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MOLO[1,3] R. AND OGWANG[1] J. A.

[1]*Namulonge Agricultural and Animal Production Research Institute (NAARI), P. O. Box 7084, Kampala, Uganda.*
Email: naari@naro-ug.org

[3]*Present address: Imperial College, Silwood Park campus, Ascot, Berkshire, SL5 7PY, United Kingdom*

Address editorial correspondence to:
Richard Molo, Imperial College, Silwood Park campus, Ascot, Berkshire, SL5 7PY, United Kingdom.
+44 077 92808857 (phone);
+44 02075942339 (fax);
Email r.molo@imperial.ac.uk

ABSTRACT

Surveys were undertaken in lakes Victoria, Kyoga and River Nile in Uganda between 2000 and 2001 to determine fungal pathogens associated with water hyacinth. Several potential pathogen isolates including *Alternaria eichhorniae*, *Cercospora* sp. and *Acremonium zonatum* were identified. Isolates of *Cercospora* sp. and *A. eichhorniae* were evaluated for their effectiveness on water hyacinth plants in the screen house. The disease incidence and severity progressed with time in both isolates although there was no significant difference ($P>0.05$) between the isolates and overall disease incidence and severity was not significant ($P>0.05$). There was no significant ($P>0.05$) effect of pathogens on overall plant fresh weight, number of living leaves and daughter plants, but linear regression analysis showed a significant ($P<0.01$) decrease in fresh weight of water hyacinth plants. Disease infection increased with increasing conidia concentration and was significantly ($P<0.05$) lower at the lower conidia doses. Host range tests on 9 cultivated plant species showed that sorghum was highly susceptible to *Cercospora* sp. while *A. eichhorniae* did not cause disease symptoms on any of the plants. This study suggests that both pathogens have potential for biological control of water hyacinth, but *A. eichhorniae* is safer to use than *Cercospora* sp.

Keywords: Fungal pathogens, water hyacinth control.

INTRODUCTION

Water hyacinth is an aquatic weed problem worldwide and rated as the world's worst weed (Holm *et al.*, 1977). It occurs in tropical regions between latitudes 40°N and 45°S and has been spread by man as an ornamental plant from its area of origin in Brazilian Amazonia. The weed was reported in Africa during the ancient Egyptian times between 1879 and 1892 (Gopal and Sharma, 1990) and its detrimental effects on the livelihood of people have been extensively reviewed (Gopal, 1987); (Pieterse *et al.*, 1996).

Successful control of the water hyacinth has been carried out using two weevils, *Neochetina eichhorniae* Warner and *N. bruchi* Haustache that have already established in various countries (Julien, 1992). The use of weevils was adopted in Uganda in 1993 through importation of both species from the International Institute of Tropical Agriculture, Benin station. The weevils became widely distributed throughout the range of water hyacinth and contributed to the decline of the weed problem in most locations (Ogwang and Molo, 1999). Efforts have been made to integrate weevils with different weed control measures to improve on the control strategy. Studies conducted in the United States indicate increased effectiveness of the weevils when used in combination with herbicides (Charudattan, 1986).

The potential of fungal pathogens as myco-herbicides for integration in biological control of water hyacinth has also been extensively investigated. In Egypt, promising fungal strains attacking water hyacinth have been identified (Shabana *et al.*, 1995); Elwakil *et al.*, 1988). In East Africa, adequate knowledge about potential pathogens of water hyacinth is still lacking. Surveys have been conducted in Kenya and several pathogens of water hyacinth have been identified, but their potential role have not been fully investigated (KARI, 1997). Similar surveys have been initiated in Uganda. This study was aimed at identifying and evaluating potential pathogens associated with water hyacinth in Uganda and determining their host range on cultivated plant species.

MATERIALS AND METHODS

Surveys and isolation of fungi

Surveys were conducted in lakes Victoria, Kyoga and River Nile by boat during July 2000 and May 2001. Water hyacinth plants in weed mats were examined for disease symptoms like necrotic spots, leaf lesions, browning and

wilting. The leaves showing disease symptoms were collected from three sampling points per site and for each disease symptom, 3-5 leaves were collected. The leaves were put in envelopes and taken to the laboratory for isolation of pathogens. Four infected tissues (about 5 mm diameter) were cut from each leaf and surface sterilized using 70% alcohol. They were plated on Potato dextrose agar (PDA) and isolates were pure cultured on 25% strength PDA.

