Pertanika J. Trop. Agric. Sci. **31(1)**: 19 - 26 (2008)
ISSN: 1511-3701

Effect of *Exserohilum monoceras* (Drechslera) Leonard & Suggs on the Competitiveness of *Echinocloa cruss-galli* (L.) P. Beauv.

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ABSTRACT

The use of bioherbicide to reduce interference by barnyard grass in rice cropping system has been suggested but has not been reported. Against this conceptual background, a mini-plot study was conducted to simulate the efficacy of *Exserohilum monoceras* to reduce competitiveness of barnyard grass in rice using replacement series experiment. The effect of *E. monoceras* on rice was negligible, as it did not cause any infection. Severe infection was observed on barnyard grass inoculated with this fungus at all plant densities as indicated by high AUDPC values (ranges from 610.35-468.28 unit^2) and fast disease progress rates (r_L= 0.48 logit/day). Rice biomass in mixture with diseased weed was higher than in the presence of healthy weed, and is not significantly different from rice biomass in the non-weedy control. In the inoculated experiment, at lower weed density, competition between barnyard grass and rice was not apparent despite the fact that the weed growth was reduced. As the weed density increased, rice continued to grow, but barnyard grass was suppressed; the growth difference was bigger and more measureable. In the non-inoculated control, the interaction between barnyard grass and rice was observed at 2:2 ratio, but at 3:1 in the inoculated experiment, indicating that rice was more competitive over barnyard grass. It took three barnyard grass to equal the shoot dry weight of one rice plant. This study provides strong evidence of the ability of *E. monoceras* in reducing the competitive ability of barnyard grass and thus provides new opportunities for the future of biological weed control in Malaysia.

Keywords: Barnyard grass, *Echinocloa crus-galli*, *Exserohilum monoceras*, weed competition, biological control

INTRODUCTION

Bioherbicide has been proposed as one of the components of Integrated Weed Management (IWM), but little research has been done on this aspect. The efficacy of a potential bioherbicide needs to be established and this is normally done after the study on inoculum production and epidemiological studies (Morin *et al.*, 1990; Charudattan, 2001). The efficacy of any bioherbicide can be measured in terms of weed control, the level of disease stress or increase in crop yield resulting from reduced weed competition (Charudattan, 1988). The use of bioherbicides to reduce interference by barnyard grass in rice cropping systems has only recently started in Malaysia. However, there are several reports of successes in reducing weed interference in different cropping systems (Kennedy *et al.*, 1991; Jacobs *et al.*, 1996; Kadir *et al.*, 2000b).

Tasrif *et al.* (2003) has indicated the potential of *Exserohilum* monoceras as a bioherbicide for barnyard grass, but its control efficacy has not been determined in a rice cropping system. Therefore the objective of this study was to determine the potential of using *E. monoceras* in reducing the interference of barnyard grass on rice in the field and its role in controlling barnyard grass.

MATERIALS AND METHODS

Location

The experiment was conducted in 2003 at Field 2 of the University Research Farm, Faculty of Agriculture, Universiti Putra Malaysia, Serdang, Selangor, Malaysia. The location of the plot was 03°00559' N, 101°42.195' E, and the daily average temperature was 30 ± 2°C with an average annual rainfall of about 2500 mm.

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Soil Preparation

Soil taken from Tanjung Karang (Malaysia) rice field estate was filled into 1 m^3 fiberglass boxes until three-quarters full. The soil was flooded to 10 cm deep for 3 to 4 days so as to simulate real field conditions, and any emerging weed seedlings were killed. Thereafter, water level was reduced to 1-2 cm for planting.

