INFORMATION TO USERS

This manuscript has been reproduced from the microfilm master. UMI films the text directly from the original or copy submitted. Thus, some thesis and dissertation copies are in typewriter face, while others may be from any type of computer printer.

The quality of this reproduction is dependent upon the quality of the copy submitted. Broken or indistinct print, colored or poor quality illustrations and photographs, print bleedthrough, substandard margins, and improper alignment can adversely affect reproduction.

In the unlikely event that the author did not send UMI a complete manuscript and there are missing pages, these will be noted. Also, if unauthorized copyright material had to be removed, a note will indicate the deletion.

Oversize materials (e.g., maps, drawings, charts) are reproduced by sectioning the original, beginning at the upper left-hand corner and continuing from left to right in equal sections with small overlaps.

Photographs included in the original manuscript have been reproduced xerographically in this copy. Higher quality 6" x 9" black and white photographic prints are available for any photographs or illustrations appearing in this copy for an additional charge. Contact UMI directly to order.

Bell & Howell Information and Learning
300 North Zeeb Road, Ann Arbor, MI 48106-1346 USA
800-521-0600

UMI®

Pathogenicity of Three *Curvularia* Isolates to Cyperaceae Weeds and Rice (*Oryza sativa* L.)

Lilian Z. de Luna

Department of Plant Science

Macdonald Campus of McGill University

Montréal, Québec, Canada

March 1999

A Thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements of the degree of Doctor of Philosophy

Short title:

Pathogenicity of *Curvularia* Isolates to Sedge Weeds and Rice

Lilian Z. de Luna

ABSTRACT

Three isolates of *Curvularia* belonging to *Curvularia tuberculata* (isolates 93-020 and 93-022) and *Curvularia oryzae* (isolate 93-061) were obtained from diseased *Cyperus difformis*, *Cyperus iria*, and *Fimbristylis miliacea*, respectively, in the Philippines in 1993. Under greenhouse conditions, these fungal isolates caused high mortality and significant plant dry weight reduction in *C. difformis*, *C. iria*, and *F. miliacea* when sprayed at the rate of 1 x 10^8 spores/m^2. Cross-pathogenicity of the isolates was demonstrated in three other sedge weed species. *C. difformis*, *C. iria*, and *F. miliacea* were killed but *C. rotundus* was resistant. Most of the thirteen rice varieties tested were resistant to the fungal isolates. The order of decreasing pathogenicity to rice was *C. oryzae* (93-061), *C. tuberculata* (93-020), and *C. tuberculata* (93-022). The infection process of *C. tuberculata* and *C. oryzae* was similar. Spore germination was polar for *C. tuberculata* and bipolar for *C. oryzae*. Germ tube growth was random and branching. Appressoria were formed preferentially over epidermal cell wall junctions on sedge hosts and over stomatal apertures in rice. Complex infection cushions were observed only on sedge hosts. Infection hyphae developed inter- and intracellularly, causing epidermal cell walls to separate and mesophyll cells to shrink and collapse. The vascular bundles were not invaded. Colonization of susceptible weeds was rapid and conidiophores emerged from the stomatal aperture between 96 to 120 hours post inoculation (HPI). Resistance to *C. tuberculata* and *C. oryzae* in *C. rotundus* and rice was expressed as a delay in appressorial formation, inhibition of fungal growth after penetration, and lack of sporulation.

Résumé

Trois souches de *Curvularia* ont été obtenus des Philippines en 1993 à partir de plants infectés de *Cyperus difformis*, de *Cyperus iria* et de *Fimbristylis miliacea*. Ce sont respectivement les souches 93-020 et 93-022 de *Curvularia tuberculata* et la souche 93-061 de *Curvularia oryzae*. En serre, ces souches fongiques ont causé une haute mortalité et une réduction signifiante du poids sec des plants de *C. difformis*, *C. iria* et *F. miliacea* lorsque 1 x 10^8 spores/m^2 étaient appliquées. Ces souches ont montré un pouvoir pathogène croisé pour trois autres espèces de souchet. *C. rotundus* était résistant mais les plants de *C. difformis*, *C. iria* et *F. miliacea* ont été tués. La majorité des treize variétés de riz testées étaient résistantes aux souches fongiques. Chez le riz, le pouvoir pathogène en ordre décroissant a été *C. oryzae* (93-061), *C. tuberculata* (93-020) et *C. tuberculata* (93-022). Les processus d'infection de *C. tuberculata* et de *C. oryzae* étaient similaires. La germination des spores se faisaient de façon polaire pour *C. tuberculata* and de façon bipolaire pour *C. oryzae*. La croissance du tube germinatif était irrégulière et branchée. Les appressoria se formaient préférentiellement au-dessus des jonctions des parois des cellules épidermiques chez le souchet et au-dessus des ouvertures des stomates chez le riz. Des complexes d'infection ont été observés seulement chez le souchet. Les hyphes se développaient de façon inter et intracellulaire amenant la séparation des parois des cellules épidermiques et la diminution et l'effondrement des cellules mésophylliques. Les faisceaux vasculaires n'étaient pas envahis. La colonisation des mauvaises herbes susceptibles était rapide. Les conidiophores émergeaient à partir des ouvertures des stomates entre 96 et 120 heures après l'inoculation. La résistance à *C. tuberculata* et à *C.*

oryzae chez *C. rotundus* et chez le riz était exprimée par un retard dans la formation de l'appressorium, par l'inhibition de la croissance fongique après la pénétration et par l'absence de sporulation.

Acknowledgements

I would like to express my gratitude to Dr. Alan K. Watson, my supervisor, for the opportunity to study at McGill University and for his support, guidance, trust, and patience; Dr. Timothy C. Paulitz for valuable suggestions on how to improve my thesis; Dr. Antonio DiTommaso for his encouraging words; and Dr. Steven Hallett for his guidance in the initial part of this thesis. I am also grateful to Dr. Danielle Donnelly for her generosity in allowing me to do some histology work in her laboratory.

I am indebted to the International Rice Research Institute (IRRI) for an affiliate research scholarship that enabled me to conduct my research. My sincere appreciation goes to the Weed Science Department and to the Plant Pathology Department at IRRI for the use of laboratory facilities.

I treasure all the memories I have of the IRRI Weed Biocontrol Group, my partners and friends with whom I shared the ups and downs while doing our experiments. I thank Max Mabbayad, who isolated *Curvularia oryzae* in 1993, and for countless favors granted; Camille Yandoc, who isolated the *C. tuberculata* isolates, Angeli Galon-Maghuyop and Rowena Mercado, for their cheerfulness and optimism, who managed to help me harvest spores despite their own heavy workload; Danilo Lucillo, our devoted and generous lab technician, who was always ready to help even if sampling had to be done during unholy hours of the night; Pablo Lucillo and Emil Sandoval, extremely hard-working lab aides, who collected soil, weed seeds, and transplanted the seedlings.

I also thank Joel Janiya, Domingo Navarez, and Rosauro Lubigan, weed scientists at IRRI for valuable advice, Ellen Sibal, Ate Fanny, Kuya Mando, Tito Max, Willie

Apolinario, Meklay Sta. Cruz (EM lab), Litbong Soriano, Nancy Castilla, all members of the Plant Pathology Department at IRRI for various favors and their gift of friendship, and Lingkod Sayo ("Mang Lingkod") for assistance in photography at IRRI.

A special thank you is also extended to my friends, Raymond Pamplona, Florizel Dugay, Doreen Darvin-Ligot, Wendy Asbil, Luz Montesclaros, Jocelyn Mendez, Luzette Teruel, Clarissa Cosico and Ofelia Francisco-Pabalan for their faith in me and for keeping me going with their encouraging e-mail messages; Weedies, past and present, especially Dr. Miron Teshler, Dr. Inna Teshler, Marie Ciotola, Hameed Baloch and Sophie St. Louis for always being ready to lend a helping hand; Diane Valois for the French translation of my thesis abstract; Roslyn James, Carolyn Bowes and Louise Mineau for their kind help during my years as a student at Mac; and Helen Cohen-Rimmer for her generosity and for her patience in teaching me how to prepare the figures using various graphics software. I am also grateful to Guy Rimmer and to Dr. S. Hare's laboratory for assistance in thesis printing. I also thank Mario Lira, Jr. for his assistance in saving my thesis from two computer crashes.

My sincere appreciation goes to Mrs. Constancia K. Magalit, for her motherly concern during my stay in Los Baños, and to all brothers and sisters at Lingkod ng Panginoon (Los Baños) who showed me God's loving ways by their presence in my life.

This work would not have been completed without the love and support of my family in the Philippines, Mommy and Daddy, my sister Juvy (Rumel), my brothers, Buds (Jenny), John, and Darius, my nephews Jor-el, Joco and Jafar and my nieces, Julia and Jamie, who give me joy, and my family in Canada, Mum, Dad and Jay.

Finally, I would like to thank and honor, Scott, my husband and best friend, whose belief in me never wavered, to him I dedicate this thesis.

Table of Contents

List of Tables

List of Figures

Description of Thesis Format

This thesis is comprised of original papers that will be submitted to appropriate scientific journals for publication. In accordance with Part B, section 2 of the "Guidelines Concerning Thesis Preparation" from the Faculty of Graduate Studies and Research, McGill University, I quote the entire text that applies to this format:

> **"2/ Manuscripts and authorship**: *Candidates have the option, subject to the approval of their Department, of including, as part of their thesis, copies of the text of a paper(s) submitted for publication, or the clearly-duplicated text of a published paper(s). If this option is chosen, connecting texts, providing logical bridges between the different papers are mandatory. The thesis must still conform to all other requirements of the "Guidelines Concerning Thesis Preparation" and should be in a literary form that is more than a collection of manuscripts published or to be published. The thesis must include, as separate chapters or sections: (1) table of contents, (2) a general abstract in English and French, (3) an introduction which clearly states the rationale and objectives of the study, (4) a comprehensive general review of the background literature to the subject of the thesis, when this review is appropriate, and (5) a final overall conclusion and/or summary. Additional material (procedural and design data, as well as descriptions of equipment used) must be provided where appropriate and in sufficient detail (e.g. in appendices) to allow a clear and precise judgement to be made of the importance and originality of the research reported in the thesis. In the case of manuscripts, co-authored by the candidate and others, the*

candidate is required to make an explicit statement in the thesis of who contributed to such work and to what extent; supervisors must attest to the accuracy of such claims at the Ph. D. oral defense. Since the task of the examiners is made much more difficult in these cases, it is in the candidate's best interest to make perfectly clear the responsibilities of the different authors of co-authored papers."

In order for this thesis to be consistent with the above statement, it is structured in the following manner:

The thesis begins with abstracts in English and French, followed by a table of contents. Chapter 1 comprises a general introduction in which background knowledge and the current status of research on the thesis subject is presented. This section concludes with an outline of the specific objectives of the thesis. The next four chapters constitute the body of the thesis, each chapter being a complete manuscript.

Chapter 2 manuscript is to be submitted for publication to *Biological Control.*

Chapter 3 manuscript is to be submitted for publication to *Plant Disease.*

Chapter 4 manuscript is to be submitted for publication to *Phytopathology.*

Chapter 5 manuscript is to be submitted for publication to *Phytopathology.*

The various manuscript chapters are linked via connecting texts so as to establish logical bridges between the different papers.

A general discussion and synthesis of the major conclusions of the thesis are presented in Chapter 6. The main contributions to knowledge of this research are outlined in Chapter 7.

Manuscripts from chapters 2, 3, 4, and 5 are co-authored by Dr. A.K. Watson and Dr. T.C. Paulitz. The candidate (Lilian Z. de Luna) performed all the experimental research, statistical analysis, and is the primary author of all four manuscripts. Dr. A.K. Watson provided supervisory guidance and assisted in manuscript preparation. Dr. T.C. Paulitz assisted in manuscript preparation.

Chapter 1. General Introduction

1.1 Abstract

Cyperus difformis, *Cyperus iria*, *Cyperus rotundus*, and *Fimbristylis miliacea* are serious weeds that limit rice production in Southeast Asia. Current methods of control are inadequate and biological control using indigenous fungal pathogens has good potential as a component of an integrated weed management program for rice (Watson, 1994). The biology of these weeds is discussed and research on biological control efforts involving the Family Cyperaceae is reviewed. The specific objectives of this thesis are also presented.

1.2. Family Cyperaceae

The sedge family Cyperaceae is closely related to the Poaceae family and has a worldwide distribution. The number of genera and species included in this family varies in the literature. Gleason (1963) reported that it consists of 75 genera and over 4000 species, while Taylor (1983), included 90 genera in his list.

Sedges are annual or perennial herbs that can be differentiated from grasses by their three-sided, usually solid stems, three-ranked leaves with one-third phyllotaxy, and leaves that have closed leaf sheaths (Mohlenbrock, 1976). Sedges do not possess ligules, and exhibit terminal or axillary inflorescences. A single glume or scale subtends the flowers. The mature fruit, either a trigonous or lenticular achene, is required for positive species identification.

Approximately 220 species within the Cyperaceae have been identified as weeds (Bendixen and Nandihalli, 1987). Nearly 42% of these are found in the genus *Cyperus*,

with an additional 43% located in of the genera *Eleocharis*, *Scirpus*, and *Fimbristylis*. The genera *Scleria*, *Kylllingia*, *Rhyncospora*, *Bulbostylis*, *Fuira*, and *Dichromena* comprise the remaining 15%.

Sedge weeds have been reported in various crops cultivated in many areas of the world including, but not limited to, corn (*Zea mays* L.), sugarcane (*Saccharum officinale* L.), banana (*Musa paradisiaca* L.), taro (*Calocasia esculenta* (L.) Schott), pineapple (*Ananas sativus* Schultz), cassava (*Manihot esculenta* L.), sweet potato (*Ipomoea batatas* (L.) Poir.), vegetables, cotton (*Gossypium hirsutum* L.), and rice (*Oryza sativa* L.) (Holm et al., 1977).

The impact of these weeds in the rice-growing areas of Asia is considerable. Rice is the world's most important food crop (Chandler, 1979), providing 35% of the daily caloric intake of an estimated three billion people (IRRI, 1993). Weeds are a major limiting factor in rice production systems in Southeast Asia (Watson, 1992), including the Philippines (Bayot et al., 1992). Chandrasena (1988) conducted a survey of rice weeds in Sri Lanka and found that grass and sedge weeds were by far the most abundant and troublesome weeds in rice. Field trials at the International Rice Research Institute (IRRI) in Los Baños, Laguna, Philippines, indicated rice yield reductions due to sedges were 78% with IR36 and 54% with UPLR$_i$-5 (IRRI, 1987).

The major sedge weeds infesting rice production areas around the world are *Fimbristylis miliacea* (L.) Vahl, *Cyperus difformis* L., *C. iria* L., and *C. rotundus* L. (Holm et al, 1977). *C. difformis, C. iria* and *F. miliacea* have been identified as major weeds in lowland or irrigated rice in the Philippines and other parts of tropical Asia (Bayot et al., 1992; Ampong-Nyarko and De Datta, 1991). *C. rotundus* has been

identified as the most serious weed in upland rice (Ampong-Nyarko and De Datta, 1991; Padhi et al., 1991).

1.2.1. Cyperus difformis

Cyperus difformis L. or small flower umbrella sedge (Fig. 1.1A) is a tufted annual sedge native to the Old World tropics (Holm et al., 1977). The plant is 10 to 75 cm in height with tender, triangular stems that are 1 to 3 mm thick. The leaves are linear, 2 to 3 mm wide, rather abruptly acuminate and are usually two-thirds of the plant height. The inflorescence, subtended by leaf-like bracts, consists of dense, globose heads with stellately spreading spikelets. The achenes are elliptic or obovate, triangular and straw-colored, about 6 mm long. This species can be distinguished by dense globose heads with many radiating spikelets; and the small, orbicular glumes, which are brown at first but later, become variegated with pale yellow or white margins.

The distribution of *C. difformis* includes Southern Europe, Asia, Central America, North America, and many islands of the Indian and Pacific Oceans, where it is reported as a weed of rice in 46 countries (Holm et al., 1977). Mainly a tropical and subtropical weed, it is adapted to flooded conditions or very moist soils, and has been identified primarily as a weed of flooded or paddy rice (Biswas et al., 1990). However, it can also become a problem in other crops such as corn, banana, tea (*Camellia sinensis* (L.) O. Kuntze), lowland taro, pineapple, and in pasturelands (Holm et al., 1977). Optimum growth is achieved in rich, fertile soils, however considerable growth also occurs in poorer soils, in waste areas, or in fallow rice fields.

Sanders (1994) recently reviewed the life cycle and ecology of *C. difformis* in temperate Australia. Information about its biology is patchy, with most reports

concentrating on the negative effects on rice yields due to competition from *C. difformis*. Rice yield was most significantly affected when *C. difformis* competed during pre-tillering and tillering stages of rice growth (Swain et al., 1975).

C. difformis has become a widespread and serious weed of paddy rice and other crops due in part to its abundant seed production, enabling it to achieve dominant status (Holm et al., 1977). *C. difformis* produces between 5100 seeds/plant (Kusanagi 1981, citing Kasahara, 1968) and 50,000 seeds/plant (Jacometti, 1912), although this range may simply reflect a difference in intra- or interspecific competition. The seeds are long-lived and may retain 90% viability after six years of dry storage (Sanders, 1994).

In tropical areas with sufficient soil moisture such as Hawaii and the Philippines, inflorescences and seeds are produced year round. Massive seedling densities result in the formation of dense, solid mats of vegetation in fields of young crops. The plant completes its vegetative and reproductive cycle in 30 days (Vaillant, 1967), making it especially competitive in a crop that requires 90 days or more to reach maturity. *C. difformis* competes with rice for moisture and nutrients, often causing rice yield reductions of 12-50% (Ampong-Nyarko and De Datta, 1991). *C. difformis*, however, cannot withstand deep flooding. Harrowing a wet-seeded crop when it is about three to four weeks old will press down both the rice and weeds into the water, but after several days the rice plants recover and become erect while the weeds remain under the water and die (Anon., 1952). A similar technique is used by farmers in Iloilo province in the Philippines to control *C. difformis, C. iria*, and *F. miliacea* (Moody, 1990).

1.2.2. Cyperus iria

Cyperus iria L. or rice flatsedge (Fig. 1.1B) also originated from the Old World tropics. It is a tall, annual, tufted sedge that is a problem mainly in rice fields in Asia (Holm et al., 1977). The culms are 20 to 60 cm long, while the leaves are linear-lanceolate, 3 to 6 mm wide, often shorter than the culm. There may be three to five leaf bracts, with the lower one longer than the inflorescence. The inflorescence itself may be simple or compound, usually open, and may be 20 cm long, consisting of dense, elongated spikes. The spikelets are erect, spreading, crowded and covered with six to 24 yellow flowers. The fruit is a small achene, obovate, brown, and triangular in cross-section. This species is distinguishable by its yellowish red fibrous roots, its yellowish, usually open inflorescence, and by the lowest bract of the flower, which is always longer than the inflorescence.

The biology of *C. iria* has not been extensively studied although its distribution and occurrence as a weed in many crops has been documented (Holm et al., 1977). It is now known to occur mainly in an area from Japan south to the Pacific Islands and Australia, and west through India. It has also been reported from southern and western Africa and in the southern United States. The plant is propagated by seed and one plant produces up to 5,000 seeds (Holm et al., 1977). There is some evidence of dormancy in the seeds, which can germinate about 75 days after shedding (Ampong-Nyarko and De Datta, 1991). Although *C. iria* is principally a weed of rice, it has also been reported as a weed in tea, vegetables, pasture, peanut (*Arachis hypogaea* L.), soybean (*Glycine max* L.), banana, corn, pineapple, sugarcane, cassava, and sweet potato (Holm et al. 1977).

C. iria competes with rice for nutrients and can reduce rice yields by as much as 50% (Ampong-Nyarko and De Datta, 1991).

1.2.3. Cyperus rotundus

The world's worst weed, *Cyperus rotundus* L. or purple nutsedge, is native to India (Holm et al., 1977). The biology and lifecycle of *C. rotundus* have been the subject of many studies (e.g. Wills, 1987; Stoller and Sweet, 1987). It is an erect, glabrous, perennial herb with dark green leaves, triangular stems, and branched, fibrous roots (Fig. 1.1C). *C. rotundus* rarely reproduces by seed (Thullen and Keeley, 1979) but produces extensive, horizontal, slender rhizomes, which are white and fleshy when young, but turn brown, ligneous and "wiry" with increasing age (Wills, 1987; Fig. 1.1E). Rhizomes either extend upward, horizontally, or downward. Those that extend upward swell upon reaching the soil surface and form a basal bulb, a tuberous bulb, or a corm that is about 0.3 to 1 cm in diameter (Fig. 1.1E). This structure is then capable of producing shoots, roots and other rhizomes. Those that extend horizontally or downward give rise to underground tubers at intervals of 5 to 25 cm, forming tuber chains that extend to a considerable depth in the soil. The tubers are irregularly shaped or nearly round and white and fleshy when young and a coarse dark brown or black when old (Holm, 1977; Fig. 1.1E).

Tubers consist of rhizomatous tissue with numerous buds (Wills and Briscoe, 1970). These buds sprout and initiate rhizomatous growth, which develop into seedlings that eventually grow into mature plants. Tuber formation begins four to six weeks after seedling emergence (Stoller and Sweet, 1987), 95% of which are usually formed in the upper 45 cm of the soil (Tripathi, 1969). *C. rotundus* tubers exhibit apical dominance

since the apical buds sprout first and inhibit sprouting of the more basipetal buds (Smith and Fick, 1937).

The inflorescence is a loose umbel subtended by two to four leaf-like bracts that are as long, or slightly longer than the flower-bearing rays. The spikelets bear 10 to 40 flowers and are red, reddish brown, or purplish brown. The achene is ovate or oblong-ovate, three-angled, granular, olive-grey to brown or black, and covered with a network of grey lines. The distinguishing characteristics of this plant include its tuber-bearing rhizomes, its red, reddish brown or purplish brown inflorescence, and its basal leaves that are shorter than the inflorescence. Flowering commences three to eight weeks post-emergence, and is stimulated by short photoperiods of six to eight hours. Flowers are cross-pollinated, mainly by wind, and although many seeds are produced, most have a shrunken and shrivelled appearance. Seed germination rarely exceeds five percent (Justice and Whitehead, 1946).

C. rotundus is a weed in 52 crops in 92 countries covering both hemispheres with its range being limited only by cold temperatures (Holm et al., 1977). Otherwise, it can grow profusely in almost any soil type, at any elevation, humidity, soil moisture and pH, and can survive extremely high temperatures. Growth of *C. rotundus* is best under high soil moisture, high fertility (Bhardwaj and Verma, 1968; Nyahoza, 1973), and high temperatures (Davis, 1942; Wills, 1978; Horowitz, 1972).

Despite its vigour in most circumstances, *C. rotundus* cannot tolerate saline soils, and does not grow well under shaded conditions (Ranade and Burns, 1925). When light penetration is extremely limited by crop canopy, the leaves of *C. rotundus* yellow and die. Growth is then resumed by dormant tubers that sprout as soon as an opening in the

canopy appears. *C. rotundus* is found in cultivated fields, on roadsides, in neglected areas, and at the forest edges. It may also cover the banks of irrigation canals and streams. It's success as a weed is due to its prolific production of tubers that can remain dormant and carry the population through extremes of heat, drought, flooding, or lack of aeration. *C. rotundus* limits upland rice production by competing for light, water, and nutrients (Okafor and De Datta, 1976), and can reduce grain yields by 50% (Ampong-Nyarko and De Datta, 1991) or more (Okafor and De Datta, 1974).

1.2.4. Fimbristylis miliacea

Fimbristylis miliacea (L.) Vahl (= *F. littoralis* Gaudich.), or globe fingerush, has become yet another increasingly troublesome weed in paddy rice (Holm et al., 1977). It is a tufted, erect plant that may grow as an annual or perennial (Fig. 1.1D). Originally from tropical America, it is now a weed in 21 countries, infesting fields of rice, taro, abaca (*Musa textilis* Née), banana, corn, sugarcane, and sorghum (*Sorghum vulgare* L.= *S. bicolor*). It has fibrous roots and four-angled, somewhat flattened, slender culms that are 40 to 60 cm tall. The leaves are two-ranked, thread-like and stiff, 1.5 to 2.5 mm wide and up to 40 cm long. The inflorescence is a reddish-brown compound umbel, 6 to 10 cm long. The seed is a white or yellowish achene, triangular, with a sugar-coated appearance.

The high competitive ability of *F. miliacea* in rice is due to several factors. Plants flower all year round in the Philippines and produce as many as 10,000 seeds per plant (Holm et al., 1977). Seeds exhibit little dormancy and germinate quickly when moisture and light are present (Ampong-Nyarko and De Datta, 1991). In a study conducted in the Philippines, Vega and Sierra (1970) took a soil sample with weed seeds from a rice field

and recorded the seedlings that emerged over a three-year period. They found that *Fimbristylis* emerged in all periods and constituted 70% of all the seedlings that appeared. Noda and Eguchi (1965) confirmed these findings when they reported that in Japan, *F. miliacea* seedlings also emerged from the field throughout the entire culture period, thus, any single application of a herbicide only affects the present crop of weed seedlings. Those that appear later escape the effects of the herbicide and will grow and produce more seeds.

In the absence of other weed species, *F. miliacea* can compete more effectively with the crop and tolerate soil moisture conditions below field capacity (Noda and Eguchi, 1965). A shortage of irrigation water triggers the heavy germination of this weed, whereas keeping the water layer at 15 cm suppresses germination completely (Holm et al., 1977). The hardiness of the seed also contributes to its success as a weed. *F. miliacea* seeds eaten by cattle pass through their digestive tracts, remain viable, and later germinate near the animal droppings (Burkill, 1935). Finally, rapid growth of the fibrous roots of this species make *F. miliacea* a serious competitor for soil nutrients (Holm et al., 1977), capable of reducing rice grain yields by up to 50% (Ampong-Nyarko and De Datta, 1991).

1.3. Strategies for the management of Cyperaceae weeds in rice

1.3.1. Control of C. difformis, C. iria, and F. miliacea

Traditionally, manual labour has been used to remove weeds from production systems (Moody, 1992). This procedure is not only costly and time consuming, but relatively ineffective unless the weeds are removed within 20 days after crop emergence.

If hand weeding is performed after this time, irreparable damage is done to the crop, and significant yield reductions may result because during early establishment, the weeds accomplish 20 to 30% of their total growth compared to only 2 to 3% of total growth for the crop (Moody, 1990). In many parts of Asia, an animal-drawn spike-toothed harrow or rotary weeder is used to manage weeds (Mukhopadhyay, 1983; Moody, 1992).

Cultural and mechanical methods have been employed to minimize adverse effects on crop yield due to the presence of weeds. In the Philippines, *C. difformis, C. iria,* and *F. miliacea* may be controlled by harrowing, and subsequently submerging these weeds in the flooded rice fields (Moody, 1990).

Janiya and Moody (1984) used *Azolla pinnata* R. Br. to suppress four species of weeds in transplanted rice. They report that growth of *C. difformis* was reduced by 100% at rice flowering, and by 93.9% at crop harvest, when *Azolla* was inoculated in the plots immediately after transplanting. Furthermore, the yield obtained in plots where *Azolla* suppressed the weeds is equivalent to that observed from hand-weeded plots and significantly higher than that of the non-weeded control. The phytotoxic potential of crop residues has also been exploited in management of weeds. Lin et al. (1992) found that straw incorporated into the soil from rice germplasm with high allelopathic activity controlled *C. iria* almost as effectively as a tank mixture of propanil (N- (3,4-dichlorophenyl) propanamide) + bentazon (3- (1-methylethyl)- (1H)-2,1,3-benzothiadiazin-4 (3H)-one 2,2-dioxide).

In Asia, the use of herbicides has increased due to the pressing need to raise rice yields and maintain profits on a progressively limited land base (Moody, 1995). Reliance on herbicides has grown due in part to the shift to direct-seeded rice, as well as

significant increases in labor and irrigation costs. Among the most widely used pre-plant or pre-emergence herbicides are the chloroacetamides including butachlor (N-(buthoxymethyl)-2-chloro-N- (2,6-diethylphenyl) acetamide), pretilachlor (a-chloro-2, 6-diethyl-N-(2-propoxyethyl) acetanilide), mefenacet (2-(2-benzothiazolyloxy)-N-methyl-N-phenyl acetamide), metolachlor (2-chloro-N-(2-ethyl-6-methylphenyl)-N-(2-methoxy-1-methyethyl) acetamide), and the thiocarbamates such as thiobencarb (S-[(4-chlorophenyl)methyl] diethylcarbamothioate), molinate (S-ethyl hexahydro-1 *H*-azepine-1-carbothioate). Propanil, bentazon, and bensulfuron (methyl 2-[[(4,6-dimethoxypyrimidin-2-yl) aminocarbonyl aminosulfonylmethyl] benzoate) are some of the popular post-emergence herbicides (Baltazar, 1995; De Datta, 1995). Herbicide injury to rice, lack of technical knowledge and prohibitive cost to small farmers limit the widespread use of herbicides in Asia (Baltazar, 1995). Most herbicides are also ineffective on weed seeds and propagules, which are the important survival mechanisms of a number of weeds (Yamasue and Ueki, 1983).

The continued use of the same herbicide for long periods of time has resulted in the evolution of herbicide-resistant weeds. In Malaysia, a resistant form of *F. miliacea* has been found in rice fields where 2,4-D has been used for 25 years, and the weed cannot be controlled with six times the recommended rate of 2,4-D (Watanabe et al., 1994). *C. difformis* resistant to bensulfuron has been reported in the USA in 1993, and in Australia in 1994 (Heap, 1997).

1.3.2. Control of C. rotundus

The persistence and aggressiveness of *C. rotundus* is well known wherever upland rice is grown and several methods of control are currently being used (Ampong-Nyarko

and De Datta, 1991). Hand weeding must be performed at frequent intervals to effectively prevent the smothering of rice by *C. rotundus*, and should continue until the rice canopy closes, at which time the shade will suppress weed growth. Managerial practices such as using adequate, but not excessive fertilizer, observing the optimum planting density, and planting date help tip the balance in favour of the rice crop. Preplant tillage can also be used to stimulate weed propagule germination and move the tubers to the soil surface where they are subject to desiccation (Wax, 1975).

A number of reports suggest desiccation and temperature extremes can kill the tubers. Reducing tuber moisture levels to 15% killed the tubers and intermediate moisture contents resulted in reduced viability (Rao and Nagarajan, 1962; Smith and Fick, 1937). Sun-drying in the field for seven to 14 days or burial at depths greater than 40 cm would effectively control *C. rotundus* tubers (Smith and Fick, 1937; Tripathi, 1969; Marambe et al., 1995).

Common herbicides such as butachlor and propanil are ineffective against *C. rotundus* (Gupta and O'Toole, 1986). Bentazon and 2,4-D may be applied post-emergence, three weeks after rice seeding but control is temporary (Ampong-Nyarko and De Datta, 1991). Pre-plant application of glyphosate is the best way to control *C. rotundus* using chemicals. However, it appears that an integrated control method for *C. rotundus* combining tillage with chemical methods is more effective than tillage or herbicides alone (Ampong-Nyarko and De Datta, 1991). In this scheme, plowing and harrowing stimulate dormant tubers to grow. After three to four weeks, *C. rotundus* is then sprayed with a systemic herbicide with no residual soil activity, such as glyphosate. Rice is then planted after a week with no additional land preparation.

Although some herbicides selectively kill *C. rotundus*, most provide only poor or temporary control (Pereira et al., 1987; Ampong-Nyarko and De Datta, 1991). Reasons for failure include marginal translocation of herbicides to sites of action, temporary inhibition of tuber sprouting and control of new tuber formation, or inconsistent control when applied at different stages of growth and under various environmental conditions (Pereira et al., 1987).

1.4. Biological weed control

The challenge of the next millennium is how to feed a rapidly growing human population while maintaining the sustainability of increased agricultural productivity (Ehui and Hertel, 1989). Intensification of rice production has resulted in undesirable weed shifts and emergence of new weed problems, including weedy forms of rice (Moody, 1995). There is also increasing concern about environmental contamination and food quality due to augmented herbicide use (Moody, 1995). These issues have contributed to a greater interest in alternative weed control methods, such as biological control, that can be used within an integrated weed management system.

Biological control is the deliberate use of natural enemies to reduce the growth or suppress the population of a target weed to economically acceptable levels (Watson, 1985). There are three general approaches to biological control. The classical approach involves the importation and/or inoculative release of one or more natural enemies that attack the target plant in its native range, into areas where it has been introduced, is troublesome, and where its natural enemies are absent (Watson, 1991). In the bioherbicide or inundative approach, large numbers of a biological control agent are reared and released at appropriate times in the region where the weed is found at noxious

levels (Wapshere et al., 1989). Most of the earlier studies involved fungal pathogens so that the term bioherbicide and mycoherbicide were used interchangeably (Watson, 1985). The herbivore management approach makes use of polyphagous aquatic grazers such as the grass carp (*Ctenopharyngodon idella* Valenciennes), tilapia (*Tilapia zilli* Gervais), and manatees (*Trichechus* sp.); and polyphagous terrestrial grazers such as cattle, sheep, horses, and goats.

The inundative approach is most applicable to weeds in short cropping cycles. Furthermore, native agents present a lower risk factor because all the crops in the region of utilisation have already been exposed to the agent. Thus, if a crop has not been recorded as one of the hosts of the agent, it can be considered safe from attack (Wapshere et al., 1989).

For this approach to be successful, abundant and durable inoculation must be produced in artificial culture, the pathogen must be genetically stable and specific to the target weed, and it must be possible to infect and kill the weed in environments of reasonably wide latitude (Daniel et al., 1973).

Biological control research programmes have been established in many parts of the world. Julien (1992) recorded 679 releases of exotic agents for the control of weeds, while Charudattan (1990) identified over 20 potential mycoherbicide candidates for economically important weeds. In various parts of Asia, interest in biological control is growing as well. Waterhouse (1992) reported partial or completely successful biological control programmes for some Southeast Asian aquatic weeds such as *Chromolaena odorata, Eichornnia crassipes, Mimosa invisa, Mimosa pigra,* and *Pistia striatoides.*

Five pathogens are currently registered as bioherbicides worldwide (Charudattan, 1998). DeVine® is a "fresh-milk" formulation of chlamydospores of *Phytophthora palmivora* which kills seedlings and adult stranglervine, *Morrenia odorata* in citrus groves of Florida (Burnett et al., 1974; Kenney, 1986; Ridings, 1986). Collego® is a wettable powder formulation of *Colletotrichum gloeosporioides* f. sp. *aeschynomene*, an anthracnose-inciting pathogen of northern joint vetch, *Aeschynomene virginica* used in rice and soybeans cropping systems (Daniel et al., 1973; Smith, 1986). BioMal® is a dry formulation of *Colletotrichum gloeosporioides* f. sp. *malvae* registered in Canada since 1992 for the control of round-leaved mallow, *Malva pusilla* Sm. in flax and lentils (Makowski and Mortensen, 1992) but has not yet been commercially available. Dr. BioSedge®, a *Puccinia canaliculata* isolate, is registered to control yellow nutsedge in all crops in the United States. Camperico® (*Xanthomonas campestris* pv. *poae*) is registered in Japan for the control of annual bluegrass (*Poa annua*) in golf courses and turf.

Two pathogens have been approved for selective stump-treatment to prevent regrowth of weedy trees (Charudattan, 1998). BioChon® (*Chondrostreum purpureum*) is used as a natural decay-promoter in cut hardwood tree stumps in the Netherlands, and StumpOut® (*Cylindrobasidium laeve*), to prevent re-sprouting of cut trees in South Africa. In addition, Luboa® 1 S_{22} (*Colletotrichum gloeosporioides* f.sp. *cuscutae*) has been registered in China for the control of dodder (*Cuscuta chinensis* and *C. australis*) in soybean (Wan et al., 1994).

1.5. Fungal pathogens of weedy sedges

Most reports of fungal pathogens used on weeds in the Cyperaceae family were those associated with *C. rotundus* and *C. esculentus* (Phatak et al., 1987). The rust, *Puccinia canaliculata* was identified as a potential biological control agent of *C. esculentus* in 1942 (Castellani. 1942 cited in Callaway et al.. 1985). Both *Xanthium* sp. and *Ambrosia trifida* L. are alternate hosts of this rust (Callaway et al., 1985). When released in early spring, the rust inhibits flowering and new tuber formation, and dehydrates and kills the plant. Since the form used by Phatak et al. (1983) did not attack either *C. rotundus* or a variety of crop plants, it was suggested that *P. canaliculata* could be a valuable component of an integrated management system for *C. esculentus*.

Cyperus rotundus infected with the systemic fungus, *Balansia cyperi* was observed in Louisiana in 1984 (Clay, 1986). A deformed inflorescence densely covered with white mycelium may be observed but infected plants are often asymptomatic. It is not clear whether infection is detrimental to the host since infected shoots produce diseased inflorescences but otherwise appear quite healthy. In addition, *C. rotundus* rarely produces viable seed, so diseased inflorescences have little effect on its overall reproductive potential. In a subsequent study, Stovall and Clay (1988) found that infected plants produced significantly more, yet smaller, tubers than uninfected plants. Thus, it is doubtful that *B. cyperi* represents a potential biocontrol agent.

In 1985 and 1986, a fungus was isolated from diseased leaves of *C. esculentus* which was later identified as *Cercospora caricis* (Blaney et al., 1988). Disease symptoms produced by the fungus consisted of dark brown lesions on leaves surrounded,

in some cases, by a yellow halo. This type of lesion suggests that a diffusable toxin is involved in disease development. Purification by column chromatography indicated that the toxin cercosporin was produced. Cercosporin, a red pigment, is a non-host specific toxin implicated in disease development in plants (Assante et al., 1977)

A species of *Ascochyta* was isolated from *C. rotundus* in Banaras, India (Upadhyay et al., 1991). Reddish-brown sunken spots on stems, sheaths and involucral leaves were produced on severely infected plants. Sometimes a lesion completely girdled the stem resulting in death of the upper portion of the plant. Severely infected plants exhibited bronzing, browning and death of all aerial plant parts. Cyperine, a new and extremely active phytotoxin from *A. cypericola* was later obtained (Sterle et al., 1991).

A survey of fungi associated with *C. rotundus* conducted in Brazil showed that *Cercospora caricis, Cintractia limitata, Dactylaria higinsii, Duosporium cyperi* and *Puccinia canaliculata* were part of the pathogenic mycobiota (Baretto and Evans, 1995). The dematiaceous hyphomycetes *C. caricis, D. higinsii*, and *D. cyperi* deserves attention for possible mycoherbicide development while the rust pathogens could be exploited for classical introductions (Evans, 1990). Pathogens infecting the inflorescence such as *C. limitata* have little importance as a biocontrol agent since sedges are propagated mainly by vegetative means (Evans, 1990).

Recently, Kadir et al. (1996a) reported that an isolate of *Dactylaria higginsii* was obtained from *C. rotundus*. The fungus was highly pathogenic to *C. rotundus*, inoculated plants developing disease symptoms within five days and secondary infections developing 10 days later. Higher disease rates were observed with multiple inoculations of 10^6 conidia ml^{-1} was used. Kadir et al. (1996b) also tested the performance of *D.*

higginsii in *C. rotundus* grown with pepper and tomatoes and found that when the fungus was sprayed at the rate of 10^6 conidia ml^{-1}, the yield of the two crops were equal to those of weed-free controls.

Upadhyay et al. (1990) isolated *Phoma cyperi*, from *C. iria*. *P. cyperi* was specific to nutsedges and produced at least one phytotoxin. Infected leaves of *C. iria* exhibited small, yellow to light brown lesions on either side of the leaf blade. Ultimately these blotches turned into large dark brown-black necrotic lesions with a dull-grey necrotic centre. Numerous pycnidia were seen embedded in leaf tissues. Under glasshouse conditions, these lesions developed after five to seven days on wounded leaves and after 10 to 12 days on unwounded leaves.

There are few reports on possible fungal biological control agents of *C. iria* and *C. difformis*, and none on *F. miliacea*. Most of the research has focused on *C. rotundus* and *C. esculentus* (Table 1.1). However, in 1993, *Curvularia* spp. were isolated from naturally infested *C. difformis*, *C. iria*, and *F. miliacea* plants in the Philippines. The Commonwealth Mycological Institute (CMI) identified isolates 93-020 from *C. difformis* and 93-022 from *C. iria* as *Curvularia tuberculata* Jain and isolate 93-061 from *F. miliacea* as *C. oryzae* Bugnicourt. These three isolates are the subject of this study.

1.6. *Curvularia* spp. as plant pathogens

The genus *Curvularia* Boedijn is a group of dematiaceous hyphomycetes related to *Bipolaris*, *Drechslera*, and *Exserohilum*, all of which occur mostly as tropical and subtropical facultative plant pathogens. Members of the group form brown, gray or black colonies that may be hairy, cottony or velvety (Ellis, 1966; 1971). The mycelium consists of branched, septate hyphae that are hyaline or brown and smooth or verrucose.

Septate, brown conidiophores bear multicellular conidia, straight or curved with one or more cells larger and darker than the rest. Boedijn first described the type species, *Curvularia lunata* in 1933 (Sivanesan, 1987), while Sivanesan (1987) reported a total of 32 graminicolous species of *Curvularia*. The teleomorphic state belonging to the genus *Cochliobolus* is known for only 10 out of the 32 species reported (Sivanesan, 1987).

