Potential use of *Exserohilum monoceras* as a biological control agent for *Echinochloa* spp. (barnyard grass) in Vietnam

Nguyen Van Tuat[A], Tran Hu Hanh[A], Shane D. Hetherington[B], Duong Van Chin[C] and **Bruce A. Auld[B]**

[A] National Institute of Plant Protection, Chem, Tu Liem, Hanoi, Vietnam.
[B] NSW Agriculture, Orange Agricultural Institute, Forest Road, Orange, New South Wales 2800, Australia.
[C] Cuu Long Rice Research Institute, Omon, Cantho, Vietnam.

Summary

***Exserohilum monoceras* causes a leaf blight on *Echinochloa crus-galli* a grass weed of rice cultivation. Indigenous isolates of this fungus were collected from north, central and south Vietnam. Two isolates were chosen to examine the severity of this disease on *E. crus-galli* and to determine if the fungus could infect rice. Following inoculation, rice and *E. crus-galli* were exposed to moist periods of 8, 12 and 24 hours and their height and mortality measured 5, 10 and 15 days after inoculation (DAI). The above ground dry weight of all plants was measured 15 DAI. Rice height, above ground weight and mortality did not differ from uninoculated controls after any treatment. All *E. crus-galli* plants were dead 15 DAI. All *E. crus-galli* plants were dead 10 DAI when they had been subject to post-inoculation high humidity (80–90%) periods of 12 or 24 hours. *E. crus-galli* height was significantly reduced by inoculation followed by dew periods of 8, 12 and 24 hours. The possibility of using *E. monoceras* as a biological control agent for *E. crus-galli* is discussed.**

Introduction

Echinochloa crus-galli (L.) Beauv. (barnyard grass, barnyard millet, cocksfoot) and *Echinochloa colonum* (L.) Link (jungle rice) are amongst the world's most troublesome weeds and are significant weeds in all areas of rice cultivation (Holm *et al.* 1977). In Vietnam, seven species of *Echinochloa* are reported including both of these weed species (Ho 1993). Surveys of the weed flora of rice cultivation in two principal rice growing regions of Vietnam—the Red River and Mekong Deltas—identified *E. crus-galli* as the principal grass weed (Tan *et al.* 1997, Chin *et al.* 1998)

Hand weeding of *E. crus-galli* is still widely practised although use of herbicides is increasing especially in the Mekong Delta. While providing good control, sustained reliance on chemical herbicides poses a number of threats to Vietnamese agriculture. Rice paddies are frequently used for aquaculture and a number of 'wild' foods are also gathered (e.g. frogs and snails). The accumulation of pesticide residues in the flesh of animals is well documented and in this case constitutes an unmeasured but real hazard. Additionally, up to three rice crops are grown annually in the highly fertile delta areas. Where herbicides are used, each of these crops is sprayed with a limited suite of chemicals and the potential for herbicide resistance exists in weed populations. Propanil resistant *E. crus-galli* populations in Colombia (Baker 1991), the United States (Baltazar and Smith 1994) and Costa Rica (Leah *et al.* 1994) and populations resistant to propanil and quinclorac in Spain and Greece (De Prado *et al.* 1997) have been recorded. This problem also exists in Asia, butachlor resistant *Echinochloa* populations cover an estimated area of two million hectares of rice growing area in China (Huang and Gressel 1997). Baker (1991) concludes that *E. crus-galli* is the most likely tropical grass weed to cause herbicide resistance problems. Pressure is mounting for the diversification of control strategies for this weed.

Fungal agents, to date, have proven ineffective as biological control agents of grass weeds. Early attempts to control *E. crus-galli* with the fungus *Cochliobolus lunatus* Nelson and Haasis required that use of the fungus be integrated with the chemical agent atrazine. Reliance on the fungus alone was ineffective and the plant was able to 'grow away' from the infection having reached a certain stage (Scheepens 1987).

Recently a number of southeast Asian projects have studied the fungus *Exserohilum monoceras* (Drechsler) Leonard & Suggs (teleomorph: *Setosphaeria monoceras* Alcorn) for the control of species of *Echinochloa* (Auld *et al.* 1998). Fungal isolates causing disease on *E. crus-galli* were collected from northern, central and southern Vietnamese provinces. Preliminary testing of these isolates established *E. monoceras* as the most likely pathogen for inclusion into a biological control scheme in Vietnam. Work outlined in this paper details studies designed to identify and test promising isolates of this fungus based on their selective infection of *Echinochloa* and the severity of the disease they cause on this weed.