Preparation of Inoculum

Inoculum of *E. monoceras* was produced using a biphasic culturing technique (Chandramohan, 2000) with modifications. Five mycelium plugs were transferred in a 250 mL flask containing 100 mL V8 broth. The inoculated flask was then shaken at 100 rpm on a rotary shaker for 2 days at 28°C, after which it was transferred into a 1L flask containing 400 mL of v8 broth. The resultant broth was blended with a Waring blender at low speed for 30-60 sec; 25 ml of the suspension was poured onto a layer of V8 agar (250 ml) in a tray (35x 26 x 2.5 cm), exposed to 24 h light at 30±2°C. The conidia was gently scraped off with sterile rubber spatula and transferred into sterile water. After filtering through two layers of cheese cloth, the spores were rinsed with sterile water. The conidial suspensions were pooled and the concentration was determined with a hemocytometer.

Plant Preparation

The substitutive or replacement series approach that was used in this study was designed basically for mini-plot trials. By this method, plant densities remain constant and the proportions of both species were varied from monoculture of one species at that given density to the monoculture of the other species (Radosevich, 1988).

The rice and barnyard grass seedlings were planted randomly in the fiberglass boxes using the approach in the proportions of 4:0, 3:1, 2:2, 1:3, and 0:4 (rice : barnyard) with a final total of 250 seedlings; a 4:0 means that 250 rice seedlings were planted as a monoculture.

Plant Inoculation

Barnyard grass and rice at three to four-leaf stage were sprayed with 15 ml of conidial suspension (6.5×10^5 $conidial^{-1}$ mL) in a 10% oil emulsion using an AiFA pressure sprayer (Winstar Enterprice, Model 8505). The oil emulsion was used to maintain uniform conidial distribution on the leaves and to break through the protective cuticle layers for better penetration of the fungus. Each fiberglass box was therefore sprayed with 9.7 million spores. The control was sprayed with 10% oil emulsion only. Disease incidence and severity were recorded daily for seven days. The plants were harvested for dry weight biomass assessment 30 days after inoculation.

Disease Assessment

Disease incidence was assessed based on the proportion of plants affected out of the total number inoculated and expressed as the percentage of diseased plants (James, 1974; Horsfall and Cowling, 1978; Kranz, 1988). The disease severity was based on the percentage area of plant tissues showing symptoms of the disease (Kranz, 1988). The disease progress was assessed by monitoring the disease development. The disease severity was scored on a scale of 1-10 whereby 0 = healthy, 5 = 50% diseased, and 10 = plant death (Kadir *et al.,* 2000a).

Measured Variables

For the dry weight, shoots were harvested by cutting all plants just atop the soil level. The plants were oven dried for four days at 75°C and the dry biomass was determined.

Assessment for competitive interaction was carried out by the resulting models on replacement series (Radosevich, 1988). The "Relative Yield Total" was adopted to draw conclusions from the data collected; it determines the relative amounts of biomass produced by any two species by adding the relative yields (RY) of both species within each proportion used. Both variables were obtained from the equations below as explained by Willard and Shilling (1990).

RYT = RY_a + RY_b (relative yield total)

RY_a = $Biomass_{ap}/Biomass_{am}$ (relative yield)

RY_b = Relative yield of species *b.*

$Biomass_{ap}$ = Biomass production of species *a* at *a* particular proportion *p.*

$Biomass_{am}$: Biomass production of species *a* as *a* monoculture.

STATISTICAL ANALYSIS OF DATA

The field studies were carried out with two trials; one in early January and another in mid August 2003, arranged in a randomized complete block design with four replications. The average temperatures for these trial dates were 32°C and 30°C respectively. Data were pooled since the individual trials did not show significant difference. A two-way ANOVA set to 5% significant level was performed to test treatment effects and interaction between factors. When interactions between proportions were significant, each possible combination was considered as an independent treatment. Means were compared with a Fisher's Protected LSD test. Standard error bars were calculated to show differences in relative yield graphs.

RESULTS

Disease Progress

Exserohilum monoceras was very pathogenic to barnyard grass and the disease started with specks that became numerous as the disease progressed. The margins around the lesions turned grayish and eventually the areas turned necrotic. The infected leaves then turned dark green to brown and eventually shrivelled and dried.