Curvularia tuberculata was first described by Jain (1962) when it was isolated from maize leaves and paddy grains. Ellis (1966) described colonies on potato dextrose agar (PDA) as grey, dark blackish brown or black and cottony or woolly, and sometimes zonate. The mycelium consists of branched, septate, subhyaline to pale brown and smooth hyphae. The conidiophores arise singly or in groups, from the tips or lateral branches of older hyphae with the conidia borne spirally or alternately towards the tip. They are 23 to 52 μm long, 13 to 20 μm thick at the widest part, brown to dark brown, and covered with tubercles all over the surface. The spores are straight or very rarely slightly curved and are three- to five-septate. The end cells are usually paler and smaller than the middle cells and the second cell from the base is the largest and darkest. Germination was found to be bipolar although the middle cells may also germinate. *C. tuberculata* is heterothallic and its teleomorphic state, *Cochliobolus tuberculatus* Sivan., could be produced by pairing monoconidial compatible isolates (Sivanesan, 1985). Ascospores are filiform, hyaline, helically coiled in the ascus and 13-to 23-septate.

In 1968, *C. tuberculata* was reported to be the causal agent in die-back disease of citrus in India (Lele at al., 1968). Lambat and Asha Ram (1969) attributed a blight disease of sorghum or *Sorghum vulgare* (L.) Pers. to *C. tuberculata*. The fungus was found on the seed coats and when the seeds germinated, both the radicle and plumule

were infected and within one week the sprouts died. When tested further on seedlings, they found symptoms of a severe blight disease. Four days after inoculation, small isolated brown spots appeared and within seven to 12 days, lesions rapidly expanded and coalesced to form irregular patches on the leaves of inoculated seedlings. Leaf spot of (*Cyperus rotundus*) caused by *C. tuberculata* was reported by Misra et al. (1973). In the field, small, translucent, water-soaked spots appear on the leaves that later turn brown and cause the plants to die prematurely. The same type of lesions were found on cosmos (*Cosmos* sp.) plants infected by *C. tuberculata* which cause severely affected leaves to die after six to 10 days (Ghosh and Gupta, 1980).

A new blight disease of sugarcane (*Saccharum officinale*) caused by *C. tuberculata* was reported by Seshadri et al. (1980). Necrotic spots appear on the leaves and with time become oval, lenticular, or irregular in shape. Lesions have a dull white or greyish center with a well-defined dark brown margin. Later, the spots coalesce to form large necrotic areas causing extensive blighting of the leaves. In the field, dense spotting and extensive blighting of leaves were only observed on some severely affected plants leading Seshadri et al. (1980) to conclude that the disease appeared to be of negligible importance. Succeeding experiments showed that in inoculated detached leaf segments, discolored, reddish-brown stomata occurred near germ tubes or hyphae. Discoloration and necrosis of neighboring epidermal cells and the underlying mesophyll cells soon followed.

C. tuberculata also infects mango (*Mangifera indica* L.), with initial symptoms consisting of small, isolated dark brown irregular spots on leaves (Lele et al., 1981). Enlargement and coalescence of the lesions results in necrotic areas that are concentrated

on the leaf tips and margins. Mishra and Singh (1987) reported leaf spot and leaf blight of coconut (*Cocos nucifera* L.) caused by *C. tuberculata*. Isolates were apparently pathogenic to wounded one-year old seedlings. *C. tuberculata* has also been reported on guava fruits (Kapoor and Tandon, 1971), dune soil (Rama Rao, 1964), wood, fabric, and air (Sivanesan, 1987). *C. tuberculata* has been isolated from rice with the black kernel disease (Webster and Gunnell, 1992) together with 13 other *Curvularia* species. Damping-off of Philippine pine species (*Pinus* sp.) has also been reported (Militante and De Guzman, 1987, cited in Tangonan and Quebral, 1992).

Curvularia oryzae isolated from rice grains was first reported by Bugnicourt (1950) and later included in descriptions by Ellis (1966, 1971) and Sivanesan (1987). This species produces colonies that are blackish brown to mid gray. Conidia are 24 to 41 μm long, 13 to 23 μm at the widest part, straight, smooth, three-septate, ovoid, obclavate, or almost elliptical. The second cell from the base is the largest and the cell at each end pale brown, intermediate cells brown or dark brown with the largest cell often also the darkest. No teleomorphic state has been discovered so far.

There are few reports on plant diseases caused by *C. oryzae*. Rotting of okra (*Abelmoschus esculentus* (L.) Moench. fruits due to *C. oryzae* was reported by Lal and Goel (1989). Black kernel disease of rice has been associated with 14 species of *Curvularia*, including *C. tuberculata* and *C. oryzae*. The disease is characterized by a blackish discoloration of the grain and infection of rice kernels is thought to occur at the flowering stage (Webster and Gunnell, 1992). *C. oryzae* was also reported to be the causative agent of seedborne rot of rice in the Philippines (Mendoza and Molina, 1980, cited in Tangonan and Quebral, 1992).

1.7. Infection process of fungal weed biocontrol agents

Although factors such as conidial production, dew and temperature requirements, use of surfactants and adjuvants have been routinely investigated in the evaluation of potential weed biocontrol agents, aspects of the infection process are also important considerations in the development of mycoherbicides (Van Dyke, 1989). From a plant pathologist's point of view, factors affecting the host-surface interface are important areas of study for the ultimate purpose of reducing or eliminating disease, but from a weed biocontrol perspective, information about these are needed in order to enhance the disease progress in the target weed.

TeBeest et al. (1978) studied the histopathological relationship of *Colletotrichum gloeosporioides* f. sp. *aeschynomene* with its host, *Aeschynomene virginica* (northern jointvetch). The fungus is used as a biocontrol agent for northern jointvetch in Arkansas rice fields because it is host specific and rapidly kills seedlings of the weed (Daniel et al., 1973; Templeton et al., 1984). From microscope examination of diseased seedlings, TeBeest et al. (1978) found that spores germinated and formed appressoria within four to five hours after inoculation, but that penetration of the plants via the trichome bases did not occur until after 48 hours. Infection of the host produced stem lesions, which coalesced and girdled the stem, resulting in death of the plant within 10 days. Sections of diseased tissues revealed that the intracellular mycelium grew within the cortex, cambium, xylem, and pith rays. The fungus also sporulated abundantly on the necrotic areas.

The uredinial stage of *Puccinia canaliculata* on yellow nutsedge (*Cyperus esculentus*) was studied by scanning electron microscopy in order to gain a better understanding of how infection occurs in this pathogen (Wetzstein and Phatak, 1987). Phatak et al. (1983) established that the narrow and specific host range of this rust species gives it potential as a biocontrol agent against yellow nutsedge. The urediniospores are the reinfecting, vegetative spores that are responsible for the epiphytotics on this host (Wetzstein and Phatak, 1987). Germination occurred via the emergence of germ tubes through one of two equatorial germ pores. Germ tube growth was found to be perpendicular to the longitudinal axis of epidermal cells and appressoria formed over and completely covered the stomata. The linearly aligned cavities in the substomatal chambers were the sites of uredinial development. At later stages, the swollen epidermis on abaxial leaf surfaces ruptures to expose the uredinia and associated paraphyses.

The interaction of *Alternaria cassiae* with sicklepod (*Cassia obtusifolia* L.) was studied by Van Dyke and Trigiano (1987) using light and scanning electron microscopy. Conidia germinated within two to three hours after inoculation and formed an average of six germ tubes/conidium. Terminal and intercalary appressoria were formed both over stomata and directly on the cuticle. Cells of cotyledons and leaves became necrotic prior to fungal penetration suggesting the presence of a diffusable toxin.

The infection process of *Colletotrichum gloeosporioides* f. sp. *malvae* (BioMal) on Malvaceae weeds was investigated by Morin et al. (1996). They found that this *Colletotrichum* species is highly pathogenic on *Malva pusilla* Sm. and *M. parviflora* L. but not on *M. neglecta* Wallr. or *Abutilon theophrasti* Medik. Spore germination and pre-penetration structures were similar on the four weeds and safflower but lower penetration

frequencies in *M. neglecta* and *M. parvifolia* were observed. The fungus was observed to penetrate plant cuticles directly and infection structures were produced within 31 to 36 hours after inoculation. In susceptible tissues, the fungus produced infection vesicles, primary and secondary hyphae while in moderately resistant hosts, colonization was arrested by a hypersensitive reaction of the cells near the initial infection site and no secondary hyphae were observed. Morin et al. (1996) concluded that compatibility and incompatibility mechanisms in this pathosystem likely do not operate during the pre-penetration phase but are activated after successful penetration has occurred. Their work demonstrated that the infection strategy of *C. gloesporioides* f. sp. *malvae* was consistent with intracellular hemibiotrophic species of *Colletotrichum* where an initial biotrophic phase was followed by a necrotrophic phase accompanied by symptom development. Succeeding experiments by Wei et al. (1997) showed that the duration of the biotrophic stage decreased as mallow leaves became older or were senesced in the dark and that symptom development was delayed by application of thiol reagents and promoted by antioxidants.

1.8. Thesis objectives

The extent of weed problems in rice ecosystems was underscored by Moody (1989) when he reported that 1800 weed species appear in the literature as growing in association with rice. Waterhouse (1992) further stated that only 14 out of the 232 major weeds are not included in Moody's (1989) compilation, most of them being problems in orchards and plantations. In the same survey, members of the family Cyperaceae were rated as the second worst group of weeds in Southeast Asia. This is unfortunate because no single method has successfully controlled these troublesome weeds.

Presently, there are no reports on potential biocontrol agents of *C. difformis, C. iria*, and *F. miliacea* in rice. In 1991, a collaborative research project was initiated between McGill University and the International Rice Research Institute in the Philippines to find potential biocontrol agents for 10 major weeds in rice, including *C. difformis, C. iria, F. miliacea*, and *C. rotundus*. This research focuses on aspects of the biocontrol of these four weeds using three *Curvularia* isolates. Specifically, the objectives of this work were to:

1. determine the efficacy of two *C. tuberculata* isolates and one *C. oryzae* isolate on their primary hosts;
2. determine cross-pathogenicity of the three *Curvularia* isolates on Cyperaceae weeds;
3. evaluate responses of rice varieties to the *Curvularia* isolates;
4. describe the infection processes of *C. tuberculata* and *C. oryzae*, and find histological indicators of susceptibility and resistance in the four weed hosts; and
5. determine the histological responses of rice to *C. tuberculata* and *C. oryzae*.

1.9. Literature cited

Alcorn, J.L. and W. Pont. 1973. Races of *Drechslera maydis* in Queensland. Aust. J. Exp. Agric. Anim. Husb. 13:213-215.

Ampong-Nyarko, A. and S.K. De Datta. 1991. A Handbook for Weed Control in Rice. International Rice Research Institute, Manila, Philippines. 113 pp.

Anonymous. 1952. Weed control in paddy. Tropical Agriculture 198:196-199.

Arthur, J.C. 1920. North American Flora 7:334-335.

Arthur, J.C. 1922. Urediniales collected by Fred J. Seaver in Trinidad. Mycologia 14:12-13, 240-241.

Arthur, J.C. 1934. Manual of the Rusts in United States and Canada. Purdue Research Foundation. Lafayette, IN, USA. 438 pp.

Assante, G., L. Locci, L. Camarda, and G. Nasini. 1977. Screening of the genus *Cercospora* for secondary metabolites. Phytochemistry 16:243-247.

Bain, D.C. 1964. *Sclerotinia* blight on nutgrass in Mississippi. Plant Dis. Reptr. 48:742.

Baltazar, A. 1995. Weed management in wet-seeded rice in Asia. Pages 656-661 *in* Proc. 15th Asian Pacific Weed Science Society Conference. Vol. IB. July 24-28, 1995, Tsukuba, Japan.

Baretto, R.W. and H.C. Evans. 1995. Mycobiota of the weed *Cyperus rotundus* in the state of Rio de Janeiro, with an elucidation of its associated *Puccinia* complex. Mycol. Res. 99:407-419.

Bayot, R.G., A.K. Watson and K. Moody. 1992. Control of paddy weeds by plant pathogens in the Philippines. Pages 259-272 *in* Proc. International Symposium on Biological Control and Integrated Management of Paddy and Aquatic Weeds in Asia. National Agriculture Research Center, Tsukuba, Japan.

Bendixen, L.E. and U.B. Nandihalli. 1987. Worldwide distribution of purple and yellow nutsedge (*Cyperus rotundus* L. and *C. esculentus* L.) Weed Technol. 1:61-65.

Betria, A.I. 1973. Biology of the purple nutsedge (*Cyperus rotundus*). Rev. Fac. Agron., Univ. Nac. La Plata 49:181-199.

Bhardwaj, R.B.L. and R.D. Verma. 1968. Seasonal development of nutgrass (*Cyperus rotundus* L.) under Delhi conditions. Indian J. Agric. Sci. 38:950-957.

Biswas, J.C., S.K.A. Sattar, and S.B. Siddique. 1990. Critical crop-weed competition in wet season transplanted rice. Bangladesh Rice J. 1:17-22.

Blaney, C. L. and C. G. Van Dyke. 1986. Conidial reprodcution in culture by a *Cercospora* sp. from yellow nutsedge (*Cyperus esculentus* L.). Page 386 *in* Proc. Southern Weed Science Society.

Blaney, C. L., C. G. Van Dyke, and L. F. Grand. 1988. *Cercospora caricis* from *Cyperus esculentus* (yellow nutsedge): Morphology and cercosporin production. Mycologia 80:418-421.

Bugnicourt, F. 1950. Les espèces du genre *Curvularia* isolées des semences de riz. Rev. gén. Bot. 57:65-77.

Burkill, I. 1935. A Dictionary of the Economic Products of the Malay Peninsula. Vols. I and II. Governments of the Straits Settlements and Federated Malay States, Crown Agents for the Colonies, London, UK. 2402 pp.

Burnett, H.C., D.P.H. Tucker, and W.H. Ridings. 1974. *Phytophthora* root and stem rot of milkweed vine. Plant Dis. Reptr. 58:355-357.

Callaway, M. B., S. C. Phatak, and H. D. Wells. 1985. Studies on alternate hosts of the rust *Puccinia canaliculata*, a potential biological control agent for nutsedges. Plant Dis. 69:924-926.

Castellani, E. 1941. On the presence of pluricellular teleutospores in *Puccinia canaliculata* (Schw.) Lagerh. Nuovo G. Bot. Ital. (Nuovo Ser.) 48:658-661.

Castellani, E. 1946. On two diseases of *Cyperus rotundus* L. Agricoltura colon. 36:7.

Chandler, R.F., Jr. 1979. Rice in the Tropics: A Guide to the Development of National Programs. Westview Press. Boulder, CO, USA. 256 pp.

Charudattan, R. 1990. Pathogens with potential for weed control. Pages 132-154 *in* Microbes and Microbial Products as Herbicides. R.E. Hoagland (Ed.) ACS Symposium Series 349. American Chemical Society, Washington, DC, USA.

Charudattan, R. 1998. Indigenous pathogens and commercial development: An overview. (unpublished paper).

Chandrasena, J.P.N.R. 1988. Floristic composition and abundance of rice-field weeds in four low-country wet zone districts of Sri Lanka. Trop. Pest Management (UK). 34: 278-287.

Chupp, C. 1954. A monograph of the fungus *Cercospora*. Privately printed. Ithaca, NY, USA. 667 pp.

Clay, K. 1986.New disease (*Balansia cyperi*) of purple nutsedge (*Cyperus rotundus*). Plant Dis. 70:597-599.

Daniel, J.T., G.E. Templeton, R.J. Smith, and W.T. Fox. 1973. Biological control of northern joint vetch in rice with an endemic fungal disease. Weeds Sci. 21:303-307.

Das, N.P. and S.K. Mukherji. 1968. A stem rot and leaf drying of *Cyperus tagetum* Roxb. (matgrass) and its control. Z. Pflanzenlr. Pflanzenpathol. Pflanzenschutz. 75:683-686.

Davis, C.H. 1942. Response of *Cyperus rotundus* L. to five moisture levels. Plant Physiol. 17:311-316.

De Datta, S.K. 1995. Weed management perspectives for sustainable agriculture in rice-based systems. Pages 17-27 *in* Proc. 15th Asian-Pacific Weed Science Society Conference. Vol. I (A). July 24-28, 1995. Tsukuba, Japan.

Diehl, W.W. 1950. *Balansia* and the Balansiae in America. Agricultural Monograph 4. United States Department of Agriculture, WA, USA. 82 pp.

Edgerton, C.W. 1919. A new *Balansia* on *Cyperus*. Mycologia 11:259-261.

Ehui, S.K. and T.W. Hertel. 1989. Deforestation and agricultural productivity in the Côte d' Ivoire. Am. J. Agric. Econ. 71:703-711.

Ellis, M.B. 1966. Dematiaceous Hyphomycetes. VII. *Curvularia, Brachysporium*, etc. Mycol. Pap. 106:1-57.

Ellis, M.B. 1971. Dematiaceous Hyphomycetes. Commonwealth Mycological Institute. Kew, Surrey, England. 608 pp.

Ellis, M.B. 1976. More Dematiaceous Hyphomycetes. Commonwealth Mycological Institute. Kew, Surrey, England. 507 pp.

Evans, H.C. 1987. Fungal pathogens of some subtropical and tropical weeds and the possibilities for biological control. Biocontrol News and Information 8:7-30.

Evans, H.C. 1990. Biological control of tropical grassy weeds. Pages 52-72 *in* Tropical Grassy Weeds. F.W.G. Baker and P. Terry (Eds.) CAB International. Wallingford, UK.

Farr, D.F., G.F. Bills, G.P. Chamuris, and A.Y. Rossman. 1989. Fungi of Plants and Plant Products in the United States. APS Press. St. Paul, MN, USA.

Gleason, H.A.1963. Illustrated Flora of the Northeastern United States and Adjacent Canada. Vol. 1. Hafner Publishing Co., Inc. NY. 482 pp.

Ghosh, R.N. and S. Gupta. 1980. Two new host records from India. Indian Phytopath. 33:498-500.

Gjaerum, H.B. 1984. East African rusts (Uredinales), mainly from Uganda. 2. on Cyperaceae. Mycotaxon 20:53-63.

Gupta, P.C. and J.C. O'Toole. 1986. Upland Rice: A Global Perspective. International Rice Research Institute, Manila, Philippines. 360 pp.

Heap, I.M. 1997. The occurrence of herbicide-resistant weeds worldwide. Pestic. Sci. 51:235-243.

Holm, L.G., D.L. Plucknett, J.V. Pancho, and J.P. Herberger. 1977. The World's Worst Weeds. The University Press of Hawaii. Honolulu, Hawaii. 609 pp.

Horowitz, M. 1972. Effect of growth regulators on *Cynodon dactylon* (L.) Pers., *Sorghum halapense* (L.) Pers. and *Cyperus rotundus* L. Weed Res. 12:11-20.

International Rice Research Institute. 1987. Annual Report for 1986. International Rice Research Institute. Manila, Philippines. 639 pp.

International Rice Research Institute.1993. Rice Almanac (1993-1995). International Rice Research Institute. Manila, Philippines. 142 pp.

Ito, S. and D. Murayama. 1943. Notae mycologicae Asia Orientalis. IV. Trans. Sap. Nat. His. Soc. 17:160-172.

Jain, B.L. 1962. Two new species of *Curvularia*. Trans. Brit. Mycol. Soc. 45:539-544.

Jackson, H.S. 1926. The rusts of South America based on the Holway collections. Mycologia 18:139-144.

Jacometti, G. 1912. Le erbe che infestano le risaie italiano. Atti del Congresso Risicolo Internazionale, Vercelli 4:57-91.

Janiya, J. D. and K. Moody. 1984. Use of *Azolla* to suppress weeds in transplanted rice. Trop. Pest Management (UK) 30:1-6.

Johnson, W.M. and L.A. Brinkerhoff. 1976. Susceptibility of some crops and weeds to *Verticillium dahliae* Kleb. isolated from cotton. Pages 21-22 *in* Proc. Beltwide Cotton Producers Research Conference.

Julien, M.H. 1992. Biological Control of Weeds: A World Catalogue of Agents and their Target Weeds. 3rd edition. CAB International, Wellington in association with Australian Center for International Agriculture Research (ACIAR). Canberra, Australia. 186 pp.

Justice, O.L. and M.D. Whitehead. 1946. Seed production, viability, and dormancy in the nutgrasses *Cyperus rotundus* and *C. esculentus*. J. Agric. Res. 73:303-318.

Kadir, J.B., R. Charudattan, R.D. Berger, W.M. Stall, and B.J. Brecke. 1996a. Field efficacy of *Dactylaria higginsii* for control of purple nutsedge. Phytopathology 87:S49.

Kadir, J.B., R. Charudattan, W.M. Stall, and T.A. Bewick. 1996b. Effect of *Dactylaria higginsii* on the interference of purple nutsedge with tomato and pepper. Phytopathology 87:S50.

Kapoor, I.J. and R.N. Tandon. 1971. Occurrence of *Curvularia tuberculata* Jain on stored fruits of *Psidium guajava* L. Sydowia 24:201-202.

Kenney, D.S. 1986. DeVine - the way it was developed - an industrialist's view. Weed Sci. 34 (Suppl. 1):15-16.

Kern, F.D. 1919. North American rusts on *Cyperus* and *Eleocharis*. Mycologia 11:134-147.

Kusanagi, T. 1981. Ecological aspects of weeds on paddy fields. Pages 68-88 *in* Weeds and Weed Control in Asia. M.H. Tetangco (Ed.). FFTC Book Series 20. Food and Fertilizer Center for Asia and Pacific Regions, Taipei.

Lal, B. and D. Goel. 1989. A new rot of *Abelmoschus esculentus*. Indian Phytopath. 42:482.

Lambat, A.K. and Ram, A. 1969. Seed-borne infection of *Curvularia* causing a new blight disease of Jowar. Indian Phytopath. 22:282-284.

Lele, V.C., S.P. Raychaudhuri, R.B. Bhalla, and A. Ram. 1968. *Curvularia tuberculata*, a new fungus causing die-back disease of citrus in India. Indian Phytopath. 21:66-72.

Lele, V.C., J. Singh, S.N. Rai, and J. Kandhari. 1981. Occurrence of a new blight disease of mango caused by *Curvularia*. Curr. Sci. 50:464-465.

Lin, J., R.J. Smith, Jr., and R.H. Dilday. 1992. Allelopathic activity of rice germplasm on weeds. Page 99 *in* Proc. 45th Annual Meeting of the Southern Weed Science Society, Little Rock, Arkansas, USA.

Ling, L. 1950. Studies on the genus *Cintractia*. II. *C. axicola* and related species. Mycologia 42:646-653.

Loveless, A.R. 1967. A new species of *Claviceps* on Cyperaceae. Trans. Br. Mycol. Soc. 50:19-22.

Luttrell, E.S. 1954. An undescribed species of *Pyricularia* on sedges. Mycologia 46:810-814.

Mailum, N.P. and G.G. Divinagracia. 1969. Leaf spot of ginger in the Philippines. Philipp. Agric. 53:202-217.

Makowski, R.M.D. and K. Mortensen.1992. The first mycoherbicide in Canada: *Colletotrichum gloeosporioides* f. sp. *malvae* for round-leaved mallow control. Pages 298-300 *in* Proc. 1st International Weed Control Congress. Vol. II. Weed Science Society of Victoria, Inc., Melbourne, Australia.

Marambe, B., U.R. Sangakkara, and S.K. Ratnayaka. 1995. Control of tuber growth of purple nutsedge (*Cyperus rotundus*): Effects of drying and depth of burying. Pages 562-566 *in* Proc. 15th Asian Pacific Weed Science Society Conference. Vol. IB. July 24-28, 1995, Tsukuba, Japan.

Mishra, D. and N. Singh. 1987. Two new leaf diseases of coconut seedlings caused by *Curvularia* spp. Indian J. Plant Pathol. 5: 208-209.

Misra, A.P., O. Prakash, B. Mishra, and K.K. Dutta. 1972. A note on a new leaf spot disease of motha (*Cyperus rotundus* L.) caused by *Curvularia tuberculata* Jain. Indian J. Weed Sci. 4:57-59.

Misra, A.P., O. Prakash, B. Mishra, and K.K. Dutta. 1973. A new leaf spot disease of motha (*Cyperus rotundus* L.) caused by *Curvularia tuberculata*. Indian Phytopath. 26:165-167.

Mohlenbrock, R.H. 1976. The Illustrated Flora of Illinois: Sedges, *Cyperus* to *Scleria*. Southern Illinois University Press. Carbondale, IL. 192 pp.

Moody, K. 1989. Weeds reported in rice in South and Southeast Asia. International Rice Research Institute, Manila, Philippines. 442 pp.

Moody, K. 1990. Postplanting weed control in direct-seeded rice. Paper presented at a Rice Symposium, 25-27 September 1990, MARDI, Penang, Malaysia.

Moody, K. 1992. Weed management in wet-seeded rice in tropical Asia. Pages 1-20 *in* Proc. International Symposium on Biological Control and Integrated Management of Paddy and Aquatic Weeds in Asia. National Agriculture Research Center. Tsukuba, Japan.

Moody, K. 1995. Sustainability in rice weed management. Pages 93-103 *in* Proc. 15th Asian Pacific Weed Science Society Conference. Vol. IA. July 24-28, 1995, Tsukuba, Japan.

Morin, L., J.L. Derby, and E.G. Kokko. 1996. Infection process of *Colletotrichum gloeosporioides* f. sp. *malvae* on Malvaceae weeds. Mycol. Res. 100:165-172.

Mukhopadhyay, S.K.1983. Weed control technology in rainfed wetland rice. Pages 109-118 *in* Proc. 1981 Weed Control in Rice Conference. International Rice Research Institute. Manila, Philippines.

Noda, K. and S. Eguchi. 1965. Studies on the ecology of weeds on arable lands. I: Emergence patterns of annual representative weeds which are commonly found on the paddy rice fields of south-western Japan. Bull. Kyushu Agric. Expt. Stn. 11:345-374.

Nyahoza, F. 1973. Studies of the biology of *Cyperus rotundus* L. early growth and vegetative reproduction strategy. E. Afr. Agr. For. J. 17:120-130.

Okafor, L.I. and S.K. De Datta, 1974. Competition between weeds in upland rice in monsoon – Asia. Phil. Weed Sci. Bull. 1:39-45.

Okafor, L.I. and S.K. De Datta, 1976. Competition between upland rice and purple nutsedge for nitrogen, moisture, and light. Weed Sci. 24:43-46.

Padhi, A.K., B.K. Sahoo, and K.C. Das. 1991. Effect of weed management on yield of rainfed, direct-seeded, upland rice (*Oryza sativa*). Indian J. Agric. Sci. 61:27-30.

Pereira, W., G. Crabtree, and R. D. William. 1987. Herbicide action on purple and yellow nutsedge (*Cyperus rotundus* and *C. esculentus*) Weed Technol. 1:92-98.

Phatak, S.C., H.D. Wells, D.R. Sumner, D.K. Bell, and N.C. Glaze. 1981. Observations on rust on yellow nutsedge (*Cyperus esculentus* L.). Phytopathology 71:899.

Phatak, S. C., D. R. Summer, H. D. Wells, D. K. Bell, and N. C. Glaze. 1983. Biological control of yellow nutsedge with the indigenous rust fungus Puccinia *canaliculata*. Science 219:1446-1447.

Phatak, S. C., M. B. Callaway, and C. S. Vavrina. 1987. Biological control and its integration in weed management systems for purple and yellow nutsedge (*Cyperus rotundus* and *C. esculentus*). Weed Technol. 1:84-91.

Purohit, S.D., K.G. Ramawat, and H.C. Arya. 1979. Polyphenols and some oxidative enzymes in rust infected leaves of *Cyperus rotundus*. Indian Phytopath. 32:255-259.

Rama Rao, P. 1964. *Curvularia tuberculata* from dune soil. Curr. Sci. 33:121.

Ranade, S. and W. Burns. 1925. The eradication of *Cyperus rotundus* L. Memoirs of the Department of Agriculture in India, Botanical Series, 13:99-192.

Rao, J.S. and M. Nagarajan. 1962. Relationship between moisture levels and viability of nutgrass tubers. Madras Agric. J. 49:120-123.

Ridings, W.H. 1986. Biological control of stranglervine in citrus - a researcher's view. Weed Sci. 34 (Suppl.1):31-32.

Safeeulla, K.M. and H.C. Govindu. 1950. *Cintractia minor* on three species of *Cyperus* in Mysore. Curr. Sci. 19:325-326.

Sawada, K. 1927. Descriptive catalogue of Formosan fungi. Part III. Rep. Dep. Agric. Res. Inst. Formosa. 27:73.

Sawada, K. 1959. Descriptive catalogue of Taiwan (Formosan) fungi. Part XI. College of Agriculture, Government Research Institute of Formosa, Report 51.

Seethalakshmi, K.V. 1953. Blight of *Cyperus rotundus* L. and *C. bulbosus* Vahl. Indian Phytopath. 6:57-62.

Sirohi, H.S. and P.K. Dublish. 1981. *Eudarluca caricis* on *Puccinia conclusa* – a new host record for India. Indian Phytopath. 34:84.

Standley, P.C. 1916. Fungi of New Mexico. Mycologia 8:142, 157.

Sanders, B.A. 1994. The life cycle and ecology of *Cyperus difformis* (rice weed) in temperate Australia: a review. Aust. J. Exp. Agric. 34:1031-1038.

Seshadri, K., P. Padmanaban, and K.C. Alexander. 1980. A new leaf blight disease of sugarcane. Indian Phytopath. 33:325-326.

Sivanesan, A. 1985. The teleomorph of *Curvularia tuberculata*. Trans. Brit. Mycol. Soc. 84:548-551.

Sivanesan, A. 1987. Graminicolous species of *Bipolaris, Curvularia, Drechslera, Exserohilum* and their teleomorphs. Mycological Papers, No. 158. CAB International Mycological Institute. Wallingford, UK. 261 pp.

Smith, R.J. Jr.1986. Biological control of northern joint vetch in rice and soybeans - a researcher's view. Weed Sci. 34 (Suppl.1):17-23.

Smith, E.V. and G.L. Fick. 1937. Nutgrass eradication studies. I. Relation of the life history of nutgrass, *Cyperus rotundus* L., to possible methods of control. J. Am. Soc. Agron. 29:1007-1013.

Sterle, A., R. Upadhyary, and G. Strobel. 1991. Cyperine, a phytotoxin produced by *Aschochyta cypericola*, a fungal pathogen of *Cyperus rotundus*. Phytochemistry 30:2191-2192.

Stevens, F.L. 1932. Additional Philippine Uredinae. Nat. Appl. Sci. Bull. (Philippines) 2:441-447.

Stoller. E.W. and R.D. Sweet. 1987. Biology and life cycle of purple and yellow nutsedges. Weed Technol. 1:66-73.

Stovall, M. E. and K. Clay. 1988. The effect of the fungus, *Balansia cyperi* Edg., on growth and reproduction of purple nutsedge. New Phytol. 109:351-359.

Swain, D. J. Nott, and R. B. Trounce. 1975. Competition between *Cyperus difformis* and rice: the effect of time of weed removal. Weed Res. 15:149-152.

Sydow, H. and P. Sydow. 1906. Neue und kritische Uredineen – IV. Annales Mycologici 4:28-32.

Tangonan, N.G. and F.C. Quebral. 1992. Host Index of Plant Diseases in the Philippines. 2nd Edition. The Department of Science and Technology, Bicutan, Taguig, Manila, Philippines. 273 pp.

Tarr, S.A.J. 1955. The fungi and plant diseases of the Sudan. Commonwealth Mycological Institute. Kew, Surrey, England. 127 pp.

Tarr, S.A.J. 1963. A supplementary list of Sudan fungi and plant diseases. Mycol. Pap. 85, 31 pp.

Taylor, T.M.C. 1983. The Sedge Family of British Columbia. British Columbia Provincial Museum, Handbook 43. British Columbia Provincial Museum. Victoria, BC. 375 pp.

TeBeest, D.O., G.E. Templeton, and R.J. Smith, Jr. 1978. Histopathology of *Colletotrichum gloeosporioides* f. sp. *aeschynomene* on northern jointvetch. Phytopathology 68:1271-1275.

Templeton, G.E., D.O. TeBeest, and R.J. Smith, Jr. 1984. Biological weed control in rice with a strain of *Colletotrichum gloeosporioides* (Penz.) Sacc. used as a mycoherbicide. Crop Prot. 3:409-422.

Thind, K.S. and G.S. Rawla. 1961. A new fungus on *Cyperus iria*. Am. J. Bot. 48:859-862.

Thullen, R.J. and P.E. Keeley. 1979. Seed production and germination in *Cyperus esculentus* and *C. rotundus*. Weed Sci. 27:502-505.

Tripathi, R.S. 1969. Ecology of *Cyperus rotundus* L. III. Population of tubers at different depths of the soil and their sprouting response to air-drying. Proc. Nat Acad. Sci. India 39:140-142.

Upadhyay, R. K., G. A. Strobel, and W. M. Hess. 1990. *Phoma cyperi* sp. nov., a new pathogen of *Cyperus iria* , its vegetative and reproduction structures and production of phytotoxins. Can J. Bot. 68:2059-2064.

Upadhyay, R. K., D. Kenfield, and G. A. Strobel 1991. *Ascochyta cypericola* sp. nov. causing leaf blight of purple nutsedge (*Cyperus rotundus*). Can. J. Bot. 69:797-802.

Vaillant, A. 1967. Chemical control of annual weeds in rice. World Crops 19:38-44.

Van Dyke, C.G. 1989. Factors in the infection process of fungal pathogens for biological control of weeds. Pages 559-563 *in* Proc. VII International Symposium on Biological Control of Weeds. Delfosse, E.S. (Ed.). Ist. Sper. Patol. Veg. (MAF). Rome, Italy.

Van Dyke, C.G. and R. N. Trigiano. 1987. Light and scanning electron microscopy of the interaction of the biocontrol fungus *Alternaria cassiae* with sicklepod (*Cassia obtusifolia*). Can. J. Plant Pathol. 9:230-235.

Vega, M. and J. Sierra. 1970. Population of weed seeds in a lowland rice field. Philippine Agriculturist 54:1-7.

Wan, F.H., R. Wang, and S.B. Qui. 1994. Biological weed control in China: Current status and prospects (Mimeograph, unpublished paper).

Wapshere, A. J., E. S. Delfosse, and J. M. Cullen. 1989. Recent developments in biological control of weeds. Crop Prot. 8:227-250.

Watanabe, H., I. Md. Zuki, and N.K. Ho. 1994. 2,4-D resistance of *Fimbristylis miliacea* in direct seeded rice fields in the Muda area. Pages 353-356 *in* Proc. 4th International Conference on Plant Protection in the Tropics. A. Rajan and Y. Ibrahim (Eds.). Kuala Lumpur, Malaysia.

Waterhouse, G.M. 1970. The genus *Phytophthora* De Barry. Mycol. Pap. 122, 59 pp.

Waterhouse, D.F. 1992. Prospects in biological control of paddy weeds in Southeast Asia and some recent successes in the biological control of aquatic weeds. Pages 21-42 *in* Proc. International Symposium on Biological Control and Integrated Management of Paddy and Aquatic Weeds in Asia. 20-23 Oct. 992. National Agriculture Research Center, Tsukuba, Japan.

Watson, A. K. 1985. Host specificity of plant pathogens in biological weed control. Pages 577-586 *in* Proc. VI International Symposium on Biological Control of Weeds. E. D. Delfosse, (Ed.). Vancouver, Canada.

Watson, A.K. 1991. The classical approach with plant pathogens. Pages 3-23 *in* Microbial Control of Weeds. T.O. TeBeest (Ed.). Chapman and Hall. New York, NY, USA.

Watson, A.K. 1992. Current status of bioherbicide development and prospects for rice in Asia. Pages 367-379 *in* Proc. International Symposium on Biological Control and Integrated Management of Paddy and Aquatic Weeds in Asia. 20-23 Oct. 1992. National Agriculture Center, Tsukuba, Japan.

Watson, A.K. 1994. Current status of bioherbicide development and prospects for rice in Asia. Pages 195-201 *in* Integrated Management of Paddy and Aquatic Weeds in Asia. Proc. International Seminar " Biological Control and Integrated Management of Paddy and Aquatic Weeds in Asia". H. Shibayama, K. Kiritani, and J. Bay-Petersen (Eds.) Oct. 19-25, 1992. Tsukuba, Japan.

Wax, L. M. 1975. Control of yellow nutsedge in field crops. Proc. North Central Weed Control Conference. 30:125-128.

Webster, R.K. and P.S. Gunnell (Eds.). 1992. Compendium of Rice Diseases. The American Phytopathological Society. APS Press. St. Paul, MN, USA. 62 pp.

Wei, Y.D., K.N. Beyer, and P.H. Goodwin. 1997. Hemibiotrophic infection of round-leaved mallow by *Colletotrichum gloeosporioides* f. sp. *malvae* in relation to leaf senescence and reducing agents. Mycol. Res. 101:357-364.

Weiss, F. 1950. Index of plant diseases in the United States. Special Publication of the Plant Disease Survey. U.S. Dep. Agric., No. 1, Part II. pp. 291-292.

Welles, C.G. 1922. A provisional list of the parasitic fungi of the Philippine Islands. Philipp. Agric. Prev. 15:149-202.

Wetzstein, H.Y. and S.C. Phatak. 1987. Scanning electron microscopy of the uredinial stage of *Puccinia canaliculata* on yellow nutsedge, *Cyperus esculentus* (Cyperaceae). Amer. J. Bot. 74:100-106.

Wills, G.D. 1978. Initial evaluations of purple nutsedge (*Cyperus rotundus* L.) ecotypes (Abstract, 40). Weed Science Society of America Annual Meeting.

Wills, G.D. 1987. Description of purple and yellow nutsedge (*Cyperus rotundus* and *C. esculentus*). Weed Technol. 1:2-9.

Wills, G.D. and G.A. Briscoe. 1970. Anatomy of purple nutsedge. Weed Sci. 18:631-635.

Yamasue, Y. and K. Ueki. 1983. Biology of paddy weeds and their control in wetland rice. Pages 227-242 *in* Proc. 1981 Weed Control in Rice Conference. International Rice Research Institute, Manila, Philippines.

Yen, J.M. 1974. A study on the parasitic fungi of Southeast Asia. XXIII. Uredinales of the Philippines. Bull. Trimest. Soc. Mycol. France. 90:195-200.

Fig. 1.1. A. *Cyperus difformis*, mature plant. **B.** *Cyperus iria*, mature plant. **C.** *Cyperus rotundus*, mature plant. **D.** *Fimbristylis miliacea*, mature plant. **E.** Vegetative propagation in *Cyperus rotundus* showing tubers (T), basal bulb (B), and rhizomes (R). A to D taken from Ampong-Nyarko and De Datta (1991).