The culture of each isolate was made into a strong suspension containing $1.2x10^7$ conidia/ml using distilled water. The suspensions were applied on water hyacinth plants grown in plastic buckets filled with 5 litres of tap water using hand sprayer, until the foliage was fully wetted. The plants were covered with polythene bags for 48 hours to maintain humidity and observed for 10 days for appearance of disease symptoms on the leaves. The isolates which proved pathogenic were rated on the basis of severity of the disease symptoms as follows: + = small and few spots, light infection; ++ = small and many spots, moderate infection; and +++ = many large spots, severe infection.

Comparative evaluation studies of fungal isolates

Two isolates of *Cercospora* sp. and *Alternaria eichhorniae* (Nag Raj and Ponnappa) were selected and 3-week old cultures were made into suspensions containing $1.2x10^7$ conidia/ml in distilled water and Tween 80 was added to the suspensions (one drop/litre). The suspensions were applied on water hyacinth plants grown in plastic buckets and the plants were maintained in the screen house as described in the previous experiment. Disease incidence was measured weekly for 4 weeks by counting the number of infected leaves on each plant and expressed as a percentage of infected leaves. The disease severity was also measured by scoring lesion development on each leaf on a scale 0-9 as in (Charudattan *et al.*, 1985). Control plants were sprayed with sterile distilled water only. All treatments were replicated three times. The plants in each bucket were also drained of excess water and weighed individually, and the number of living leaves and number of daughter plants produced by each plant in each treatment was also counted.

In a separate experiment, 3-week old cultures of each isolate were made into suspensions containing $1.2x10^7$, $1.2x10^6$, $1.2x10^5$ and $1.2x10^4$ conidia/ml using distilled water and Tween 80. The respective concentrations were applied on the water hyacinth plants grown in plastic buckets and the plants were maintained as described in the previous experiment. The disease infection caused by

each conidial concentration was estimated weekly for 4 weeks after inoculation. The treatments were replicated three times.

Effect of isolates on cultivated plant species

Seeds of 8 cultivated plant species were planted in plastic bowls (30 cm diameter) in the screen house in 1000 g of heat sterilised soil media supplemented with 10 g of N.P.K fertilizer. After sprouting, the seedlings were thinned to leave 6 plants per bowl. Banana suckers were also planted singly in polythene bags and left to establish for three weeks. About 5 mm diameter agar blocks of inoculum of each isolate of *A. eichhorniae* and *Cercospora* sp. were obtained from 3-week old cultures grown on PDA and placed on the upper leaf surface of 2-week old seedlings and banana plants. Some leaves were inoculated with sterile agar blocks as control. The treated plants were covered with transparent polythene bags and maintained as described in the previous experiment. They were observed one week after inoculation and weekly thereafter for 4 weeks for susceptibility to the diseases.

Statistical analysis

All data were analyzed using the Statistical Analysis System program (Statistical Analysis Systems) SAS (1985). Percentage disease incidence and disease severity scores were transformed into arc sines and square roots (x+0.5) respectively before statistical analysis. Levels of significance were determined by 'F' values using Proc ANOVA and means compared using Tukey's test at a probability level of 0.05. The relationship between plant growth parameters and time was estimated by linear regression.

RESULTS

Identification of fungal pathogens

A total of six fungal pathogens were isolated from water hyacinth, of which *Alternaria eichhorniae* , *Cercospora* sp. and *Acremonium zonatum* (Saw.) were found to be highly pathogenic (Table I). They caused necrotic spots, which enlarged to various sizes on the leaves of water hyacinth plants and symptoms appeared between 5 and 6 days after inoculation. Other isolates which proved pathogenic are still awaiting identification.

Table I: List of fungal pathogens collected and disease reaction to water hyacinth plants in the screen house.

Isolate Code	Scientific name	Disease Reaction
MP-N-001	*Cercospora sp.*	+++
KA-N-001	Awaiting identification	+
NA-V-001	*Alternaria eichhorniae*	+++
G-V-002	Awaiting identification	+
MA-V-012	Awaiting identification	+
DR-V-004	*Acremonium zonatum*	+++

Disease incidence and severity of *A. eichhorniae* and *Cercospora* sp. applied as suspensions of the isolates on water hyacinth plants are presented in Figures 1 and 2. Both pathogens caused substantial disease infection during the study period and the infected plants showed clear necrotic disease symptoms on the leaves, which progressed with time. No disease symptoms were observed on the control plants.

Alternaria eichhorniae caused higher disease incidence than *Cercospora* sp. in the third week of inoculation (Fig. 1), although there was no significant difference ($P>0.05$, $df=11$, $F=1.2$) in disease incidence between these isolates. After 28 days, the disease incidence increased to over 80 % and was the same for both isolates. Overall there was no significant ($P>0.05$, $df=24$, $F=0.94$) difference in disease incidence between the isolates.