Materials and methods

Seed origins

Echinochloa crus-galli seed was collected from Hoai Duc district Ha Tay province in the summer of 1997. Two batches of seed were collected representing a morphotype with a pink panicle (*E. crus-galli* 1) and one with a green panicle (*E. crus-galli* 2). Seed of two rice cultivars, CR203 and IR50404 were obtained from the collections of the National Institute of Plant Protection (NIPP) and the Cuu Long Rice Research Institute (CLRRI) respectively. These cultivars are currently the most commonly planted in the north and south of Vietnam.

Seeds of all plants were germinated in petri dishes on moistened filter paper in an incubator at 35°C for 48 hours. Ten germinated seeds (coleoptile and radicle visible) were planted in 12 cm ceramic pots filled with a peat soil collected from a paddy field at NIPP. Seeded pots were placed in a shallow cement trough in a screen-house and a 2–3 cm water level was maintained in the trough throughout the experiment. The seedlings were thinned before inoculation to five plants per pot. Screen house conditions during the course of the experiment were 22.9°C to 27.9°C average daily temperature and there was an approximate photoperiod of 12 hours.

Isolate origins, maintenance, growth and spore production

Single conidial isolates of *E. monoceras* were obtained from CLRRI and NIPP; these isolates were designated E1097 and 85.1 respectively and identified as *E. monoceras* by Dr. John Alcorn, Queensland Department of Primary Industries. The latter isolate was also identified as *E. monoceras* by the International Mycological Institute (Kew, UK) and given the accession number IMI380328. The isolates were grown on half strength Potato Carrot Agar (20 g potato, 20 g carrots, 1 L water) at room temperature (25–28°C) for 15 days.

Conidia were harvested from the colonies by flooding the plates with 5–10 mL of distilled water and scraping the surface of the colonies with a glass slide. The resulting suspension was filtered through a layer of cheesecloth. The inoculum concentration was determined using a haemocytometer and adjusted to 1×10^6 conidia mL^{-1} by dilution with distilled water.

Inoculation procedure

Plants were inoculated at the two true leaf stage. One drop of Tween 80 (polyoxyethlenesorbitan monoleate) was added to each 100 mL of conidial suspension to act as a wetting agent. The suspension was

sprayed through a small volume sprayer with a 200 mL capacity. Plants were sprayed to the point of run-off. Immediately after inoculation plants were transferred to a moist chamber where they were held in darkness at 25±2.5°C and 85±4% relative humidity for 8, 12 or 24 hours.

One drop of Tween 80 was added to 100 mL of water and this was sprayed onto a second group of plants which acted as controls. They were then treated identically to inoculated plants.

Assessment

Plant height and mortality were measured 5, 10 and 15 days after inoculation (DAI). The above ground dry weight of living tissue was assessed 15 DAI. Completely collapsed, necrotic seedlings were considered dead. Seedlings were cut at soil level, aerial parts placed in a paper bag and then in an oven at 80°C for 24 hours before weighing. Dead tissue was not included in dry weight measurements.

Analysis

All experiments were performed three times. Treatments were replicated four times in each experiment. The mean value of the five plants in each pot was used for statistical analysis. The significance of treatment effects and interactions were determined using the analysis of variance, general treatment structure procedure of the Genstat statistical package (IACR-Rothamsted, Harpenden). Treatment means were separated using Fisher's Least Significance Test at the 1% level of significance.

Results

Disease symptoms were seen on all *E. crus-galli* plants as reduced height (when compared to uninoculated controls) and necrotic leaf spots one to two days after inoculation. Areas of necrosis coalesced and leaves became fully blighted. There was no fungal isolate or weed morphotype effect and the results represent the mean of pooled values for both weed morphotypes and fungal isolates. Within five days many of the *E. crus-galli* plants were dead. The wilting and collapse associated with these symptoms made meaningful measurement of weed height impossible after five days. Over 80% of those given an eight hour moist period were dead ten DAI. All weeds given 12 and 24 hour dew periods were dead after the same time period (Figure 1). All *Echinochloa* plants were dead 15 DAI regardless of the moist period experienced at inoculation. No treatment resulted in the death of any rice plants. At no stage during the experiment was rice height significantly reduced by any treatment.