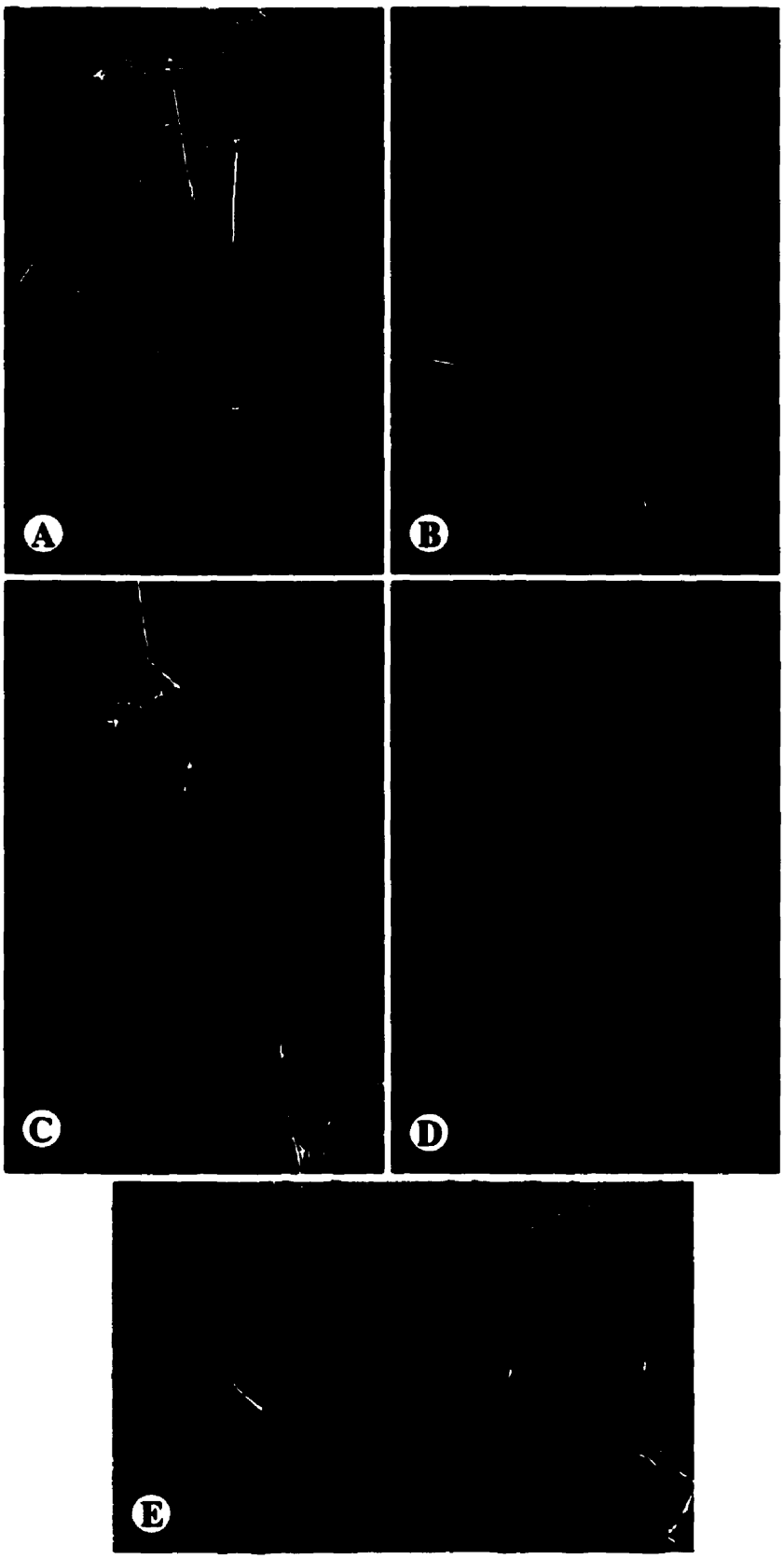

A
B
C
D
E

Table 1.1. Cyperaceae weeds and their fungal pathogens.*

Fungal pathogen	*Host*	Reference(s)
Mastigomycotina		
Phytophthora cyperi (Ideta) Ido	*C. rotundus*	Waterhouse, 1970; Tarr, 1963
P. cyperi complex		
P. cyperi-iriae Sawada	*C. iria*	Waterhouse, 1970
P. cyperi-rotundati Sawada	*C. rotundus*	Sawada, 1927; Seethalakshmi, 1953; Waterhouse, 1970
Ascomycotina		
Balansia cyperi Edgerton	*Cyperus* spp., *C. esculentus*	Diehl, 1950; Clay, 1986
B. cyperacearum	*C. rotundus*	Farr et al., 1989
Claviceps cyperi	*C. rotundus*, *C. esculentus*	Loveless, 1967
Phyllachora cyperi	*C. rotundus*, *C. esculentus*	Weiss, 1950
Sclerotinia homeocarpa	*C. rotundus*	Bain, 1964

Basidiomycotina		
Puccinia canaliculata (Schw.) Lagerh.	*C. rotundus*, *C. esculentus*	Standley, 1916; Kern, 1919; Arthur, 1920, 1922; Jackson, 1926; Castellani, 1941, 1946; Weiss, 1950; Phatak et al., 1981, 1983; Barretto and Evans, 1995; Herb-IMI[a]
	C. difformis	Herb-IMI
P. conclusa Thum = *P. romagnoliana* Maire & Saccardo	*C. difformis*, *C. iria*, *C. esculentus*, *C. rotundus*	Tarr, 1955; Purohit et al., 1979; Sirohi and Dublish, 1981; Gjaerum, 1984; Herb-IMI
P. philippinensis H. & P. Sydow	*C. rotundus*	Stevens, 1932; Yen, 1974
	Cyperus spp.	Kern, 1919; Arthur, 1934
P. cyperi Arthur, *P. cyperi-tagetiformis* (P. Henn.) Kern	*C. rotundus*	Weiss, 1950; Herb-IMI
Cintractia limitata Clinton	*C. rotundus*	Ling, 1950; Tarr, 1955; Barretto and Evans, 1995
	Cyperus spp.	Ling, 1950
C. minor (Clint.) Jacks.	*C. rotundus*	Safeeulla and Govindu, 1950; Weiss, 1950

Deuteromycotina		
Alternaria tenuissima	*C. rotundus*	Betria, 1973
Ascochyta sp.	*C. esculentus*	Weiss, 1950
	C. rotundus	Upadhyay et al., 1991
Cercospora caricis Oud. = *C.*	*C. esculentus.*	Chupp, 1954; Barretto and
ugandensis Hansford	*C. rotundus*	Evans, 1995
Cercospora sp.	*C. esculentus*	Blaney et al., 1986, 1988
Curvularia tuberculata Jain	*C. rotundus*	Misra et al., 1972, 1973
Dactylaria higginsii (Lutrell) =	*Cyperus spp. including*	Lutrell, 1954; Barretto and
Pyricularia higginsii Lutrell	*C. iria and C. rotundus*	Evans, 1995; Kadir et al., 1996a,b
Drechslera maydis	*C. rotundus*	Alcorn and Pont, 1973
Duosporium cyperi Thind and	*C. iria*	Thind and Rawla, 1961;
Rawla		Barretto and Evans, 1965
Fusarium oxysporum	*C. esculentus*	D.K. Bell[b]
F. lateritum	*C. esculentus*	D.K. Bell[b]
Phoma cyperi	*C. iria*	Upadhyay et al., 1990
Phyllosticta zingiberi	*C. rotundus*	Mailum and Divinagracia, 1969
P. cypericola Sawada	*C. rotundus*	Sawada, 1959
Rhizoctonia solani Kuhn	*C. rotundus*	Weiss, 1950

R. bataticola (= *Macrophomina phaseolina*)	*C. rotundus*	Das and Mukherji, 1968
Verticillium dahliae	*C. esculentus*	Johnson and Brinkerhoff, 1976

* adapted from Evans, 1987; Phatak et al., 1987; and Barretto and Evans, 1995.

[a] Herbarium Collection. International Mycological Institute. Kew. Surrrey. England.

[b] personal communication, cited in Phatak et al., 1987.

CONNECTING TEXT

The review of literature in the previous chapter described the impact of sedge weeds on rice and other important crops and the measures used to control them. The use of biological agents to control weeds is gaining wider acceptance. Indigenous fungal weed pathogens have good potential as components of integrated weed management programs for rice. Three *Curvularia* isolates identified as *Curvularia tuberculata* (isolates 93-020 and 93-022) and *Curvularia oryzae* (isolate 93-061) were obtained from diseased *Cyperus difformis*, *Cyperus iria*, and *Fimbristylis miliacea* in the Philippines. This chapter presents results of the virulence of these isolates to their respective weed hosts and describes the symptoms associated with the disease. Cross-pathogenicity of the *Curvularia* isolates to the other sedge weeds was also determined.

Chapter 2. Seedling blights of Cyperaceae weeds caused by *Curvularia tuberculata* and *C. oryzae*

2.1. Abstract

Two isolates of *Curvularia tuberculata* (isolate 93-020 and 93-022) and one isolate of *C. oryzae* (isolate 93-061) were obtained from diseased *Cyperus difformis*, *C. iria*, and *Fimbristylis miliacea*, respectively, in the Philippines in 1993. When inoculated onto their hosts at 1 x 10^8 spores/m^2, and provided with 24 h dew at 28° C, a severe and rapid blighting of the leaves caused the weed seedlings to die within two weeks. The three *Curvularia* isolates were cross-pathogenic to *C. difformis*, *C. iria*, *F. miliacea*, and *C. rotundus* but exhibited varying degrees of virulence depending on the weed species being attacked. *C. tuberculata* isolates 93-020 and 93-022 effectively controlled *C. difformis* and *C. iria*, but were less effective on *F. miliacea*. *C. oryzae* was equally virulent on *C. difformis*, *C. iria*, and *F. miliacea*. *C. rotundus* was not killed by any of the three isolates and exhibited only minor flecking and leaf tip die-back. Sporulation of the three *Curvularia* isolates on necrotic leaf tissues occurred on susceptible (*C. difformis*, *C. iria*, and *F. miliacea*) but not on resistant (*C. rotundus*) weed hosts.

2.2. Introduction

Members of the family Cyperaceae are frequently represented in the weed flora of rice crops of Southeast Asia, including the Philippines. *Cyperus difformis* L. (small flower umbrella sedge), *Cyperus iria* L. (rice flatsedge), and *Fimbristylis miliacea* (L.) Vahl (globe fingerush), are common in irrigated rice fields, and are known to cause up to a 50% reduction in rice yields by competing with rice for nutrients (Ampong-Nyarko and De Datta, 1991). *Cyperus rotundus* L. (purple nutsedge) is the most serious weed of

upland rice in all of tropical Asia, Africa, and Latin America (De Datta, 1983). It competes with rice for sunlight, water, and nutrients (Okafor and De Datta, 1974) and is a major limiting factor in upland rice production. Yield losses up to 50% in rice have been reported in cases of heavy infestation (Ampong-Nyarko and De Datta, 1991).

Control strategies for *C. difformis*, *C. iria*, and *F. miliacea* include handweeding (Moody, 1992), cultivation by an animal-drawn, spike-toothed harrow or rotary weeder (Moody, 1992; Mukhopadhyay, 1983), flooding (Moody, 1990), and use of phytotoxic crop residues (Lin et al., 1992). Preplant, pre-emergence, and post-emergence herbicides have also been used (Baltazar, 1995; De Datta, 1995), but the prohibitive cost to small farmers, lack of technical knowledge, and herbicide injury to rice limit their widespread use in Asia (Baltazar, 1995). There are also reports of developing herbicide resistance by *F. miliacea* to 2,4-D (2,4-dichlorophenoxyacetic acid) (Watanabe et al., 1994) and *C. difformis* to bensulfuron (methyl 2- [[(4,6-dimethoxypyrimidin-2-yl) aminocarbonyl] aminosulfonylmethyl] benzoate) (Heap, 1997). An integrated weed management strategy is recommended to help avoid the development of herbicide resistance of weeds in rice (Powles and Holtum, 1990).

Weeds have a strong impact on rice production (Moody, 1995). Current estimates report that annual rough rice production would have to increase by 70% in the next 30 to 35 years to keep up with population growth and income-demand for food (Watson et al., 1997). To ensure long-term food supply, sustainable agriculture technology must be integrated into rice-based cropping systems. Biological control of weeds has good potential as a component of an integrated weed management program for rice (Watson, 1994).

Pathogens from major sedge weeds of irrigated rice were obtained in the Philippines in 1993. Isolate 93-020 from *C. difformis* and isolate 93-022 from *C. iria* was identified by the International Mycological Institute (IMI) as *Curvularia tuberculata* Jain, and isolate 93-061 from *F. miliacea*, as *Curvularia oryzae* Bugnicourt. This study describes the disease progress and reports mortality of these weed species as a result of inoculation with conidial suspensions of their respective *Curvularia* pathogen. Cross-infectivity of the isolates to closely related weedy sedges were also demonstrated and the implications on biocontrol potential is discussed.

2.3. Materials and methods

All experiments were conducted at the International Rice Research Institute (IRRI), Los Baños, Laguna, Philippines.

2.3.1. Fungal isolates

Three isolates belonging to two species of *Curvularia* were used in the experiments. *Curvularia tuberculata* was isolated from diseased *Cyperus difformis* (isolate 93-020) and from diseased *Cyperus iria* (isolate 93-022) from Lian, Batangas, Philippines. *Curvularia oryzae* (isolate 93-061) was obtained from diseased *Fimbristylis miliacea* from Manicahan, Zamboanga, Philippines. All fungal isolates were cultured on half-strength potato dextrose agar (½ PDA) plates or slants for initial observation and later stored in agar-oil slants and/or soil cultures.

2.3.2. Colony morphology and spore development of C. tuberculata and C. oryzae

The slide culture technique described by Dhingra and Sinclair (1985) was used to study spore development and morphology. A bent glass rod was placed on a filter-paper-lined bottom of a culture plate and a clean glass slide on top of it. The filter paper was moistened with 5% glycerin and autoclaved. Blocks of solidified ½ PDA, approximately 1-cm^2, were cut with a sterile scalpel and placed on the sterile slide. The center of each edge of the agar block was then seeded with small agar pieces from a one-week-old culture of the isolate. A cover slip was then placed on top of the seeded agar block. The top of the dish was replaced and the dish sealed with a strip of parafilm. The slide cultures of isolates 93-020 and 93-022 were then incubated at 28° C in the dark while those of *C. oryzae* were incubated under lighted conditions (30 $\mu E\ m^{-1}\ s^{-1}$) at 28° C. After four to five days, the cover slips were carefully lifted and mounted in a drop of plain lactophenol on a clean glass slide. The fungal growth on the culture slide was similarly mounted. Observations were made through a compound microscope at 10 to 100x magnification and photographically recorded using a 35-mm Nikon camera.

2.3.3. Preparation of inoculum

Agar plugs were used to seed ½ PDA plates for the mass production of the inoculum. Seeded plates of isolates 93-020 and 93-022 were incubated in the dark at 28° C while cultures of 93-061 were maintained under lighted conditions (30 $\mu E\ m^{-1}\ s^{-1}$) at 28° C. High levels of spore production was observed in both lighted and dark conditions for *C. tuberculata* but very few spores were formed by *C. oryzae* when it was incubated in the dark (de Luna, unpublished data). Hence, for these experiments, *C. oryzae* was grown under lighted conditions. Spore suspensions were prepared by flooding each dish

with distilled water and lightly scraping the surface of the colony with a clean glass slide or camel's hairbrush. The suspension was filtered using a piece of cheesecloth to separate mycelial fragments from the spores. Spore concentration was determined using a hemacytometer. Two drops of Tween 20 or oxysorbic (20 POE) (polyoxyethylene sorbitan monolaurate) were added for every 100 ml of spore suspension. The conidial suspension was then sprayed at the rate of 1×10^8 spores/m^2.

2.3.4. Cross-infectivity experiment

This experiment was designed to determine whether the three *Curvularia* isolates were cross-pathogenic to three closely related weeds other than their primary host. Seeds of *Cyperus difformis*, *C. iria*, and *Fimbristylis miliacea* were obtained from mature plants in rice fields in and around the International Rice Research Institute (IRRI), Los Baños, Laguna, Philippines. They were then germinated in petri dishes lined with moist filter paper. One-week old seedlings of approximately the same height and vigor were transplanted into pots filled with soil (Maahas clay, Suborder Haplustic, Order Alfisol) and maintained in the greenhouse. Tubers of *C. rotundus* were also obtained from the IRRI rice fields and sown directly into potted soil. There were four plants per pot in the first trial and five plants per pot in the second trial. The experiment was a 4 x 4 factorial experiment in a randomized complete block design with six replications. The experiment was performed twice.

Two-week-old plants were inoculated with the spore suspension using an atomizer connected to a pressurized air source (A.H. Thomas Co., Scientific Apparatus, Philadelphia, USA.). At this stage, *C. difformis* and *C. iria* had three to four expanded

leaves, *F. miliacea*, five to six leaves, and *C. rotundus*, six to eight leaves. Distilled water with Tween 20 was employed as the control.

The control and inoculated seedlings were incubated for 24h in a dew chamber set at 28° C and 100% RH in the dark, after which they were moved to the mist room of the greenhouse. The mist room is a humid room in the greenhouse with an automatic mister and kept at 24 to 28° C and 85 to 95% RH (Yeh and Bonman, 1986). Plants were observed daily for the development of disease symptoms, and photographs were taken using a Nikon 35-mm camera. Three to five days after inoculation, leaves with young lesions were collected from treated plants and re-isolation of the fungal pathogen was performed to confirm Koch's postulates.

2.3.4.1.Disease development

Disease development was evaluated after 14 days in inoculated plants by using a key modified from Mardinus (1983). Control plants were healthy and did not show any visible symptoms.

2.3.4.2. Plant mortality and dry weight reduction

Plant mortality was assessed after 14 days by counting the number of dead plants per pot and expressed as percent mortality. Dry matter production was determined by cutting live aboveground plant material from each pot and drying to constant weight at 65 to 70° C. Data were expressed as percent dry weight reduction compared to the controls.

2.3.4.3. Sporulation of *C. tuberculata* and *C. oryzae* on necrotic leaf tissues

Sporulation of the *Curvularia* isolates was assessed by observing cut leaf pieces with lesions at five (*C. differmis*, *C. iria*, and *F. miliacea*) or seven (*C. rotundus*) DAI, following incubation in a moist chamber. After 24 to 72 h, leaf segments were examined and scored using the following key: - no sporulation; + light sporulation; ++ moderate sporulation; +++ heavy sporulation.

2.3.5. Data analysis

An arcsine transformation was performed on plant mortality and dry weight reduction data prior to performing the analysis of variance using the GLM procedure in the Statistical Analysis System Package (SAS Institute, 1987). Data for two trials were combined since homogeneity of variances was confirmed by Bartlett's test (Gomez and Gomez, 1984). Means were separated using Fischer's Least Significant Difference (LSD).

2.4. Results

2.4.1. Colony morphology and spore development of C. tuberculata and C. oryzae

Colonies of the two *C. tuberculata* isolates were cottony and dark charcoal grey to black, while those of *C. oryzae* were a lighter charcoal grey to black with an olive tinge (Fig. 2.1A). Growth of *C. tuberculata* on ½ PDA was rapid, with an average radial colony growth of 11.1 mm day^{-1} for isolate 93-020, and 10.4 mm day^{-1} for isolate 93-022. The expanding colony usually reached the edge of the petri dish after eight days. In contrast, growth of *C. oryzae* on ½ PDA plates was much slower, averaging 5.4 mm

day^{-1}, usually taking two weeks for the growing colony to reach the edge of the dish. Isolates 93-020 and 93-022 (*C. tuberculata*) grew evenly and margins followed the initial round shape of the agar plug. *C. oryzae*, however, tended to have highly irregular margins even if the same size agar plug was used as the seed inoculum (Fig. 2.1B). At the end of 14 days, colonies of isolate 93-020 (*C. tuberculata*) developed small, white areas devoid of spores while colonies of 93-022 were uniformly black in color (Figs. 2.1C and D). *C. oryzae* colonies were velvety and zonate (Fig. 2.1E).

Spores of the two isolates of *C. tuberculata* (93-020 and 93-022) cannot be differentiated morphologically. Both isolates produce straight, dark brown conidia, with the middle cells darker than the end cells (Figs. 2.2A and 2.3A). At maturity, the conidia are mostly three-septate (Figs. 2.2B, 2.3B) and their surfaces are covered with tubercles (Figs. 2.2C and 2.3D). Tri-radiate stauroconidia (Ellis, 1966) were sometimes observed in isolate 93-022 (Fig. 2.3C). The hilum is dark and flush with the conidiophore (Figs. 2.2C and 2.3D).

Conidiophores of *C. tuberculata* arise singly and laterally from the vegetative hyphae (Figs. 2.2A and 2.3A). The immature conidium is hyaline and smooth-walled (Figs. 2.2C and 2.3A). As the spore develops, the median septum is laid down first (Fig. 2.2B). The second septum is distal and delimits the apical cell of the conidium (Fig. 2.2B). At this time, the beginnings of wall ornamentation are evident and the conidial wall appears rough and dark (Fig. 2.2B). The third septum delimits the basal cell. The conidiophores of *C. tuberculata* are indeterminate and produce additional spores borne spirally or alternately at successive growing points (Figs. 2.2B and 2.3B).

Vegetative hyphae of *C. oryzae* are pale brown (Fig. 2.4A) and give rise to lateral conidiophores. Spores of *C. oryzae* are a lighter brown, straight, ovoid, ellipsoidal, or obclavate and smooth walled (Fig. 2.4B). Mature conidia are generally three-septate, with the two middle cells darker than the end cells (Fig. 2.4B). Conidiophores are straight and geniculate, with swollen nodes (Fig. 2.4C) at the point of attachment of the conidia.

The two isolates of *C. tuberculata* did not differ in spore length, width or number of cells per conidium (Table 2.1). Occasionally, tri-radiate stauroconidia were observed in cultures of isolate 93-022, otherwise the two isolates were morphologically indistinguishable. *C. oryzae* spores had lower length:width ratios than the *C. tuberculata* isolates but did not differ in number of cells per conidium.

2.4.2. Cross-infectivity Experiment

2.4.2.1. Disease development

C. difformis, C. iria, and *F. miliacea* were all susceptible to the fungal isolates (Table 2.2). The three *Curvularia* isolates are capable of killing their respective weed hosts within seven days after inoculation (7 DAI) when sprayed at the rate of 1×10^8 spores/m^2, and provided with 24 h-dew period at 28° C. Isolate 93-020 produced small brown spots on *C. difformis* that were round to irregular in shape three days after inoculation (Fig. 2.5A). Leaf tips also became brown and wilted. During the following 48 hours, the lesions increased in size and coalesced, forming blighted areas on the leaves (Fig. 2.5B). Necrotic areas were also present on the leaf sheaths and at the base of

the plant where the leaves originate (Fig. 2.5B). This rapid blighting reaction caused death of *C. difformis* seedlings within one week (Fig. 2.5C).

C. iria inoculated with 93-022 developed small, round to irregular brown spots, which were similar to the spots formed by 93-020 on *C. difformis*. These spots were visible on the leaves three to four days after inoculation (Fig. 2.5D). This response was accompanied by browning and drying of the leaf tips (Fig. 2.5E). Lesions were also observed on leaf sheaths and the leaf blade. Enlargement of the lesions caused either the leaf to fold (Fig 2.5E), or the entire plant to collapse (Fig. 2.5F). Coalescence of the lesions produced extensive blighting of the leaves and death occurred within one week.

The first symptom of infection in *F. miliacea* plants inoculated with 93-061 was the browning of leaf tips. In the next two days, dark brown spots appeared on the leaf (Fig. 2.6A) and on the leaf sheaths, near the base of the plant. The lesions enlarged rapidly and coalesced, forming blighted areas on the leaves (Fig. 2.6B). Necrotic spots at the base of the plant also enlarged, causing the whole plant to dry up and die within one week (Fig. 2.6C).

The three *Curvularia* isolates infected and killed two other closely related sedge weeds included in the experiment. For example, isolate 93-020 (*C. tuberculata*) from *C. difformis*, infected *C. iria* and *F. miliacea*. Isolate 93-022 (*C. tuberculata*) from *C. iria* was pathogenic on *C. difformis and F. miliacea* as well. Isolate 93-061 (*C. oryzae*) from *F. miliacea* was equally virulent on *C. iria* and *C. difformis*. Leaf spots identical to typical lesions observed in the primary hosts developed on the secondary hosts within three to five days. Enlargement and coalescing of lesions occurred in all weed-pathogen combinations.

All three isolates (93-020, 93-022, and 93-061) were cross-infective to *C. rotundus* (Table 2.2). *C. rotundus* plants treated with 93-020 exhibited minute (1 mm or less in diameter), brown, flecks on the leaf surface and dried leaf tips four days after inoculation (Fig. 2.7A and B). These spots however, did not grow in size during the succeeding days and the plants did not suffer any ill effects resulting from the infection. Plants continued to grow vigorously despite the presence of brown leaf tips and margins and flecks on the older (inoculated) leaves. No plant death occurred by the end of the 14-day observation period. Disease development in *C. rotundus* due to isolate 93-022 was identical to the effects of isolate 93-020 (Fig. 2.7E).

In *C. rotundus* plants treated with isolate 93-061, lesions, approximately 1 mm in size and elliptical, oval to irregular in shape, were formed near the leaf midrib, and on some leaf sheaths. Leaf tips were brown and dried up (Figs. 2.7C and D). In a few leaves where the lesions occurred in close proximity, the necrotic spots coalesced during the first week after treatment, producing a blotchy appearance. During the second week after inoculation, no growth in the necrotic areas of *C. rotundus* was observed, and the plants continued to grow vigorously. No *C. rotundus* plants died but yellowing of older leaves was more pronounced than in control plants (Fig. 2.7F).

2.4.2.2. Plant mortality and dry weight reduction

Isolate 93-020 killed 100% of *C. difformis*, 93% of *C. iria*, and 39% of *F. miliacea* seedlings after fourteen days (Table 2.3). The responses of the four sedge weeds to 93-022 were similar to the effects of 93-020, although mortality levels were generally lower. Drying and browning of the leaf tips accompanied the appearance of lesions on the leaves and leaf sheaths. Lesions caused by 93-022 in *C. difformis* rapidly

enlarged and killed 94% of the seedlings within two weeks. After 14 days, only 24% of *F. miliacea* seedlings were killed by 93-022 compared to 89% mortality for *C. iria* seedlings. Non-sporulating pinhead lesions with chlorotic halos were observed on *C. rotundus* (Fig. 2.7A) but these remained small and did not affect plant growth (Fig. 2.7E).

Curvularia oryzae (isolate 93-061) was pathogenic and virulent on *C. difformis*, *C. iria*, and *F. miliacea*. One hundred percent mortality was achieved in *F. miliacea* within two weeks while 97% and 98% of *C. difformis* and *C. iria*, respectively, were killed in the same time period.

Dry weight reduction due to inoculations of the three *Curvularia* isolates followed the same trend as described previously (Table 2.3). *C. tuberculata* isolates 93-020 and 93-022 reduced dry matter production in *C. difformis* and *C. iria* by 89 to 100% and *F. miliacea* by 49 to 52%. *C. oryzae* (isolate 93-061) was equally effective on these three weeds and reduced dry matter from 97 to 100%. However, only a slight reduction (7 to 13%) in dry matter of *C. rotundus* was observed when inoculated with any of the three *Curvularia* isolates.

2.4.2.3. Sporulation of *C. tuberculata* and *C. oryzae* on necrotic leaf tissues

Sporulation of the three *Curvularia* isolates occurred on necrotic tissues of susceptible sedge hosts, *C. difformis*, *C. iria*, and *F. miliacea* (Table 2.4). For the three isolates, sporulation on susceptible hosts occurred after 24 h, and by 48 h profuse sporulation on necrotic tissues was observed (Figs. 2.8A to F). Each stomatal aperture supported more than one conidiophore with usually more than one conidium per conidiophore and both conidiophores and conidia were dark (Figs. 2.8A, C, and E).

Sporulation was delayed by 24 hours in *F. miliacea* treated with the *C. tuberculata* isolates, however dark spores and conidiophores were present after 48 and 72 hours (Fig. 2.8G). On *C. rotundus*, no sporulation of *C. tuberculata* or of *C. oryzae* (Fig. 2.8H) occurred even after 72 h in the humid chamber.

2.5. Discussion

This study has demonstrated the pathogenicity of isolates of *C. tuberculata* to *C. difformis* and *C. iria* and *C. oryzae* to *F. miliacea*. These are new host records for these two pathogens. Members of the genus *Curvularia* occur mostly as tropical or subtropical facultative plant pathogens (Rossman et al., 1987). The species *C. tuberculata* is not well studied. It has been reported as the causal agent of leaf spots, leaf blights, and leaf tip die-back in several tropical plants (Lele et al., 1968; Lambat and Asha Ram, 1969; Seshadri et al., 1980; Lele et al., 1981; Misra and Singh, 1987). Misra et al. (1973) reported leaf spots on *C. rotundus* caused by *C. tuberculata*, however there are no succeeding accounts to date. Information about *C. oryzae* is restricted to reports of fruit rot in okra (*Abelmoschus esculentus* (L.) Moensch.) and its association with black kernel disease of rice, together with 13 other *Curvularia* species (Lal and Goel, 1989; Webster and Gunnell, 1992). The exact role that *C. oryzae* plays in black kernel disease remains to be confirmed.

Cultural characteristics, spore development, and morphology of *C. tuberculata* and *C. oryzae* followed previous reports (Ellis, 1966; 1971; Sivanesan, 1987). *C. tuberculata* isolates 93-020 and 93-022 are morphologically indistinguishable. *C. oryzae* can be readily differentiated from *C. tuberculata* by its smooth-walled, ellipsoid conidia.

The symptoms exhibited by susceptible weedy sedges are similar to those

reported for sorghum (Lambat and Asha Ram, 1969), sugarcane (Seshadri et al., 1980), and mango (Lele et al., 1980). At a concentration of 1 x 10^8 spores/m^2 and a 24-hour dew period, disease progress was rapid and blighted plants were killed one to two weeks after inoculation. In non-blighted plants, lesions that girdled the leaf caused it to bend downwards and eventually wilt. Lesions girdling the stem caused the whole plant to wilt and fall over despite the presence of some green plant parts. These contributed greatly to reducing host plant vigor, which will reduce the weed's competitiveness. Plants infected by fungi typically are weakened compared to uninfected conspecifics, resulting in decreased growth rates and/or reduced competitive abilities (Stovall and Clay, 1988 citing Harper, 1977; Paul and Ayres, 1986).

Cross-infectivity of the three *Curvularia* isolates to three other closely related weedy sedges was also demonstrated. Isolates caused high mortality on and significantly reduced dry matter production in *C. difformis*, *C. iria*, and *F. miliacea*. The fact that these pathogens are capable of controlling more than one species is distinctly advantageous because *C. difformis*, *C. iria*, and *F. miliacea* often occur together in irrigated rice fields. This broader range of activity is important since the high specificity exhibited by many biocontrol fungi is a deterrent to their practicality and commercialization (Boyette et al., 1993).

Extreme host specificity is not needed in the case of native pathogens that are to be used for site-directed, inundative applications (Watson, 1985). In fact, a certain level of non-specificity is desirable to develop mycoherbicides capable of controlling more than one weed species (Charudattan, 1990). A number of fungal weed pathogens with potential to control several weed species have been studied or are currently under

investigation. *Rhynchosporium alismatis* is being evaluated for its potential to control several weed species in the Alismataceae in Australia (Cother and Gilbert, 1994 a, b). A *Fusarium oxysporum* isolate has been shown to effectively control sicklepod (*Cassia obtusifolia*), coffee senna (*Cassia occidentalis*) and hemp sesbania (*Sesbania exaltata*) (Boyette et al., 1993), while an isolate of *Alternaria cassiae* was reported to be a potential mycoherbicide for sicklepod, coffee senna, and showy crotalaria (*Crotalaria spectabilis*) (Boyette, 1988). *Fusarium lateritum* from spurred anoda (*Anoda cristata*) and prickly sida (*Sida spinosa*) plants was also pathogenic to velvetleaf (*Abutilon theophrasti*) (Walker, 1981). *Colletotrichum gloeosporioides* f. sp. *malvae* from round-leaved mallow (*Malva pusilla*) also caused girdling lesions on leaf petioles and stems of velvetleaf (Mortensen, 1988). In many of these studies control of secondary weed hosts, although significant from the control, were not as high as in the primary weed host. However, our experiments with *C. tuberculata* isolates 93-020 and 93-022 showed that both fungal pathogens can cause over 90% mortality and dry weight reduction in either *C. difformis* or *C. iria* while *C. oryzae* killed between 97 to 100% of *C. difformis*, *C. iria*, and *F. miliacea*.

Infection of *C. rotundus* by each of the three *Curvularia* isolates occurred but did not cause mortality and only slightly reduced dry matter production. At best, only leaf tip die-back was observed in addition to small leaf spots on the lamina. Resistance in this species was confirmed since inoculated plants grew and flowered as well as controls but this should not be taken to mean that these isolates are completely ineffective on *C. rotundus*. Perhaps a second application of the inoculum could hasten disease development and severity. The use of a second fungal treatment to control aggressive

weeds has been reported previously. Two applications of conidial suspensions of *Alternaria cassiae* resulted in higher mortality of coffee senna and sicklepod when compared to a single dose (Boyette et al., 1993). Higher disease rates were observed in *C. rotundus* when multiple inoculations of *Dactylaria higginsii* were performed (Kadir et al, 1996). Even if treated plants are not killed, a severe reduction in photosynthetic leaf area will ultimately reduce its competitiveness and perhaps, tuber production as well. Early studies have shown that clipping the shoots of *C. rotundus* at two-week intervals reduced tuber number by 60% (Horowitz, 1965), while weekly clippings effectively controlled *C. rotundus* (Nyahoza, 1973; Ranade and Burns, 1925). The use of a foliar pathogen that causes extensive leaf necrosis with repeated inoculations might reduce tuber formation also.

Combinations of biocontrol agents and herbicides may improve and broaden the spectra of control (Charudattan and De Loach, 1988). Round-leaved mallow (*Malva pusilla*) control was improved by applying recommended rates of the herbicides bromoxynil (3,5-dibromo-4-hydroxybenzonitrile ester of octanoic acid) plus MCPA ((4-chloro-2-methylphenoxy)acetic acid), imazetaphyr ((±)-2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1*H*-imidazol-2-yl]-5-ethyl-3-pyridinecarboxylic acid), metribuzin (4-amino-6-(1,1-dimethylethyl)-3-(methylthio)-1,2,4-triazin-5(4*H*)-one, and with certain restrictions, sethoxydim (2-[1-(ethoxyimino)butyl-5-[-2-(ethylthio)propyl]-3-hydroxy-2-cyclohexen-1-one) in combination with *Colletotrichum gloeosporioides* f. sp. *malvae* (Grant et al., 1990). Treatments with *Colletotrichum gloeosporioides* f. sp. *aeschynomeme* and propanil increased disease levels in northern jointvetch (*Aeschynomene virginica*) (Klerk et al., 1985). Rust-herbicide combinations were more

effective at controlling yellow nutsedge (Cyperus esculentus L) than rust or herbicide alone (Phatak, 1984) while sequential applications of rust followed by herbicides reduced number of live plants, total tuber weight, and tuber number of yellow nutsedge (Callaway et al., 1985). Applications of the biocontrol agent in combination with recommended or reduced rates of glyphosate, bentazon, or 2,4-D may improve control of *C. rotundus*.

Integration of insects that feed on *C. rotundus* with applications of the fungal pathogen could also be done. Previous studies have shown that control of water hyacinth (*Eichhornia crassipes* (Mart.) Solms) in nutrient-rich lakes is more effective when the fungal pathogens *Cercospora rodmanii* and *Acremonium zonatum* were combined with the insects *Neochetina eichorniae* and *Arzama densa* (Addor, 1977; Charudattan et al., 1978). Nine species of moths belonging to the genus *Bactra* attack plants only in the Family Cyperaceae (Frick and Garcia, 1975). Of these, *Bactra verutana* Zeller, *B. mimima* Meyrick and *B. venosana* Zeller as well as the weevil, *Athesapeuta cyperi* Marshall, have been studied in detail and although all are adequately host-specific, none have proved effective as classical biological control agents of *C. rotundus* (Phatak et al., 1987). Perhaps the release of the moth *B. venosana*, which has been tested previously in the Philippines (Frick and Garcia, 1975), combined with inoculations of a fungal pathogen might be effective in controlling *C. rotundus*.

Both *C. tuberculata* isolates exhibited varying degrees of virulence on different sedge weeds. This confirms earlier reports of genetic variability in this species. In fact, an isolate of *C. tuberculata* causing die-back of Rangpur lime (*Citrus limonia* Osbeck) did not infect four out of nine additional *Citrus* species tested and was also avirulent on rice (Lele et al., 1968). A *C. tuberculata* isolate from mango was not cross-pathogenic

on citrus (*C. karan* Rafinesque) and *vice versa* (Lele at al., 1981). A host range test conducted on a *C. tuberculata* isolate from *C. rotundus* showed that only sorghum (*Sorghum vulgare* Pers.), johnsongrass (*Sorghum halapense* (L.) Pers.), and foxtail (*Setaria italica* (L.) P. Beauv.) were susceptible (Misra et al., 1973). Eight other graminaceous species, including corn (*Zea mays* L.), barley (*Hordeum vulgare* L.), wheat (*Triticum aestivum* L.), and oats (*Avena sativa* L.) were unaffected. Thus, it seems very likely that different host-limited strains of this fungus exist in nature.

Reports on *C. oryzae* are few. While it effectively controlled *C. difformis, C. iria,* and *F. miliacea, C. rotundus* was resistant. More studies should be done to determine its host range and to obtain information about its genetic variability.

2.6. Literature Cited

Addor, E.E. 1977. Experiments on biological control of water hyacinth with multiple agents. Miscellaneous Series, Botanical Society of America, Publication 154:24.

Ampong-Nyarko, A. and S.K. De Datta. 1991. A Handbook for Weed Control in Rice. International Rice Research Institute, Manila, Philippines. 113 pp.

Baltazar, A. 1995. Weed management in wet-seeded rice in Asia. Pages 656-661 *in* Proc. 15th Asian Pacific Weed Science Society Conference. Vol. IB. July 24-28, 1995, Tsukuba, Japan.

Boyette, C.D. 1988. Biocontrol of three leguminous weed species with *Alternaria cassiae*. Weed Sci. 2:414-417.

Boyette, C.M., H.K. Abbas, and W.J. Connick, Jr. 1993. Evaluation of *Fusarium oxysporum* as a potential bioherbicide for sicklepod (*Cassia obtusifolia*), coffee senna (*C. occidentalis*), and hemp sesbania (*Sesbania exaltata*). Weed Sci. 41:678-681.

Callaway, M.B., S.C. Phatak, and H.D. Wells. 1985. Effect of rust and rust-herbicide combinations on yellow nutsedge. Page 131 *in* Proceedings of the Southern Weed Science Society. Volume 38.

Charudattan, R. 1990. Pathogens with potential for weed control. Pages 132-154 *in* Microbes and Microbial Products as Herbicides. R.E. Hoagland (Ed.) ACS Symposium Series 349. American Chemical Society, Washington, DC, USA.

Charudattan, R. and C.J. De Loach. 1988. Management of pathogens and insects for weed control in agroecosystems. Pages 245-264 *in* Weed Management in Agroecosystems: Ecological Approaches. M.A. Altieri and M. Liebman (Eds.). CRC Press, Boca Raton, FL, USA.

Charudattan, R., B.D. Perkins, and R.C. Littell. 1978. Effects of fungi and bacteria on the decline of arthropod-damaged water hyacinth (*Eichhornia crassipes*) in Florida. Weed Sci. 26:101-107.

Cother, E.J. and R.L. Gilbert. 1994a. Pathogenicity of *Rhyncosporium alismatis* and its potential as a mycoherbicide on several weed species in the Alismataceae. Aust. J. Exp. Agr. 34:1039-1042.

Cother, E.J. and R.L. Gilbert. 1994b. Efficacy of a potential mycoherbicide for control of *Alisma lanceolatum* and *Damasonium minus* in Australian rice crops. Aust. J. Exp. Agr. 34:1043-1050.

De Datta, S.K. 1983. Perennial weeds and their control in rice in the tropics. Pages 255-272 *in* Weed Control in Rice. International Rice Research Institute, Manila, Philippines.

De Datta, S.K. 1995. Weed management perspectives for sustainable agriculture in rice-based systems. Pages 17-27 *in* Proc. 15th Asian-Pacific Weed Science Society Conference. Vol. I (A). July 24-28, 1995. Tsukuba, Japan.

Dhingra, O.D. and J.B. Sinclair. 1985. Basic Plant Pathology Methods. CRC Press, Boca Raton, FL, USA. 355 pp.

Ellis, M.B. 1966. Dematiaceous Hyphomycetes. VII. *Curvularia, Brachysporium*, etc. Mycol. Pap. 106:1-57.

Ellis, M.B. 1971. Dematiaceous Hyphomycetes. Kew, Surrey, England: Commonwealth Mycological Institute. Kew, England. 608 pp.

Frick, K.E. and C. Garcia, Jr. 1975. *Bactra verutana* as a biological control agent for purple nutsedge. Ann. Entom. Soc. Am. 68:7-18.

Gomez, K.A. and A.A. Gomez. 1984. Statistical Procedures for Agricultural Research. 2nd Edition. John Wiley & Sons, New York, NY, USA. 680 pp.

Grant, N.T., E. Prusinkiewicz, K. Mortensen, and R.M.D. Makowski. 1990. Herbicide interactions with *Colletotrichum gloeosporioides* f. sp. *malvae*, a bioherbicide for round-leaved mallow (*Malva pusilla*) control. Weed Technol. 4:716-723.

Heap, I.M. 1997. The occurrence of herbicide-resistant weeds worldwide. Pestic. Sci. 51:235-243.

Horowitz, M. 1965. Data on the biology and chemical control of the nutsedge (*Cyperus rotundus*) in Israel. PANS (Pest Artic. News Summ.) 11:389-416.

Kadir, J.B., R. Charudattan, and R.D. Berger. 1996. Field efficacy of *Dactylaria higginsii* for control of purple nutsedge. Phytopathology 87:S49.

Kapoor, I.J. and R.N. Tandon. 1971. Occurrence of *Curvularia tuberculata* Jain on stored fruits of *Psidium guajava* L. Sydowia 24:201-202.

Klerk, R.A., R.J. Smith, Jr., and D. O. TeBeest. 1985. Integration of a microbial herbicide into weed and pest control programs in rice. Weed Sci. 33:95-99.

Lal, B. and D. Goel. 1989. A new rot of *Abelmoschus esculentus*. Indian Phytopathology 42:482.

Lambat, A.K. and Ram, A. 1969. Seed-borne infection of *Curvularia* causing a new blight disease of Jowar. Indian Phytopath. 22:282-284.

Lele, V.C., S.P. Raychaudhuri, R.B. Bhalla and A. Ram. 1968. *Curvularia tuberculata*, a new fungus causing die-back disease of citrus in India. Indian Phytopath. 21:66-72.

Lele, V.C., J. Singh, S.N. Rai, and J. Kandhari. 1981. Occurrence of a new blight disease of mango caused by *Curvularia*. Curr. Sci. 50:464-465.

Lin, J., R.J. Smith, Jr., and R.H. Dilday. 1992. Allelopathic activity of rice germplasm on weeds. Page 99 *in* Proc. 45th Annual Meeting of the Southern Weed Science Society, Little Rock, AR, USA.

Mardinus. 1983. Pathogenicity test of three isolates of *Drechslera oryzae* (Breda de Haan) Subram. & Jain on rice seedlings. Pages 215-218 *in* Proc of the Symposium on Pest Ecology and Pest Management. 18-20 Sept. 1979. BIOTROP Special Publication no. 18. SEAMEO Regional Center for Tropical Biology, Bogor, Indonesia.

Misra, A.P., O. Prakash, B. Mishra and K.K. Dutta. 1973. A new leaf spot disease of motha (*Cyperus rotundus* L.) caused by *Curvularia tuberculata*. Indian Phytopath. 26:165-167.

Moody, K. 1990. Post-planting weed control in direct-seeded rice. Paper presented at a Rice Symposium, 25-27 September 1990, MARDI, Penang, Malaysia.

Moody, K. 1992. Weed management in wet-seeded rice in tropical Asia. Pages 1-20 *in* Proc. International Symposium on Biological Control and Integrated Management of Paddy and Aquatic Weeds in Asia. National Agriculture Research Center. Tsukuba, Japan.

Moody, K. 1995. Sustainability in rice weed management. Pages 93-103 *in* Proc. 15th Asian Pacific Weed Science Society Conference. Vol. IA. July 24-28, 1995, Tsukuba, Japan.

Mortensen, K. 1988. The potential of an endemic fungus, *Colletotrichum gloeosporioides*, for the biological control of round-leaved mallow (*Malva pusilla*) and velvetleaf (*Abutilon theophrasti*). Weed Sci. 36:473-478.

Mukhopadhyay, S.K.1983. Weed control technology in rainfed wetland rice. Pages 109-118 *in* Proc. 1981 Weed Control in Rice Conference. International Rice Research Institute, Manila, Philippines.