The older lower leaves were more afflicted with larger necrotic areas lined with dark watery borders. Most of the leaf blade was blighted within 24 h. The control plants sprayed with 10% oil emulsion remained healthy and asymptomatic throughout. The disease progress could be best described by the logistic growth model (*Fig. 1*) with the overall apparent infection

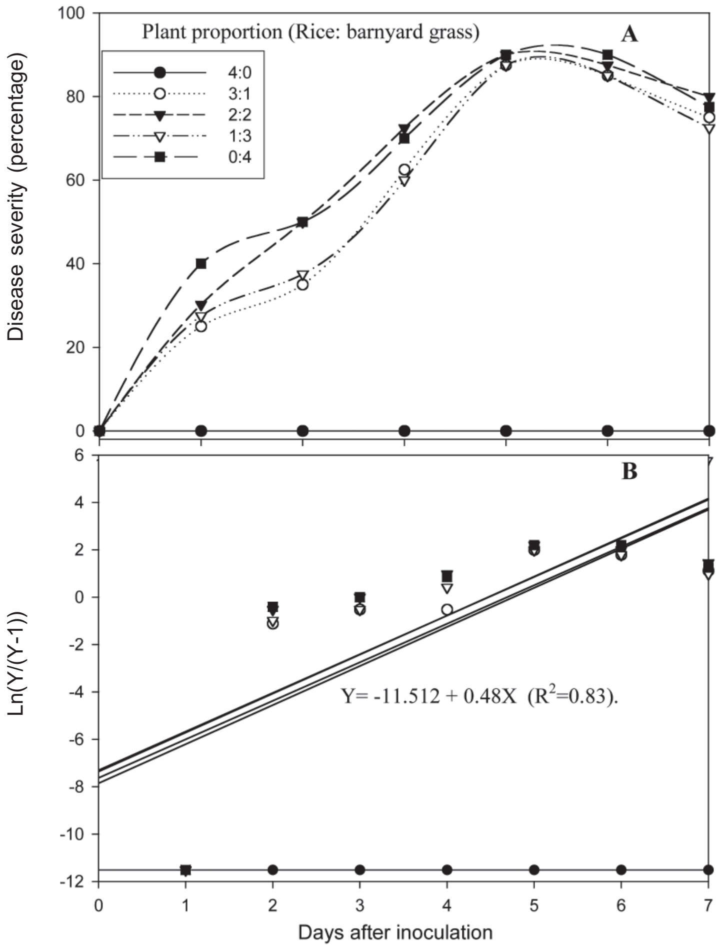

Fig. 1: Disease progress of leaf blight by E. monoceras *on barnyard grass seedlings; (A) Untransformed diseased severity value. (B) Regression of transformed disease severity using the logistic model ln (Y/(1-Y))*

TABLE 1
The effect of *E. monoceras* on relative yield (RY) and relative yield total (RYT) within proportions of rice and barnyard grass

Treatment	Proportion (*O. sativa: E. crusgalli*) 4:0	3:1	2:2	1:3	0:4
Non Inoculated					
RY Rice	1.00a	0.85a	0.49b	0.21c	0d
RYBarnyard grass	0e	0.21d	0.51c	0.78b	1.00a
RYT	1.00a	1.05a	1.00a	0.99a	1.00a
Inoculated					
RY Rice	1.00a	0.88a	0.79a	0.78a	0c
RYBarnyard grass	0c	0.20b	0.16b	0.21b	1.00a
RYT	1.00a	1.08a	0.95a	0.99a	1.00a

Values for RY and RYT within rows followed by the same letters are not significantly different at P<0.05 according to Fisher's Protected LSD test.