Dry weight was measured 15 days after inoculation. At this time all weed plants had died and many of their leaves had come into contact with the moist soil in the pots and begun to decompose. The weight of living tissue was effectively zero. The above ground dry weight of the necrotic remains of the weeds was measured but is not presented. Neither fungal isolate reduced the above ground dry weight of either rice cultivar 15 DAI (Table 1).

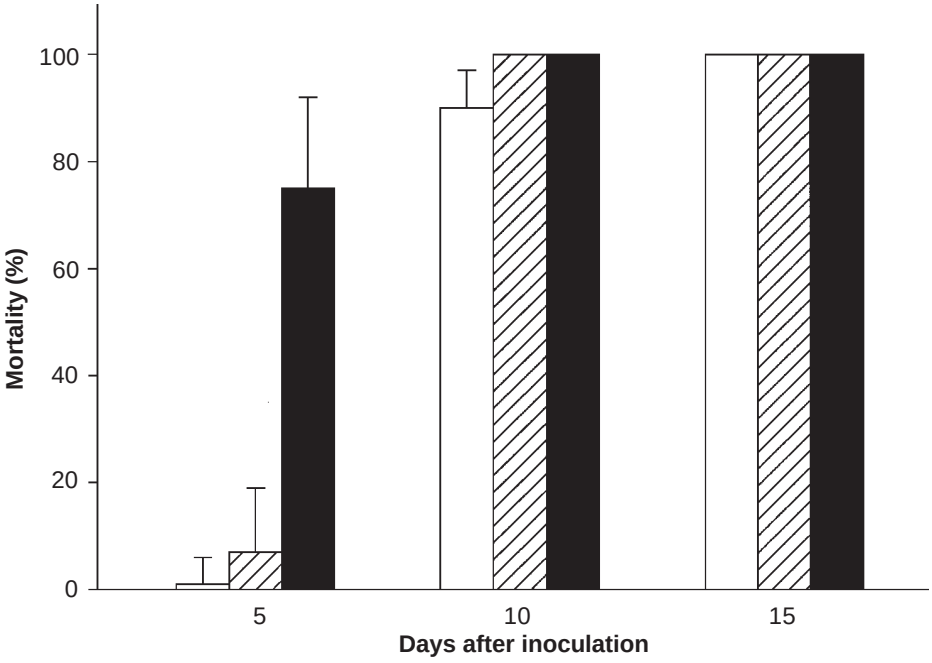

Figure 1. Mortality of *Echinochloa crus-galli* 5, 10 and 15 days after inoculation with the fungal pathogen *Exserohilum monoceras* following an 8 hour moist period □; a 12 hour dew period ▨; and a 24 hour dew period ■. Data points represent means of 16 replicated, error bars represent the standard deviations of those means.

Table 1. The above ground dry weight (mg) of two rice cultivars fifteen days after inoculation with two isolates of *Exserohilum monoceras* and an uninoculated control. Figures represent the mean weight of four replicates, where a replicate was five seedlings grown in a single pot. No treatment resulted in a significant (P<0.01) reduction in rice weight.

	Rice cultivar	
Isolate	CR203	IR50404
85.1	127	140
E1097	154	162
Uninoculated control	116	134

Discussion

Vietnamese isolates of *E. monoceras* infected *E. crus-galli* and did not infect either of the two cultivars of rice tested. Further experiments have shown that these isolates are similarly pathogenic to *E. colonum* (data not shown). The high level of specificity displayed by these two Vietnamese isolates is a character shared by the Philippine isolates of Zhang *et al.* (1996). In southeast Asian countries a wide range of agricultural and wild plants are part of the diet, host specificity is therefore very important. A wide variety of plants need to be tested to ensure that no valuable species are susceptible. Certain Korean isolates of this fungus infect rice (Chung *et al.* 1990). This sounds a note of caution about the use of *E. monoceras* and directs future research toward making extensive host range testing a priority.

In this study infection invariably led to weed death after 15 days regardless of the dew period which the plant and pathogen had been subject to following inoculation. Our findings are in agreement with those of Zhang *et al.* (1996) in which 100% weed mortality was recorded 10 days after inoculation where plants had been given 12 and 24 hour dew periods. Our study also examined the effect of shorter dew periods of 8 hours. Weed mortality was invariably over 80% 10 days after inoculation following an 8 hour dew period. In the humid tropics, with an infection court suspended above a rice paddy the dew period may be less critical than in the context of dryland agriculture. Nonetheless this short dew period requirement is an encouraging trait.