Nyahoza, F. 1973. Studies of the biology of *Cyperus rotundus* L. Early growth and vegetative reproduction strategy. E. Afr. Agr. For. J. 17:120-130.

Okafor, L.I. and S.K. De Datta, 1974. Competition between weeds in upland rice in monsoon – Asia. Phil. Weed Sci. Bull. 1:39-45.

Paul, N.D. and P.G. Ayres. 1986. Interference between healthy and rusted groundsel (*Senecio vulgaris* L.) within mixed populations of different densities and proportions. New Phytol.104:257-269.

Phatak, S.C. 1984. Knock out nutsedge. Am. Veg. Grower 32(6):44-46.

Powles, S.B. and J.A.M. Holtum. 1990. Herbicide resistant weeds in Australia. Pages 185-193 *in* Proc.9th Australian Weeds Conference, Adelaide, Australia.

Ranade, S. and W. Burns. 1925. The eradication of *Cyperus rotundus* L. (A study in pure and applied botany). Memoirs of the Department of Agriculture in India, Botanical Series, 13:99-192.

Rossman, A.Y., M.E. Palm, and L.J. Spielman. 1987. A Literature Guide for the Identification of Plant Pathogenic Fungi. APS Press, St. Paul, MN, USA. 252 pp.

Sanders, B.A. 1994. The life cycle and ecology of *Cyperus difformis* (rice weed) in temperate Australia: a review. Aust. J. Exp. Agric. 34:1031-1038.

SAS Institute, Inc. 1987. SAS/STAT User's guide for personal computers. 6th Edition. SAS Institute, Inc. Cary, NC, USA.

Seshadri, K., P. Padmanaban, and K.C. Alexander. 1980. A new leaf blight disease of sugarcane. Indian Phytopath. 33:325-326.

Sivanesan, A. 1987. Graminicolous species of *Bipolaris, Curvularia, Drechslera* and *Exserohilum* and their teleomorphs. Mycol. Pap. 158:1-261.

Stovall, M.E. and K. Clay. 1988. The effect of a fungus, *Balansia cyperi* Edg., on growth and reproduction of purple nutsedge. New Phytol.109:351-359.

Walker, H.L. 1981. *Fusarium lateritum*: A pathogen of spurred anoda (*Anoda cristata*), prickly sida (*Sida spinosa*), and velvetleaf (*Abutilon theophrasti*). Weed Sci. 29:629-631.

Watanabe, H., I. Md. Zuki, and N.K. Ho. 1994. 2,4-D resistance of *Fimbristylis miliacea* in direct seeded rice fields in the Muda area. Pages 353-356 *in* Proc. 4th International Conference on Plant Protection in the Tropics. A. Rajan and Y. Ibrahim (Eds.). Kuala Lumpur, Malaysia.

Watson, A. K. 1985. Host specificity of plant pathogens in biological weed control. Pages 577-586 *in* Proc. VI International Symposium on Biological Control of Weeds. E. D. Delfosse, (Ed.). Vancouver, Canada.

Watson, A.K. 1991. Prospects for bioherbicide development in Southeast Asia. Pages 65-73 *in* Proc.13th Asian Pacific Weed Science Society Conference. Jakarta, Indonesia.

Watson, A.K. 1994. Current status of bioherbicide development and prospects for rice in Asia. Pages 195-201 *in* Integrated Management of Paddy and Aquatic Weeds in Asia. Proc. International Seminar " Biological Control and Integrated Management of Paddy and Aquatic Weeds in Asia". H. Shibayama, K. Kiritani, and J. Bay-Petersen (Eds.) Oct. 19-25, 1992. Tsukuba, Japan.

Watson, A.K., M.O. Mabbayad, W. Zhang, R.F. Masangkay-Watson, L.Z. de Luna-Couture, C.B. Yandoc, T.C. Paulitz, and A.M. Mortimer. 1997. Progress of a biological weed control project in rice-based cropping systems in Southeast Asia. Pages 342-244 *in* Proc. 16th Asian Pacific Weed Science Society Conference. A. Rajan (Ed.) Malaysian Plant Protection Society. Kuala Lumpur, Malaysia.

Webster, R.K. and P.S. Gunnell (Eds.). 1992. Compendium of Rice Diseases. APS Press. St. Paul, MN, USA. 62 pp.

Yeh, W.H. and J.M. Bonman. 1986. Assessment of partial resistance to *Pyricularia oryzae* in six rice cultivars. Plant Pathol. 35:319-323.

Figure 2.1. Cultural characteristics of *Curvularia tuberculata* (93-020 and 93-022) and *Curvularia oryzae* (93-061) on half-strength potato dextrose agar (½ PDA). **A.** 14-day-old colonies on ½ PDA. **B.** Irregular colony growth by *C. oryzae* (93-061) two days after inoculation. **C.** Patches of white, aerial mycelia in a two-week-old culture of *C. tuberculata* (93-020). **D.** Uniformly dark colony of *C. tuberculata* (93-022) after 14 days. **E.** Velvety colony of *C. oryzae* (93-061) after 14 days.

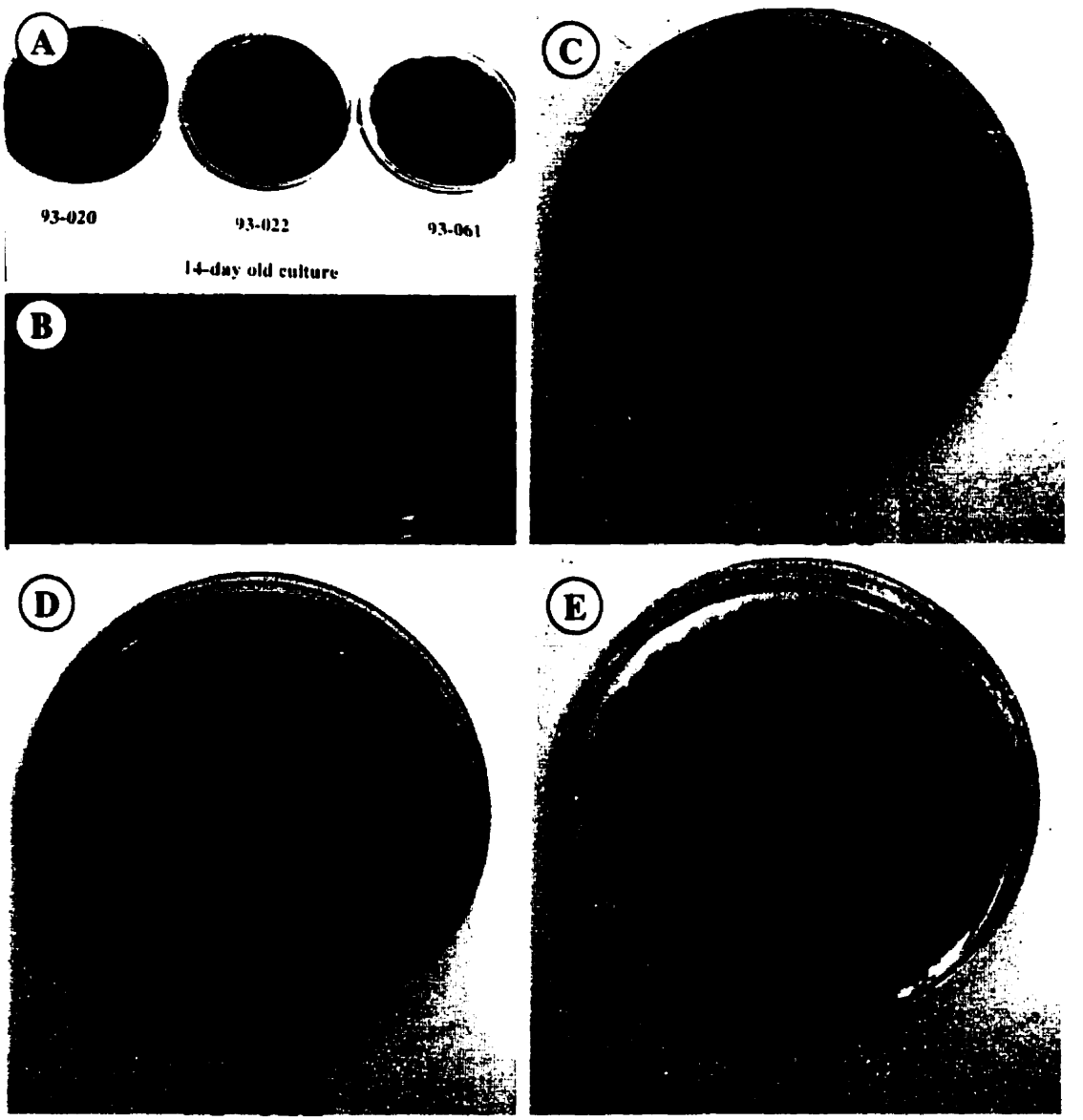

A
93-020
93-022
93-061
14-day old culture
B
C
D
E

Figure 2.2. Spore development and morphology of *Curvularia tuberculata* isolate 93-020. **A.** Conidiophores (CP) arise singly and laterally from vegetative hyphae (VH). A mature conidium (MC) is three-septate and the middle cells are darker brown in color than the end cells. A two-septate young conidium (YC) showing the beginnings of ornamentation on the conidium wall. **B.** Successive conidium formation results from the repeated branching and elongation of the conidiophore (CP). The median septum (S) is the first to form in an immature conidium (IC). Its walls are still devoid of tubercles. **C.** Walls of mature conidia (MC) are rough and covered with tubercles. The hilum (H) is flush and dark. An immature conidium (IC) is hyaline, has smooth walls and has not developed septa.

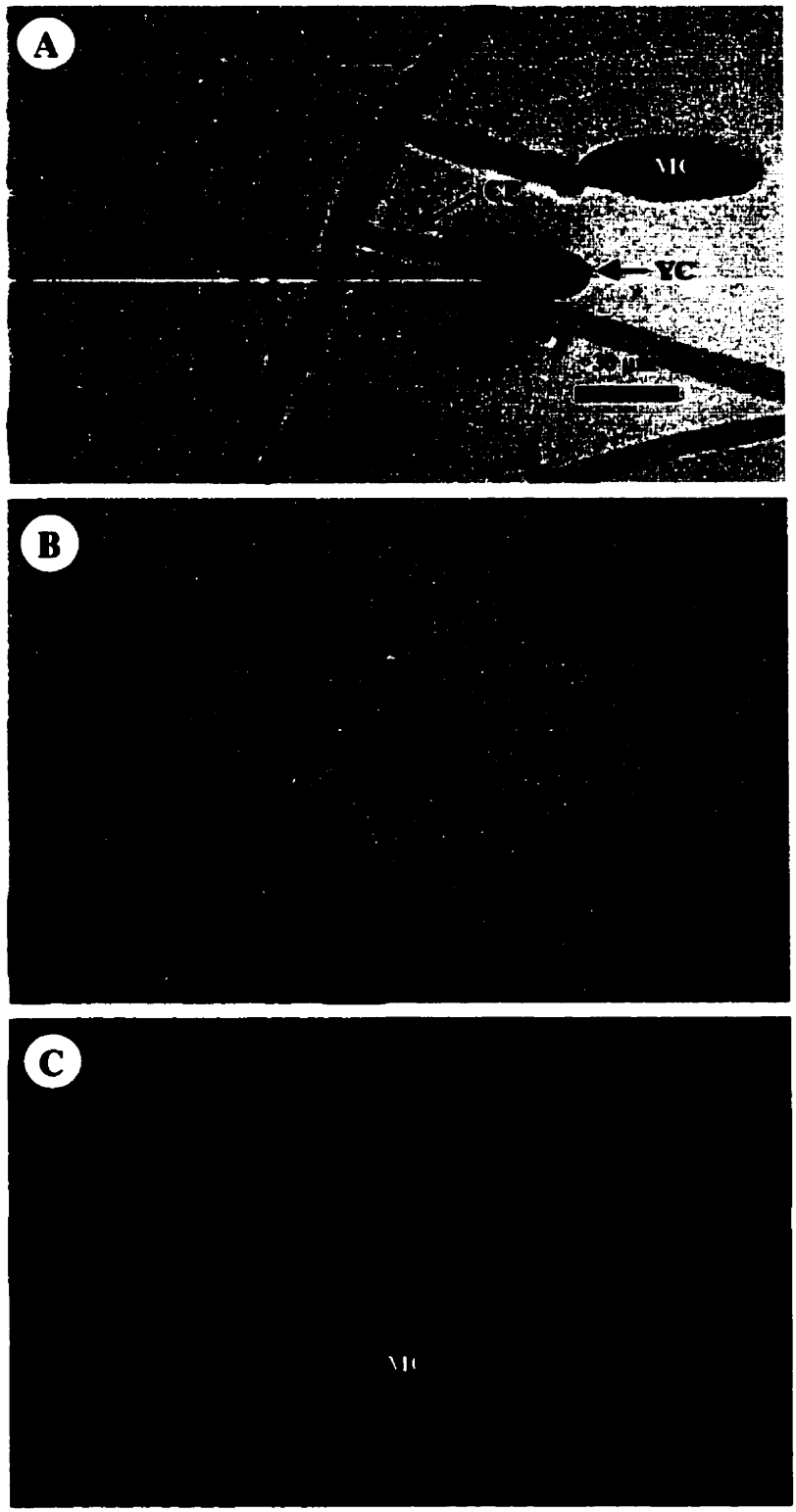

A
VC
YC
B
C
VC

Figure 2.3. Spore development and morphology of *Curvularia tuberculata* isolate 93-022. **A.** Conidiophores (CP) arise laterally and singly from vegetative hyphae (VH). An immature conidium (IC) is shown with smooth spore walls and no septa. **B.** Mature conidia (MC) are borne spirally or alternately at the tip of the conidiophore (CP) and its successive growing points.

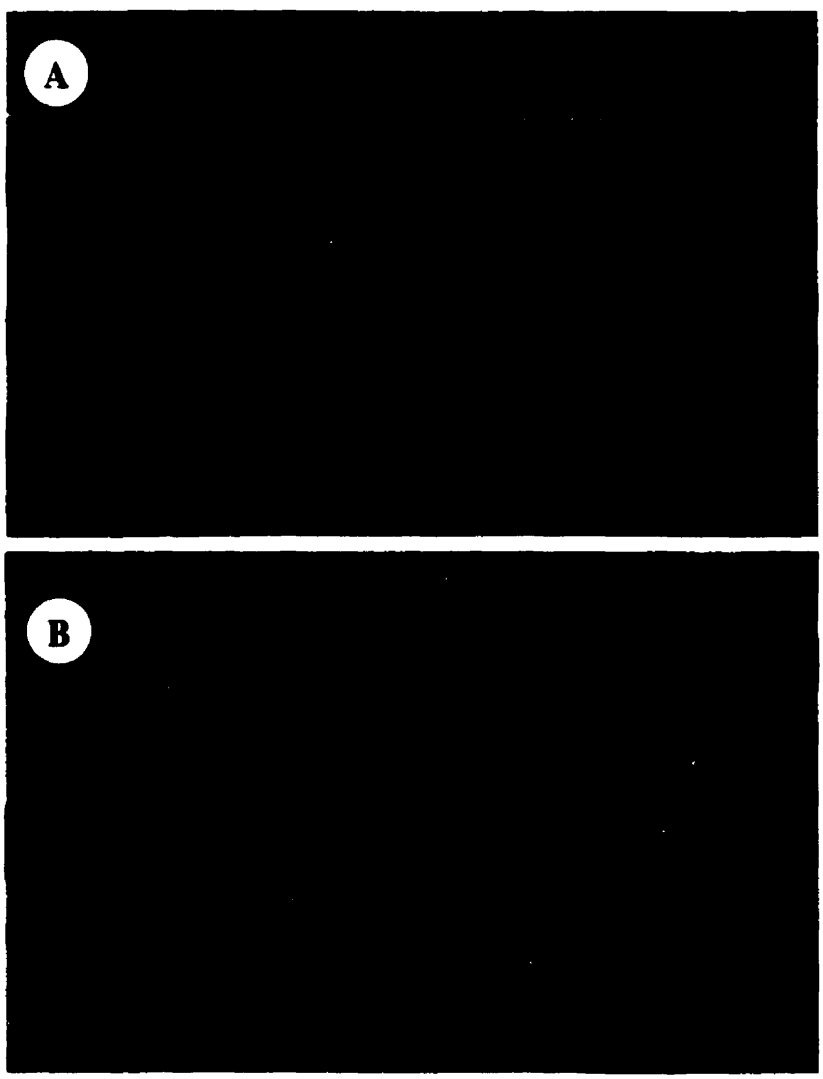

A
B

Figure 2.3. Spore development and morphology of *Curvularia tuberculata* isolate 93-022. Continued. **C.** Triradiate stauroconidia (SC) were sometimes observed. **D**. The walls of mature conidia (MC) are ornamented with tubercles (T) of varying sizes. The hilum (H) is flush and dark.

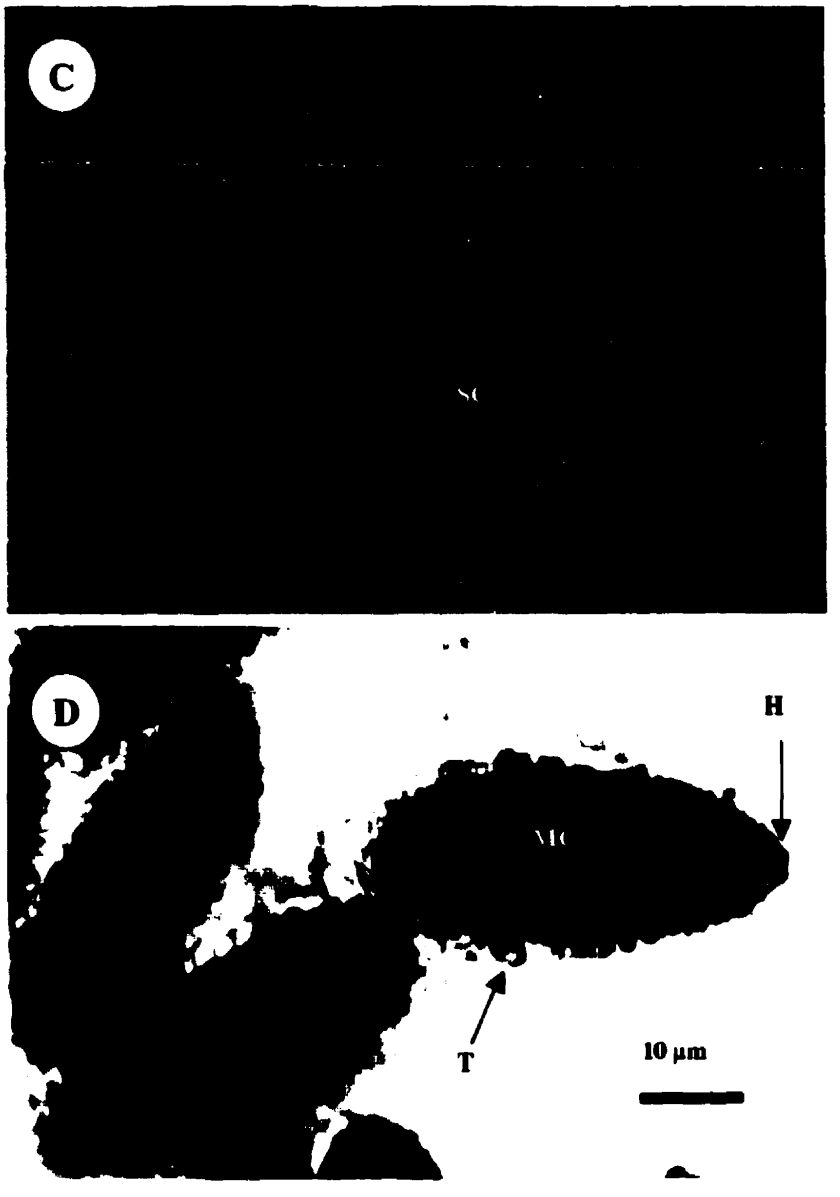

C
D
H
T
10 µm

Figure 2.4. Spore development and morphology of *Curvularia oryzae* isolate 93-061. **A.** The mycelium of *C. oryzae* consisted of pale brown vegetative hyphae. **B.** Mature conidia (MC) were straight, ovoid, ellipsoidal or obclavate and had smooth walls. CP, conidiophore; VH, vegetative hyphae. **C.** Conidiophores (CP) were geniculate and had swollen nodes (N).

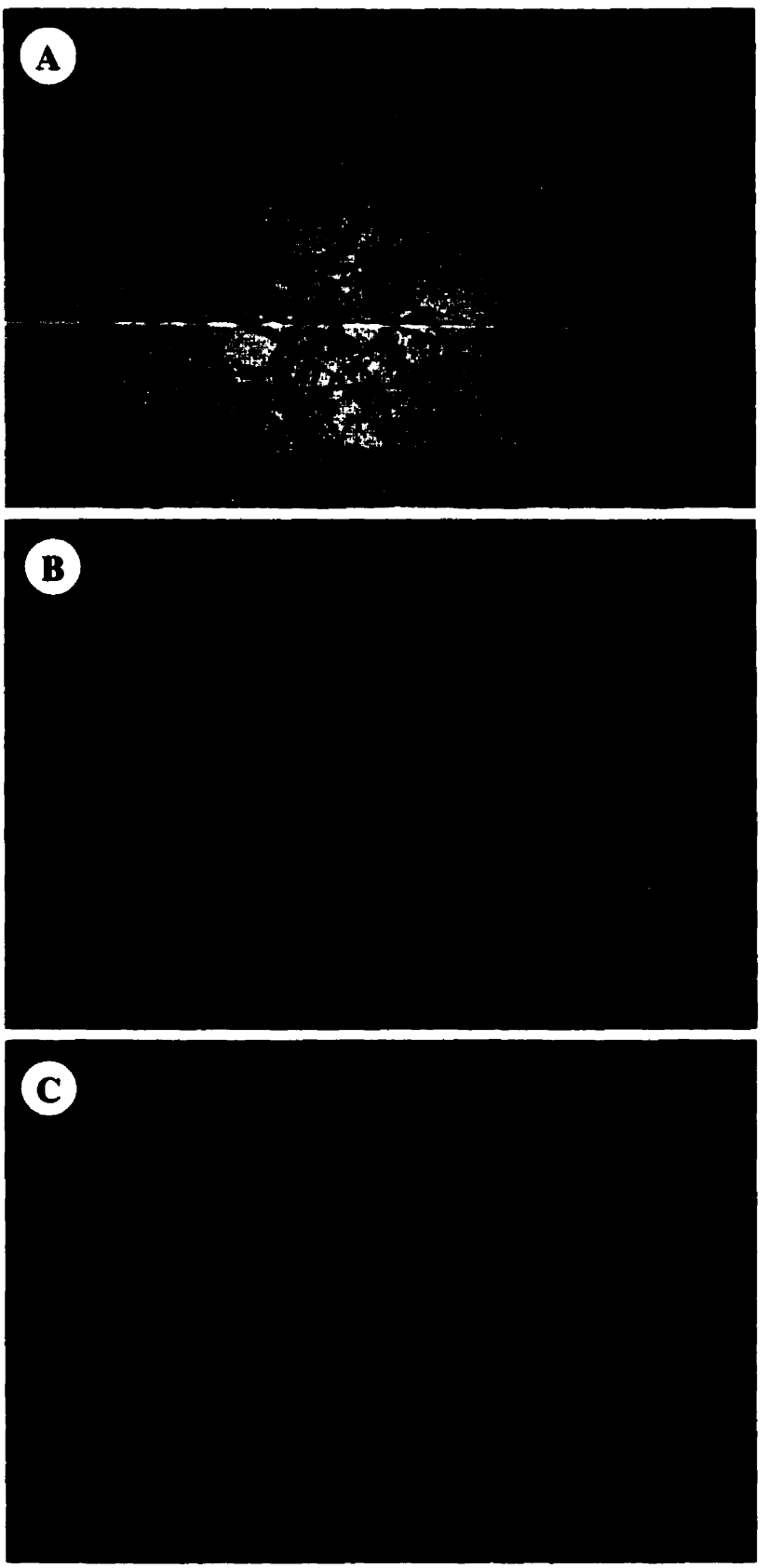

A
B
C

Figure 2.5. Effect of *Curvularia tuberculata* isolate 93-020 on *Cyperus difformis* and isolate 93-022 on *Cyperus iria*. A to C. *C. tuberculata* (93-020) on *C. difformis* **A.** Irregularly shaped leaf spots on *C. difformis* produced by *C. tuberculata* 93-020 at four days after inoculation (DAI). **B.** Lesions enlarged rapidly and coalesced to produce a blighting reaction on seedlings. **C.** Plants died from the infection within one week after inoculation. D and E. *C. tuberculata* (93-022) on *C. iria*. **D.** A leaf spot on *C. iria* at four DAI. **E.** Leaf spots frequently enlarged to cover the entire width of the leaf blade, causing the whole leaf to fold (LS). Leaf tips (LT) turned brown. Plants eventually developed blighted leaves (BL) and died.

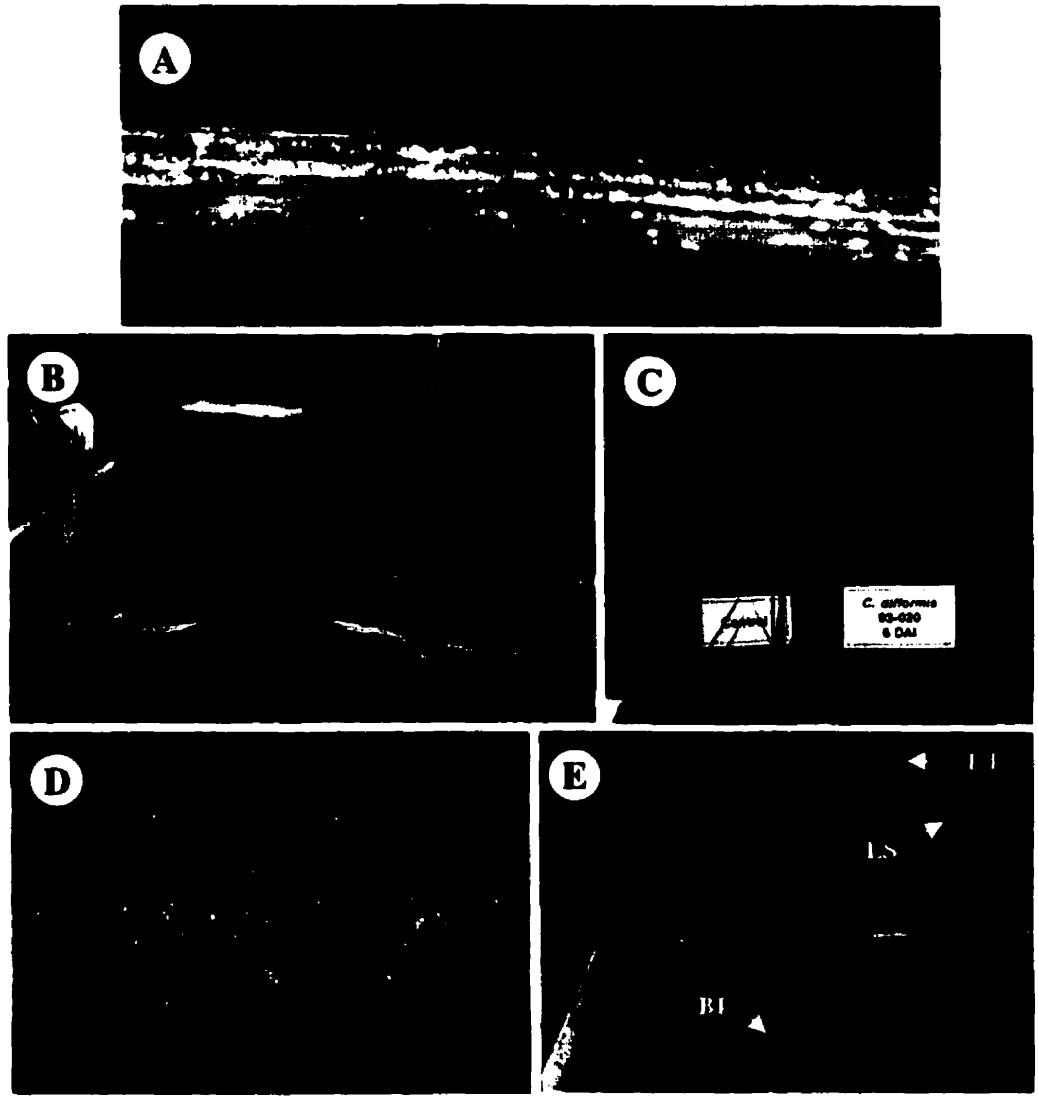

A
B
C
D
E
LS
BI

Figure 2.6. Effect of *Curvularia oryzae* (93-061) on *Fimbristylis miliacea*. **A.** A lesion on the leaf blade at three days after inoculation (DAI). **B.** A rapid blighting reaction occurred causing *F. miliacea* to dry up within five days after inoculation. **C.** Plant death occurred within one week after inoculation.

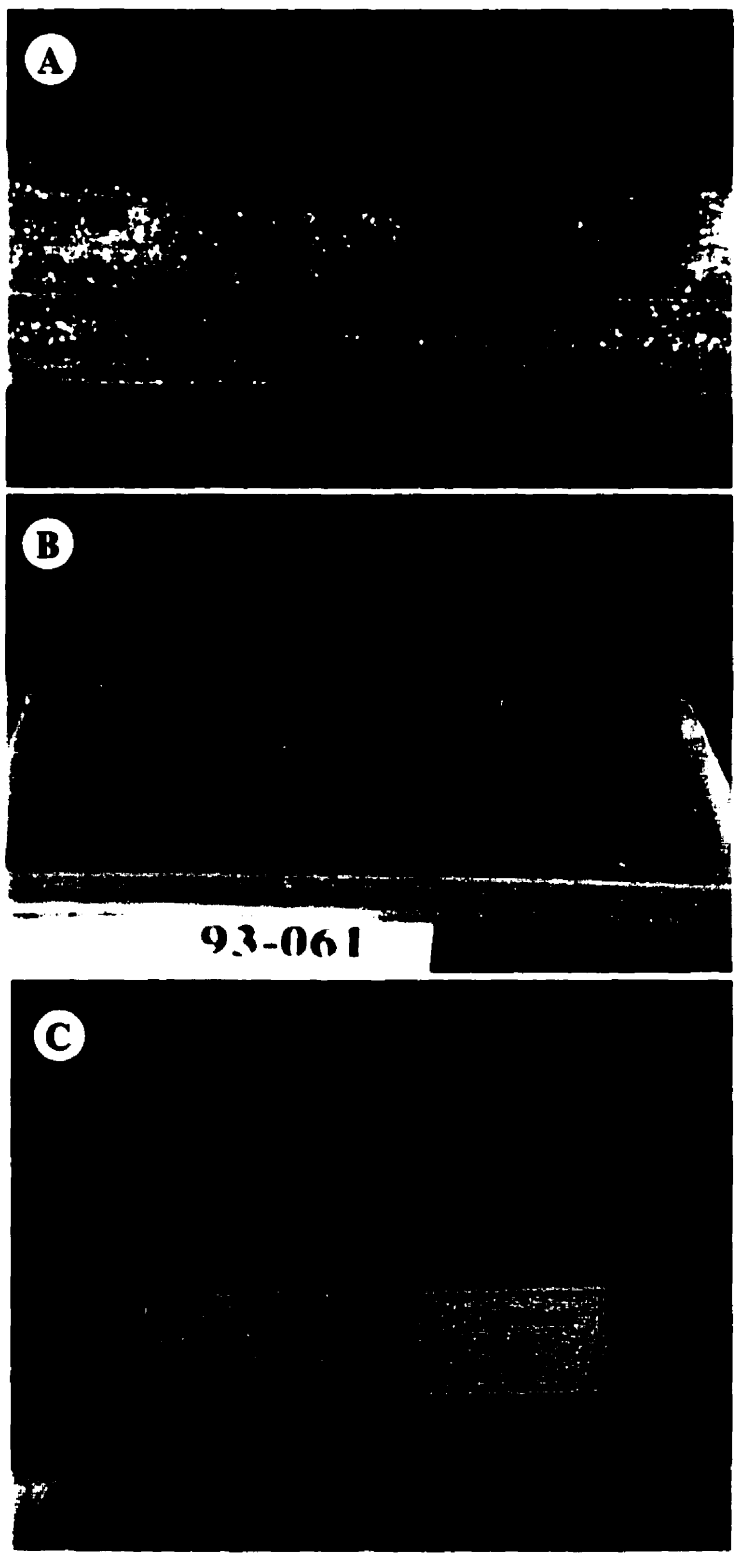

A
B
93-061
C

Figure 2.7. Reaction of *Cyperus rotundus* to *Curvularia tuberculata* (93-020; A and B and 93-022; E) and *Curvularia oryzae* (93-061; C, D, and F). **A.** Pinhead lesions with chlorotic haloes produced by *C. tuberculata* (93-020) at five days after inoculation (DAI). **B, C.** Dried leaf tips on *C. rotundus* treated with *C. tuberculata* (93-020) (B) and *C. oryzae* (93-061) (C) at seven DAI. **D.** Older leaves of *C. rotundus* were more susceptible to damage by *C. oryzae* (93-061). **E.** *C. rotundus* treated with *C. tuberculata* (93-022) did not differ from the control. **F.** *C. rotundus* inoculated with *C. oryzae* (93-061) grew vigorously despite some leaf tip damage. Slightly fewer flower heads were observed on treated plants.

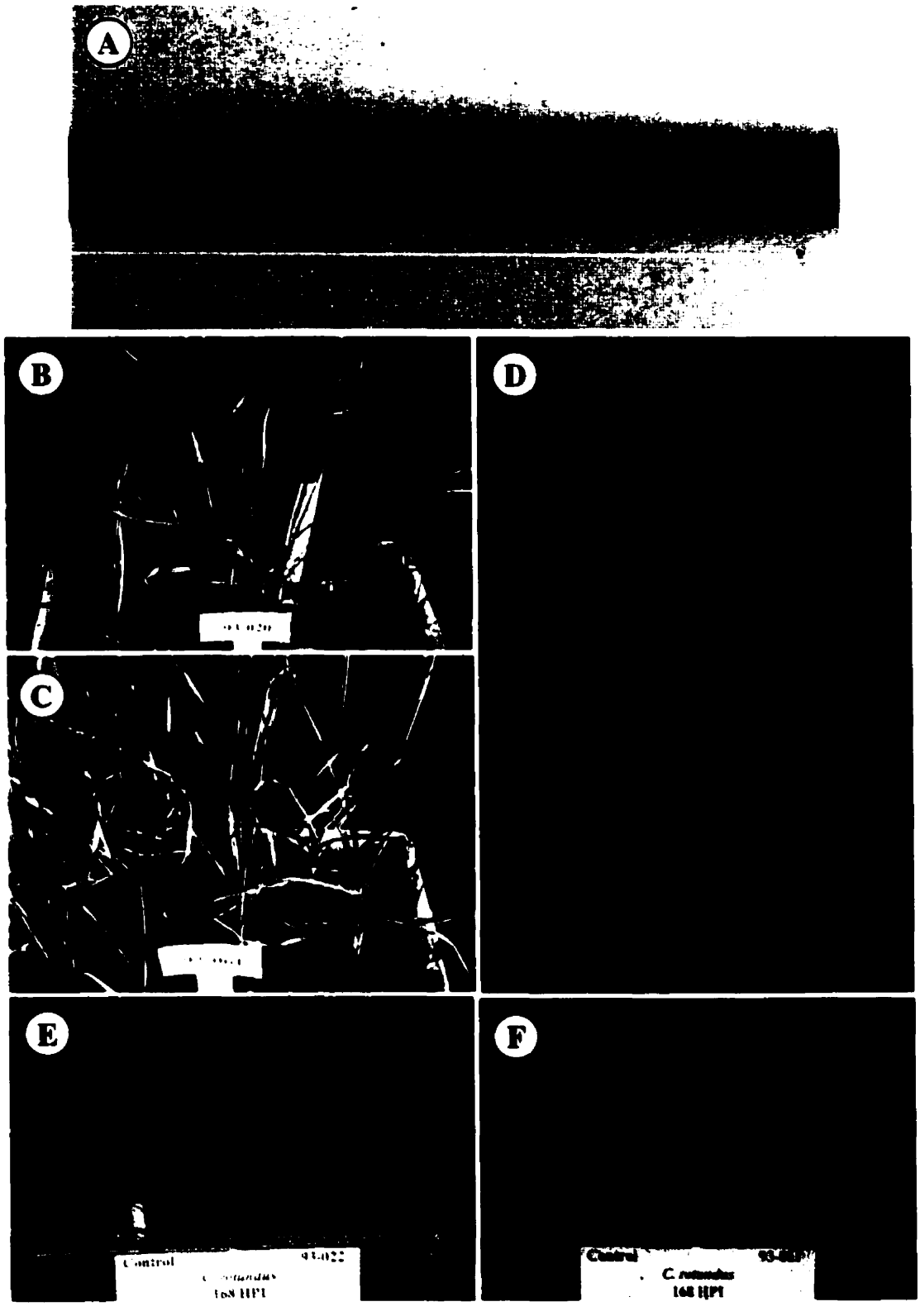

A
B
C
D
E
F
Control
168 HPI

Figure 2.8. Sporulation of *Curvularia tuberculata* (93-020 and 93-022) and *Curvularia oryzae* (93-061) on four sedge hosts after 72 h incubation. **A.** *C. tuberculata* (93-020) on *Cyperus difformis*. **B.** *C. tuberculata* (93-022) on *Cyperus iria*. **C.** *C. oryzae* (93-061) on *Fimbristylis miliacea*. **D.** *C. oryzae* (93-061) on *C. difformis*. **E.** *C. tuberculata* (93-020) on *C. iria*. **F.** *C. oryzae* (93-061) on *C. iria*. **G.** *C. tuberculata* (93-020) on *F. miliacea*. **H.** *C. oryzae* (93-061) did not sporulate on *Cyperus rotundus*.

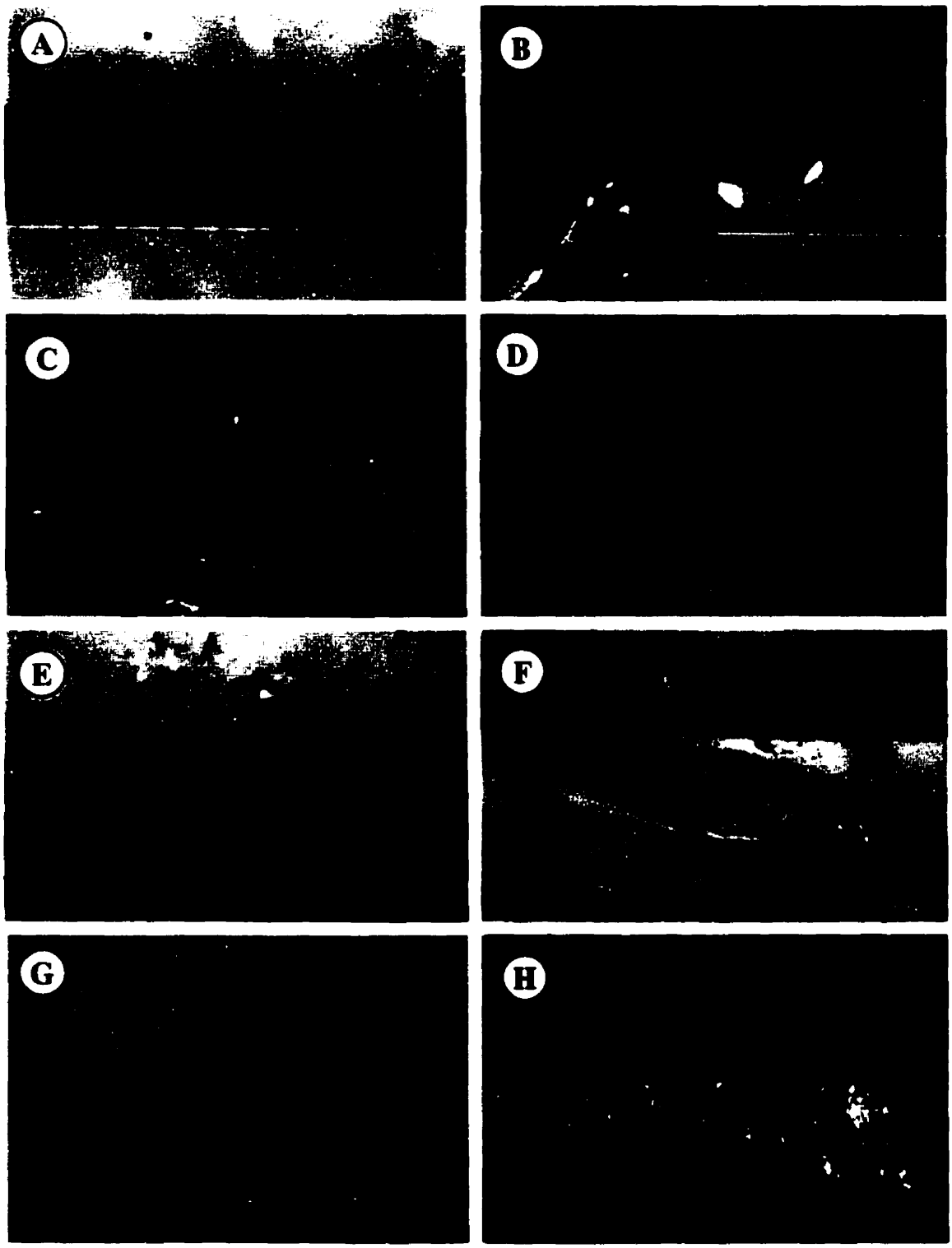

A
B
C
D
E
F
G
H

Table 2.1. Spore measurements of three *Curvularia* isolates from slide cultures incubated at 28°C for five days.