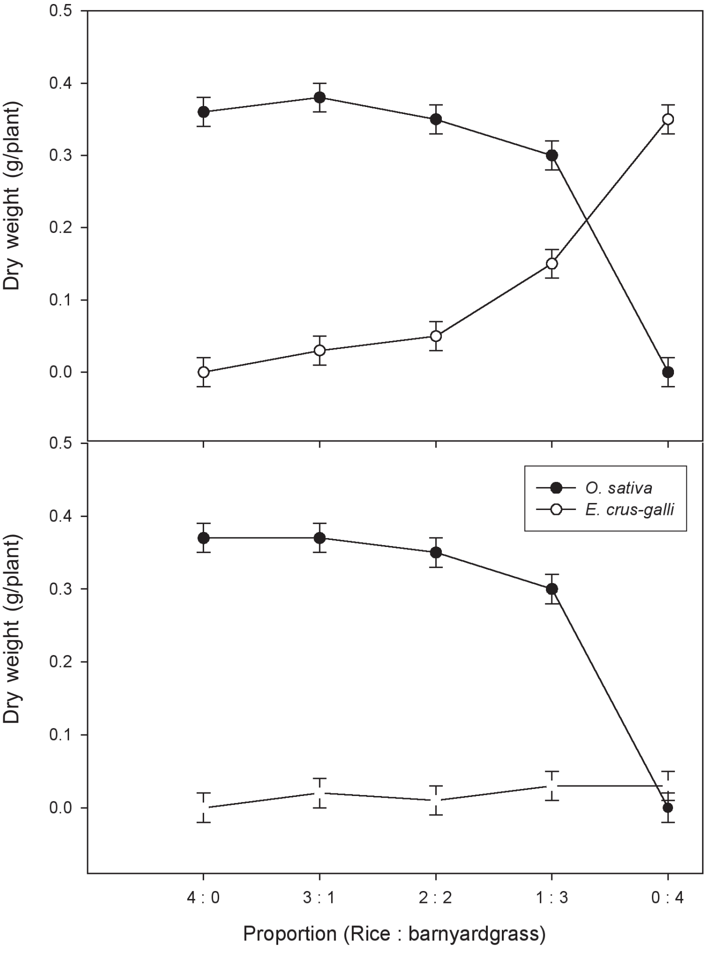

Fig. 2: Effect of E. monoceras *on dry weight per plant of "replacement series-proportion" between rice and barnyard grass; (A) uninoculated treatment and (B) inoculated treatment. Bars represent the standard deviation of the difference between means*

rate of the two inoculated trials averaging r_L = 0.48 logit/day (SE = 0.001, R^2 = 0.83; P <0.005). Although initially *E. monoceras* caused severe infection on barnyard grass, 100% mortality by *E. monoceras* was not recorded. However, the infected plants remain stunted and never resume normal growth.

Plant Dry Weight

In the non-inoculated control, rice grown as a monoculture (4:0) and 3:1 proportion produced the highest dry weight per plant (*Fig. 2A*). Dry weights within these proportions were not significantly different. Barnyard grass as a monoculture and at 1:3 proportions produced significantly greater dry weight per plant compared to other proportions (*Fig. 2A*). In the inoculated experiment, the dry weight of rice remained consistently high for all proportions except for the 0:4 ratio (*Fig. 2B*). The dry weight of barnyard grass was significantly reduced in all plant proportions in the inoculated experiment but the difference in dry weight according to plant proportions was not significant (*Fig. 2B*).

Derived Variables: Relative Yield (RY) and Relative Yield Total (RYT)

The RY is the yield of each species in a mixture as a percentage of its monoculture yield produced under the same growing condition. RYT is the result of adding relative yield for each species within a given proportion. Both variables are coefficients indicating species that is more competitive, without partitioning intra-specific or inter-specific effects (Radosevich, 1988; Willard and Schilling, 1990). Plant proportion significantly affected (P <0.01) RY of rice and barnyard grass (Table 1; *Fig. 2*); however, RYT did not differ statistically among proportions. At all proportions, RY of both species in mixture

