J. Aquat. Plant Manage. 43: 105-107

Natural Suppression of the Aquatic Weed, *Salvinia molesta* D. S. Mitchell, by Two Previously Unreported Fungal Pathogens

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INTRODUCTION

Salvinia molesta D. S. Mitchell (Salviniaceae), variously called giant salvinia, water fern or African payal, is a vegetatively reproducing, perennial, free-floating, aquatic weed, native to southeastern Brazil (Waterhouse and Norris 1987). It (hereafter called salvinia) is a very serious weed in most regions outside its native range (Harley and Mitchell 1981) including India. Although there are different views on the entry and introduction of salvinia into India, it was first observed in the 1950s in Veli Lake, Trivandrum (now Thiruvananthapuram, Kerala), and in 1964 it assumed pest status (Joy 1978). It has both direct and indirect effects on the aquatic environment, especially due to its habit of choking rivers, canals, lagoons and other water bodies. The most-affected crop plant is rice, where salvinia infestations can interfere with cultivation and reduce yields by competing for available nutrients (Singh 1989). As a classical biological control measure, two insect species were introduced into India to suppress salvinia. Although the performance of the grasshopper, *Paulinia acuminata* De Geer, imported from Trinidad in 1974, was not satisfactory (Joy et al. 1981), *Cyrtobagous salviniae* Calder & Sands of Brazilian origin introduced in 1982 from insect populations in Australia has been successful (Jayanth 1987). Notwithstanding this, the need for additional agents is highly desirable because salvinia remains problematic in India. Intensive surveys were conducted in different parts of the country to identify pathogens with potential to act as biological control agents of the weed. The purpose of this paper is to report on two fungal pathogens that were found to be the cause of a sudden decline in salvinia in Bangalore.

MATERIALS AND METHODS

In the process of maintaining *C. salviniae* colonies at the Project Directorate of Biological Control, Bangalore, healthy salvinia plants were brought from water bodies in and around

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Thrissur, Kerala, and from the Indian Institute of Horticultural Research, Bangalore in August 1998. During June 1999, the cultures (most of them 'tertiary forms') were suppressed due to extensive fungal growth. This ultimately brought about a total decline of the mats in all the culture tanks.

A close observation of the affected plants revealed the presence of extensive fungal hyphae along leaf margins as well as on the laminae. Plant samples were collected on 11 June 1999 for isolating and studying the associated organism(s). Plating of surface-sterilized, excised diseased leaf tissues on tap-water agar (agar, 18 g; streptomycin sulphate, 0.2 g; tap water, 1 L) yielded two different fungi when incubated at 25°C in an incubator set to a 12-h photoperiod. Single-conidial isolates of the two species were propagated on Czapek-Dox agar (HiMedia, Mumbai, India) to study morphological and growth characteristics in culture for preliminary identification of the species.

The two fungal species were assayed separately for pathogenicity. Mycelial and conidial suspensions of each pathogen were prepared by adding 20% w/v or 1×10^6 and 1×10^7 conidia/ml, respectively to sterile distilled water containing 0.05% Tween 20. Mycelia of each species were grown separately in 250-ml Erlenmeyer flasks each containing 100 ml of potato dextrose broth (HiMedia) for 5 days on a rotary shaker at 200 rpm under room conditions (28 ± 2°C). The submerged mycelium thus obtained was filtered through four layers of cheesecloth and blended in a mixer for 30 seconds and used for preparing the mycelial suspension. Conidia were obtained from sporulating cultures grown on homemade potato dextrose agar in 90-mm petri plates by flooding them with sterile distilled water. Final conidial counts were assessed with a haemocytometer. Salvinia plants used in the experiments were 'secondary forms' that were grown under partial shade conditions in 17-cm diameter (inner brim) plastic pots containing tap water up to 5 cm from the brim. The test plants were removed from the water and spread over a sheet of filter paper before spraying the inoculum from a close distance till run off. Sprayed plants were put back into the respective pots for incubation and further observations. Plants sprayed with only sterile water containing the surfactant served as controls. Each treatment was replicated 10 times. Fifteen days post inoculation, 30 pairs of opposite leaves per pot were randomly assessed for percent disease incidence. A pair of floating leaves was considered to be diseased only when both leaves were totally covered with lesions as originally observed under natural conditions. Observations were continued further for another 15 days. Pots were replenished with tap water as needed to maintain the original level.

RESULTS AND DISCUSSION

Mycelial suspensions of both the fungi were ineffective in causing disease in salvinia (Table 1). In all likelihood the mycelium dried on the plant surfaces and rendered it inactive before it could invade the host tissues. The two conidial concentrations were infective and induced disease. The two fungi induced at first water-soaked lesions, which later expanded to form larger patches. As disease progressed mycelial growth was evident along the leaf margins leading to drying of leaf tissues. By 30 days post inoculation, severely infected plants started to disintegrate and collapse into the water. Both fungi could be reisolated and were able to subsequently induce disease upon fresh inoculation to healthy salvinia plants, thus satisfying Koch's postulates. However, the disease induced artificially was not as severe as that observed in the culture tanks in 1999. It is conjectured that lower concentrations of inoculum combined with incubation conditions were less than optimum for disease development.

The two isolates designated as WF(Sm)37 and WF(Sm)38 were identified as *Phoma glomerata* (Cda) Wollenw. & Hochapf. and *Nigrospora sphaerica* (Sacc.) Mason, respectively. The same fungal species were isolated in subsequent years, though their impact on the weed was not as damaging as in 1999. There is no previous record of these two fungi as pathogens of salvinia anywhere in the world. Prior to the present work, the association of only two fungi, *Myrothecium roridum* Tode ex Fries with decaying mats in Kakki Reservoir, Sabarigiri, Kerala and *Verticillium nigrescens* Pethybridge with living salvinia leaves in Bangalore (Ponnappa 1977) was known in India. Recently, *Rhizoctonia solani* Kühn has been implicated as a pathogen of both *S. molesta* and *S. minima* Baker in Florida, USA (Rayachhetry et al. 2002).

The pathogens reported herein merit further consideration and evaluation, in spite of their ubiquitousness. However, commercialization of *P. glomerata* or *N. sphaerica* as mycoherbicides for salvinia will require additional research on host-specificity, strain variations, efficacy, mass production and formulation.

TABLE 1. EVALUATION OF TWO NEWLY IDENTIFIED PATHOGENS AGAINST *SALVINIA MOLESTA*.

Inoculum	Percent disease incidence	
	Phoma glomerata	*Nigrospora sphaerica*
Mycelial suspension (20% w/v)	3.3 (7.2)[a]	3.0 (7.6)
Conidial suspension (1×10^6 conidia/ml)	29.3 (32.8)	25.0 (29.9)
Conidial suspension (1×10^7 conidia/ml)	62.0 (52.1)	53.3 (47.0)
SEM[b] (±)	2.1	2.0
CD[c] ($P = 0.05$)	6.2	5.8

[a]Data in parentheses are arcsine-transformed values.
[b]Standard error of mean.
[c]Critical difference.

ACKNOWLEDGMENTS

The financial help provided by the Department of Biotechnology, Government of India, in the form of a project for establishing a National Repository of Natural Enemies of Crop Pests and Weeds is gratefully acknowledged. Thanks are due to Dr. Alaka Pande, Agharkar Research Institute, Pune, for the identification of the two fungal species.

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