

# CONTROL OF PADDY WEEDS BY PLANT PATHOGENS IN THE PHILIPPINES

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## ABSTRACT

*Bioherbicide research in the Philippines is still in the discovery and development phase. Survey, isolation and pathogenicity tests of isolates revealed the presence of indigenous fungal pathogens with great potential as bioherbicides to control some paddy weeds. The present emphasis of the research is on a leaf blight pathogen which infects gooseweed (*Sphenoclea zeylanica*).*

## INTRODUCTION

Rice (*Oryza sativa* L.) is the staple food of most Filipinos. The land area planted in rice in the Philippines is about 3.2 million ha, which is roughly 23% of the total agricultural land in the country. About 1.66 million ha (52%) of rice are irrigated, while about 1.3 million ha (44%) are rainfed. The remaining 4% is upland rice (Medina 1990).

Weeds are a major limiting factor in rice production in the Philippines. Depending on the kinds of weeds present, their abundance, the type of rice culture, and the time of competition, yield losses ranging from 16 to 100% have been observed (Lubigan and Vega 1971, Madrid *et al.* 1972, Mercado and Talatala 1977, Sarkar and Moody 1981). Commonly used weed control strategies are water management, hand weeding, mechanical weeding and chemical herbicides. Water management can control certain weed species in irrigated lowland rice. However, as mentioned above, only half the total area planted in rice is irrigated. Hand weeding is time-consuming and is becoming expensive, while the use of mechanical weeders is known to reduce yields. Chemical herbicides, on the other hand, not only are becoming more expensive, but also contribute to environmental pollution. Continuous use of chemical herbicides can result in the development of herbicide-tolerant weed populations. There are indications that certain

populations of *Sphenoclea zeylanica* in the Philippines have already developed tolerance to 2,4-D (Sy and Mercado 1983, Migo *et al.* 1986, Mercado *et al.* 1990). These shortcomings or limitations of the existing weed control strategies have rekindled interest in biocontrol, using indigenous control agents. The use of indigenous biocontrol agents will promote self-reliance, and help alleviate the difficult economic situation in the Philippines.

Plant pathogens are biocontrol agents with tremendous potential, as shown by the success of DeVine and COLLEGO in controlling specific target weeds in the U.S.A. DeVine, a liquid formulation of *Phytophthora palmivora* (Butl.) Butl., was registered in 1981 for the control of strangler vine (*Morrenia odorata* (H. & A.) Lindl.) in Florida citrus groves. COLLEGO, a powder formulation of *Colletotrichum gloeosporioides* (Penz.) Sacc. f. sp. *aeschynomene*, was registered in 1982 for the control of northern jointvetch (*Aeschynomene virginica* (L.) B.S.P.) in rice and soybeans in Arkansas, Louisiana, and Mississippi (Watson 1991).

While the prospect of using plant pathogens as bioherbicides in the Philippines is excellent, since environmental conditions in the country are relatively favorable, some sectors of the scientific community are still skeptical about the effectiveness and environmental safety of bioherbicides.

Key words: Bioherbicide, biological control, paddy weeds, plant pathogens, weed management

## DEVELOPMENT OF BIOHERBICIDE RESEARCH IN THE PHILIPPINES

Studies on the biocontrol of weeds using plant pathogens in the Philippines are still very limited. Research by the principal author, Dr. Bayot, on biological control of selected weeds using endemic plant pathogens started in 1989, at the National Crop Protection Center (NCPC), University of the Philippines at Los Baños (UPLB), with funding from the UPLB Basic Research Program. A co-author, Dr. Alan Watson came to the International Rice Research Institute (IRRI) in the middle of 1990 and established a collaborative research program with appropriate organizations and institutes.

Major paddy weeds such as jungle rice (*Echinochloa colona*) (L.) Link), barnyard grass (*E. crus-galli* (L.) P. Beauv.), pickerel weed (*Monochoria vaginalis* (Burm. f.) Kunth), small flower umbrella sedge (*Cyperus difformis* L.), rice flatsedge (*C. iria* L.), gooseweed (*Sphenoclea zeylanica* Gaertn.) globe fingerush (*Fimbristylis miliacea* (L.) Vahl), and giant sensitive plant (*Mimosa invisa* Mart.) were selected as primary targets for this research.

This paper presents the highlights of our collaborative research on the control of paddy weeds by plant pathogens in the Philippines, with an emphasis on *S. zeylanica*.

The objectives of this study were: 1) to survey paddy weeds for disease symptoms and to isolate the causal organisms, and 2) to evaluate selected pathogens of weeds for their biocontrol potential.