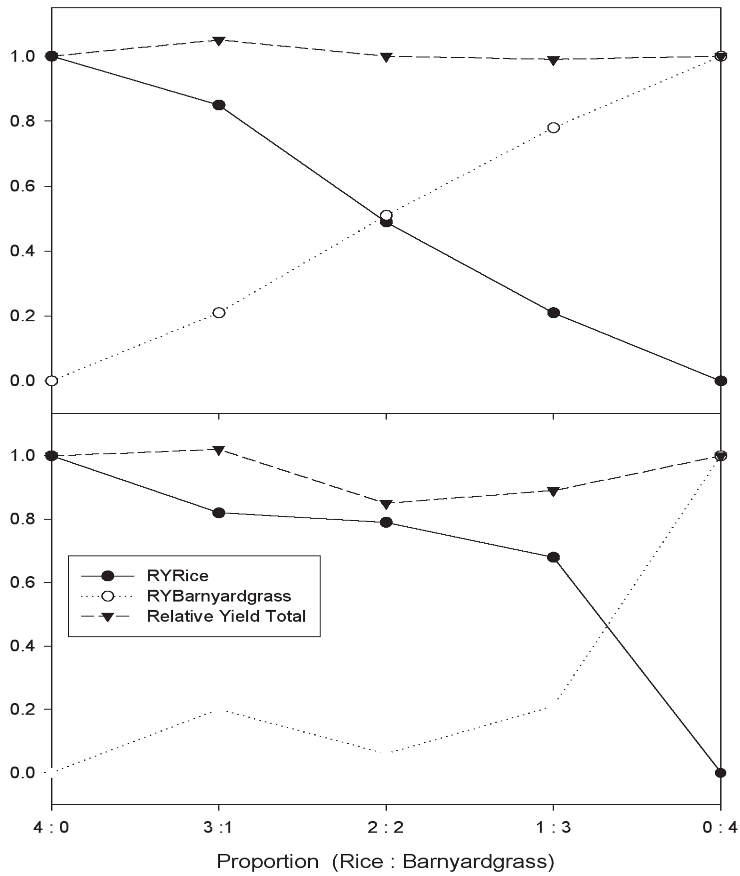

Fig. 3: The relative yield total results of rice and barnyard grass grown in replacement series: (A) non inoculated control and (B) E. monoceras *inoculated treatment*

differed from monoculture of each species, except at 3:1 (Table 1; *Fig. 3*) where the RY rice was not significantly different from RY rice as a monoculture (4:0). The interaction between rice and barnyard grass was observed at 2:2 for the non-inoculated experiment (*Fig. 3*), which implied that rice and barnyard grass were both equally competitive.

In the inoculated experiment, similar results were observed. The RYT was not significantly different among proportions. The RY of barnyard was not significantly different among proportions except at 0:4; the RY barnyard grass was significantly lower compared to the RY rice at all proportion except at 0:4. The RY rice for all proportions was not significantly different from RY rice as a monoculture. The interaction point for the inoculated experiment was reduced to between 1:3 and 0:4 (*Fig. 3*). The results implied that it took one rice plant or less to equal the shoot dry weight production of three barnyard grasses.

This study revealed that the application of *E. monoceras* reduced barnyard grass growth, hence having the advantage of rendering rice more competitive. The RYT values in non inoculated mixtures were equal to the monoculture at all proportions. These results indicated that both species in a mixture produced the same dry weight that would be expected if they had been planted separately. The mutual antagonism had resulted in both species producing less than their respective monoculture biomass and the result obtained was similar to Model III of Radosevich (1988) based on the replacement series experiments.

DISCUSSION

The infection of *E. monoceras* on rice was negligible and was expected as this fungus had been previously determined to be host specific to barnyard grass (Juraimi *et al.*, 2006). Severe infection was observed on inoculated barnyard grass although seedlings mortality was negligible; however, the growth of the infected plants was retarded.

The inability of *E. monoceras* to kill barnyard grass under field conditions compared to glasshouse may be attributed to the inadequate conidial concentration used as well as the environmental factors, especially the leaf wetness (dew). Researchers working on other mycoherbicides have reported that extended dew period requirement was responsible for the poor efficacy of many weed bioherbicides in the field (Daigle *et al.*, 1990; TeBeest *et al.*, 1992; Zhang *et al.*, 1996; Kadir *et al.*, 2000a). However, the length of the dew period required by most effective bioherbicides can be reduced by appropriate timing of the application to take advantage of the humidity provided by rain, dew and irrigation in the field.