Alternaria eichhorniae instead caused lower disease severity than *Cercospora* sp. after 21 days (Fig. 2), but the disease severity increased to maximum score over 1.6 after 28 days and were the same both isolates. By this time, infected leaves of some water hyacinth plants were beginning to die. The overall disease severity caused by the isolates was however not significant ($P>0.05$, $df=24$, $F=0.62$).

The effects of isolates on growth characteristics of inoculated water hyacinth plants are presented in Table II and Fig. 3. The plants treated with isolates showed significant ($p<0.05$) reduction in overall fresh weight and no significant effects on number of living leaves and number of daughter plants (Table II). The fitted regression lines of mean plant weight versus time after inoculation showed linear relationships in all treatments, and regression analysis showed

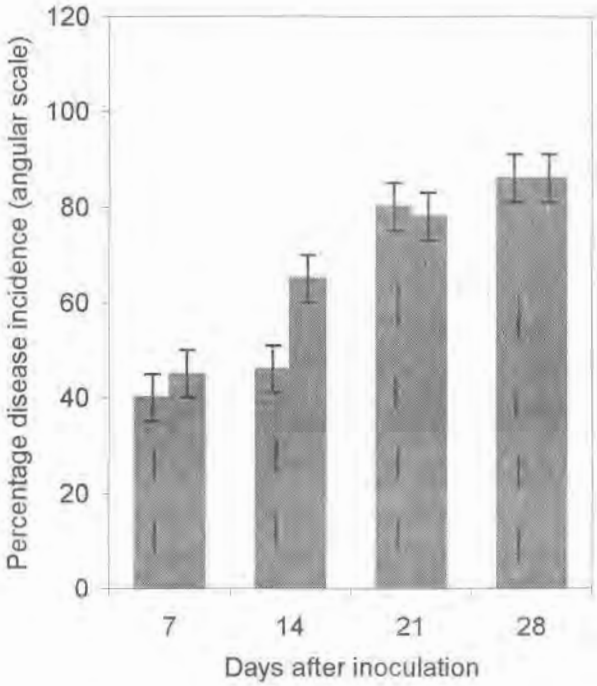

Figure 1. Percentage disease incidence after inoculation of water hyacinth plants with isolates (= *Alternaria eichhorniae*; = *Cercospora* sp.) at conidia concentration $1.2x10^7$ conidia ml^{-1}. Vertical bars indicate ± standard errors

significant ($p<0.05$) decrease in fresh plant weight with time in inoculated compared to the control plants (Fig. 3), and there was no ($p>0.05$) significant difference in plant weight between the treated plants.

Disease incidence increased with increasing conidia concentration in plants treated with either *A. eichhorniae* or *Cercospora* sp. (Fig. 4). The disease incidence was significantly ($P<0.05$) greater at the two highest concentrations than in the two lowest conidia concentrations on plants treated with *A. eichhorniae* or *Cercospora* sp. There was no significant difference ($P>0.05$) between the two highest conidia concentrations or the two lowest conidia concentrations. A similar trend was observed for disease severity. There was no significant difference ($P>0.05$) in disease severity between the two highest concentrations or the two lowest concentrations, although it was significantly ($P<0.05$) greater at the two highest concentrations than in the two lowest conidia concentrations (Fig. 5).

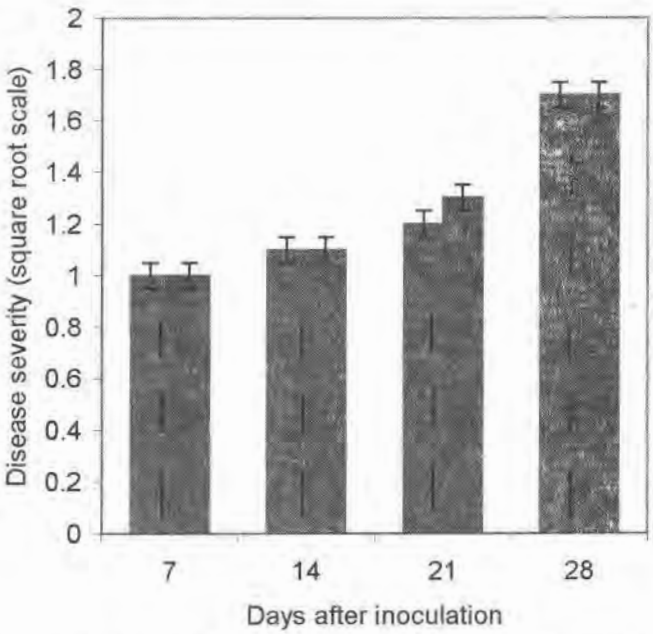

Figure 2. Disease severity after inoculation of water hyacinth plants with isolates (= *Alternaria eichhorniae*; =*Cercospora* sp.) at conidia concentration $1.2x10^7$ conidia ml^{-1}. Vertical bars indicate ± standard errors.

