the mycelia suspension was daubed in by dilution 1:9 (v/v) with water. Also, a mutant Nal1 which the conidial yield declined significantly was obtained by *Agrobacterium tumefaciens*-mediated transformation of *N. alternantherae* strain SF-193. We compared the conidial production of the wild type SF-193 to that of Nal1 by the optimal condition of conidial production. It showed that the quantity of the conidial production of SF-193 was 6.4 times of that of Nal1. Furthermore, the hygromycin-resistant DNA of the transformant was amplified, and an about 1.4kb specific fragment was obtained but the wild type. The result of Southern blot indicated that T-DNA integrated into the DNA of SF-193 randomly by the way of single copy. These results will provide a reliable technology to scale conidial production and reveal molecular mechanism in conidial production of *N. alternantherae*

Key words: *Alternanthera philoxeroides*; *Nimbya alternantherae*; conidial production; conidiation-deficient mutant

Bio-control of *Sporobolus fertilis* with *Nigrospora oryzae*: effect of season and method of inoculation

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Abstract: *Sporobolus fertilis* (GPG: giant Parramatta grass) is a perennial unpalatable weed found in more than 450,000 ha of pasture land along the coastal strip of eastern Australia. Control has to date relied on both selective and unselective herbicides and where possible removal via a cropping phase. For a large majority of infested land these methods are either not practical or uneconomic. A local bio-control agent *Nigrospora oryzae* has been found to cause crown rot symptoms in GPG and slowly kill the plant over a 2 or more year period. An experiment was conducted to compare inoculation time (4 seasons) and method (spore solution or transplantation of diseased plants) on the expression of disease symptoms and decline of GPG. The results from the first 78 weeks of the experiment from 3 sites located in the Tweed, Richmond and Clarence valleys of northern NSW are reported. The proportion of diseased plants has increased over time from 0 to 69%. The average tussock diameter has declined by 34% (9.7 to 6.4 cm). Both methods of inoculation have produced disease symptoms with the use of diseased plants tending to be slightly better and less variable. Season of planting or inoculation has not altered the efficacy of bio-control so far. Artificial inoculation of GPG with *Nigrospora oryzae* was successful in producing disease and is now being adopted as a control technique. Future work will investigate management techniques that can speed up the rate of disease expression and the commercialization of spore production in the laboratory.

Key words: *Nigrospora oryzae*; *Sporobolus fertilis*; giant Parramatta grass; Bio-control

The Perspectives of Herbicide Registration and Management in China

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The progress and public concerns of herbicide registration and management in China were discussed. The registered products of herbicides, productive capacity, and productive yields have satisfied with the demand of agricultural production in China and increased so quickly that export amount exceeded import amount in recent years. Through 30 years, China has developed a sound herbicide management system, especially herbicide registration system. China has taken some serious motions not only on registration, but also law enforcement, illegal trade commerce, application, treatment of herbicide damages and disposals, etc.

However, Chinese herbicide management also meet many new challenges or higher requirements from the public, such as farmer training, safer foods, better environments, stronger market supervision, globalization of management, etc. There are increasing public concerns especially focusing on contaminations of herbicides to environments and agricultural foods. China herbicide management should be upgraded to solve the problems.

Regulations on the Control of Agricultural Chemicals issued in 1997 is being updated and revised with the development of the modern agriculture and new status in countryside at the moment. Comparing to the former version, the data requirements for herbicide registration, distributions and managements of high risk herbicides, punishments for illegal manufacture, distribution and application in the new version are improved significantly.

It is very important to cooperate among nations and regions to harmonize herbicide registration and promote the global joint reviews of herbicides. Herbicides scientific management and reasonable application, and effective farmer training could facilitate to make sure the safety of ecological environment and agricultural products and to strengthen the export of domestic high qualified herbicides and agricultural products.

Combined effect of herbicides ZJ0273 and haloxyfop on weed control, yield components and seed quality in transplanted *Brassica napus*

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Abstract: Prior to promoting and marketing a novel herbicide, it should be investigated for all the possible effects that it can pose on the desired crops, weeds and to the environment. Propyl4-(2-(4,6-dimethoxypyrimidin-2-yloxy) benzylamino) benzoate (ZJ0273), a derivative of 2-pyrimidinyloxy-N-aryl benzoate, is a new herbicide. Therefore, this field study was planned to determine the effect of new herbicide ZJ0273 with its recommended dosages in combination with Haloxyfop in different concentrations. Oilseed rape (*Brassica napus* L.) cv. ZS 758 was tested with herbicides ZJ 0273 and Haloxyfop for three years in the field and each herbicide was used at the rate of 0, 50 mg/L, 100 mg/L and 200 mg/L at the seedling stage. In all three years, maximum weed reduction was observed when we applied both herbicides at 200 mg/L as combined after 2 and 6 weeks of treatment. This weed reduction was 72.25 % after 2 weeks of treatment as compared with initial weed population within the same treatment, and 75.33 % weed reduction was found when compared to herbicide control. The second best combination of herbicides (in terms of weed control) was observed as 200 mg/L ZJ0273 + 100 mg/L Haloxyfop. At this level, 57.82 % weed reduction was observed after 2 weeks of treatments, as compared with the initial weed population within the same treatment. Data showed that weed population was increased by 56.58 % in the herbicide control conditions. Moreover, it was noticed that highest 1000-seed weight was recorded with the application of 200 mg/L ZJ0273 alone. The results showed that when we applied Butachlor and Acetochlor (at recommended dosage i.e. 4.8 mg/L and 30 mg/L, respectively) as pre-emergence herbicides in 2010 and 2011, the rapeseed quality parameters i.e. oil content, glucosinolate content, erucic acid content and protein content were found not much affected under different combinations of herbicides as compared to control. From the present study, we can conclude that application of different herbicides at suitable concentrations might increase the seed yield and seed quality of *Brassica napus* by decreasing the weed populations in crop fields.

Keywords: *Brassica napus* L., Oilseed rape, Herbicide ZJ0273, Haloxyfop, Weed population, Yield, Yield quality

Field evaluation of “Junkekuo”, a biological control agent for broadleaf weeds in dry direct-seeded rice

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Abstract:The fungal pathogen *Scerotium rolfsii* isolate SC64 is being assessed as the basis for a mycoherbicide for biological control of broadleaf weeds in dry-seeded rice. It is our first attempt at combining “Kebaimei”(*Helminthosporium gramineum* Rabenh f. sp. *echinochloae*) and rice vinegar with “Junkekuo” separately to control weed in dry direct-seeded rice field, then we would detect the sclerotia circumstance of residue and germination in soil. The test would be conducted in Jiangpu test sites of Nanjing which a wheat-rice rotation field. Plant mortality was recorded 7 and 14 days after inoculation (DAI). The field tests contain two parts , they are “Junkekuo + Kebaimei” and “Junkekuo + Rice vinegar” separately control the weeds in the dry direct-seeded rice field. The result will confirm weed control efficacy of “Junkekuo” working with “Kebaimei” respectively, at the same time, we would test that if is sclerotia residue good security to crop of next year after using *Scerotium rolfsii* isolate SC64.

Key words: *Scerotium rolfsii*, bioherbicide, “Junkekuo + Kebaimei”, “Junkekuo + Rice vinegar”, weeds, dry direct-seeded rice

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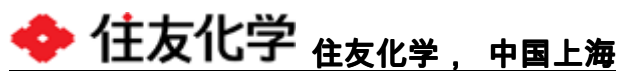
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