The formulation of foliar pathogens with water-retaining materials is another promising approach to make pathogens less dependent on available water to initiate infections. Recent research on formulation had shown the potential of materials such as surfactants (Zhang and Watson, 1997), invert emulsions (Amsellem *et al.*, 1991; vegetable oils (Kadir and Ng, 2004); humectants (Boyette *et al.*, 1996; Kadir *et al.*, 2000a) to overcome dew requirements, which will broaden the application strategies for bioherbicides.

The results of this research confirmed the presence of inter-specific and possibly intra-specific competition in rice-barnyard grass system affecting the plant growth and biomass production. Competition increases with increasing plant density. Application of *E. monoceras* reduces the growth of barnyard grass, in terms of biomass, and hence decreased the level of competition by the weed. At lower weed proportion, competition was not apparent despite the fact that weed growth was reduced. As the weed density increased, the rice continued to grow but the barnyard grass showed suppressed growth hence the difference was bigger and more measurable. The rice biomass in a mixture with diseased weeds was higher than in the presence of healthy weeds, and was not significantly different from rice as a monoculture.

Inoculation of *E. momoceras* caused the highest growth suppression in barnyard grass at the highest density. At the proportion of 1:3 (rice:barnyard grass), which is equivalent to approximately 50 rice plants to 200 barnyard grass plants m^{-1}, it is expected that plants experience more inter- and/or intra-specific competition; however, this phenomenon was not observed in the inoculated treatments. This result was consistent with other findings that fungal pathogens gave greater negative effects on host plants in higher plant density situations (Ditommaso and Watson, 1995; Kadir *et al.*, 1999; Jahromi *et al.*, 2001).

Paul and Ayres (1987) found that lettuce (*Lactuca scariola* L.) showed a competitive advantage when in a mixture with grounsel (*Senecio vulgaris* L.). This advantage was further exaggerated if the groundsel was infected by rust fungus (*Puccinia langenophorae* Cooke). They reported that the effect of the rust on the weed was expressed by reduction in the dry weight yield of groundsel, which also appeared to be the case with the barnyard grass-rice competition examined here. Kadir *et al.* (1999) reported that the top biomass and tuber production of purple nutsedge (*Cyperus rotundus* L.) was reduced drastically when this weed was inoculated with *Dactylaria higginsii* (Luttrel) MB Ellis, thus reducing competitiveness of this weed in tomato-nutsedge system.

Given the high genetic variability between barnyard grass populations (Juraimi *et al.*, 2006), the different responses of the weed population to the pathogen may become a major concern; however, this constraint can be circumvented by increasing the virulence of the pathogen through addition of additives in the formulation. Juraimi *et al.* (2006) reported the water-oil-water (WOW) formulation of *E. monoceras* had increased the efficacy of this fungus in the presence of biotype difference of barnyard grass. The use of a rice cultivar that is fast growing can provide a more successful competitor and therefore would enhance the effectiveness of the inoculation.

In this study *E. monoceras* did not cause 100% mortality of barnyard grass. It should be emphasized that any consideration of the efficacy of a biocontrol agent should be based on crop yield rather than injury or mortality of the weed (Paul and Ayres, 1987). The difference in relative yield of rice in the non inoculated and inoculated treatments was not apparent; however, the relative yield of barnyard grass was higher in the non inoculated compared to the inoculated experiments. Although the results of the glasshouse study did not concur with the field study in terms of plant mortality, it was concluded that *E. monoceras* was capable of suppressing the barnyard grass growth based on the strong evidence of its ability to reduce the competitive ability of the latter. This study has provided a new hope for the future of biological weed control in Malaysia.

ACKNOWLEDGEMENTS

The facilities provided by UPM are gratefully acknowledged. This report which forms a part of the second author's Master of Science Thesis, was supported by the IRPA grant, 01-02-04-0786.

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