### METHODOLOGY

#### Collection of Diseased Materials and Isolation of Pathogens

Major target weeds with disease symptoms were collected from rice fields on the IRRI experimental farm and in Central Luzon. The pathogens were isolated using standard isolation techniques. Pure cultures were maintained in storage.

#### Pathogenicity Testing of Isolates

##### Testing of *S. zeylanica* Pathogens

Healthy young seedlings (at the 4- to 6- leaf stage) of *S. zeylanica* were collected from paddy fields, transplanted into pots and maintained in the greenhouse. A spore suspension of the blight pathogen was prepared from 10- to 12-day-old

cultures, filtered through nylon cloth, and the concentration adjusted to  $3 \times 10^4$  and  $1 \times 10^7$  spores per mL. Spores of the leafmold pathogen were collected from heavily infected leaves, because the pathogen does not sporulate in culture. Healthy seedlings were inoculated with the spore suspension either by rubbing the inoculum onto leaves with the fingers, or by removing the waxy leaf covering with the fingers and applying the spore suspension by dripping it or spraying it with an atomizer onto the leaves. Fifty percent of inoculated seedlings were placed in a moist chamber for 24 hours, and the rest were kept under natural conditions. Uninoculated plants served as a check. Plants were then observed for the appearance of symptoms.

##### Testing of Other Pathogens on Their Respective Hosts

Weed seedlings were either grown from seeds or collected directly from paddy fields and maintained in the greenhouse. Host weed species were inoculated with cultures of microorganisms isolated from target weeds, using a spore concentration of about  $10^7$  spores per mL. Grasses and sedges were inoculated by spraying with a hand sprayer, while broadleaved weeds were inoculated by spraying or dripping spore suspension onto the leaves. After inoculation, plants were placed in a dew chamber for 24 hours and then transferred to the mist room for disease development.

#### Determining the Specificity of the Leaf Blight Pathogen

Seedlings of rice (IR 56), corn, wheat, sorghum, okra, tomato, potato, mungbean, soybean, *Monochoria vaginalis* and *S. zeylanica* were sprayed with  $10^5$  spores per mL of the leaf blight pathogen, using a hand sprayer. Inoculated plants were placed in a moist chamber for 2 days, and then transferred to the greenhouse for observation of disease symptoms.

The pathogen was also tested on IR 58 and IR 68 rice varieties, under natural conditions and in a controlled environment at IRRI. The seedlings were sprayed with a spore suspension of the pathogen at a rate of  $10^7$  spores per mL, in the presence of flowering and non-flowering *S. zeylanica* plants. IR 56 seedlings were inoculated when they were 30 and 45 days old, while IR 68 seedlings were inoculated when they were 21 days old. After inoculation, 50% of the plants were kept under natural conditions, and the remaining 50% were

placed in a dew chamber for 24 hours at 25°C, and then transferred to the mist room for possible disease development.

### Field Testing of the Leaf Blight Pathogen for Herbicidal Activity

A paddy field belonging to the IRRI Central Research Farm, where there was a dense population of *S. zeylanica*, was selected as the test site. The area was divided into plots of 50 cm x 50 cm. The concentration of the spore suspension was determined using a hemacytometer, and adjusted to 10<sup>3</sup>, 10<sup>5</sup>, and 10<sup>7</sup> spores per mL. Tween 40 was added to the spore suspension to give a final concentration of 0.02%. Each treatment was replicated 4 times, and the plots were arranged in a randomized complete block design. Each plot of 0.25 m<sup>2</sup> was sprayed with 150 mL of inoculum at 5:00 P.M. Control plots were sprayed with water. Disease severity was assessed 3 days after inoculation, and weed mortality 7 days after inoculation. After four weeks, the parts of the *S. zeylanica* plants above the soil surface, were harvested, dried inside paper bags for 48 hours at 80°C, and weighed.

## RESULTS AND DISCUSSION

### Pathogenicity of Isolates

The results of pathogenicity tests of fungi isolated from target weeds are summarized in Table 1. Some isolates show tremendous potential as bioherbicides. Among the paddy weeds studied, *S. zeylanica* was observed to be the most vulnerable to infection, so there is probably a good chance of

being able to control it with a pathogen.

Leaf blight and leaf mold are two fungal diseases which are commonly observed attacking *S. zeylanica* in the Philippines. The leaf blight pathogen is the most promising biocontrol agent, because of its aggressiveness. Its spores germinate within six hours, and have penetrated into leaf tissue within 12 to 16 hours after inoculation. *S. zeylanica* seedlings developed blight symptoms only 1 to 2 days after being sprayed with a solution containing approximately 30,000 spores per mL. Inoculated seedlings were dead after 5 days. Even when *S. zeylanica* plants have reached the flowering stage, they can still be killed by the blight pathogen.