Isolate	Species	Spore Measurements		
		Length (μm)[1]	Width (μm)[1]	No. of cells/spore[2]
93-020	*Curvularia tuberculata*[x]	36.4	13.4	4
		(25.0-54.4)[z]	(10.0-18.75)	(3-4)
93-022	*Curvularia tuberculata*[x]	35.8	13.5	4
		(25.0-52.5)	(10.0-18.8)	(3-6)
93-061	*Curvularia oryzae*[y]	27.6	13.1	4
		(19.2-33.3)	(9.2-17.5)	(3-4)

[x] Agar cultures grown in the dark.

[y] Agar cultures grown under lighted conditions (30 $\mu E\ m^{-1}\ s^{-1}$)

[z] Range

[1] Mean value

[2] Median value

Table 2.2. Reactions of Cyperaceae weeds to three *Curvularia* isolates at 14 days after inoculation (DAI).

Weed Host	*Curvularia* Isolates					
	93-020 (*C. tuberculata*)		93-022 (*C. tuberculata*)		93-061 (*C. oryzae*)	
	Score[1x]	Host Reaction[2]	Score	Host Reaction	Score	Host Reaction
Cyperus difformis	5.0	HS	5.0	HS	5.0	HS
Cyperus iria	5.0	HS	5.0	HS	5.0	HS
Fimbristylis miliacea	3.0	MS	3.0	MS	5.0	HS
Cyperus rotundus	2.0	MR	2.0	MR	2.0	MR

[x] Median disease severity score

[1] Disease severity score: 0 – no visible reaction; 1 – few to many brown specks, 0.5 to1.0 mm diameter, grayish-green or light brown leaf tips; 2 – discrete brown lesions with or without chlorotic halo, 1.0 to 2.0 mm long, brown leaf tips and margins; 3 – many brown spots, 2.0 to 3.0 mm long, few lesions coalescing, brown leaf tips and margins; 4 – many lesions with necrotic centers, 3 to 5 mm long, 10-50% leaf area killed; 5 – numerous large and expanding lesions, more than 50% leaf area killed, plant collapse and/or death.

[2]Host Reaction: 0 – Immune (I); 1 – Highly Resistant (HR); 2 – Moderately Resistant (MR); 3 – Moderately Susceptible (MS); 4 – Susceptible (S); 5 – Highly Susceptible (HS).

Table 2.3. Effect of *Curvularia* isolates on percentage mortality and dry weight reduction of four Cyperaceae weeds 14 days after inoculation with a conidial suspension at 1 x 10^8 spores/m^2.*

***Curvularia* Isolate**	**Weed Host**	**Plant Mortality (%)**	**Dry Weight Reduction (%)**
Control	*Cyperus difformis*	0 g**	0 f**
	C. iria	0 g	0 f
	Fimbristylis miliacea	0 g	0 f
	Cyperus rotundus	0 g	0 f
C. tuberculata (93-020)	*Cyperus difformis*	100 a	100 a
	Cyperus iria	93 cd	97 ab
	Fimbristylis miliacea	39 e	52 d
	Cyperus rotundus	0 g	10 ef
C. tuberculata (93-022)	*Cyperus difformis*	94 bcd	96 b
	Cyperus iria	89 d	89 c
	Fimbristylis miliacea	24 f	49 d
	Cyperus rotundus	0 g	7 ef
C. oryzae (93-061)	*Cyperus difformis*	97 abc	98 ab
	Cyperus iria	98 ab	98 ab
	Fimbristylis miliacea	100 a	100 a
	Cyperus rotundus	0 g	13 e

* Results are from pooled experiments.

** Means in each column followed by the same letter are not significantly different according to Least Significant Difference Test ($P \leq 0.05$).

Table 2.4. Sporulation of three *Curvularia* isolates on four Cyperaceae weeds inoculated with a conidial suspension of 1 x 10^8 spores/m^2 after 24, 48, and 72 hours incubation in a moist chamber.*

Weed Host	**93-020**			**93-022**			**93-061**		
	(***C. tuberculata***)			(***C. tuberculata***)			(***C. oryzae***)		
	24 h	48 h	72 h	24 h	48 h	72 h	24 h	48 h	72 h
C. differmis	++[a]	+++	+++	++	+++	+++	++	+++	+++
C. iria	++	+++	+++	++	+++	+++	++	+++	+++
F. miliacea	–	+	++	–	+	++	++	+++	+++
C. rotundus	–	–	–	–	–	–	–	–	–

[a] - no sporulation; + light sporulation; ++ moderate sporulation; +++ heavy sporulation.

CONNECTING TEXT

The previous chapter demonstrated that three *Curvularia* isolates belonging to *Curvularia tuberculata* and *C. oryzae* are pathogenic on three species of sedge weeds other than their primary host. However, the virulence of these isolates varies according to the weed species being attacked. *Cyperus difformis, C. iria,* and *Fimbristylis miliacea* were susceptible to the fungal isolates but *Cyperus rotundus* was resistant. This indicates that the *Curvularia* isolates have potential as biocontrol agents of these rice weeds. Safety of the biocontrol agent to the crop where it will be used is one of the critical aspects of bioherbicide development. Thus, it is important to determine the responses of rice to the *Curvularia* isolates. The next chapter presents results on the reactions of 13 varieties of rice from the indica, javanica, and japonica types to inoculations of the three fungal isolates.

Chapter 3. Reaction of rice (*Oryza sativa* L.) seedling varieties to *Curvularia tuberculata* and *C. oryzae*

3.1. Abstract

Thirteen varieties of rice (*Oryza sativa* L.) consisting of indica, japonica, and javanica types, as well as IRRI varieties, were tested against two isolates of *Curvularia tuberculata* (93-020 and 93-022) and one isolate of *C. oryzae* (93-061). Twelve, thirteen, and seven rice varieties expressed resistance to isolates 93-020, 93-022, and 93-061, respectively. In moderately and highly resistant varieties, lesions on the leaf laminae were small, light to dark brown in color, and had a dry appearance. Spots at the leaf margins and leaf tips were light brown to cream colored and dry. Susceptible varieties had coalescing brown areas with necrotic centers. Sporulation was observed in susceptible but not in resistant varieties. The order of decreasing pathogenicity of the three isolates was 93-061 (*C. oryzae*), 93-020 (*C. tuberculata*), and 93-022 (*C. tuberculata*).

3.2. Introduction

Species of *Curvularia* occur mostly as tropical and subtropical facultative plant pathogens with teleomorphic states in *Cochliobolus* and *Pseudocochliobolus* (Rossman, 1987). *C. tuberculata* is the causal agent of citrus die-back in India (Lele et al., 1968), blights of sorghum (*Sorghum vulgare* L.) (Lambat and Asha Ram, 1969), sugarcane (*Saccharum officinale* L.) (Seshadri et al., 1980), coconut (*Cocos nucifera* L.) (Mishra and Singh, 1987) and mango (*Mangifera indica* L.) (Lele et al., 1981), and leaf spots of *Cosmos* (Ghosh and Gupta, 1980) and purple nutsedge (Misra et al., 1973).

C. oryzae was originally isolated from rice grains (Bugnicourt, 1950) but has not been extensively studied. Ou (1985) reported that it occurs with other *Curvularia* species in rice grains exhibiting black kernel disease. Lal and Goel (1989) have also reported rotting of okra (*Abelmoschus esculentus* (L.) Moench.) fruits due to *C. oryzae*.

Rice (*Oryza sativa* L.) is one of the most important crops in the world, being the staple food for millions of people in Asia (IRRI, 1993). Current estimates show that rice production would have to increase by 70% in the next 35 years in order to keep up with population growth and income-induced demand for food (IRRI, 1993). Farmers in the Philippines regard weeds as the most important problem of transplanted and upland rice (Elliot et al., 1984; Elliot and Moody, 1986) and yield reductions of 12 to 50% have been attributed to sedge weeds (Ampong-Nyarko and De Datta, 1991). Weed control practices need improvement due in part to increasing labor costs, environmental contamination, and increasing risk of herbicide resistance.

Biological control of weeds has good potential as a component of integrated pest management (IPM) programs for rice in the Philippines (Watson, 1994). Two isolates of *C. tuberculata* (93-020 and 93-022) and one isolate of *C. oryzae* were obtained from diseased *Cyperus difformis* L. (small flower umbrella sedge), *C. iria* L. (rice flatsedge), and *Fimbristylis miliacea* (L.) Vahl (globe fingerush) in the Philippines. These isolates are currently being evaluated for their biocontrol potential at the International Rice Research Institute (IRRI), in Los Baños, Laguna, Philippines. Although both *C. tuberculata* and *C. oryzae* have been isolated from rice grains (Jain, 1962; Bugnicourt, 1950) and implicated in the black kernel disease of rice together with 12 other *Curvularia* species (Webster and Gunnell, 1992), the manner of entry and the infection

process has not been elucidated. There is also no information on the effects of these *Curvularia* species on rice plants. This work reports on the reactions of rice seedling varieties to inoculations of *C. tuberculata* and *C. oryzae*.

3.3. Materials and methods

All experiments were conducted at the International Rice Research Institute (IRRI), Los Baños, Laguna, Philippines.

3.3.1. Fungal isolates

Two isolates of *Curvularia tuberculata* and one isolate of *C. oryzae* were used in the experiments. *C. tuberculata* was isolated from diseased *C. difformis* (isolate 93-020) and from diseased *C. iria* (isolate 93-022) from Lian, Batangas, Philippines. *C. oryzae* (isolate 93-061) was obtained from diseased *F. miliacea* from Manicahan, Zamboanga, Philippines. All fungal isolates were cultured on half-strength potato dextrose agar (½ PDA) plates or slants for initial observation and later stored on agar-oil slants and/or in soil cultures.

3.3.2. Host plants

Seeds of 13 varieties of rice were obtained from the Genetic Resources Center located at IRRI, and included indica, japonica, and javanica types as well as hybrid IRRI varieties. Seeds of *C. difformis*, *C. iria*, and *F. miliacea* were obtained from mature plants in rice fields in and around the IRRI grounds. Seeds of rice and weeds were germinated in petri dishes lined with moist filter paper. One-week-old seedlings were transplanted into 10-cm standard plastic pots filled with soil (Maahas clay, Suborder Haplustic, Order Alfisol). The pots were then placed in a large pushcart and a two to

three-cm layer of water was maintained in the pushcart to keep the soil moist. The seedlings were maintained in the greenhouse with a 35/25 ± 5°C day/night temperature regime.

3.3.3. Preparation of inoculum

Agar plugs from one-week-old cultures of *C. tuberculata* and *C. oryzae* were used to seed ½ PDA plates for the mass production of the inoculum. Seeded plates of isolates 93-020 and 93-022 were incubated in the dark at 28°C while cultures of 93-061 were maintained under lighted conditions (30 $\mu E\ m^{-1}\ s^{-1}$) at 28°C. Spore suspensions were prepared by flooding each dish with distilled water and lightly scraping the surface of the colony with a clean glass slide or camel's hair brush. The suspension was filtered through cheesecloth to separate mycelial fragments from the spores. Spore concentration was determined using a hemacytometer and sprayed at the rate of 1×10^8 spores/m^2. Tween 20 or oxysorbic (20 POE) (polyoxyethylene sorbitan monolaurate) was added at the rate of two drops per 100 ml of spore suspension.

3.3.4. Inoculation procedure

Inoculation was accomplished when the plants were two weeks old by spraying them with the spore suspension using an atomizer connected to a pressure source (A.H. Thomas Co., Scientific Apparatus, Philadelphia, U.S.A.). At this stage, rice, *C. difformis* and *C. iria* had three to four expanded leaves, and *F. miliacea* had five to six leaves. Distilled water with Tween 20 was used as the control.

The control and inoculated seedlings were incubated in dew chambers at 28°C and 100% relative humidity (RH) in the dark for 24 h and then transferred to the mist

room of the greenhouse with conditions of 24 to 28°C and 80 to 95% RH (Yeh and Bonman, 1986). The experiment was set up as a completely randomized design with six replications. The experiment was repeated once.

3.3.5. Evaluation of host response

Treated and control plants were scored at seven days after inoculation (DAI) using a key (Table 3.1) modified from Mardinus (1983). Scores for each of the three isolates were analyzed using the Kruskall-Wallis ANOVA on ranks on SigmaStat for IBM PC, version 1.0 (Jandel Scientific Corporation, 1992-1994), and reactions of rice seedlings were reported as median score. Data presented are combined results of trials 1 and 2.

Sporulation of the *Curvularia* isolates was assessed by observing cut leaf pieces with lesions after 72 h incubation in a moist chamber. The leaf segments were examined and scored using the following key: - no sporulation; + light sporulation; ++ moderate sporulation; +++ heavy sporulation. Plant height differences, if present, were evaluated visually.

3.4. Results

The response of rice varieties to *Curvularia* isolates 93-020, 93-022, and 93-061 ranged from immune to susceptible. However, most varieties were resistant to the isolates. Norin 21 did not show any visible reaction to 93-022 and appeared identical to the uninoculated control (Figs. 3.1A and B). Highly or moderately resistant rice varieties such as IR 64 and Fukuminori were characterized by small brown spots on the leaves

while moderately susceptible and susceptible rice varieties, IR 65 and PBSRC 20, exhibited few to many coalescing brown areas with necrotic centers (Figs. 3.1C to F).

Lesions were formed only on fully expanded leaves and were observed first between four and five DAI. Younger leaves that unfolded after inoculation did not show any symptoms and remained healthy throughout the experimental period. The leaf tips and margins of susceptible rice varieties were extremely vulnerable to attack since the necrotic centers were concentrated in these areas. In resistant rice varieties, these areas were also invaded but appeared dry and were cream to light brown in color.

C. oryzae (93-061) was more pathogenic to the rice varieties tested than the two isolates of *C. tuberculata*. Of the 13 varieties, two were susceptible, four were moderately susceptible, five were moderately resistant and one was highly resistant to *C. oryzae* (Fig. 3.2). In contrast, one variety exhibited susceptibility and 12, high to moderate resistance to isolate 93-020 of *C. tuberculata* (Fig. 3.3) while all of the 13 varieties showed high to moderate resistance to *C. tuberculata* isolate 93-022 (Fig. 3.4).

There was no mortality associated with disease development in these rice varieties. However, after seven days, slight stunting was evident in the varieties expressing susceptibility to the *Curvularia* isolates. Sparse to moderate sporulation from the necrotic spots was detected in susceptible but not in resistant varieties (Figs. 3.2 to 3.4).

Control rice and weed seedlings were healthy and did not develop any symptoms. However, *C. difformis*, *C. iria*, and *F. miliacea* inoculated with their respective

Curvularia pathogen developed leaf spots after three days that rapidly coalesced to form large blighted areas. The infected weeds eventually collapsed and died at seven to14 DAI.

3.5. Discussion

A high degree of resistance was exhibited by rice to *C. tuberculata* and *C. oryzae*. As a foliar pathogen, *C. oryzae* was most virulent to rice varieties, although half of the varieties tested were resistant. *C. tuberculata* was less virulent and most of the rice varieties were resistant to isolate 93-020 and all were resistant to isolate 93-022. Host specialization apparently occurs in *C. tuberculata*. A *C. tuberculata* isolate from Rangpur lime (*Citrus limonia* Osbeck) is not pathogenic to rice (Taichung Native 1) but caused severe blighting and death in corn (*Zea mays* L., local variety) (Lele et al., 1968). Results of this study showed isolate 93-022 from *C. iria* to be less pathogenic to rice compared to isolate 93-020 from *C. differmis*.

Older leaves were more susceptible than younger leaves to inoculations of *C. tuberculata* and *C. oryzae*. Resistance to pathogens is often affected by the developmental stage of the plant or of the plant organ (Eskes and Toma-Braghini, 1982), with older plants appearing more resistant (Hyde, 1977; Dickinson and Crute, 1974). Conflicting results in this area have been reported. Lesions developed more rapidly in older leaves of sunflower plants infected with *Alternaria helianthii* (Allen et al., 1983). Incubation periods were shorter and lesion expansion rates were greater in lower leaves and older potato plants infected with the early blight pathogen, *Alternaria solani* (Pelletier and Fry, 1989). Tan spot on wheat (*Triticum aestivum* L.), caused by

Pyrenophora tritici-repentis, was most severe on the oldest leaf, while the youngest leaf was the least severely spotted (Cox and Hosford, 1987). It would appear that juvenile tissues are more resistant to necrotrophic pathogens than senescent or older tissues.

Greater susceptibility of older plant tissues to certain fungal phytotoxins has been reported. Barna and Gyorgyi (1992) found that older tobacco leaves were more susceptible to *Alternaria alternata* and *Botrytis cinerea*. They also found that older tissues had a greater sensitivity to fusaric acid, a non-selective toxin, and that senescent leaves were more sensitive to cell wall-degrading enzymes. At least two non-host specific toxins have been isolated from *Curvularia lunata*, a pathogen causing leaf spot of maize (Macri and Vianello, 1976). Further characterization of the *C. lunata* phytotoxins revealed that they cause a general disruption of plasma membrane functions (Vianello et al., 1976). Host specific toxins from *Curvularia pallescens* were also obtained from culture filtrates and diseased corn leaves attacked by this species (Olufolagi, 1986). Whether toxin production occurs also in *C. tuberculata* and *C. oryzae* needs to be investigated.

Most of the lesions on infected leaves occurred at the leaf tips and margins. Clumps of spores trapped by trichomes present on these sites increased the probability of infection. Lesions on the leaf margins grew inward from the edges of the leaf. In rice, hydathodes are present at the margins and tips of leaves (Hoshikawa, 1989), and these could have facilitated the entry of the fungus into the host. Hydathodes serve to secrete excess water from the interior of leaves but may also provide an opening on the epidermis through which pathogens can enter (Campbell et al., 1980). Studies on turf grasses showed that *C. lunata* could colonize heat stressed and/or old clipped leaves but

could not colonize juvenile or mature leaves (Muchovej and Couch, 1987). Apparently, leaf clipping allowed entry of *C. lunata* into the leaf tissue (Muchovej, 1986).

Species of *Curvularia* are regarded as weak pathogens and common saprophytes (Webster and Gunnell, 1992). Seshadri et al. (1980) noted that dense spotting and extensive blighting of sugarcane leaves from a *C. tuberculata* infection was only observed on some severely affected plants, and concluded that the disease was of negligible importance. It is possible that *Curvularia* leaf spots occur on rice in fields but the disease is so minor that it has gone completely unnoticed. At the flowering stage, the spores could then enter the rice plant through the stigma or ovary wall. Entry into the seeds may also take place by colonization of the spikelets from an infected flag leaf while the panicle is still enclosed. This could result in rice grains with the black kernel disease. Early studies using *C. lunata* showed that discolored and infected grains could be produced by inoculating rice plants at flowering stage and by injecting inoculum into the seeds (Martin, 1939; Martin and Altstatt, 1940).

An interaction where symptoms are clearly expressed is a compatible disease reaction as opposed to an incompatible reaction where the symptoms do not develop and the effect on the plant is minimal (Lucas, 1998). The sedge weeds, *C. difformis*, *C. iria*, and *F. miliacea* inoculated with the their respective *Curvularia* pathogen developed coalescing lesions and a rapid blighting reaction that resulted in death within one week. However, most inoculated rice seedlings developed only small leaf spots and brown leaf tips and margins, indicating an incompatible disease reaction. Sporulation of the *Curvularia* isolates also did not occur in the resistant rice varieties.

Although sporulation is never an isolated component of resistance (Cohen and Rotem, 1987), it is often the most sensitive indicator of race-specific (vertical) and race-non-specific (horizontal) resistance (Johnson and Taylor, 1976). In most cases, inhibited infection and colonization (Cohen & Rotem, 1987) accompany reduced sporulation. *Cucumis melo* resistant to *Psuedoperonospora cubensis* and to *Sphaerotheca fuliginea* race 2 was characterized by small lesions with few spores (Cohen et al., 1984; Cohen and Cohen, 1986). Rice varieties resistant to the *Curvularia* isolates such as Norin 21 and Red Khosha Cerma were characterized by limited lesion development and inhibited or lack of sporulation. Moderately susceptible to susceptible rice varieties such as IR 65 and PSBRC 20 exhibited greater leaf necrosis and sparse to moderate sporulation.

Rice germplasm is classified into three eco-geographic races or cultivars namely, indica, japonica, and javanica using morphological traits (Chang et al., 1972; Takahashi, 1984). Japonica (type A) rices are short to intermediate in plant stature, low tillering, and have narrow, dark green leaves, short, awnless to long-awned, roundish grains that are resistant to shedding or shattering. Javanica (type B) rices are tall, medium tillering plants with broad, stiff, light green leaves and long, broad, thick, long-awned or awnless grains that resists shattering. Indica (type C) rices are characterized by profusely tillering tall to intermediate plants with broad to narrow, light green leaves and long to short, slender, mostly awnless, flat grains that shatters easily. Cultivars developed at IRRI were produced mainly from semidwarf indica parents and are thus, designated as indica varieties (Dalrymple, 1986).

Resistance to *C. tuberculata* isolate 93-022 was expressed in all rice varieties from the three eco-geographic races. Although extreme host specificity is not needed for

indigenous plant pathogens that are to be developed as mycoherbicides, the pathogen must be "sufficiently safe" to desirable plant species (Watson, 1985). Future studies of isolate 93-022 are needed to clearly determine its host range.

3.6. Literature Cited

Allen, S.J., J.F. Brown, and J.K. Kochman. 1983. Effects of leaf age, host growth stage, leaf injury, and pollen on the infection of sunflower by *Alternaria helianthii*. Phytopathology 73:896-898.

Ampong-Nyarko, A. and S.K. De Datta. 1991. A Handbook for Weed Control in Rice. International Rice Research Institute, Manila, Philippines. 113 pp.

Barna, B. and Gyorghi, B. 1992. Resistance of young versus old tobacco leaves to necrotrophs, fusaric acid, cell wall-degrading enzymes and autolysis of membrane lipids. Physiol. Molec. Plant Pathol. 40:247-257.

Bugnicourt, F. 1950. Les espèces du genre *Curvularia* isolées des semences de riz. Rev. gén. Bot. 57:65-77.

Campbell, C.L., J.S. Huang, and G.A. Payne. 1980. Defense at the perimeter: The outer walls and the gates. Pages 103-120 *in* Plant Disease: An Advanced Treatise Vol. 5. Academic Press. New York, NY, USA.

Chang, T.T., G.C. Loresto, and O. Tagumpay. 1972. Agronomic and growth characteristics of upland and lowland rices. Pages 645-661 *in* Rice Breeding. International Rice Research Institute, Manila, Philippines.

Cohen, S. and Cohen, Y. 1986. Nature and genetics of resistance to powdery mildew race 2 in *Cucumis melo* PI 124111. Phytopathology 76:1165-1167.

Cohen, Y. and J. Rotem. 1987. Sporulation of foliar pathogens. Pages 315-333 *in* Fungal Infection of Plants. G.F. Pegg and P.G. Ayres (Eds.). British Mycological Society Symposium Series 13. Cambridge University Press. Cambridge, England.

Cohen, Y., H. Eyal, A. Cohen, and C.E. Thomas. 1984. Evaluating downy mildew resistance in *Cucumis melo* L. Cucurbits Genetics Cooperative Report 7:38-40.

Cox, D.J. and R.M. Hosford. Jr. 1987. Resistant winter wheats compared at differing growth stages and leaf positions for tan spot severity. Plant Dis. 71:883-886.

Dalrymple, D.G. 1986. Development and spread of high-yielding rice varieties in developing countries. Bureau for Science and Technology, Agency for International Development. Washington, D.C., USA. 117 pp.

Dickinson, C.H. and I.R. Crute. 1974. The influence of seedling age and development on the infection of lettuce by *Bremia lactucae*. Ann. Appl. Biol. 76:47-51.

Elliot, P.C. and K. Moody. 1986. Weed control studies in upland rice-based cropping systems. Paper presented at the Claveria Cropping Systems Annual Review, 6-7 March 1986, Cagayan de Oro City, Philippines. 14 pp.

Elliot, P.C., D.C. Navarez, F.F. Fajardo, and K. Moody. 1984. Farmers' concepts of weeds and their weed control practices in transplanted rice in Guimba, Nueva Ecija. Paper presented at the Cropping Systems Research Design Workshop, 7-8 May, 1984, Muñoz, Nueva Ecija, Philippines. 7 pp.

Eskes, A.B. and M. Toma-Braghini. 1982. The effect of leaf age on incomplete resistance of coffee *to Hemileia vastatrix*. Neth. J. Plant Path. 88:219-230.

Hoshikawa, K. 1989. The Growing Rice Plant: An Anatomical Monograph. Nosan Gyosan Bunka Kyokai (Nobunkyo). Tokyo, Japan. 310 pp.

Hyde, P.M. 1977. The effect of leaf age on infection of wheat seedlings by *Erysiphe graminis* on subsequent colony development. Phytopath. Z. 88:299-305.

International Rice Research Institute.1993. Rice Almanac (1993-1995). International Rice Research Institute, Los Baños, Laguna, Philippines. 142 pp.

Jandel Scientific Corporation. 1992-1994. SigmaStat Version 1 User's Manual. San Rafael, CA, USA.

Jain, B.L. 1962. Two new species of *Curvularia*. Trans. Brit. Mycol. Soc. 45:539-544.

Johnson, R. and A.J. Taylor. 1976. Spore yield of pathogens in investigations of the race specificity of host resistance. Annu. Rev. Phytopathol. 14:97-119.

Lal, B. and D. Goel. 1989. A new rot of *Abelmoschus esculentus*. Indian Phytopath. 42:482.

Lambat, A.K. and Ram, A. 1969. Seed-borne infection of *Curvularia* causing a new blight disease of Jowar. Indian Phytopath. 22:282-284.

Lele, V.C., S.P. Raychaudhuri, R.B. Bhalla, and A. Ram. 1968. *Curvularia tuberculata*, a new fungus causing die-back disease of citrus in India. Indian Phytopath. 21:66-72.

Lucas, J.A. 1998. Plant Pathology and Plant Pathogens. 3rd Edition. Blackwell Science Ltd. London, England. 274 pp.

Macri, F. and A. Vianello. 1976. Isolation and partial characterization of phytotoxins from *Curvularia lunata* (Wakk.) Boed. Physiol. Plant Pathol. 8:325-331.

Mardinus. 1983. Pathogenicity test of three isolates of *Drechslera oryzae* (Breda de Haan) Subram. & Jain on rice seedlings. Pages 215-218 *in* Proc.Symposium on Pest Ecology and Pest Management. BIOTROP Special Publication no. 18. SEAMEO Regional Center for Tropical Biology. Bogor, Indonesia.

Martin, A.L. 1939. Possible cause of black kernels in rice. Plant Dis. Reptr. 23:247-249.

Martin. A.L. and G.E. Altstatt. 1940. Black kernel and white tip of rice. Bulletin. Texas Agricultural Experiment Station No. 584. 14 pp.

Mishra, D. and N. Singh. 1987. Two new leaf diseases of coconut seedlings caused by *Curvularia* spp. Indian J. Plant Pathol. 5: 208-209.

Misra, A.P., O. Prakash, B. Mishra, and K.K. Dutta. 1973. A new leaf spot disease of motha (*Cyperus rotundus* L.) caused by *Curvularia tuberculata*. Indian Phytopath. 26:165-167.

Muchovej, J.J. 1986. Definition of leaf health in *Agrostis palustris* at the time of infection and colonization by *Curvularia lunata*. Ann. Appl. Biol. 109:249-258.

Muchovej, J.J. and H.B. Couch. 1987. Colonization of bentgrass turf by *Curvularia lunata* after leaf clipping and heat stress. Plant Dis. 71:873-875.

Olufolaji, D.B. 1986. Production and bioassay of *Curvularia pallescens* Boedijn toxins. Cryptogamie Mycol. 7:335-342.

Ou, S.H. 1985. Rice Diseases. 2nd Edition. CAB International. Wallingford, UK. 380 pp.

Pelletier, J.R. and W.E. Fry. 1989. Characterization of resistance to early blight in three potato cultivars: Incubation period, lesion expansion rate, and spore production. Phytopathology 79:511-517.

Rossman, A.Y., M.E. Palm, and L.J. Spielman. 1987. A Literature Guide for the Identification of Plant Pathogenic Fungi. APS Press. St. Paul. MN, USA. 252 pp.

Seshadri, K., P. Padmanaban, and K.C. Alexander. 1980. A new leaf blight disease of sugarcane. Indian Phytopath. 33:325-326.

Sivanesan, A. 1987. Graminicolous species of *Bipolaris, Curvularia, Drechslera* and *Exserohilum* and their teleomorphs. Mycol. Pap. 158:1-261.

Takahashi, N. 1984. Differentiation of ecotypes in *Oryza sativa* L. Pages 31-70 in Biology of Rice. S. Tsunoda and N. Takahashi (Eds.). Elsevier Science Publishers. Amsterdam, The Netherlands.

Vianello, A., F. Macri, and C. Passera. 1976. Effect of *Curvularia lunata* phytotoxin on membrane permeability of corn roots. Can. J. Bot. 54:2918-2923.

Watson, A.K. 1985. Host specificity of plant pathogens in biological weed control. Pages 99-104 *in* Proc. VI International Symposium on Biological Control of Weeds. E.S. Delfosse (Ed.). Agriculture Canada. British Columbia.

Watson, A.K. 1994. Current status of bioherbicide development and prospects for rice in Asia. Pages 195-201 *in* Integrated Management of Paddy and Aquatic Weeds in Asia. Proc.International Seminar " Biological Control and Integrated Management of Paddy and Aquatic Weeds in Asia". H. Shibayama, K. Kiritani, and J. Bay-Petersen (Eds.) Oct. 19-25, 1992. Tsukuba, Japan.

Webster, R.K. and P.S. Gunnell (eds.). 1992. Compendium of Rice Diseases. APS Press. St. Paul, MN, USA. 62 pp.

Yeh, W.H. and J.M. Bonman. 1986. Assessment of partial resistance to *Pyricularia oryzae* in six rice cultivars. Plant Pathol. 35:319-323.

Figure 3.1. Typical reactions of rice (*Oryza sativa* L.) seedlings to *Curvularia tuberculata* (93-020 and 93-022) and *Curvularia oryzae* (93-061) seven days post inoculation (DPI). **A.** Uninoculated leaf of cv. Norin 21. **B.** Inoculated leaf of cv. Norin 21 showing spores of 93-022 on the surface and two minute lesions (L). **C.** Moderately resistant reaction of cv. IR 64 to 93-020 showing small, discrete lesions (L). **D.** Leaf tip of cv. Norin 21 inoculated with 93-061 showing moderately resistant reaction consisting of light brown to cream-colored areas (LT). **E.** Moderately susceptible cv. IR 65 inoculated with 93-061 showing many brown spots (L) on the leaf lamina. **F.** Susceptible reaction of cv. PBSRC 20 to 93-061 showing coalescing lesions (L).

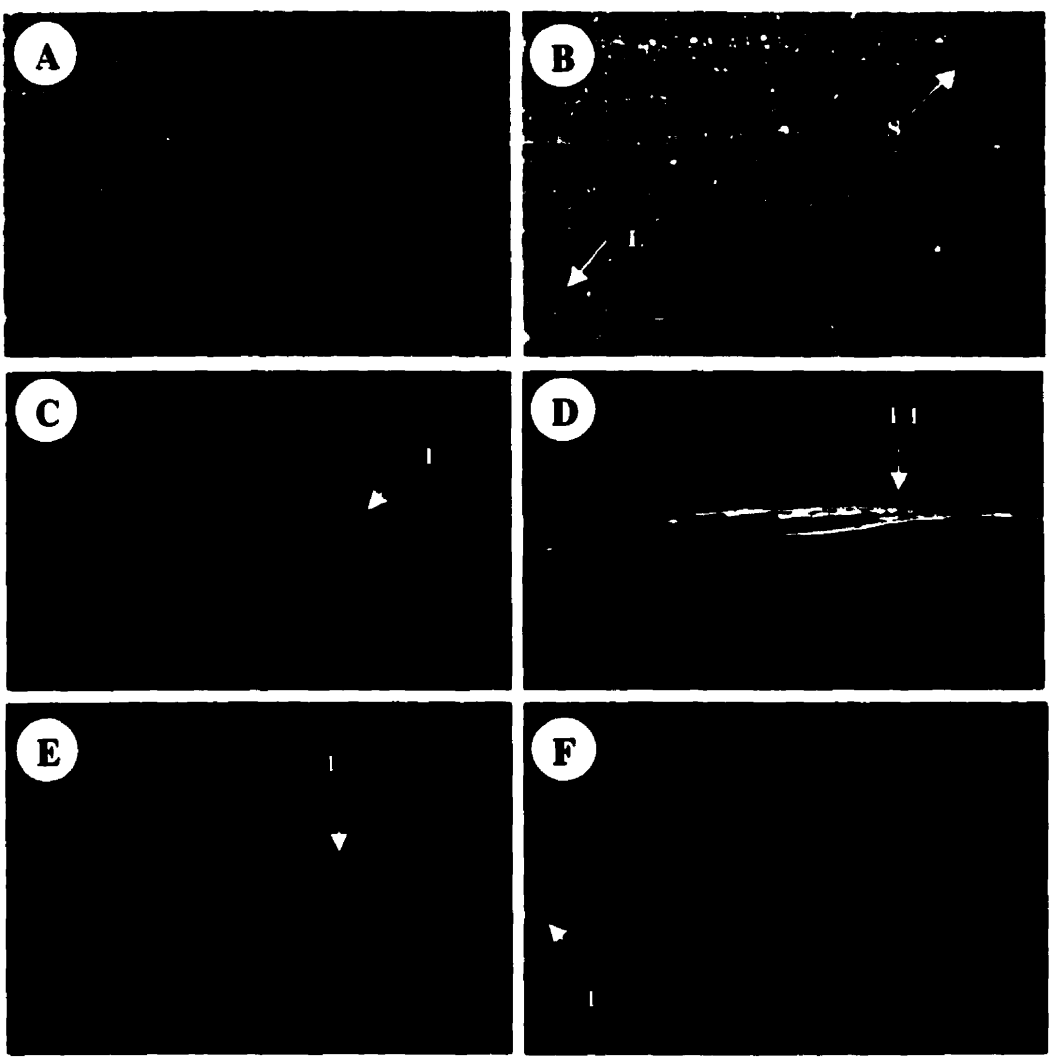
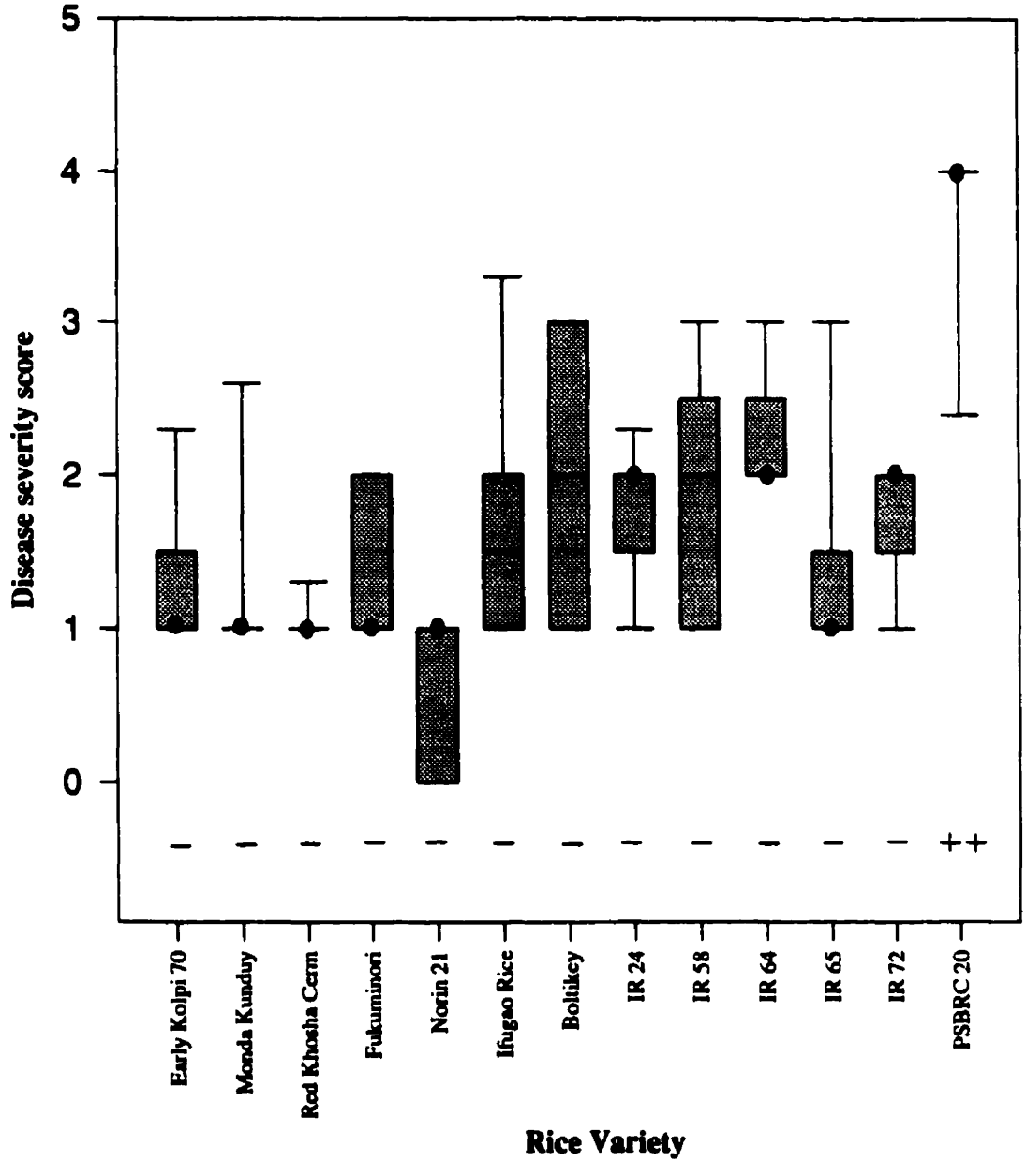
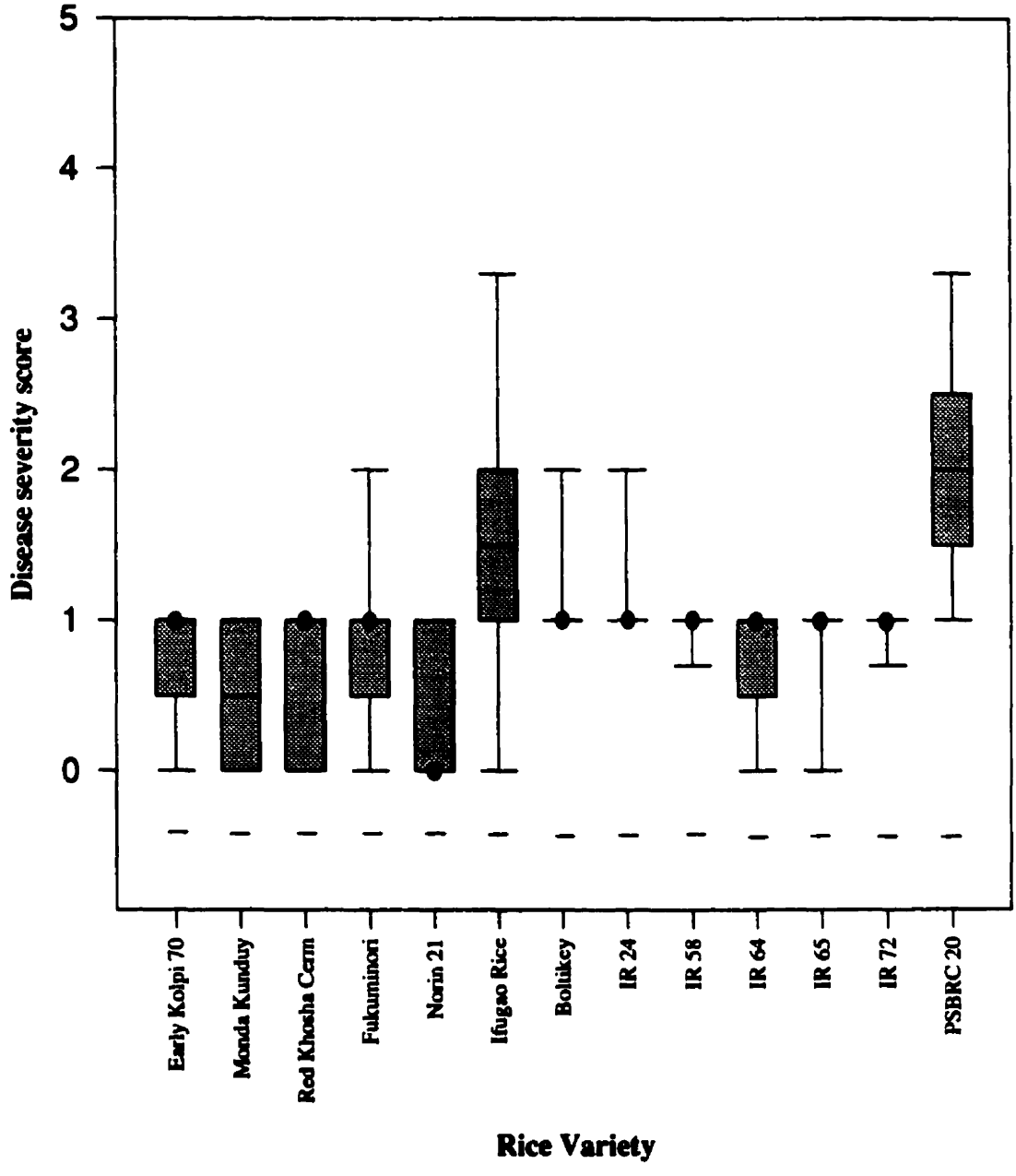

A
B
C
D
E
F

Figure 3.2. Disease severity score of rice (*Oryza sativa* L.) varieties inoculated with *Curvularia tuberculata* isolate 93-020 at 1 x 10^8 spores/m^2, seven days after inoculation (DAI). Boxed area represents the 25^{th} and 75^{th} percentile, a line within the box indicates the median and the whiskers represent the 10^{th} and 90^{th} percentile. The median is indicated by a dot when it occurs at the border of the boxed area. Sporulation is indicated by – (no sporulation), + (sparse sporulation), or ++ (moderate sporulation).