Table II: Susceptibility of some cultivated plant species to isolates of *A eichhorniae* and *Cercospora* sp. based on the appearance of disease symptoms on the leaves

Family/Species	Common name	Disease reaction[b]	
		A. eichhorniae	*Cercospora* sp.
Fabaceae			
Glycine max (L.) Merr.	Soybean	-	-
Vigna unguiculata (L.) Walp	Cowpea	-	-
Arachis hypogaea (L.)	Peanut	-	-
Pedaliaceae			
Sesamum indicum (L.)	Sesame	-	-
Poaceae			
Zea mays (L.)	Maize	-	-
Oryza sativa (L.)	Rice	-	-
Eleusine indica (L.)	Finger millet	-	-
Sorghum valgare (L.)	Sorghum	-	+
Musasea			
Musa sp.	Banana	-	-

[b] - = symptoms absent; + = symptoms present

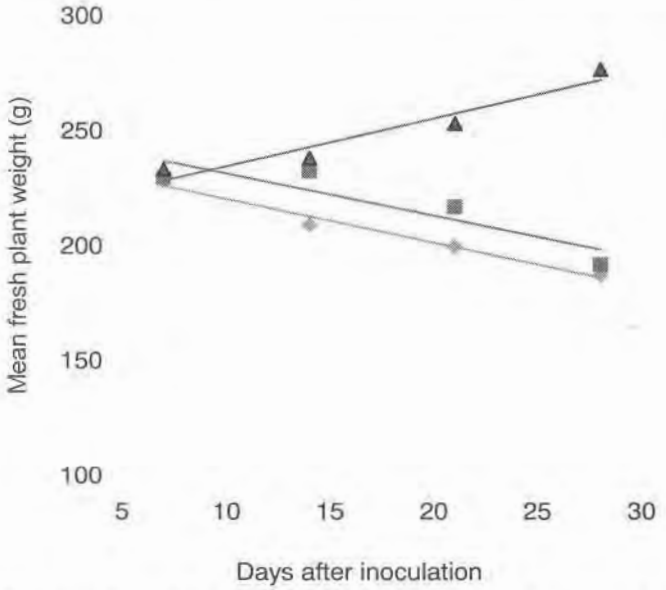

Figure 3. Relationship of fresh plant weight and time after inoculation with isolates (= *A. eichhorniae*; = *Cercospora* sp; and =healthy plants).

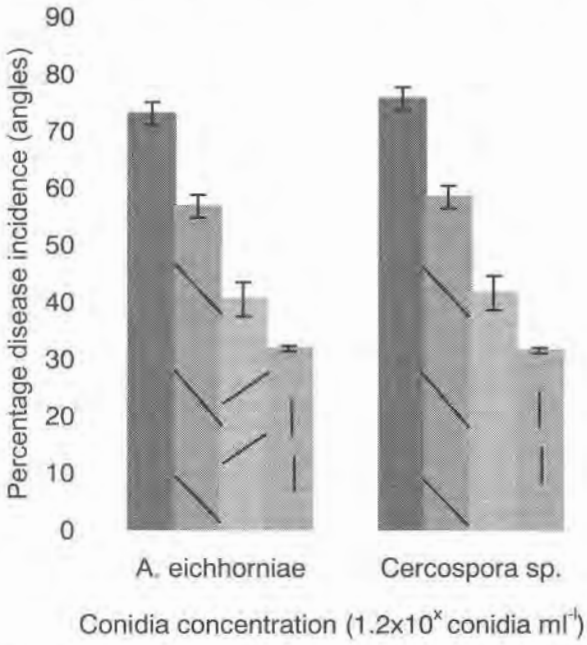

Figure 4. Dose-dose incidence relationship of isolates on water hyacinth plants 28 days after inoculation at four conidia doses (= $1.2x10^7$; = $1.2x10^6$; = $1.2x10^5$; = $1.2x10^4$). Vertical bars indicate ± standard errors