The leaf mold pathogen does not produce spores in culture, and is less aggressive than the leaf blight pathogen. It seemed to cause serious symptoms only in older or senescent plants, while disease progress in younger plants was slow. Leaf mold symptoms appeared 12 to 14 days after *S. zeylanica* seedlings were sprayed with spores gathered from heavily infected leaves.

### Host Range

Greenhouse tests showed that the leaf blight pathogen could not infect rice (IR 56), corn, wheat, sorghum, okra, tomato, potato, soybean or mungbean. It was able to infect *M. vaginalis*, causing severe leaf spot, but it was not able to kill the plants. Further testing of the leaf blight pathogen under natural conditions and in a controlled environment showed that increasing the spore concentration to 10<sup>7</sup> cells per mL still did not cause infection in IR 58 and IR 68 seedlings, although *S. zeylanica* plants died after 3 to 5 days.

The effects of the leaf blight pathogen on

Table 1. Pathogenic fungal isolates collected from major paddy weeds in the Philippines

Target weed	No. of isolates tested	No. of pathogenic isolates	No. of bioherbicide prospects
<i>Cyperus difformis</i>	2	0	0
<i>Cyperus iria</i>	21	7	1
<i>Echinochloa colona</i>	25	5	1
<i>Echinochloa crus-galli</i>	9	2	0
<i>Fimbristylis miliacea</i>	2	0	0
<i>Mimosa invisa</i>	1	1	1
<i>Monochoria vaginalis</i>	10	2	0
<i>Sphenoclea zeylanica</i>	3	1	1

direct-seeded rice still need to be determined. This will be done by inoculating very young seedlings (2 to 5 days after emergence) with a high spore concentration of the pathogen. It is also necessary to test other plants known to be susceptible to plant pathogens that closely resemble the blight pathogen *S. zeylanica*.

### Mortality from the Leaf Blight Pathogen

*S. zeylanica* plants inoculated with  $10^7$  spores per mL had already developed blight or wilt symptoms less than 24 hours after inoculation. After six days, most of the target weeds had dry or rotting stems and had died. Disease was less severe in plots sprayed with a lower spore concentration, as shown by the higher dry weight of target weeds in Table 2.

The lower disease severity and slower disease progress observed in outdoor plots sprayed with  $10^5$  spores per mL compared to plants given the same treatment in greenhouse tests is probably because of the rapid drying of inoculum in the field, even if inoculation was done late in the afternoon.

These results suggest that leaf blight pathogen has considerable potential as a biocontrol agent against *S. zeylanica*. However, more rigid tests must be undertaken to ensure that the biocontrol agent is safe to humans, economic crops and the environment. Once it is developed as a bioherbicide, its use must be viewed "not as an alternative control strategy, but as a complementary tactic in integrated weed management programs" (Watson 1992).

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Table 2. Dry weight of *S. zeylanica* four weeks after being sprayed with varying spore concentrations of leaf blight fungus

Conidia/ml.	Dry weight (g/0.25 m <sup>2</sup> plot) <sup>a</sup>
0	30.87
$1 \times 10^3$	25.87
$1 \times 10^5$	19.62
$1 \times 10^7$	0.54

a Each figure is the average of four replications

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## DISCUSSION

Dr. Bayot was asked the scientific name of the plant pathogen causing the leaf blight. He replied that the species had not yet been identified, but was thought to belong to the genus *Alternaria*. Several participants were also interested in technical points concerning mass production in culture: whether spores formed within the medium or on the surface, and how the homogenizer was used to collect spores. Dr. Bayot explained that the spores were produced in a still culture using broth made from potato dextrose or coconut water, and that the spores formed on the surface. After 10-12 days, the culture is blended, then filtered to remove the mycelia, and the spores are used to inoculate the weed plants. Asked whether there was any published data available in the literature on the plant pathogens, Dr. Bayot replied that there is none on the leaf blight pathogen, although some information is available on the leaf mold pathogen. However, the mold does not seem to be suitable as a biological control agent, because it does not produce spores in culture and the disease is not very virulent. In contrast, spores are easily produced by the leaf blight pathogen within 8-10 days and these cause rapid infection.

Dr. Kim was interested in the possibility of toxins in the medium, and asked if Dr. Bayot had attempted to sterilize the culture before spraying it onto plants. Dr. Bayot described an early series of experiments in which he had cultured the pathogen for only 2-3 weeks and sprayed the broth directly onto the plants without concentrating the broth, but this did not cause any damage. When he had subsequently prolonged the growing period to two months, and concentrated the broth by warming it, there had been some damage in leaves exposed to it, indicating some toxicity.