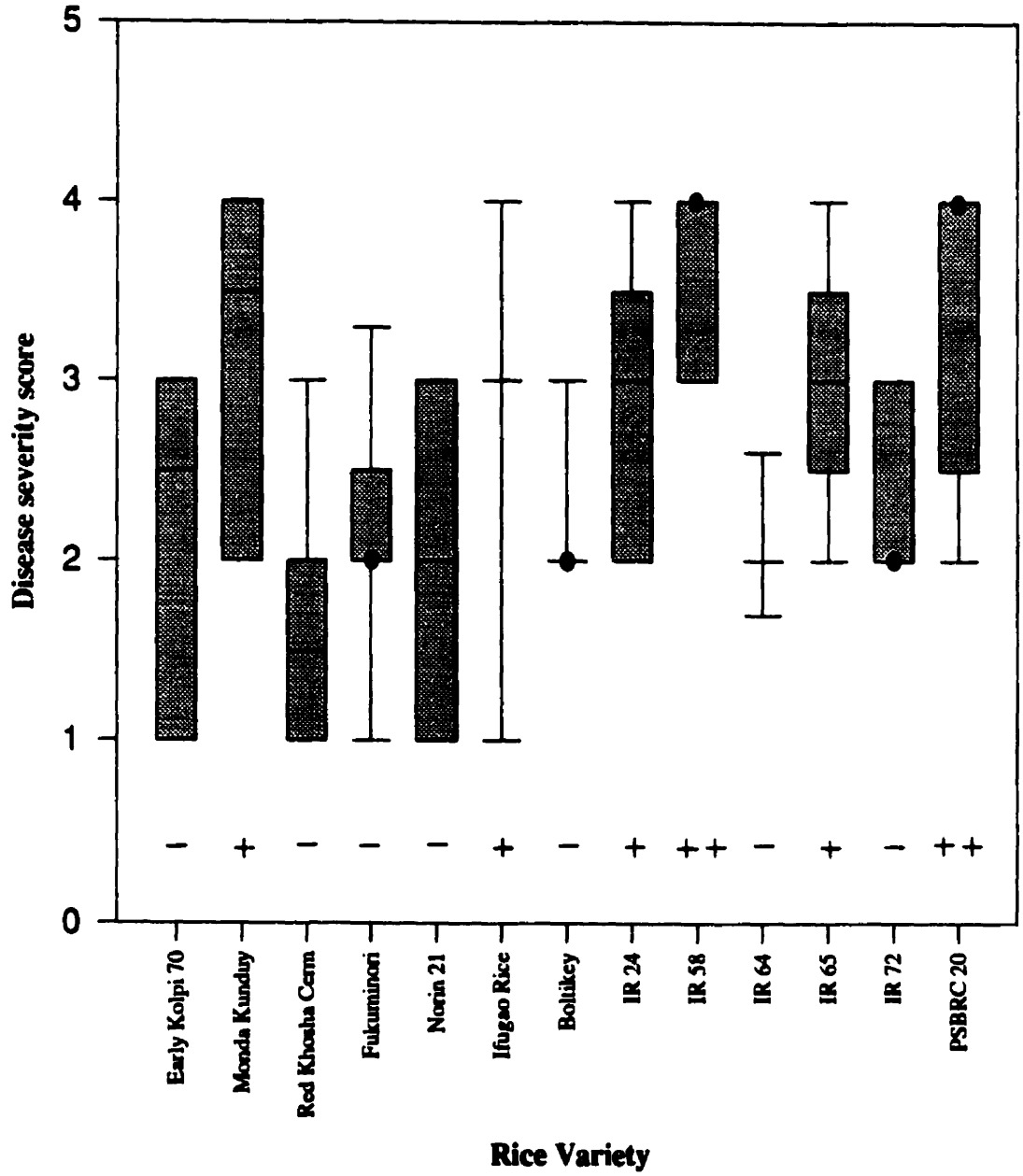
0 Immune
1 Highly Resistant
2 Moderately Resistant
3 Moderately Susceptible
4 Susceptible
5 Highly Susceptible

Figure 3.3. Disease severity score of rice (*Oryza sativa* L.) varieties inoculated with *Curvularia tuberculata* isolate 93-022 at 1 x 10^8 spores/m^2, seven days after inoculation (DAI). Boxed area represents the 25th and 75th percentile, a line within the box indicates the median and the whiskers represent the 10th and 90th percentile. The median is indicated by a dot when it occurs at the border of the boxed area. Sporulation is indicated by – (no sporulation), + (sparse sporulation), or ++ (moderate sporulation).



0 Immune
1 Highly Resistant
2 Moderately Resistant
3 Moderately Susceptible
4 Susceptible
5 Highly Susceptible

Figure 3.4. Disease severity score of rice (*Oryza sativa* L.) varieties inoculated with *Curvularia oryzae* isolate 93-061 at 1 x 10^8 spores/m^2, seven days after inoculation (DAI). Boxed area represents the 25^{th} and 75^{th} percentile, a line within the box indicates the median and the whiskers represent the 10^{th} and 90^{th} percentile. The median is indicated by a dot when it occurs at the border of the boxed area. Sporulation is indicated by – (no sporulation), + (sparse sporulation), or ++ (moderate sporulation).



0 Immune
1 Highly Resistant
2 Moderately Resistant
3 Moderately Susceptible
4 Susceptible
5 Highly Susceptible

Table 3.1. Disease severity key used to score the reaction of rice seedlings to three isolates of *Curvularia*.*

Score	Host Reaction	Description
0	I = Immune	No visible symptoms
1	HR = Highly Resistant	Few to many brown specks, 0.5 to 1.0 mm in diameter, grayish-green or light brown leaf tips
2	MR = Moderately Resistant	Discrete brown lesions with or without chlorotic halo, 1.0 to 2.0 mm long, brown leaf tips and margins
3	MS = Moderately Susceptible	Many brown spots, 2.0 to 3.0 mm long, a few lesions coalescing, brown leaf tips and margins
4	S = Susceptible	Many lesions with necrotic centers, 3 to 5 mm long, 10 to 50% of leaf area killed
5	HS = Highly Susceptible	Numerous large and expanding lesions, more than 50% of leaf area killed; plant collapsed

* modified from Mardinus, 1983.

CONNECTING TEXT

Findings presented in Chapter 2 indicate that the three *Curvularia* isolates are highly virulent on more than one weed species. This is significant because *Cyperus difformis*, *C. iria*, and *Fimbristylis miliacea* occur together as weeds of irrigated rice and it would be beneficial to have a fungal pathogen that can attack all three weed species. However, *Cyperus rotundus*, the major weed of upland rice, was resistant. It is important to examine this difference in susceptibility. An understanding of the host-fungal interactions may provide insights for enhancing the disease progress. This chapter consists of a histological study of the interactions between four sedge weeds and the three *Curvularia* isolates.

Chapter 4. Histopathology of *Curvularia tuberculata* and *C. oryzae* on Cyperaceae weeds

4.1. Abstract

Interactions between three susceptible (*Cyperus difformis, C. iria, Fimbristylis miliacea*) and one resistant (*C. rotundus*) sedge weeds with *Curvularia tuberculata* and *C. oryzae* were examined by light microscopy. The infection process of two isolates of *C. tuberculata* and one isolate of *C. oryzae* was generally the same. The onset of spore germination and percent germination did not differ markedly between susceptible and resistant hosts. Spore germination was polar in *C. tuberculata* and bipolar, in *C. oryzae*. Germ tube growth of both *Curvularia* pathogens was random although coiling and less branching was observed on *C. rotundus*. Appressoria and infection cushions were formed preferentially over epidermal wall junctions in both species. After successful penetration, primary and secondary infection hyphae were observed intra- and intercellularly. Colonization of susceptible host tissues was rapid, leading to shrinkage and collapse of epidermal, mesophyll, and bundle sheath cells. However, vascular bundle cells were not invaded. Conidiophores emerged from stomata in necrotic tissues by 96 h post inoculation (HPI). In contrast, colonization of *C. rotundus* was slow and growth of both *Curvularia* pathogens within the leaf tissues was sparse. By 168 HPI, infection hyphae began to show signs of degeneration. Fungal cells appeared granular and plasmolyzed. Sporulation of *C. tuberculata* and *C. oryzae* did not occur in *C. rotundus*.

4.2. Introduction

Cyperus difformis L., *C. iria* L., *Fimbristylis miliacea* (L.) Vahl, and *C. rotundus* L. are among the most serious weeds of rice in Southeast Asia, reducing yields by as much as 78% (IRRI, 1987). Control methods include hand weeding (Moody, 1992), flooding (Moody, 1990), cultivation (Mukhopadhyay, 1983; Moody, 1992), using *Azolla* spp. (Janiya and Moody, 1984) to suppress weed growth, and the use of herbicides (Baltazar, 1995; De Datta, 1995). Due to socio-economic and environmental concerns, biological control has gained wide acceptance as a viable component of integrated weed management programs. Watson (1992) included these four sedges in his list of candidate weeds for biological control in the Philippines.

The genus *Curvularia* is a group of dematiaceous hyphomycetes related to *Drechslera, Bipolaris,* and *Exserohilum,* that occur mostly as tropical and subtropical facultative plant pathogens (Ellis, 1966; 1971). They are characterized by straight or curved, brown, multicellular conidia, with one or more cells darker than the rest. In 1993, *Curvularia* spp. were isolated from diseased *C. difformis, C. iria,* and *F. miliacea* in the Philippines. Isolates 93-020 from *C. difformis* and 93-022 from *C. iria* were identified by the Commonwealth Mycological Institute (CMI) as *Curvularia tuberculata* Jain and isolate 93-061 from *F. miliacea* as *C. oryzae* Bugnicourt.

Jain first described *C. tuberculata* in 1962. It has since been reported as the causative agent of leaf spots and leaf blights in citrus (Lele et al., 1968), jowar or sorghum (Lambat and Ram, 1969), motha or purple nutsedge (Misra et al., 1973), cosmos (Ghosh and Gupta, 1980), sugarcane (Seshadri et al., 1980), mango (Lele et al., 1981), and fruit rot in guava (Kapoor and Tandon, 1971). It has also been found in dune soil

(Rama Rao, 1964) and in wood, fabric, and air (Sivanesan, 1987). *C. oryzae* was first reported by Bugnicourt (1950) and has been implicated in the black kernel disease of rice together with *C. tuberculata* and 12 other *Curvularia* species (Webster and Gunnell, 1982).

The previous chapters presented evidence that *C. tuberculata* and *C. oryzae* caused significant mortality on *C. difformis, C. iria*, and *F. miliacea* within two weeks after inoculation. Cross pathogenicity of the isolates to the other closely related weedy sedges was also demonstrated. This is significant because *C. difformis, C. iria*, and *F. miliacea* occur together in irrigated rice fields and a potential biocontrol agent that possesses activity on all of them is beneficial. However, *C. rotundus* was found to be resistant to all three isolates. Since *C. rotundus* is closely related to the three other weeds, it is important to examine this difference in susceptibility.

An understanding of the fungal-host interactions is important in biological control for the purpose of enhancing the progress of the disease (Van Dyke 1989). Several factors can affect the success of a particular fungal isolate. These include variability in spore size, spore adhesion to plant parts, number of germ tubes/spore, germ tube lengths, infection structures produced by germ tubes, penetration of plant parts, toxin production, use of inoculum adjuvants, and interaction with other phylloplane microorganisms (Van Dyke, 1989). The histopathology of *Puccinia canaliculata* in *Cyperus esculentus* (Wetzstein and Phatak, 1987) and *Colletotrichum gloesporioides* f. sp. *aeschynomene* in northern jointvetch (TeBeest et al., 1978) were studied in order to gain a better understanding of how these biocontrol agents infect their respective weed hosts and cause disease.

The objective of this study was to compare the pre- and post-penetration stages of infection by two isolates of *C. tuberculata* and one isolate of *C. oryzae* on *C. difformis*, *C. iria*, *C. rotundus* , and *F. miliacea*.

4.3. Materials and methods

All experiments were conducted at the International Rice Research Institute (IRRI), Los Baños, Laguna, Philippines. Some histology and photomicrography work was done at McGill University.

4.3.1. Fungal isolates

Three isolates belonging to two species of *Curvularia* were used in the experiments. *C. tuberculata* was isolated from diseased *C. difformis* (isolate 93-020) and from diseased *C. iria* (isolate 93-022) from Lian, Batangas, Philippines. *C. oryzae* (isolate 93-061) was obtained from diseased *F. miliacea* from Manicahan, Zamboanga, Philippines. All fungal isolates were cultured on ½ PDA plates or slants for initial observation and later stored on agar-oil slants and/or in soil cultures.

4.3.2. Host plants

Seeds of *C. difformis*, *C. iria*, and *F. miliacea* were obtained from mature plants in rice fields in and around the International Rice Research Institute, Los Baños, Laguna, Philippines. *C. rotundus* tubers were obtained from the soil in the same areas. *C. difformis*, *C. iria*, and *F. miliacea* seeds were germinated in petri dishes lined with moist filter paper. Seedlings of approximately the same height and vigor were transplanted when they were a week old into pots filled with soil (Maahas clay, Suborder Haplustic, Order Alfisol), and maintained in the greenhouse. Tubers of *C. rotundus* were sown

directly into potting soil. The seedlings were maintained in the greenhouse with a 35/25 ± 5° C day/night temperature regime.

4.3.3. Preparation of inoculum

Agar plugs were used to seed ½ PDA plates for the mass production of the inoculum. Seeded plates of isolates 93-020 and 93-022 were incubated in the dark at 28° C while cultures of 93-061 were maintained under lighted conditions (30 $\mu E\ m^{-1}\ s^{-1}$) at 28° C. Spore suspensions were prepared by flooding each dish with distilled water and lightly scraping the surface of the colony with a clean glass slide or camel's hair brush. The suspension was filtered using a piece of cheesecloth to separate mycelial fragments from the spores. Spore concentration was determined using a hemacytometer and the spore suspension was sprayed at the rate of 1×10^8 spores/m^2. Two drops of Tween 20 or oxysorbic (20 POE) (polyoxyethylene sorbitan monolaurate) was added per 100 ml of spore suspension.

4.3.4. Inoculation procedure

Inoculation was accomplished when the plants were two weeks old by spraying them with the spore suspension using an atomizer connected to a pressure source (A.H. Thomas Co., Scientific Apparatus, Philadelphia, U.S.A.). At this stage, *C. differmis* and *C. iria* had three to four expanded leaves, *F. miliacea* had five to six leaves and *C. rotundus* had approximately six to eight leaves. Distilled water with Tween 20 was used as the control.

The control and inoculated seedlings were incubated for 24h in the dew chamber set at 28° C and 100% relative humidity (RH) in the dark, after which they were moved to

the mist room of the greenhouse. The mist room is a humid room in the greenhouse with an automatic mister and kept at 24 to 28° C and 80 to 95% RH (Yeh and Bonman, 1986). The experiment was set up as a 4 x 4 factorial following a randomized complete block with five replications. The experiment was conducted three times.

4.3.5. Histopathology of infection

The third and fourth leaves of *C. differmis, C. iria, F. miliacea* and *C. rotundus* from infected and uninfected (control) plants were harvested at 2, 4, 8, 16, 20, 24, 36, 48, 72, 96, 120, 144, and 168 hours after inoculation (HPI). Two sets of leaf samples were obtained, one for whole leaf clearing and the other for the paraffin method. Leaf segments for whole leaf clearing were immersed in vials containing 70 parts absolute ethyl alcohol and 30 parts glacial acetic acid while those for cross-sectioning were fixed in formaldehyde-acetic acid-alcohol solution (FAA) (Johansen, 1940). Leaf samples obtained after two HPI were observed under the microscope for spore deposition before they were subjected to leaf clearing.

Whole leaf segments were immersed in several changes of alcohol-acetic acid clearing solution until they appeared translucent. They were stained in lactophenol-acid fuchsin (Dhingra and Sinclair, 1985) for 15 minutes or until a good contrast was achieved between the epidermal cells (colorless) and the fungal pathogen (deep pink). Stained tissues were dipped in plain lactophenol to remove excess stain and mounted on glass slides using 50% glycerin. Microscopic observations were conducted and photographs were taken using a Nikon 35-mm camera mounted on a compound microscope.

Leaves for cross sectioning were prepared following the method of Johansen (1940). Samples were first dehydrated in a graded ethanol-tertiary butyl alcohol series,

cleared and then infiltrated with Paraplast (Fischer Co.) in an oven at 56° C. After three changes, they were embedded in the same medium and sectioned using a rotary microtome. The sections (12 µm thick) were affixed to clean glass slides using egg albumin and stained with safranin-fast green. Microscopic examination was performed and photographs taken with a Nikon 35-mm camera mounted on a compound microscope.

4.4. Results

4.4.1. Spore deposition

Spore deposition patterns differed among the four weeds. In *C. differmis* and *F. miliacea*, most of the spores were deposited at the tips (Fig. 4.1D) and at the leaf bases near the leaf sheaths (Figs. 4.1A and C). In *C. iria* (Fig. 4.1B) and *C. rotundus* (Fig. 4.1E), spores were more or less evenly distributed in the middle part of the leaf lamina. Trichomes or leaf hairs sometimes trapped spores (Fig. 4.1F).

4.4.2. The Infection Process of Curvularia tuberculata (Isolates 93-020 and 93-022)

The infection process of the two C. *tuberculata* isolates were similar on *C. differmis* and *C. iria*. Spore germination was polar (Fig. 4.2A) and began at four HPI. Germ tubes were 1.5 to 2 µm in diameter and emerged from either the apical or basal cells but were never observed to emerge from the middle cells. The direction of germ tube growth was generally random, as they grew perpendicular or parallel to the long axis of leaf epidermal cells and developed branches (Fig. 4.2B).

Infection structure formation was initiated eight HPI. Appressoria and infection cushions formed over epidermal cell walls but more frequently, at the junction of

epidermal cell walls. Occasionally, stomata and guard cells were penetrated. Appressoria were club- or teardrop-shaped, and ranged in size from 5 to 15 µm by 3 to 12 µm (Figs. 4.3A to C). Simple appressoria were generally terminal on germ tube branches (Figs. 4.3A to C) although a few intercalary appressoria were also found (Fig. 4.3D). Infection cushions were also formed on epidermal cell walls and occasionally, over stomata. They were formed in either of three ways: (1) branching of a single germ tube tip (Fig. 4.3E), (2) branching and/or coiling of aggregating germ tubes (Fig. 4.3F), and (3) aggregation of several appressoria (Fig. 4.3G). Yellow haloes were present around or adjacent to the infection structures (Fig. 4.3B).

Penetration was direct and once the penetration peg gained entry to the epidermal cell, it immediately widened and developed primary infection hyphae (Fig. 4.4A). Thick, highly branching hyphae grew intracellularly, in most cases filling the entire cell (Fig. 4.4B). Intercellular growth of *C. tuberculata* was also observed, and resulted in the swelling and separation of adjoining cell wall layers (Fig. 4.4C). Infection of the neighboring cells proceeded by direct penetration of the adjoining cell wall by branches of the primary infection hyphae (Fig. 4.4D). These secondary infection hyphae quickly spread into adjacent epidermal and mesophyll cells (Fig. 4.4E) as well as through the leaf sheath cells (Fig. 4.4F).

Sections of the infected tissues revealed that by 48 to 72 HPI, *C. tuberculata* had completely colonized the epidermal cells and the underlying mesophyll cells (Figs. 4.5A and B). After 96 h, the hyphae had ramified to the bundle sheath cells surrounding the vascular bundle (Figs. 4.5C and D). Conidiophores had also emerged from stomata at this time (Figs. 4.5E and F).

In *F. miliacea* inoculated with either isolate 93-020 or 93-022, the infection process followed a pattern similar to *C. difformis* and *C. iria*. Spores of *C. tuberculata* germinated and produced germ tubes at four HPI. There was no apparent directionality in the growth of germ tubes (Fig. 4.6A) since they were observed to grow perpendicular to the long axis of leaf epidermal cells but they also grew tightly appressed to the long axis of the epidermal cells in the costal region. Many of the spores, however, produced germ tubes that immediately branched as soon as they emerged from the spore, resulting in bifurcated germ tubes (Fig. 4.6B). Infection structures developed over epidermal cell walls or rarely, on stomata between 20 to 24 HPI. Simple appressoria were intercalary (Fig. 4.6C) or terminal (Figs. 4.6D and E) and exhibited variation in size and shape. Infection cushions resulted from the enlargement of germ tube branches (Fig. 4.6F).

Infection hyphae grew within and in between epidermal and mesophyll cells (Figs. 4.7A and C) as well as leaf sheath cells (Figs. 4.7B and D). Stomatal guard cells were also infected (Fig. 4.7E). After 120 hours, necrotic leaf tissues showed conidiophores emerging from the stomata (Figs. 4.7F to H).

The infection process of *C. tuberculata* essentially remained the same on *C. rotundus*. Spores germinated by four HPI (Fig. 4.8A) and germination were generally polar (Fig. 4.8B). Germ tubes also lacked apparent directionality in their growth. There were long, unbranched germ tubes growing parallel to the long axis of epidermal cells (Fig. 4.8C) but some were oriented perpendicularly (Fig. 4.8D). A lesser degree of germ tube branching in *C. rotundus* was observed compared to the three other hosts. Germ tubes also coiled repeatedly on the epidermal cell surface without initiating infection (Fig. 4.8E), and occasionally coiling around trichomes (Fig. 4.8F).

Infection structures were first observed at 24 HPI and were preferentially formed on epidermal cell wall junctions. Intercalary or terminal appressoria (Fig. 4.9A) ranged from 7 to 15 μm by 3 to 12 μm while infection cushions resulting from the aggregation of germ tube branches were approximately 20 to 25 μm by 10 to 12 μm. (Figs. 4.9C, D). Penetration was direct although a few stomata were also penetrated (Fig. 4.9B).

Successful penetration was immediately followed by the growth of primary infection hyphae (Fig. 4.9E). However, infection hyphae were restricted to the cell initially penetrated (Fig. 4.9F) or to a few adjacent cells (Fig. 4.9G), even by 48 HPI. Many epidermal cells in inoculated plants also exhibited a granular appearance (Fig. 4.9H) at 24 HPI, with infected cells turning first yellow, and then brown. Further growth of *C. tuberculata* was restricted to the 20 to 30 cells surrounding the first infected cell. By 168 HPI, the hyphae were starting to become disorganized and appeared granular and plasmolyzed.

4.4.3. The infection process of Curvularia oryzae (Isolate 93-061)

Fimbristylis miliacea, C. difformis, and *C. iria* were highly susceptible to *C. oryzae*. Pre-penetration events were similar in these three weeds. Two hours after inoculation, spores of *C. oryzae* had germinated (Fig. 4.10A). Germination was bipolar and no intercalary germ tubes were observed. Germ tube growth was both parallel (Fig. 4.10B) and perpendicular (Fig. 4.10D) to the long axis of epidermal cells and developed many branches (Fig. 4.10C).

In the three susceptible weeds, appressoria began to form eight hours after inoculation on epidermal cell walls and rarely, on stomata (Fig. 4.11A). More than one

appressorium may develop from one conidium due to the branching of germ tubes (Fig. 4.11B). Infection cushions were observed starting 24 HPI. They appeared to be aggregations of simple appressoria (Figs. 4.11C and D) or coiled branches of germ tube tips (Figs. 4.11E and F). Narrow penetration pegs (Figs. 4.11E and 4.12A) achieved penetration and the penetration site was often marked by a yellow halo with a pore in the center (Fig. 4.12B). Primary infection hyphae developed from the infection structures and rapidly spread within the cell (Fig. 4.12C) or between cells (Fig. 4.12D).

After 48 to 72 h, the pathogen had completely colonized the leaf tissues of *C. differmis*, *C. iria*, and *F. miliacea*. In *F. miliacea*, fungal hyphae were visible within cleared necrotic leaves (Fig. 4.13A) and conidiophores had emerged from the stomata by 96 HPI (Fig. 4.13B). Cross sections of *C. differmis* and *C. iria* revealed the extent to which the epidermal and mesophyll cells had been infected (Figs. 4.13C, D, and G). Bundle sheath cells were also attacked (Figs. 4.13E and H) and conidiophores were present in stomatal openings by 96 HPI (Fig. 4.13F).

Spores also germinated after two hours in the resistant host, *C. rotundus* (Fig. 4.14A). Germ tubes were typically bipolar and developed branches (Fig. 4.14B). Infection structures were found beginning 12 HPI mainly over epidermal wall junctions but stomata and trichomes were occasionally penetrated. Appressoria (Fig. 4.14C) were either terminal or intercalary. Infection cushions were also observed, formed by the aggregation of several appressoria (Fig. 4.14D), or through the branching and coiling of germ tube tips (Fig. 4.14E). Yellowish haloes were produced in the area beneath the infection structures (Fig. 4.15A). Thin, penetration pegs emerged from the infection

structure (Fig. 4.15A) and primary infection hyphae developed after penetration (Fig. 4.15B).

Twenty-four hours after inoculation, primary and secondary infection hyphae were present in infected cells (Figs. 4.16A and B). Invasion of neighboring cells was slow and restricted to one or a few neighboring cells (Fig. 4.16C). Fungal colonies were small and limited to 20 to 50 epidermal cells (Fig. 4.16D) after 48 hours. The invaded epidermal and mesophyll cells turned brown and collapsed.

An intense yellowing reaction was observed in infected cells (Figs. 4.16E to G) and pinhead lesions (Fig. 4.16H). By 168 HPI, signs of degeneration in the fungal hyphae were evident (Figs. 4.17A to F). Plasmolysis and granulation of hyphal segments was observed (Figs. 4.17A to E). The fungal protoplasm was disorganized and stained unevenly with lactofuchsin (Figs. 4.17C to F).

4.5. Discussion

This is the first report on the histopathology of *C. tuberculata* and *C. oryzae* on sedge hosts. *Cyperus difformis, C. iria,* and *F. miliacea* were susceptible to two isolates of *C. tuberculata* and to *C. oryzae* while *C. rotundus* was resistant to *C. tuberculata* and *C. oryzae*. The infection process for the two *C. tuberculata* isolates were identical so they will be considered as one. The pathological histology of *C. tuberculata* and *C. oryzae* on the four weed hosts were essentially comparable and closely resemble the infection process of *Cochliobolus sativus* in wheat and barley (Huang and Tinline, 1976). The first requirement for interaction between a fungus and its plant host is the spore's arrival on the leaf surface. The third and fourth leaves of *C. difformis, C. iria, F. miliacea*, and *C. rotundus* differ in size, shape, waxiness, and leaf angle. In *C. difformis*

and *F. miliacea*, the spores tended to accumulate at the base of the plant near the leaf sheaths. This could be partially due to the leaf angle, which is less than 45°. *F. miliacea* leaves are also waxy, thin, and almost cylindrical in shape which facilitate run-off of the applied spore suspension. Spores are often ultimately deposited in locations on leaves and stems where run-off water collects, such as along veins, or in axils of leaves, buds, and flowers (Johnstone, 1931).

The hydrophobicity (or wettability) of both spores and leaves also affect spore retention and water run-off (Allen et al., 1991). In leaves with low wettability such as *F. miliacea*, water droplets maintain a high contact angle and are easily removed if the leaf is shaken or inclined. Spores are thus deposited at the leaf bases where lesions ultimately develop, causing girdling and plant collapse.

In *C. iria* and *C. rotundus*, the spores tended to be deposited more evenly due to longer and slightly wider leaves. In addition, leaf angle with respect to the vertical is also greater compared to *C. difformis* or *F. miliacea*. Increased deposition of conidia as a function of greater leaf angles has been reported for *Eucalyptus bicostata* sprayed with *Phaeoseptoria eucalypti* (Heather, 1967).

There is strong evidence that most fungal plant pathogens germinate equally well on host and non-host surfaces (Heath, 1974; Bird and Ride, 1981; Murray and Ye, 1986; Kumar and Sridhar, 1987). These results confirm this observation since the onset of germination or percent germination did not differ in susceptible and resistant hosts. However, two distinct differences were found between *C. tuberculata* and *C. oryzae*; 1) conidial germination in *C. tuberculata* is typically polar, while in *C. oryzae*, it is bipolar, and 2) spores of *C. oryzae* germinated two hours earlier than *C. tuberculata*. Sivanesan

(1987) reported lateral germination from the middle cells of *C. tuberculata* but this was not observed in any of the isolates tested here.

In contrast to the directional growth of rust germ tubes in graminaceous hosts (Wynn and Staples, 1981), germ tube growth in both *C. tuberculata* and *C. oryzae* did not have an apparent directionality since they grew both perpendicular and parallel to the long axis of epidermal cells and developed branches. Branching of germ tubes has also been reported for *Cochliobolus* (Huang and Tinline, 1976) and *Pyrenophora* (Hargreaves, 1982).

C. tuberculata and *C. oryzae* formed simple appressoria as well as infection cushions on the four weed hosts. Appressoria observed in the present study varied greatly in size and shape but they were always delimited by a septum and were stained darkly with lactofuchsin. They formed at the tips of the main germ tube or more frequently, at germ tube branches (terminal appressoria). Occasionally, appressoria were formed directly on the side of a germ tube (intercalary appressoria).

Emmett and Parberry (1975) reported that Broyles (1955) found a negative correlation between appressorium formation and degree of branching. Microscopic observations performed here suggest that branching might enhance appressorial formation since several appressoria were formed in branched germ tubes (Fig. 10B). Occasionally, some single germ tubes formed simple appressoria one after the other (Fig. 9B), but a single appressorium on an unbranched germ tube was extremely rare. This trait could have significance since a potential biocontrol agent will have more success if it is capable of forming several appressoria from one spore.

The manner of infection cushion formation in the isolates is similar to those reported for *Cochliobolus sativus* (Huang and Tinline, 1976), where several simple appressoria appear to be attracted to each other (Fig. 4.10C) and aggregate (Fig. 4.2G). However, infection cushions resulting from (1) branching and swelling of branches from one germ tube/hyphal tip or (2) coiling and aggregation of branches of several adjacent germ tubes/hyphae, were also found. These results typify infection cushions of *Rhizoctonia solani* (Dodman and Flentje, 1970). Variation in the complexity of infection structures seems to be related to resistance to the pathogen since more infection cushions were formed in *C. rotundus*. The initiation of appressorial formation in susceptible and resistant hosts did not differ significantly.

Infection structures were preferentially formed at the junction of epidermal cells. Stomata and trichomes were rarely penetrated. Preece et al. (1967) were among the first to report that germinating *Erysiphe polygoni* and *Peronospora parasitica* conidia formed appressoria in the junction areas between the anticlinal walls of adjoining epidermal cells. Since then, numerous investigators have reported the same in many foliar pathogens including *Cochliobolus* spp. (Hau and Rush, 1979), *Helminthosporium* spp. (Hilu and Hooker, 1964), *Botrytis* spp. (Clark and Lorbeer, 1976), and *Colletotrichum* spp. (Anderson and Walker, 1962; Lapp and Skoropad, 1978).

Other leaf surface sites have also been reported as penetration sites. *Colletotrichum lindemuthianum* appressoria commonly develop over veins (Preece and Dickinson, 1971) of bean leaves whereas stomata are the favored infection sites of the rust fungi (Wynn, 1976). Spores of *C. gloeosporioides* f. sp. *aschynomene* produced appressoria and penetrated trichome bases directly (TeBeest et al., 1978). The preferred

penetration site was the junction of epidermal cells and penetration of stomata was infrequent, similar to the reports on *Botrytis cinerea* (Clark and Lorbeer, 1976). Since penetration of epidermal cells or stomata by hyphal tips was not observed, it is likely that infection structures such as appressoria and infection cushions are essential for invasion.

Penetration of host cells by plant pathogenic fungi has been attributed to mechanical forces (Aist and Williams, 1971) and to enzymatic degradation of cell walls (Edwards and Allen, 1970; McKeen, 1974; McKeen et al., 1969; Sargent et al., 1973). In this case, the presence of thin, narrow penetration pegs (Figs.4.11A and 4.14A) suggests that mechanical force is involved. However, the presence of a well defined pore (Fig. 4.11B) and the yellowing reaction (Figs. 4.2B and 4.8C) in and around infection structures suggest that enzymatic processes are also at work. Penetration occurred in all interactions but it was not determined whether there were any differences in penetration frequency.

Following successful penetration, both *C. tuberculata* and *C. oryzae* produced primary infection hyphae that were much thicker than the penetration peg, an event that has also been reported for *Colletotrichum trifolii* on alfalfa (Porto et al., 1988). Primary infection hyphae were constricted at the point where they passed through the cell wall but widened immediately thereafter. Secondary infection hyphae that branched out from primary infection hyphae invaded neighboring cells.

The rapid colonization of epidermal and mesophyll cells was the best indicator of susceptibility. Both *C. tuberculata* and *C. oryzae* were able to invade highly susceptible host tissues from the adaxial to the abaxial leaf surface within 72 to 96 HPI. There was also a high concentration of hyphal mass just beneath the stomata prior to the emergence

of conidiophores. Epidermal and mesophyll cells were extremely vulnerable to attack but cells in the vascular bundle were not invaded. It might be that the lignified cell walls in the vascular bundle are an effective barrier to invasion. Profuse sporulation was observed in the necrotic areas of susceptible weeds five to seven days after inoculation. Conidiophores emerged from the stomata only (Figs. 4.4F; 4.6H; 4.12B, and F) similar to *Drechslera, Cercospora, Alternaria*, and *Pyricularia* species (Subramanian, 1983).

Pre-penetration phenomena of *C. tuberculata* and *C. oryzae* on *C. rotundus* did not differ from those observed on susceptible weeds. The onset of spore germination was the same and percentage germination approached 90% after 24 hours. However, germ tubes tended to branch less and some were observed to coil repeatedly without forming infection structures. This could be interpreted as a "tropic failure" (Wynn, 1981), where germ tubes seem unable to recognize the surface characteristics of a host. Fungi are remarkably sensitive to minor differences in topography (Wynn, 1981). Ridges and grooves on leaf surfaces (Staples and Macko, 1984; Staples et al., 1985a, b; Hoch and Staples, 1991) as well as the type and pattern of wax crystals (Lewis and Day, 1972) act as a thigmotropic stimulus that orient germ tube growth and affect infection structure formation of rust and powdery mildew fungi.

More infection cushions were formed on *C. rotundus* than on the susceptible weeds by *C. tuberculata* and *C. oryzae*. The combined action of several aggregated appressoria or hyphal tips, as well as the larger surface area involved during penetration, might have been required to infect *C. rotundus*. Dodman et al. (1968) reported that direct penetration of hosts by *Rhizoctonia solani* was always associated with the formation of complicated dome-shaped infection cushions while stomatal penetration was often

associated with less complicated lobate appressoria. They suggest that stomatal penetration requires less effort on the part of the fungus and thus, only necessitates the formation of simpler infection structures. These findings were confirmed by El-Samra et al. (1981) who found that the susceptible cotton cultivar supported the development of simple, hypha-like infection cushions and that progressively resistant cotton cultivars enhanced the formation of more complex types of infection cushions.

Resistance of *C. rotundus* to the *Curvularia* isolates was expressed as a delay in infection and slow lesion development. Although the time of initiation of infection structure formation in *C. rotundus* was generally similar to the other weed hosts, the peak time occurred 24 to 48 h later than in the susceptible hosts. The longer incubation period of the two *Curvularia* species could be the reason for the delay in the first appearance of lesions in *C. rotundus*. Results of the present study confirm this as ramification of infection hyphae of the two *Curvularia* species in *C. rotundus* was slow and the resulting fungal colonies were small. Sporulation did not occur in the pinhead lesions and although conidiophore emergence was observed at the tip of leaf #3 (one plant) in one trial, conidiophore development was sparse and did not result in the formation of mature conidia.

One week after inoculation, hyphae of *C. tuberculata* and *C. oryzae* began to exhibit signs of degeneration such as granulation, plasmolysis, vacuolation, and disorganization of the cell membrane. These signs suggest that plant reactions are likely involved in the delimitation of the pathogen within *C. rotundus* leaf tissues.

The resistance of *C. rotundus* to *C. tuberculata* and *C. oryzae* appears to be due mainly to plant reactions since most of the responses observed were initiated after

successful penetration. However, tropic mistakes such as those described by Wynn (1981) also play an important role in resistance. It is likely that the combined effects of tropic mistakes and plant reactions account for the failure of *C. tuberculata* and *C. oryzae* to cause high levels of disease and plant death in *C. rotundus*.

4.6. Literature cited

Aist, J.R. and P.H. Williams. 1971. The cytology and kinetics of cabbage root hair penetration by *Plasmodiophora brassicae*. Can. J. Bot. 49:2023-2034.

Allen, E.A., H.C. Hoch, J.R. Steadman, and R.J. Stavely. 1991. Influence of leaf surface features on spore deposition and the epiphytic growth of phytopathogenic fungi. Pages 87-110 *in* Microbial Ecology of Leaves. (J.H. Andrews and S.S. Hirano, (Eds.). Springer-Verlag, New York, NY, USA.

Anderson, J.L. and J.C. Walker. 1962. Histology of watermelon anthracnose. Phytopathology 52:650-653.

Baltazar, A. 1995. Weed management in wet-seeded rice in Asia. Pages 656-661 *in* Proc. 15th Asian Pacific Weed Science Society Conference. Vol. IB. July 24-28, 1995, Tsukuba, Japan.

Bird, P.M. and J.P. Ride. 1981. The resistance of wheat to *Septoria nodorum*: fungal development in relation to host lignification. Physiol. Plant Pathol. 19:289-299.

Broyles, J.W. 1955. Comparative studies of races and biotypes of *Puccinia graminis* with special reference to morphology of urediospore germination, chemical composition and factors affecting survival. Ph. D. Thesis. Univ. of Minnesota, Minneapolis. 229 pp.

Bugnicourt, F. 1950. Les espèces du genre *Curvularia* isolées des semences de riz. Rev. gén. Bot. 57:65-77.

Clark, C.A. and J.W. Lorbeer. 1976. Comparative histopathology of *Botrytis squamosa* and *B. cinerea* on onion leaves. Phytopathology 66:1279-1289.

De Datta, S.K. 1995. Weed management perspectives for sustainable agriculture in rice-based systems. Pages 17-27 *in* Proc. 15th Asian-Pacific Weed Science Society Conference. Vol. IA. July 24-28, 1995. Tsukuba, Japan.

Dhingra, O.D. and J.B. Sinclair, 1985. Basic Plant Pathological Methods. CRC Press, Inc. Boca Raton, FL, USA. 355 pp.

Dodman, R.L. and N.T. Flentje. 1970. The mechanism and physiology of plant penetration by *Rhizoctonia solani*. Pages 149-160 *in Rhizoctonia solani*: Biology and Pathology. J.R. Parmeter, Jr. (Ed.). University of California Press. Berkeley, CA, USA.

Dodman, R.L., K.R. Barker and J.C. Walker. 1968. A detailed study of the different modes of penetration by *Rhizoctonia solani*. Phytopathology 58:1271-1276.

Edwards, H.H. and P.J. Allen. 1970. A fine-structure study of the primary infection process during infection of barley by *Erysiphe graminis* f. sp. *hordei*. Phytopathology 60:1504-1509.

Ellis, M.B. 1966. Dematiaceous Hyphomycetes. VII. *Curvularia, Brachysporium*, etc. Mycol. Pap. 106:1-57.

Ellis, M.B. 1971. Dematiaceous Hyphomycetes. Commonwealth Mycological Institute. Kew, Surrey, England: 608 pp.

El-Samra, I.A., Y.M. El-Faham, and A.M. Kamara. 1981. Selective induction of infection cushions by *Rhizoctonia solani* in relation to host responses. Phytopath. Z. 102:122-126.

Emmett, R.W. and D.G. Parberry, 1975. Appressoria. Annu. Rev. Phytopathol. 13:147-167.

Ghosh, R.N. and S. Gupta. 1980. Two new host records form India. Indian Phytopath. 33:498-500.

Hargreaves, J.A. 1982. The nature of resistance of oat leaves to infection by *Pyrenophora teres*. Physiol. Plant Path. 20:165-171.

Hau, F.C. and M.C. Rush. 1979. Leaf surface interactions between *Cochliobolus miyabeanus* and susceptible and resistant rice cultivars. Phytopathology 69:527.

Heath, M.C. 1974. Light and electron microscope studies of the interactions of host and non-host plants with cowpea rust - *Uromyces phaseoli* var. *vignae*. Physiol. Plant Pathol. 4:403-414.

Heather, W.A. 1967. Susceptibility of juvenile leaves of *Eucalyptus bicostata* Maiden *et al.* to infection by *Phaeoseptoria eucalypti* (Hansf.) Walker. Aust. J. Biol. Sci. 20:769-775.

Hilu, H.M. and A.L. Hooker. 1964. Host-pathogen relationship of *Helminthosporium turcicum* in resistant and susceptible corn seedlings. Phytopathology 54:570-575.

Hoch, H.C. and R.C. Staples. 1991. Signaling for infection structure formation in fungi. Pages 26-46 *in* The Fungal Spore and Disease Initiation in Plants and Animals. G.T. Cole and H.C. Hoch (Eds.). Plenum Press. New York, NY, USA.

Huang, H.C. and R.D. Tinline. 1976. Histology of *Cochliobolus sativus* infection in subcrown internodes of wheat and barley. Can. J. Bot. 54:1344-1354.

International Rice Research Institute. 1987. Annual Report. International Rice Research Institute, Manila, Philippines. p. 281.