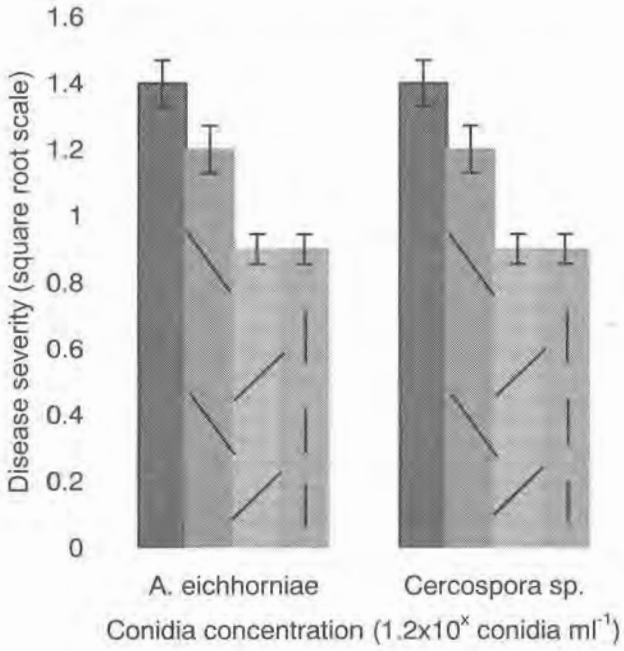

Figure 5. Dose-dose severity relationship of isolates on water hyacinth plants 28 days after inoculation at four conidia doses (= $1.2x10^7$; = $1.2x10^6$; = $1.2x10^5$; = $1.2x10^4$). Vertical bars indicate ± standard errors

Effect of isolates on cultivated plant species

The results of the host specificity tests of the two isolates are presented in Table II. The disease symptoms caused by *Cercospora* sp. appeared on sorghum leaves after 3 days of inoculation. The leaf necrosis increased with time and after 7 days, some of the infected leaves had died. There were however no apparent disease symptoms on the plant species treated with *A. eichhorniae*.

DISCUSSION

A total of six fungal pathogens were collected from Lakes Victoria, Kyoga and River Nile, but only *A. eichhorniae*, *Cercospora* sp. and *Acremonium zonatum* were found to be highly pathogenic. These pathogens have previously been reported in other countries (Nag Raj and Ponnappa, 1970; Conway, 1976; Abel and Tawfiq, 1985; Elwakil *et al.*, 1988 and Morris, 1990) and they are considered as potential myco-herbicide agents for integration in biological control of water hyacinth.

Comparative studies of efficacy conducted between *A. eichhorniae* and *Cercospora* sp. showed that both isolates were highly pathogenic to water hyacinth and the infected plants showed clear disease symptoms. The pathogens however did not cause mortality to water hyacinth plants after 28 days of inoculation, but exhibited a reduction in fresh weight of plants. This observation confirms other findings with different strains of *A. eichhorniae* (Shabana *et al.*, 1995). The reduction in plant weight was presumably attributed to the severe stress caused by the pathogens to the plants, which affected the ability of mature plants to produce fresh leaves and new plants. In virulent strains of *A. eichhorniae,* greater stress is produced by production of phyto-toxins, compounds that accelerate cell death and leaf necrosis (Shabana *et al.*, 2001). The ability to cause stress to the plants may however decline when the pathogens are repeatedly sub-cultured in the laboratory (Nyvall and Hu, 1997).

The highest conidia dose of $1.2x10^7$ was the most effective in causing the disease infection in water hyacinth plants and disease infection declined with reducing conidia concentrations. A positive linear relationship between inoculum concentration and disease infection has also been reported for other potential myco-herbicide agents (Walker and Tilley, 1997). At low conidia doses, disease infection also occurred. The susceptibility of even at low conidia doses may be attributed to the physiological state and age of the plants. These pathogens that can cause disease infection at even low conidia doses are considered potentially most promising agents for myco-herbicide development (Spotts and Cervantes, 1991).

Sorghum was found to be highly susceptible to *Cercospora* sp. when plants were inoculated using agar blocks containing mycelia. The necrosis appeared on the plants 3 days after inoculation. *Cercospora* disease has been reported in many plant species and this isolate could therefore be closely related to *Cercospora sorghi* which has been reported in sorghum (Mansuetus, 1995). Sorghum is a very important staple food crop in Eastern and South Western Uganda. The usefulness of *Cercospora* sp. to control water hyacinth has however been demonstrated in the field (Charudattan *et al.*, 1985). Therefore this pathogen could still be developed for restricted application in the water environment where water hyacinth occurs.

The plants treated with *A. eichhorniae* did not show any disease symptoms. The lack of susceptibility of cultivated plants tested in this experiment to *A. eichhorniae* confirms the findings of (Nag Raj and Ponnappa, 1970 and Shabana

et al., 1995) and suggests that the pathogen has a narrow host range. The level of host specificity exhibited by *A. eichhorniae* however makes evaluation of this fungus as a biological control agent more promising than *Cercospora* sp.

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