Philippine and Vietnamese *E. monoceras* isolates have a similar disease response toward *E. crus-galli*. This interaction is under investigation in both countries for use as a bioherbicide. Similar research is being undertaken in Korea (Chung *et al.* 1990), Japan (Tsukamoto *et al.* 1997) and Malaysia (Caunter and Wong 1988). Zhang *et al.*

(1996) raise the possibility of DNA fingerprinting as a means of comparison of isolates. Given the concurrent emergence of these programs a collaborative project involving molecular genetic comparison seems meritorious. Isolates pathogenic to rice, such as those found by Korean researchers may present a distinct genetic profile and molecular techniques would allow more efficient screening.

Acknowledgments

Financial support for this study was provided by the Australian Centre for International Agricultural Research. The authors wish to acknowledge the contribution of staff from the Orange Agricultural Institute, National Institute of Plant Protection, Cuu Long Rice Research Institute and Cantho University.

References

Auld, B.A., Watson, A.K., Mabbayad, M.O., Ciotola, M. and Hetherington, S.D. (1998). Biocontrol of weeds with fungi in developing nations: the role of bioherbicides. Proceedings 4th International Bioherbicide workshop, Glasgow, Scotland UK, Abstract p. 9.

Baltazar, A.M., and Smith (Jr.), R.J. (1994). Propanil-resistant barnyard grass (*Echinochloa crus-galli*) control in rice (*Oryza sativa*). *Weed Technology* 8, 576-81.

Baker, F.W.G. (1991). Herbicide resistance. *In* 'Tropical grassy weeds', eds F.W.G. Baker and P.J. Terry, p. 96. (CAB International, Wallingford, Oxon, UK).

Caunter, I.G. and Wong, S.H. (1988). Foliar diseases of Lalang in Penang: biocontrol potential. *MAPPS Newsletter* 12, 6.

Chin, D.V., Du, P.V., Auld, B.A., and Hetherington, S.D. (1998). A survey of the relative importance of the major weeds in wet seeded rice in the Mekong River Delta. Bioherbicide project paper No. 4. ISBN 0 7313 1507 3, 19 pp.

Chung, Y.R., Kim, B.S., Kim, H.T. and Cho, K.Y. (1990). Identification of *Exserohilum* species, a fungal pathogen causing leaf blight of barnyard grass (*Echinochloa crus-galli*). *Korean Journal of Plant Pathology* 6, 429-33.

De Prado, R., Lopez-Martinez, N., Gimenez-Espinosa, R. (1997). Herbicide resistant weeds in Europe: agricultural, physiological and biochemical aspects. *In* 'Weed and crop resistance to herbicides', eds R. De Prado, J. Jorrín and L. García-Torres, p. 17. (Kluwer Academic, Boston Mass. USA).

Ho, P.H. (1993). An illustrated flora of Vietnam, Volume 3, pp. 810-12. (Mekong printing, Santa Ana, USA).

Holm, L.G., Plucknett, D.L., Panch, J.V. and Herberger, J.P. (1977). The world's worst weeds: distribution and biology, p. 32 (University of Hawaii Press, USA).

Huang, B., and Gressel, J. (1997). Barnyard grass (*Echinochloa crus-galli*) resistance to both butachlor and thiobencarb in China. *Resistant Pest Management* 9, 5-7.

Leah, J.M., Casely, J.C., Riches, C.R., and Valverde, B. (1994). Association between elevated activity of aryl acylamidase and propanil resistance in jungle-rice, *Echinochloa colona*. *Pesticide Science* 42, 281-9

Scheepens, P.C. (1987). Joint action of *Cochliobolus lunatus* and atrazine on *Echinochloa crus-galli* (L.) Beauv. *Weed Research* 27, 43-7.

Tan, N.T., Son, N.H., Trung, H.M., Auld, B.A., Smith, H.E. and Hetherington, S.D. (1997). A survey of the weeds of water rice in the Red River Delta. Bioherbicide project paper No. 2, ISBN 0 7310 9838 2, 18 pp.

Tsukamoto, H., Gohbara, M., Tsuda, M. and Fujimori, T. (1997). Evaluation of fungal pathogens as biological control agents for the paddy weed, *Echinochloa* species by drop inoculation. *Annals of the Phytopathological Society of Japan* 63, 366-72.

Zhang, W.M., Moody, K. and Watson, A.K. (1996). Responses of *Echinochloa* species and rice (*Oryza sativa*) to indigenous pathogenic fungi. *Plant Disease* 80, 1053-8.