Jain, B.L. 1962. Two new species of *Curvularia.* Trans. Brit. Mycol. Soc. 45:539-544.

Janiya, J. D. and K. Moody. 1984. Use of *Azolla* to suppress weeds in transplanted rice. Tropical Pest Management 30:1-6.

Johansen, D.A. 1940. Plant Microtechnique. McGraw -Hill Book Co., New York. NY, USA. 523 pp.

Johnstone, K.H. 1931. Observations on the varietal resistance of the apple to scab (*Venturia inequalis* Aderh.) with special reference to its physiological aspects. J. Pom. 9:30-52.

Kapoor, I.J. and R.N. Tandon. 1971. Occurrence of *Curvularia tuberculata* Jain on stored fruits of *Psidium guajava* L. Sydowia 24:201-202.

Lambat, A.K. and Ram, A. 1969. Seed-borne infection of *Curvularia* causing a new blight disease of Jowar. Indian Phytopath. 22:282-284.

Lapp, M.S. and W.P. Skoropad. 1978. Location of appressoria of *Colletotrichum graminicola* on natural and artificial barley leaf surfaces. Trans. Br. Mycol. Soc. 70:225-228.

Lele, V.C., J. Singh, S.N. Rai, and J. Kandhari. 1981. Occurrence of a new blight disease of mango caused by *Curvularia.* Curr. Sci. 50:464-465.

Lele, V.C., S.P. Raychaudhuri, R.B. Bhalla, and A. Ram. 1968. *Curvularia tuberculata*, a new fungus causing die-back disease of citrus in India. Indian Phytopath. 21:66-72.

Lewis, B.G. and J.R. Day. 1972. Behavior of urediospore germ tubes of *Puccinia graminis tritici* in relation to the fine structure of wheat leaf surfaces. Trans. Br. Mycol. Soc. 58:139-145.

McKeen, W.E. 1974. Mode of penetration of epidermal cell walls of *Vicia faba* by *Botrytis cinerea*. Phytopathology 64:461-467.

McKeen, W.E., R. Smith, and P.K. Bhattacharya. 1969. Alterations of the host wall surrounding the infection peg of powdery mildew fungi. Can. J. Bot. 47:701-706.

Misra, A.P., O. Prakash, B. Mishra and K.K. Dutta. 1973. A new leaf spot disease of motha (*Cyperus rotundus* L.) caused by *Curvularia tuberculata*. Indian Phytopath. 26:165-167.

Moody, K. 1990. Postplanting weed control in direct-seeded rice. Paper presented at a Rice Symposium, 25-27 September 1990, MARDI, Penang, Malaysia.

Moody, K. 1992. Weed management in wet-seeded rice in tropical Asia. Pages 1-20 *in* Proc. International Symposium on Biological Control and Integrated Management of Paddy and Aquatic Weeds in Asia. National Agriculture Research Center.

Mukhopadhyay, S.K. 1983. Weed control technology in rainfed wetland rice. Pages 109-118 *in* Proc. 1981 Weed Control in Rice Conference. International Rice Research Institute. Manila, Philippines.

Murray, T.D. and H. Ye. 1986. Papilla formation and hypersensitivity at penetration sites and resistance to *Pseudocercosporella herpotrichoides* in winter wheat. Phytopathology 76:737-744.

Porto, M.D., C.R. Grau, G.A. de Zoeten, and G. Gaard. 1988. Histopathology of *Colletotrichum trifolii* on alfalfa. Phytopathology 78:345-349.

Preece, T.F. and C.H. Dickinson. 1971. Ecology of Leaf Surface Microorganisms. Academic Press. New York, NY, USA. 640 pp.

Preece, T.F., G. Barnes, and J.M. Bayley. 1967. Junctions between epidermal cells as sites of appressorium formation by plant pathogenic fungi. Plant Pathol. 16:117-118.

Rama Rao, P. 1964. *Curvularia tuberculata* from dune soil. Curr. Sci. 33:121.

Sargent, J.A., I.C. Tommerup, and D.S. Ingram. 1973. The penetration of a susceptible lettuce variety by the downy mildew fungus *Bremia lactucae* Regel. Physiol. Plant Pathol. 3:231-239.

Seshadri, K., P. Padmanaban, and K.C. Alexander. 1980. A new leaf blight disease of sugarcane. Indian Phytopath. 33:325-326.

Sivanesan, A. 1987. Graminicolous species of *Bipolaris, Curvularia, Drechslera* and *Exserohilum* and their teleomorphs. Mycol. Pap. 158:1-261.

Staples, R.C. and V. Macko. 1984. Germination of urediospores and differentiation of infection structures. Pages 255-289 *in* The Cereal Rusts. Vol. I. W.R. Bushnell and A.P. Roelfs (Eds.). Academic Press. New York, NY, USA.

Staples, R.C., H.C. Hoch, and L. Epstein. 1985a. The development of infection structures by the rusts and other fungi. Microbiological Sciences 2:193-198.

Staples, R.C., H.C. Hoch, L. Epstein, L Laccetti and S. Hassouna. 1985b. Recognition of host morphology by rust fungi: responses and mechanisms. Can. J. Plant Pathol. 7:314:322.

TeBeest, D.O., G.E. Templeton, and R.J. Smith, Jr. 1978. Histopathology of *Colletotrichum gloeosporioides* f. sp. *aeschynomene* on northern jointvetch. Phytopathology 68:1271-1275.

Van Dyke, C.G. 1989. Factors in the infection process of fungal pathogens for biological control of weeds. Pages 559-563 *in* Proc. VII International Symposium on Biological Control of Weeds. Delfosse, E.S. (Ed.). Ist. Sper. Patol. Veg. (MAF). Rome, Italy.

Watson, A.K. 1992. Current status of bioherbicide development and prospects for rice in Asia. Pages 367-379 *in* Proc. International Symposium on Biological Control and Integrated Management of Paddy and Aquatic Weeds in Asia. 20-23 Oct. 1992. National Agriculture Center, Tsukuba, Japan.

Webster, R.K. and P.S. Gunnell (Eds.). 1992. Compendium of Rice Diseases. The American Phytopathological Society. APS Press. St. Paul, MN. 62 pp.

Wetzstein, H.Y. and S.C. Phatak. 1987. Scanning electron microscopy of the uredinial stage of *Puccinia canaliculata* on yellow nutsedge, *Cyperus esculentus* (Cyperaceae). Amer. J. Bot. 74:100-106.

Wynn, W.K. 1976. Appressorium formation over stomates by the bean rust fungus: response to a surface contact stimulus. Phytopathology 66:136-146.

Wynn, W.K. and R.C. Staples. 1981. Tropisms of fungi in host recognition. Pages 45-69 *in* Plant Disease Control: Resistance and Susceptibility. R.C. Staples and G.H. Toenniessen (Eds.). John Wiley & Sons. New York, NY, USA.

Yeh, W.H. and J.M. Bonman. 1986. Assessment of partial resistance to *Pyricularia oryzae* in six rice cultivars. Plant Pathol. 35:319-323.

Figure 4.1. Spore deposition in *Cyperus difformis, Cyperus iria, Fimbristylis miliacea,* and *Cyperus rotundus*. **A.** Spores deposited at the upper leaf sheath in *C. difformis*. **B.** Spores were more evenly distributed in leaf blades of *C. iria*. **C.** Clumps of spores caught at the leaf sheath of *F. miliacea*. **D.** Spores deposited on leaf tips of *F. miliacea*.

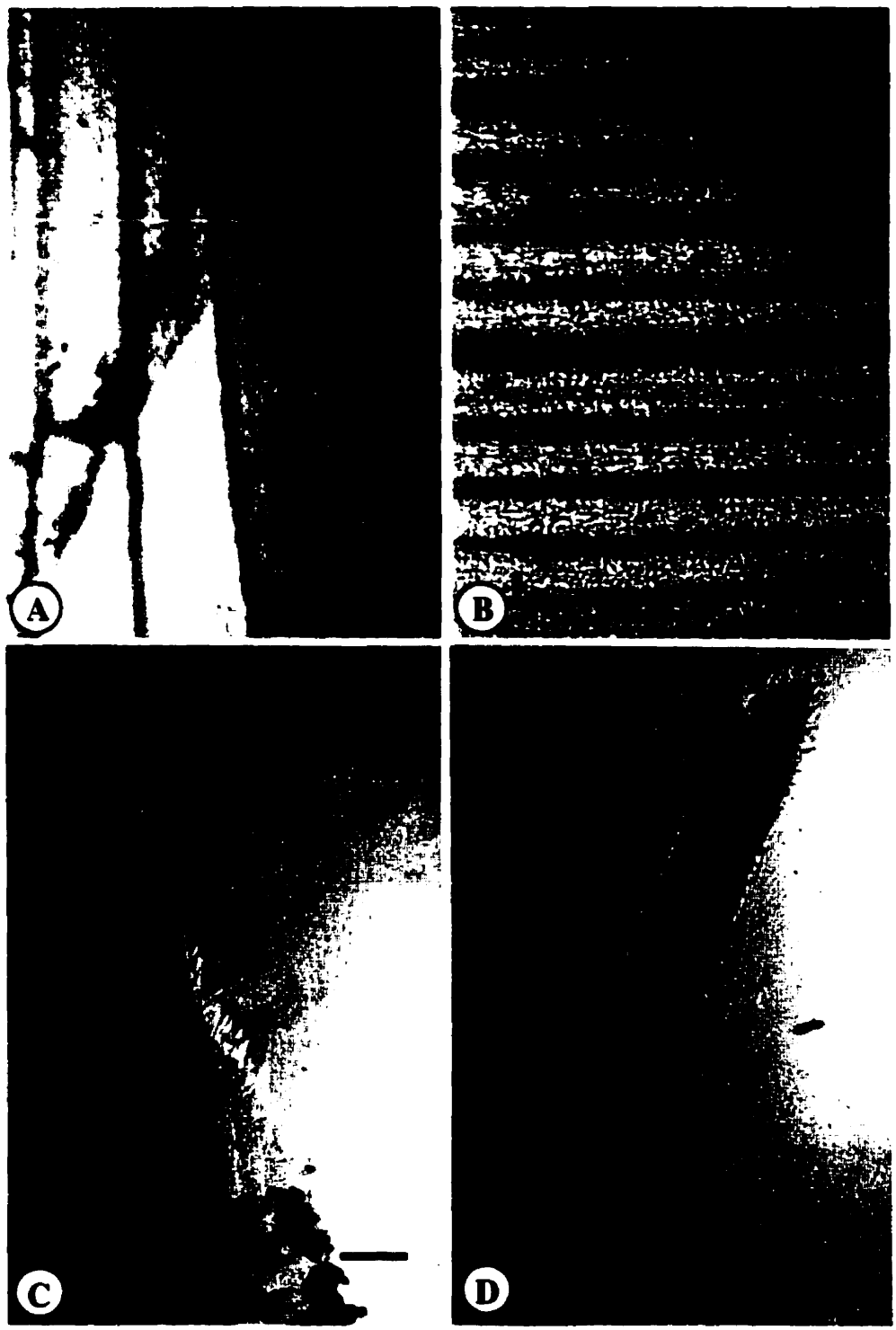

A
B
C
D

Figure 4.1. continued. **E.** Spores on the leaf blade of *C. rotundus*. **F.** Spores trapped by trichomes in *F. miliacea*. Bar = 200 μm except B and F.

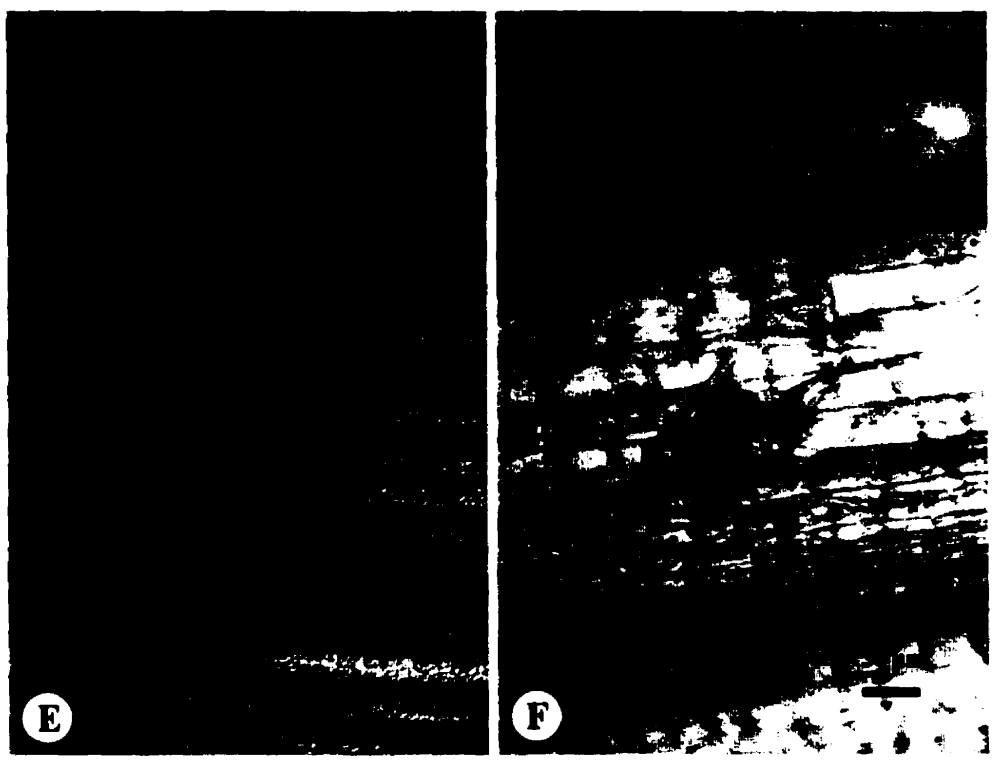

E
F

Figure 4.2. Spore germination and infection structure formation of *Curvularia tuberculata* (93-020) in *Cyperus difformis* (A) and *Cyperus iria* (B).

A. Spore germination at four hours post inoculation (HPI). Germ tubes (GT) are typically polar and emerge from either the apical or hilar end of the spore. GT stained with cotton blue. **B.** By eight HPI, germ tubes are branched and have initiated formation of appressoria (A). C, conidium. Bar = 10 μm.

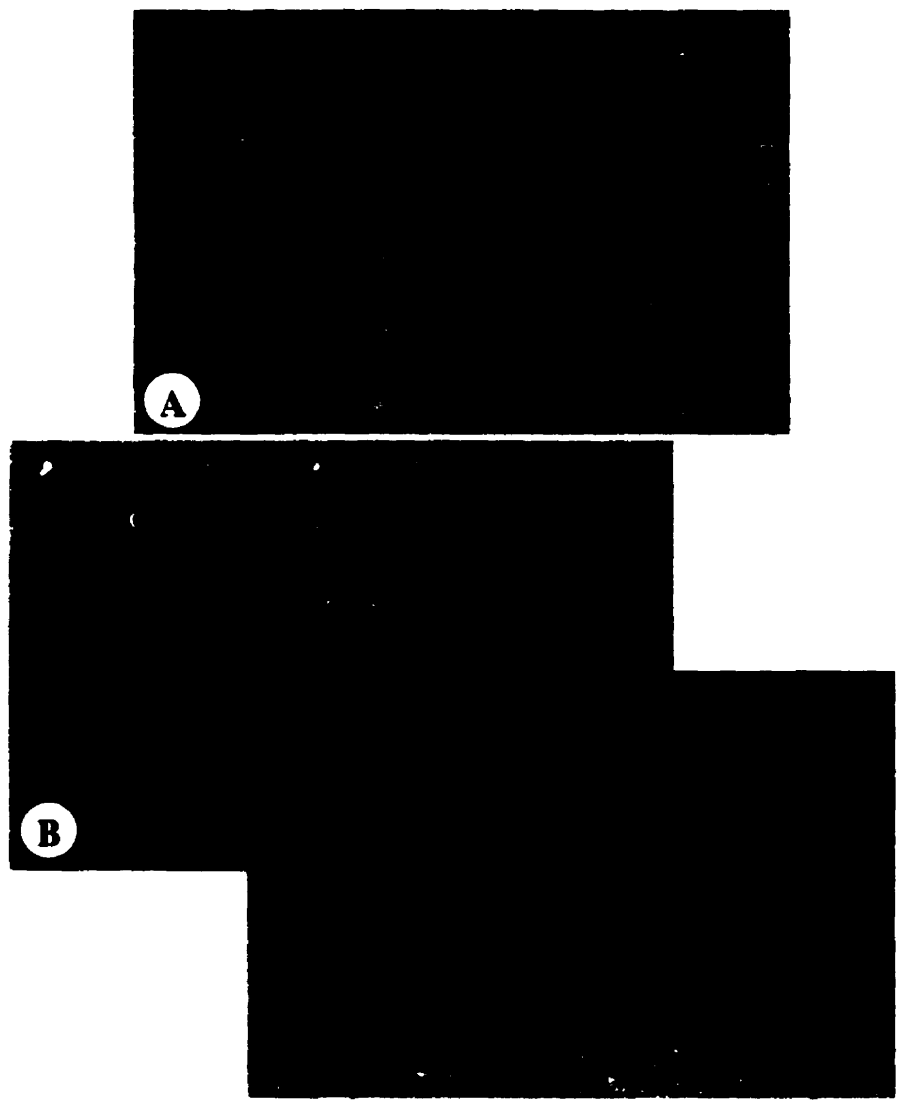

A
B

Figure 4.3. Infection structures of *Curvularia tuberculata* (93-020) on *Cyperus difformis* (A, C, D, and E) and *Cyperus iria* (B, F, and G), eight to 12 hours post inoculation (HPI). **A.** Club-shaped, terminal appressorium (A) on epidermal cell wall. **B.** Yellowing reaction (YR) around a terminal appressorium (A) arising from a germ tube (GT) branch at 12 HPI. **C.** Two simple appressoria (A) at the tips of adjacent germ tube (GT) branches. **D.** Intercalary appressorium (A) formed over a stomatal aperture (SA). **E.** Infection cushion (IC) over stomatal aperture (SA) formed by branching of a single germ tube tip. **F.** Infection cushion (IC) on epidermal cell wall formed by coiling of several germ tubes (GT). **G.** Several simple appressoria (A) seem to have aggregated to form an infection cushion (IC). Bar = 10 μm.

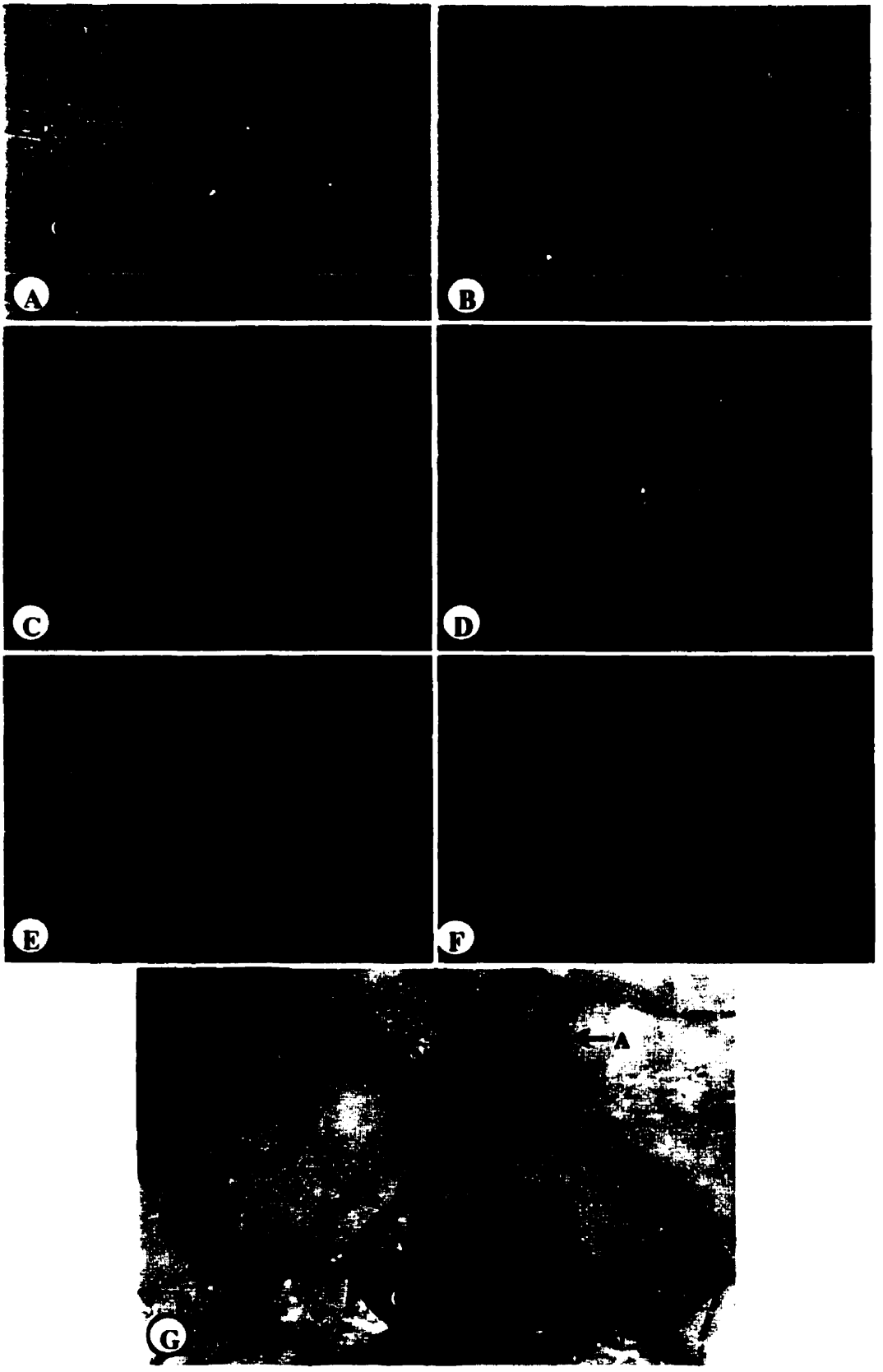

A
B
C
D
E
F
A
G

Figure 4.4. Infection of *Cyperus difformis* (C and F) by *Curvularia tuberculata* (93-020; C, D, and E) and *Cyperus iria* (A, B, D, and E) by *Curvularia tuberculata* (93-022; A, B, and F), 20 to 48 hours post inoculation (HPI). **A.** Primary infection hyphae (PIH) develop after successful penetration of an epidermal cell by an appressorium (A). Secondary infection hyphae (SIH) branch from the PIH and grow intercellularly. **B.** Primary and secondary infection hyphae (PIH, SIH) have ramified within adjacent epidermal cells. Note yellowing and separation of anticlinal wall near the appressorium (A). **C.** Rupture and swelling of cell wall during intercellular growth of an infection hypha (IH). **D.** Infection of neighboring cells by direct penetration of adjoining cell walls by infection hyphae (IH). **E.** Cleared, whole necrotic leaf showing infection hyphae (black arrowheads) among mesophyll cells (MC). **F.** Infection hyphae (IH) in leaf sheath cells. Bar = 10 μm.

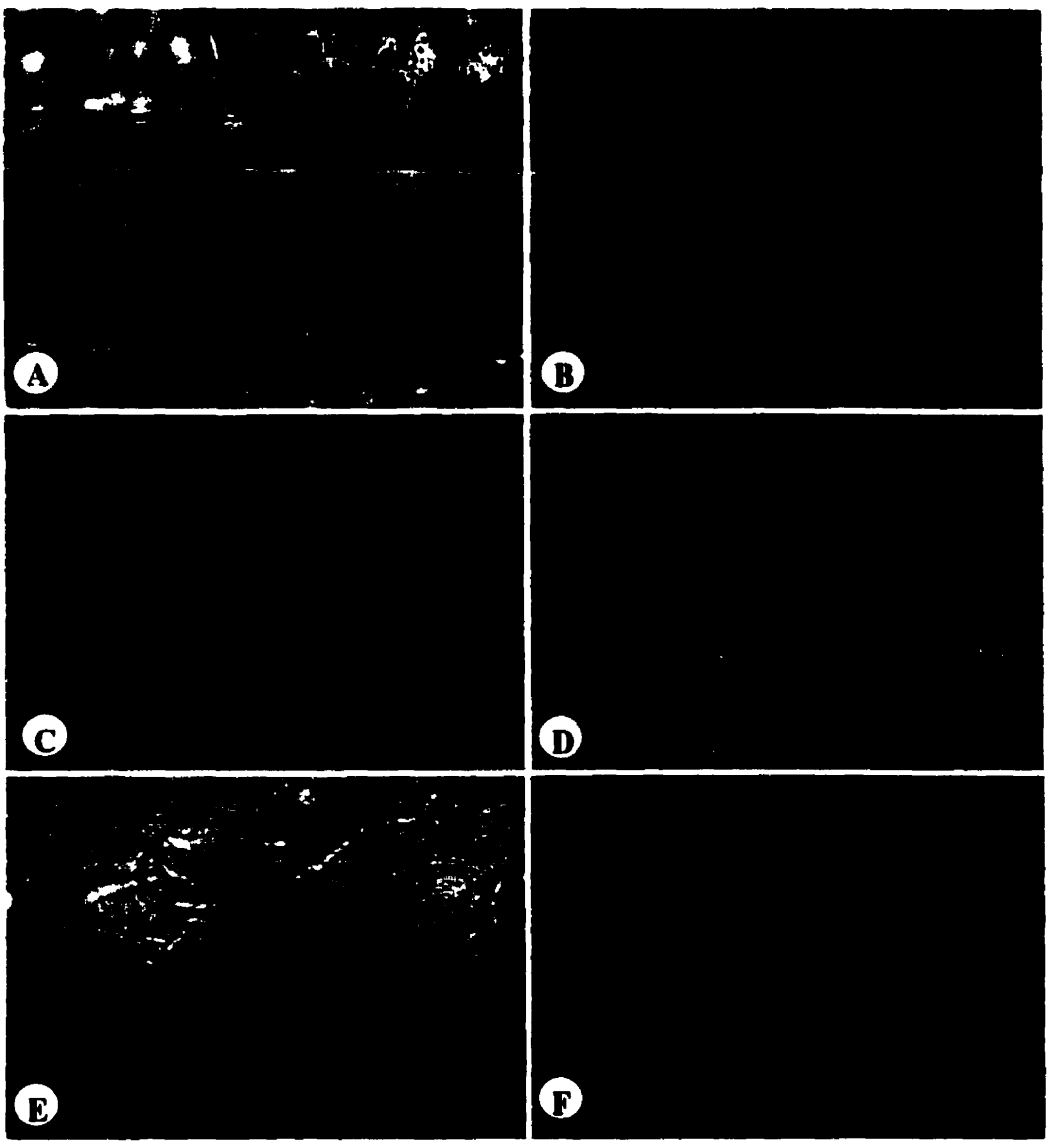

A
B
C
D
E
F

Figure 4.5. Colonization of *Cyperus difformis* (A, B, E, and F) and *Cyperus iria* (C and D) by *Curvularia tuberculata* (93-020), 48 to 96 hours post inoculation (HPI). **A.** Transverse section of a blighted leaf 48 HPI. **B.** High magnification view of a portion of the leaf in A showing the infection hyphae (red) that have ramified throughout the epidermal and mesophyll cells. **C.** Intercellular hyphae are present in infected bundle sheath cells (BS). **D.** Later stage of infection showing completely colonized bundle sheath cells around a vascular bundle (VB). Conidiophores (CP) have started to emerge from the necrotic leaf. **E.** Cleared whole necrotic leaf showing conidiophores emerging from stomatal openings (SP). **F.** High magnification view of emerging conidiophores (CP). Bar = 10 μm except A and E.

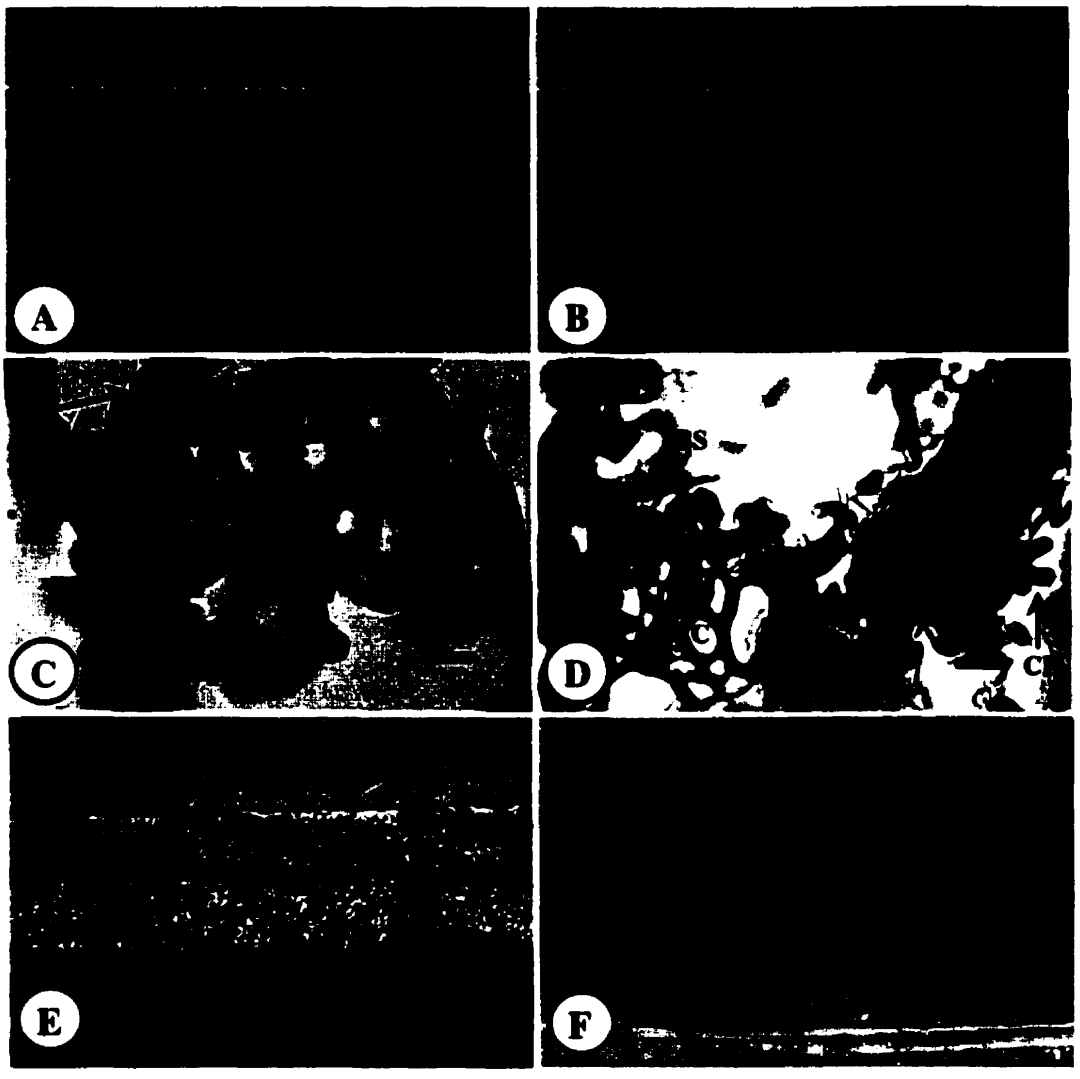

A
B
C
S
C
D
C
E
F

Figure 4.6. Spore germination and infection structure formation of *Curvularia tuberculata* (93-020, B and C; 93-022; A, D, E, and F) in *Fimbristylis miliacea*, four to 24 hours post inoculation (HPI). **A.** Germinated spores at 12 HPI. **B.** Polar germination of conidium (C) showing bifurcated germ tube (GT). C. Intercalary appressorium (A) over an epidermal cell. **D.** Simple, elongated appressorium (A) formed near a stomata (ST). **E.** Simple appressorium (A) on an epidermal cell. GT, germ tube; ST, stomata. **F.** Infection cushion (IC) over an epidermal cell. GT, germ tube. Bar = 10 μm.

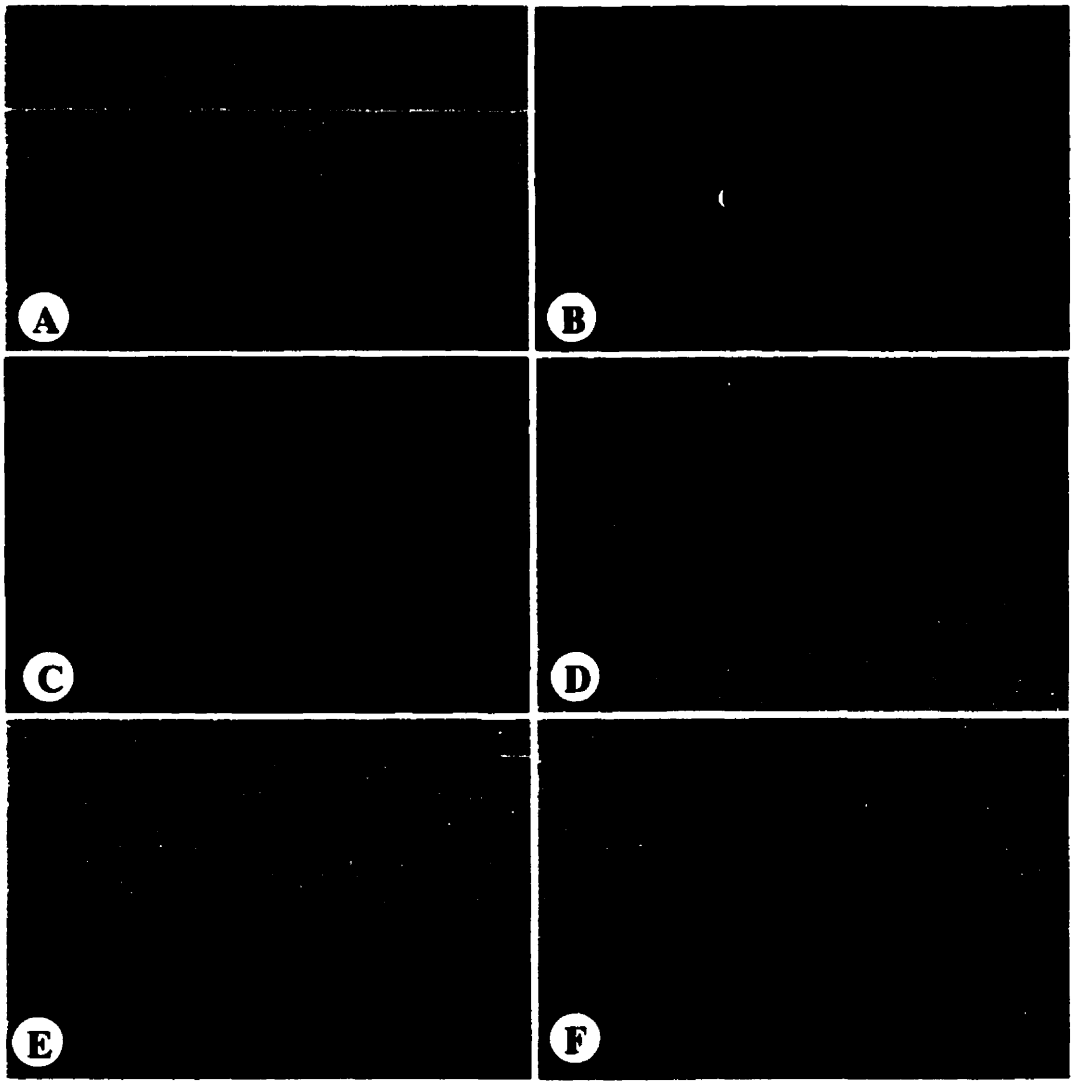

A
B
C
D
E
F

Figure 4.7. Infection and colonization of *Fimbristylis miliacea* by *Curvularia tuberculata* (93-020; C, D, F, G, and H and 93-022; A, B, and E), 24 to 144 hours post inoculation (HPI). **A.** Primary infection hyphae (PIH) within an epidermal cell, 48 HPI. **B.** Intracellular infection hyphae (IH) in leaf sheath cells, 36 HPI. **C.** Intracellular (IH1) and intercellular (IH2) infection hyphae in a necrotic area of the leaf. **D.** Infection hyphae (IH) in leaf sheath cells at 48 HPI. **E.** Infection hyphae (IH) inside guard cells (GC) of stomata. **F.** Conidiophores (CP) emerging from the stomata (ST). Infection hyphae (IH) are visible beneath the stomata and among collapsed mesophyll cells (MC). **G.** Portion of necrotic leaf tip showing emerging conidiophores (CP). **H.** High magnification view of conidiophores (CP) with young conidium (YC). Bar = 10 μm except A, B, D, and G.

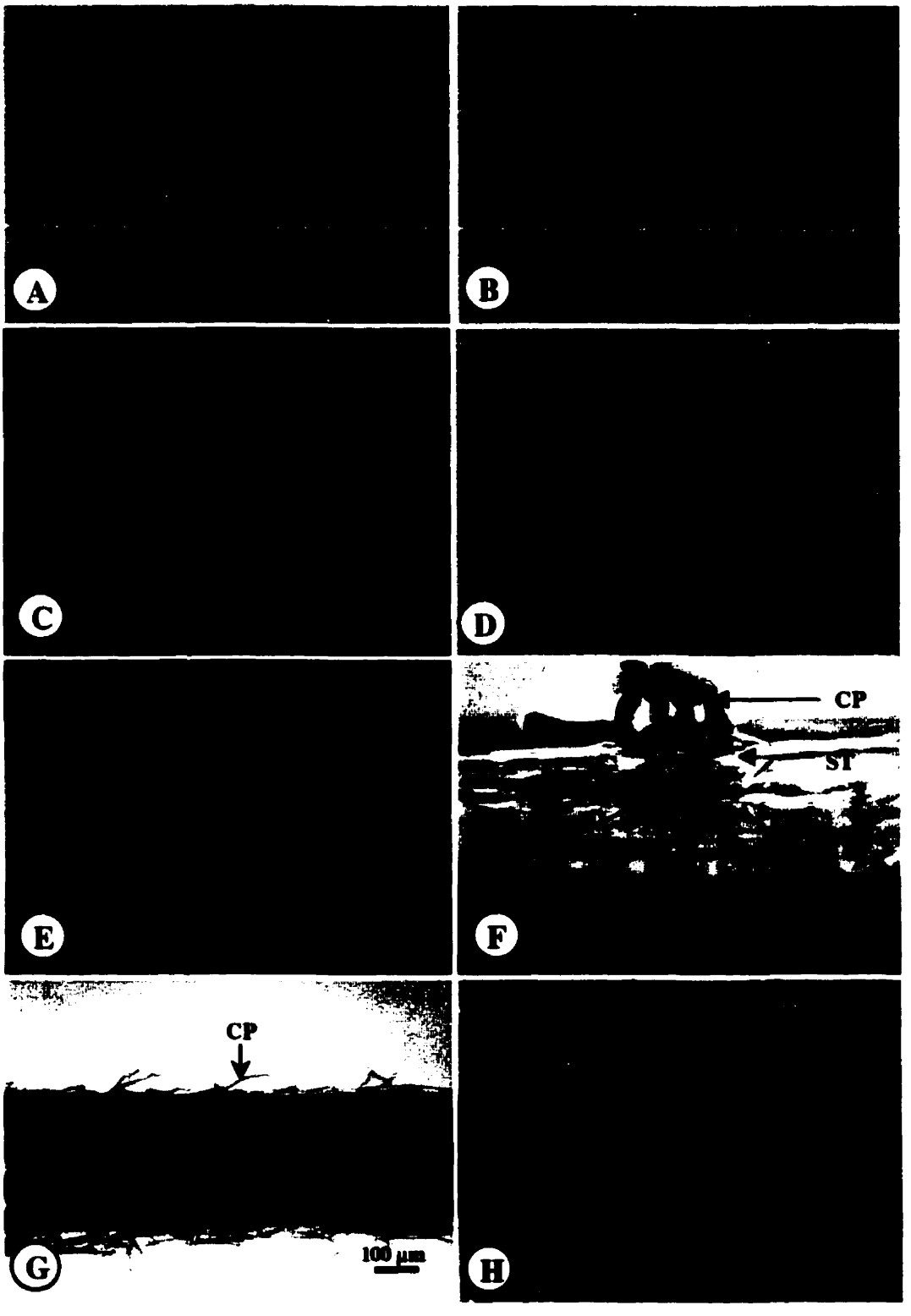

A
B
C
D
E
CP
ST
F
CP
G
100 μm
H

Figure 4.8. Spore germination and germ tube growth *Curvularia tuberculata* (93-020, B and F; 93-022, A, C, D, and E) on *Cyperus rotundus*, four to 36 hours post inoculation (HPI). **A.** Germinated conidium (C) showing short germ tube (GT) at four HPI. **B.** Germ tubes were typically polar and rarely, bipolar. **C.** Long, unbranched germ tubes (GT) growing parallel to the long axis of epidermal cells. **D.** Germ tubes (GT) growing perpendicular to the long axis of epidermal cells. **E.** Coiled germ tubes (GT) at 36 HPI. **F.** Germ tubes (GT) sometimes coiled around trichomes (TR).

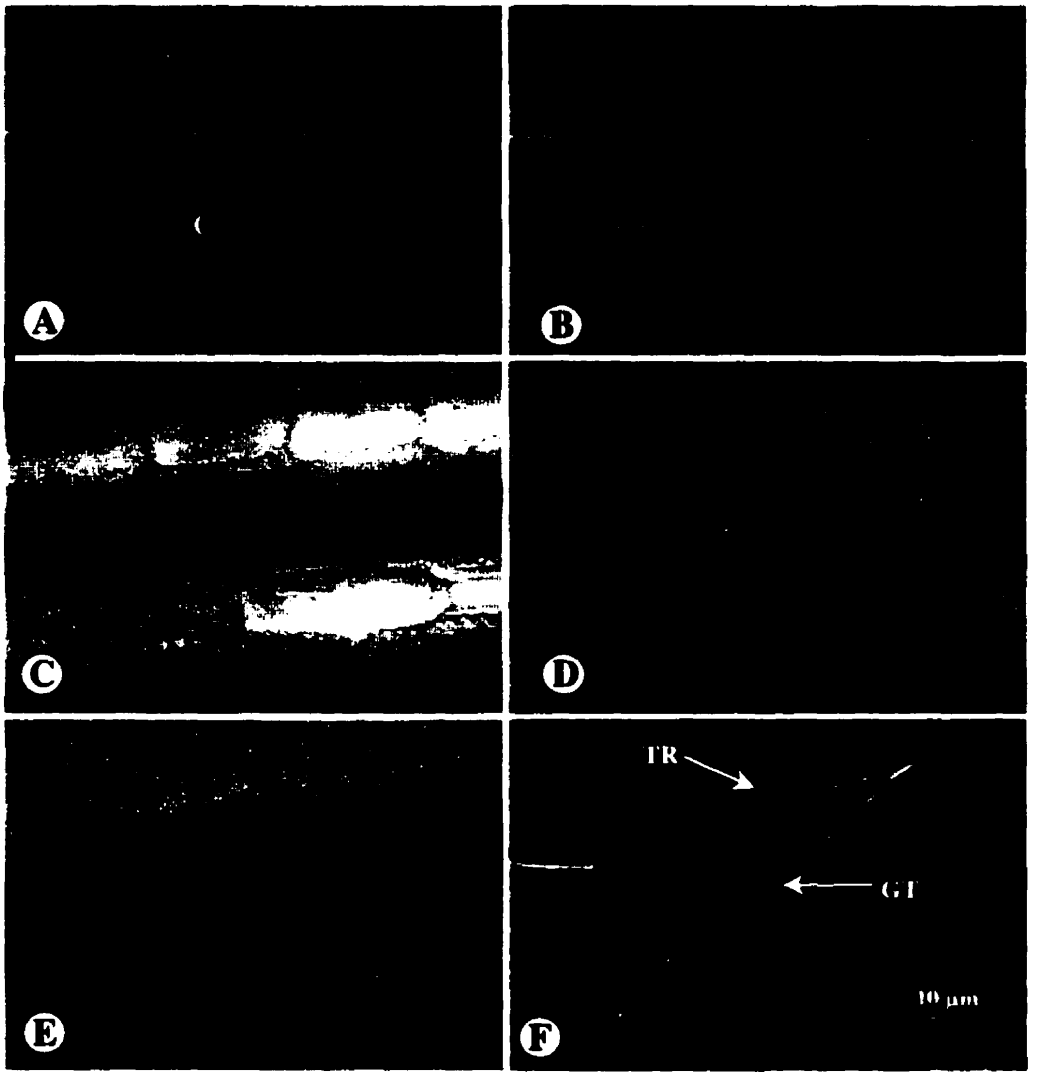

A
B
C
D
E
TR
GT
F
10 μm

Figure 4.9. Infection and colonization of *Cyperus rotundus* by *Curvularia tuberculata* (93-020; C, E, and H and 93-022; A, B, D, F, and G), 24 to 168 hours post inoculation (HPI). **A.** Appressorium (A) formed at epidermal cell wall junction at 24 HPI. **B.** Appressorium (A) formed over a stomata (ST) at 36 HPI. **C.** Infection cushion (IC) on epidermal cell wall showing contributing germ tubes and formation of yellow halo. **D.** Infection cushion (IC) over epidermal cell wall formed by branching of the germ tube (GT) tip. **E.** Infection hyphae (IH) spreading within an epidermal cell. **F.** Infection hyphae (IH) restricted to the cell initially penetrated. **G.** Limited development and spread of infection hyphae (IH) among neighboring epidermal cells, 96 HPI. **H.** Granular appearance of inoculated epidermal cells. The yellow spot indicates the penetration site. Bar =10 μm except D.

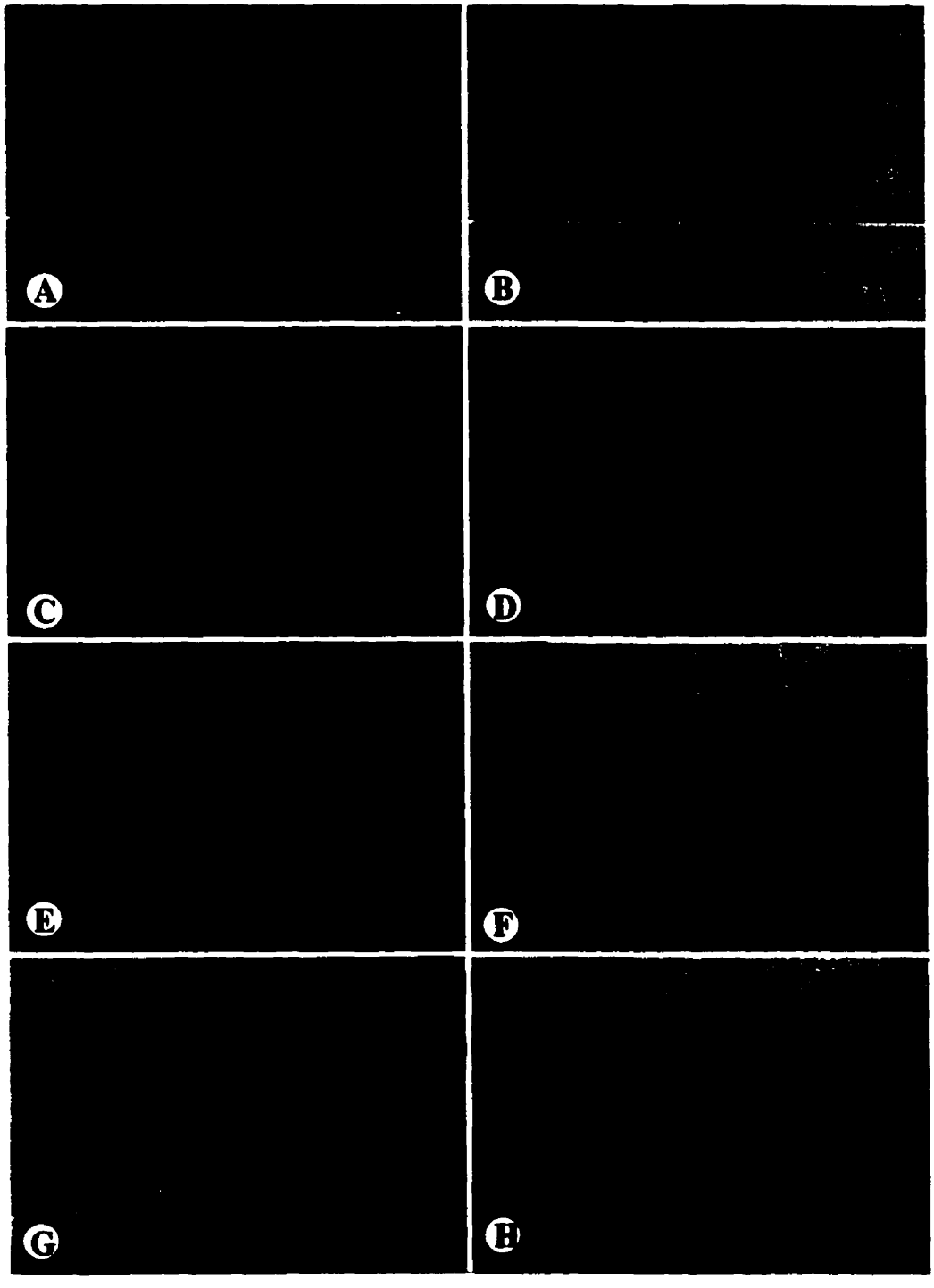

A
B
C
D
E
F
G
H

Figure 4.10. Spore germination and germ tube growth of *Curvularia oryzae* (93-061) on *Fimbristylis miliacea*, four to 12 hours post inoculation (HPI). **A.** Bipolar germination typical of *C. oryzae* at four HPI. **B, C,** and **D.** Germ tubes grew parallel (B) or perpendicular to the long axis of epidermal cells and developed branches (C). Appressoria (black arrowheads) were present at 12 HPI.

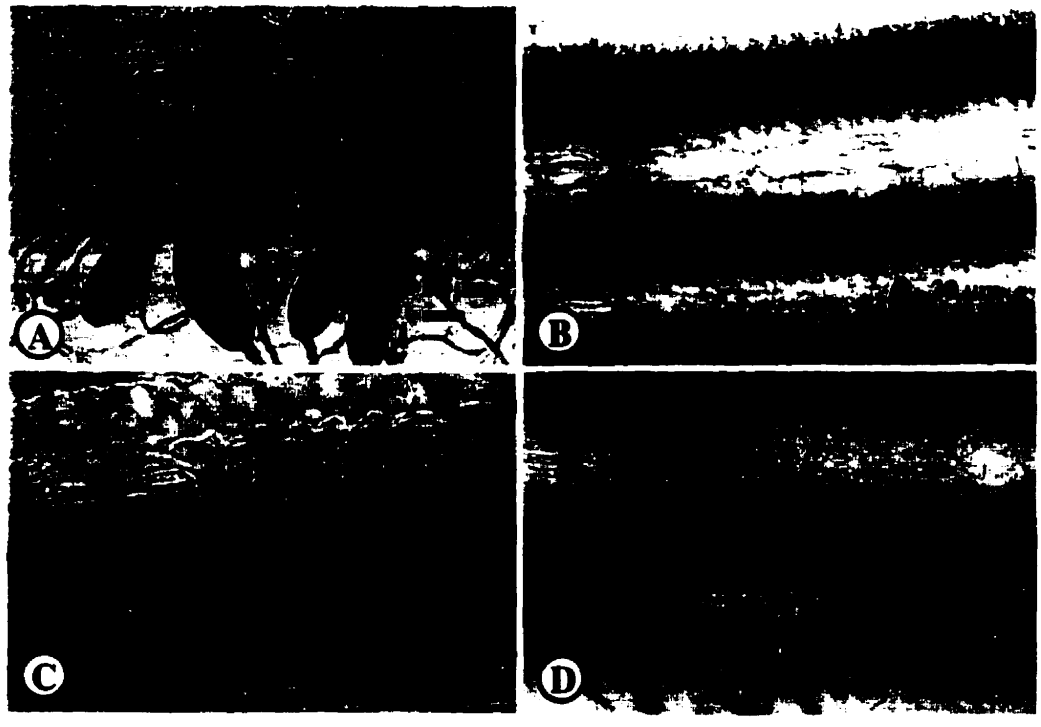

A
B
C
D

Figure 4.11. Infection structure formation of *Curvularia oryzae* (93-061) in *Cyperus difformis* (A), *Cyperus iria* (F), and *Fimbristylis miliacea* (B to E), eight to 24 hours post inoculation (HPI). **A.** Simple appressorium (A) and infection hyphae (IH) in guard cells of the stomata at eight HPI. **B.** Geminated conidium (C) showing pattern of germ tube (GT) branching and location of appressoria (A), 12 HPI. **C.** Attraction among appressoria (A) in the early stage of infection cushion formation. **D.** Infection cushion (IC) on an epidermal wall junction (EWJ) with two penetration pegs (black arrowheads). **E.** Infection cushion (IC) over an epidermal cell consisting of coiled germ tube (black arrowheads) tips. **F.** Infection cushion (IC) over stomatal opening (ST) at 24 HPI. Bar = 10 μm except B.

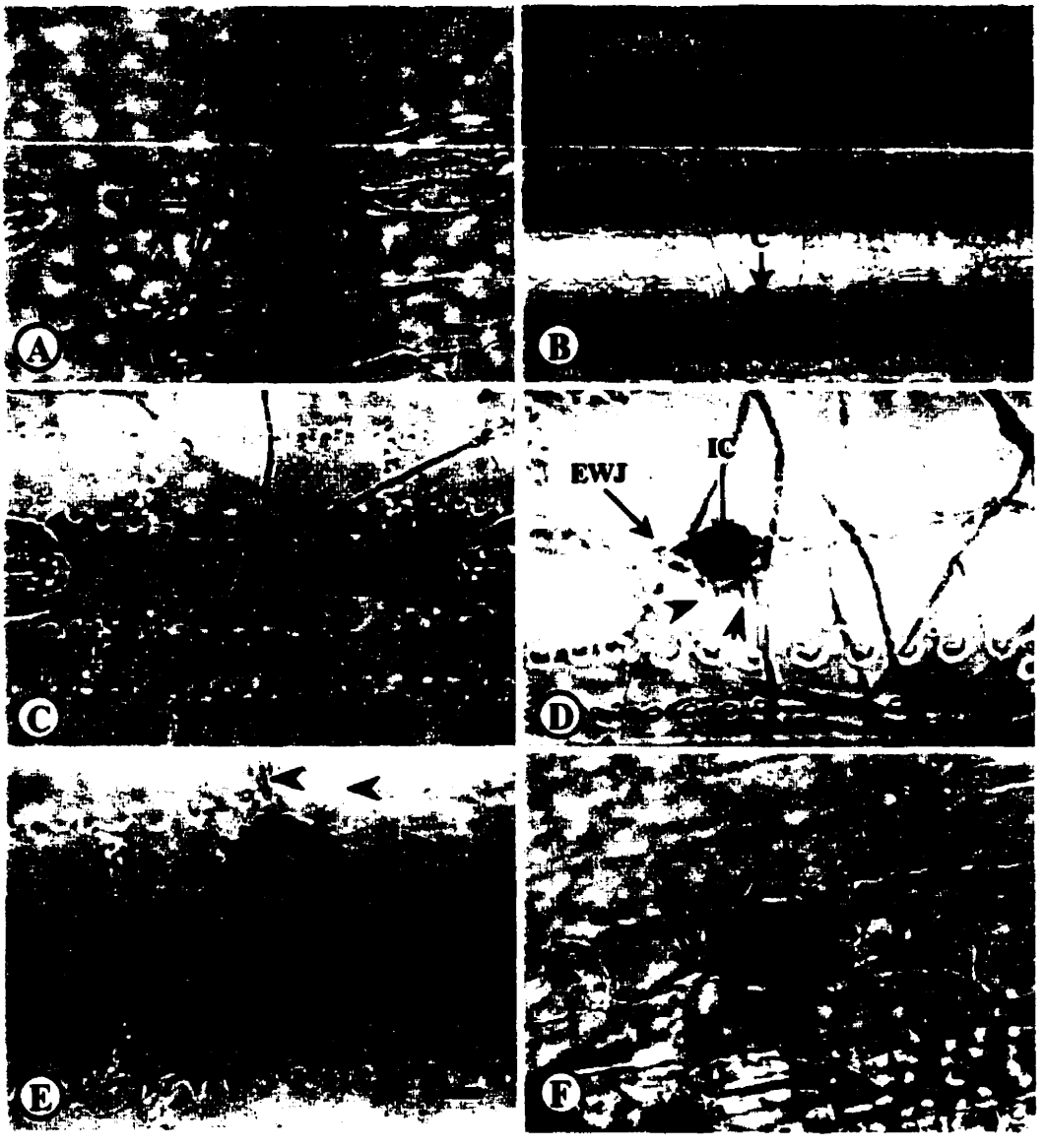

A
B
C
EWJ
IC
D
E
F

Figure 4.12. Penetration and infection of *Cyperus iria* (B and D) and *Fimbristylis miliacea* (A and C) by *Curvularia oryzae* (93-061), 20 to 48 hours post inoculation (HPI). **A.** Penetration of an epidermal cell of *F. miliacea* by an appressorium (A) with penetration peg (PP), 20 HPI. **B.** Adjacent cells are invaded by infection hyphae (IH) in *C. iria*, 36 HPI. The penetration site (PS) is bordered by a yellow halo and shows a small pore in the center. **C.** Infection of an epidermal cell by an appressorium (A). The primary infection hypha (PIH) has spread within the cell. **D.** Growth of infection hyphae (IH) from an appressorium (A) over several epidermal cells of *C. iria*. Bar = 10 μm.

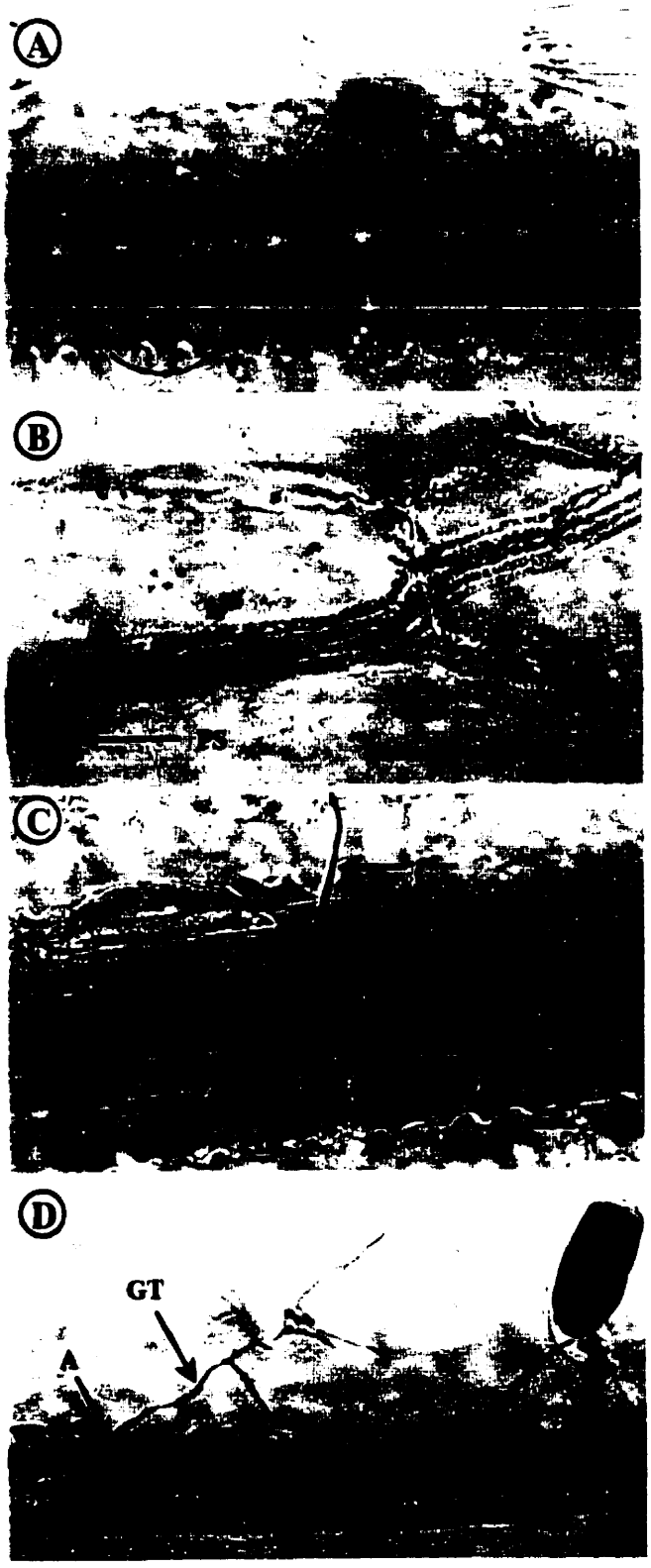

A
B
C
D
GT

Figure 4.13. Colonization of *Cyperus difformis* (C to F), *Cyperus iria* (G and H) and *Fimbristylis miliacea* (A and B) by *Curvularia oryzae* (93-061), 48 to 96 hours post inoculation (HPI). **A.** Fungal hyphae (IH) are visible within cleared necrotic leaf of *F. miliacea*. **B.** Conidiophores (CP) have emerged from the stomata (ST) in necrotic leaf tissues by 96 HPI. **C.** Cross section of a blighted leaf of *C. difformis* at 72 HPI showing infected and collapsed tissues. **D.** High magnification view of boxed area in C showing infection hyphae (IH) within epidermal and mesophyll cells. Some bundle sheath cells surrounding a vascular bundle (VB) have been invaded (black arrowheads). **E.** By 96 HPI infection hyphae (black arrowheads) have ramified to the mesophyll cells and the vascular bundle sheath. **F.** Conidiophores (CP) emerging from the stomata (ST) . Note concentration of fungal hyphae below the stomata. The bundle sheath cells have been completely colonized (black arrowhead). **G.** Cross section of a blighted leaf of *C. iria* showing necrotic area covering the main vein and five minor veins. **H.** Infection hyphae (IH) around the major vein (VB) of a *C. iria* leaf at 72 HPI.
Bar = 10 μm except C and G.

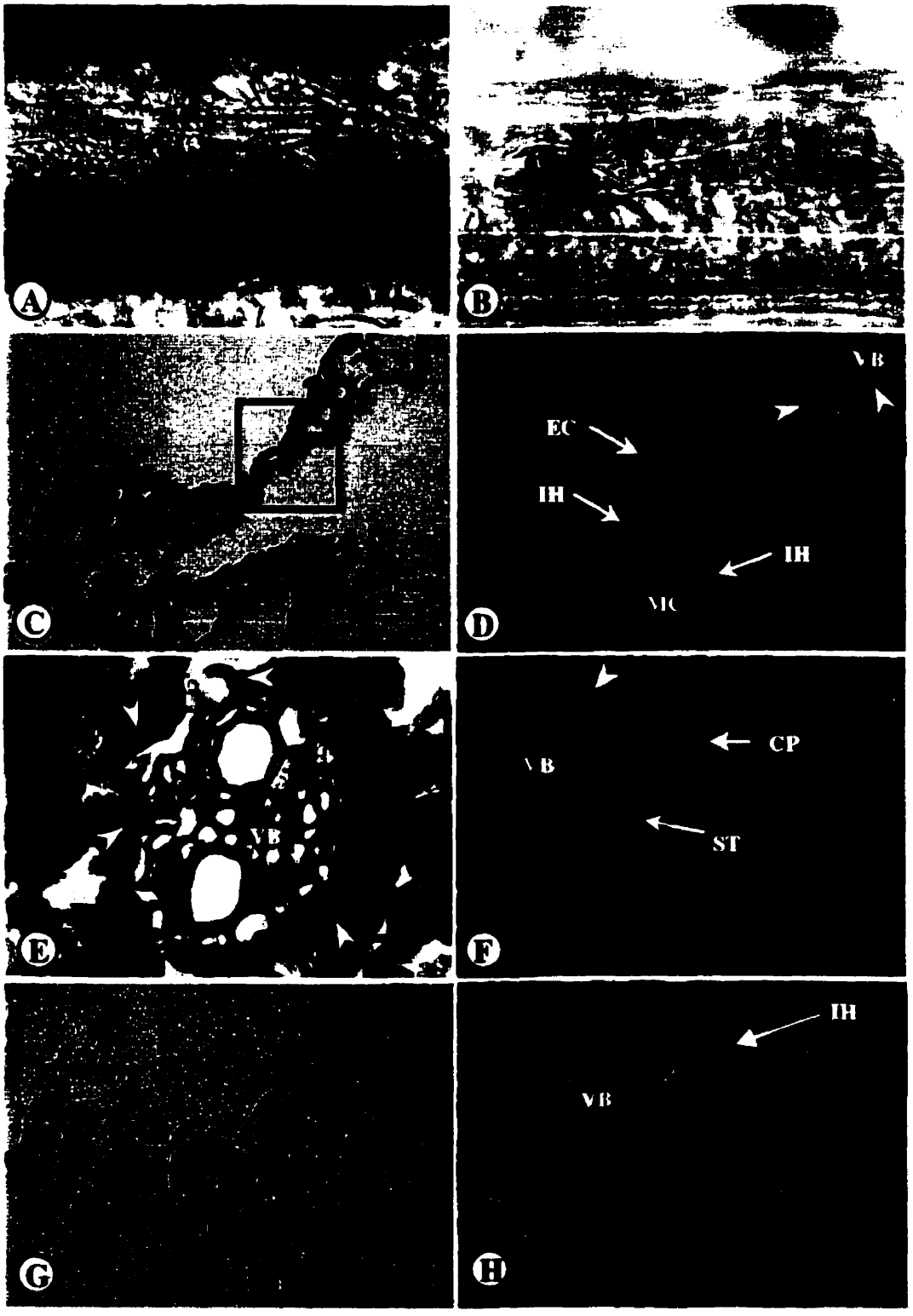

A
B
C
D
VB
EC
IH
IH
MC
E
VB
F
CP
VB
ST
G
H
IH
VB

Figure 4.14. Spore germination and infection structure formation of *Curvularia oryzae* (93-061) on *Cyperus rotundus*, two to 24 hours post inoculation (HPI). **A.** Bipolar germination of conidium (C) with branched germ tubes (GT) at eight HPI. **B.** Appressorium (A) on an epidermal cell wall junction. **C.** Infection cushion (IC) on an epidermal cell. **D.** Early stage of infection cushion formation showing aggregation of individual appressoria (A) with their respective germ tubes (GT). Bar = 10 μm except A.

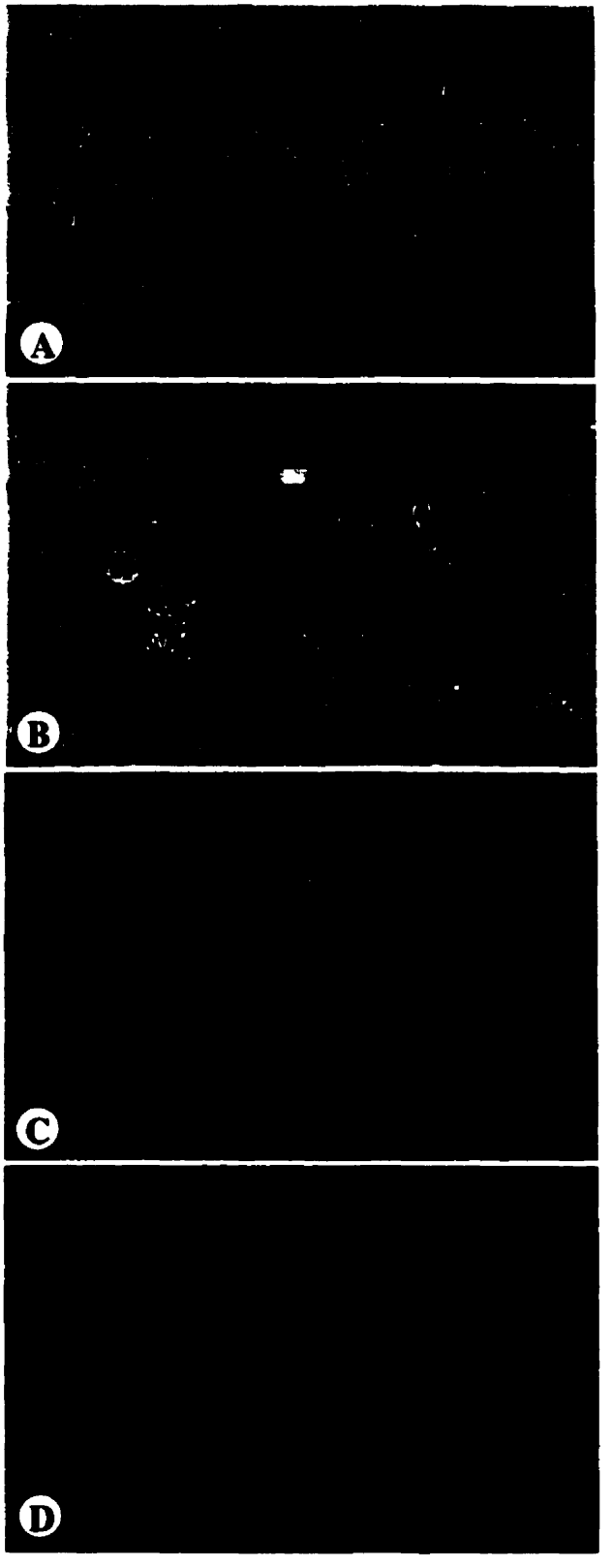

A
B
C
D

Figure 4.15. Penetration of *Cyperus rotundus* by *Curvularia oryzae* (93-061), 20 to 24 hours post inoculation (HPI). **A.** Thin and narrow penetration peg (PP) from an appressorium (A) penetrating an epidermal cell. **B.** Primary infection hyphae (PIH) developed from an infection cushion (IC) on an epidermal cell wall junction (EWJ). Bar = 10 μm.

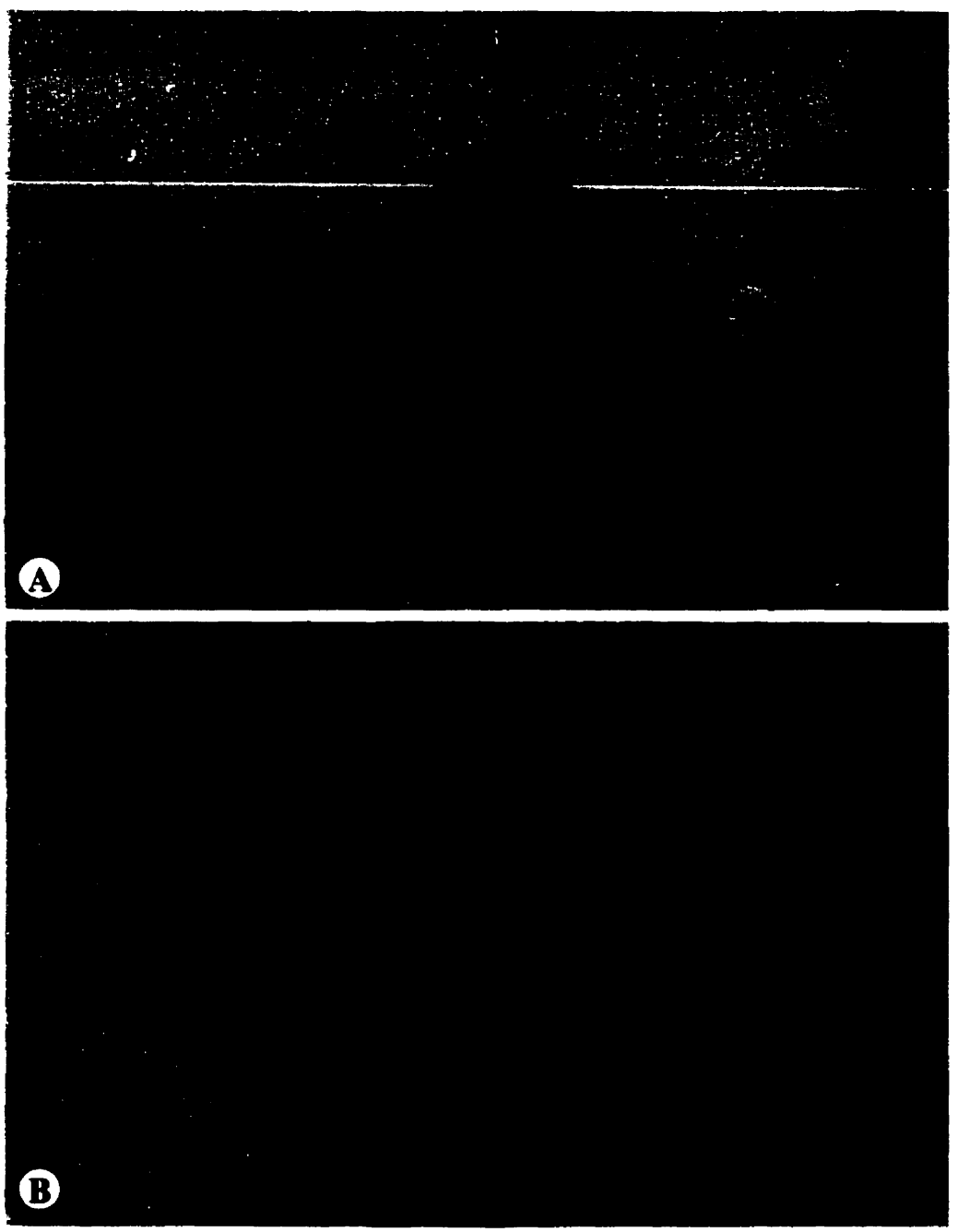

A
B

Figure 4.16. Infection and colonization of *Cyperus rotundus* by *Curvularia oryzae* (93-061), 24 to 168 hours post inoculation (HPI). **A.** Thick, primary infection hyphae (PIH) growing over epidermal cells. Thin, secondary infection hyphae (SIH) grew from PIH. **B.** Primary infection hyphae (PIH) spreading within an epidermal cell. Note yellow areas (black arrowheads) where PIH penetrated the cell wall to infect adjacent cells. **C.** Infection of neighboring cells by infection hyphae (IH). **D.** From the cell initially penetrated (black arrowhead), infection hyphae (IH) ramified and spread to neighboring cells covering an area surrounded by the black line. **E** and **F**. Intense yellowing reaction in infected epidermal cells at 168 HPI. **G.** High magnification view of **F** showing granular appearance (G) of an epidermal cell. **H.** Yellowing reaction in a small lesion at 120 HPI.

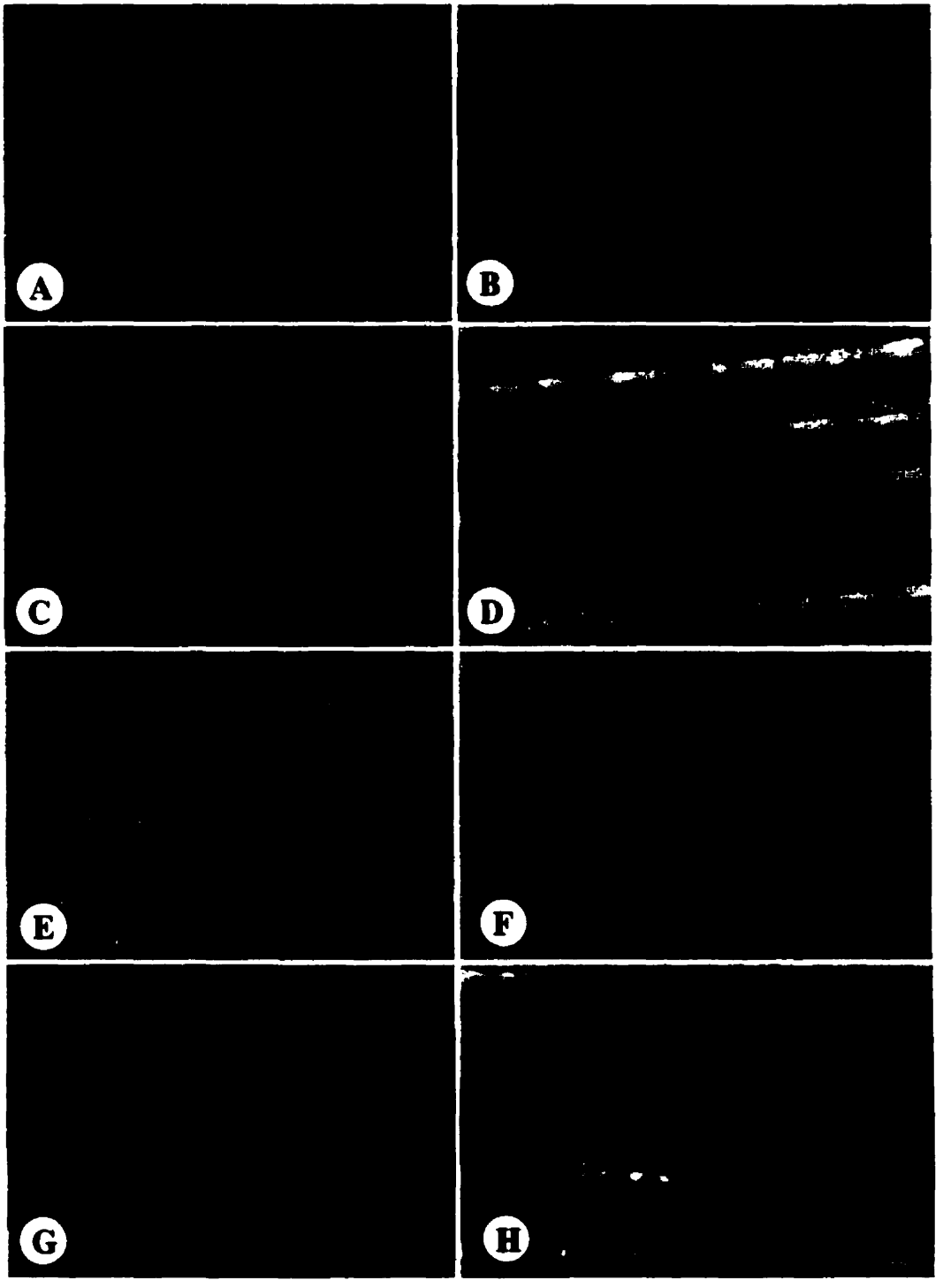
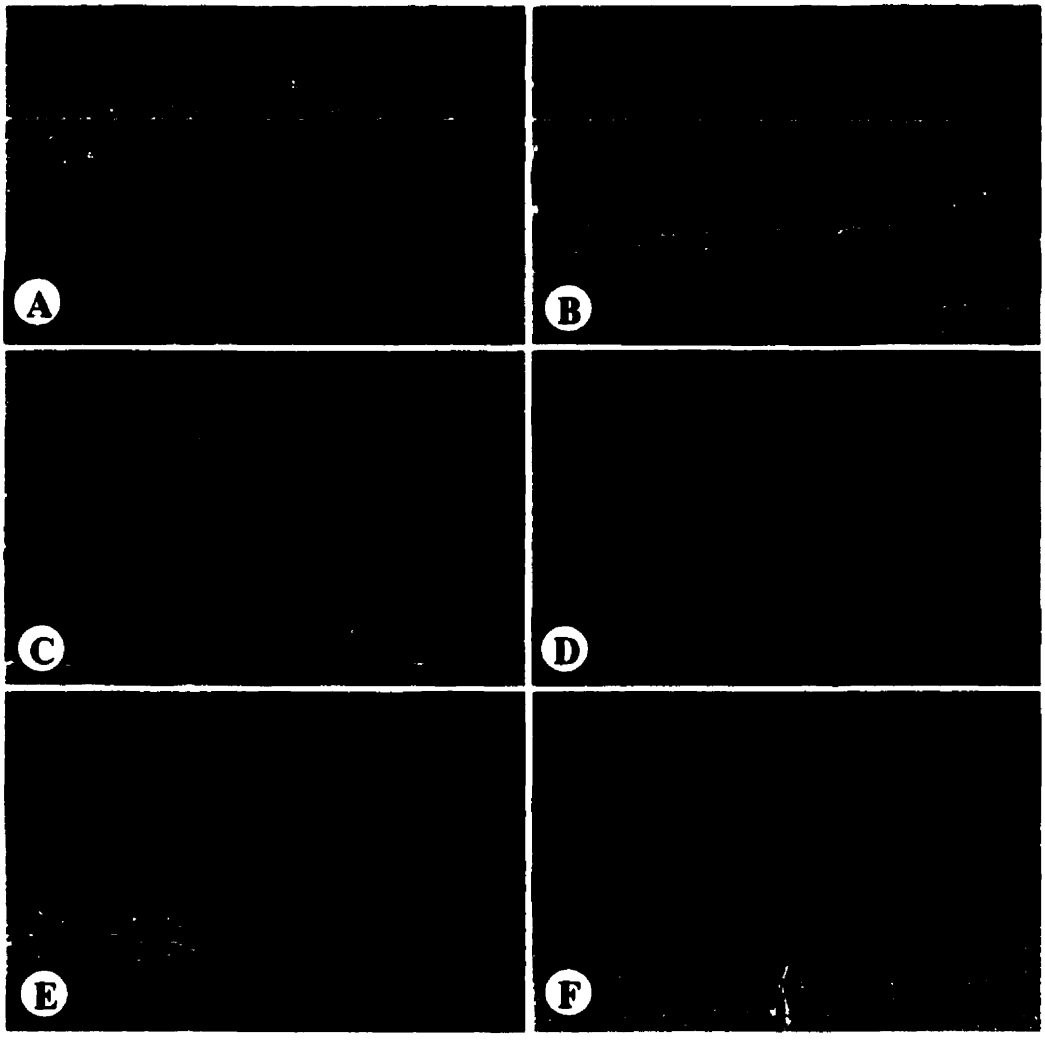

A
B
C
D
E
F
G
H

Figure 4.17. Degeneration of *Curvularia oryzae* (93-061) in *Cyperus rotundus*, 168 hours post inoculation (HPI). **A.** Infection hyphae within an epidermal cell exhibiting granular (G) appearance of hyphae. **B.** Plasmolysis (PL) of infection hyphae in epidermal cell. **C, D,** and **E.** Degenerating infection hyphae (DIH) showing plasmolysis (PL) and granulation (G) of hyphal segments. **F.** Degenerating infection hyphae (DIH) stained unevenly with lactofuchsin. Bar = 10 μm.


A
B
C
D
E
F

CONNECTING TEXT

In Chapter 3, it was demonstrated that most rice varieties tested exhibited resistance to *Curvularia tuberculata* and *Curvularia oryzae*. Although flecking was observed in moderately and highly resistant varieties, no sporulation occurred in the lesions. This indicates that resistance mechanisms are operating. This chapter presents findings on the histology of the resistant fungal-rice interaction and examines resistance mechanisms at the pre-penetration and post-penetration stages.

Chapter 5. Histological responses of *Oryza sativa* to *Curvularia tuberculata* and *C. oryzae*

5.1. Abstract

Reactions of two rice (*Oryza sativa* L.) varieties, IR 64 (IRRI Acc. #66970) and Norin 21 (IRRI Acc. #7686), with moderate to high resistance to isolates of *Curvularia tuberculata* (93-020 and 93-022) and *C. oryzae* (93-061) were studied by light microscopy. Spore deposition was even but most spores were deposited on the middle portion of the leaf blade. The infection process was similar to those reported for sedge hosts. Polar germination beginning four hours post inoculation (HPI) was typical for *C. tuberculata* while *C. oryzae* was characterized by bipolar germination starting two HPI. Infection structures consisting of simple terminal or intercalary appressoria were initiated at 24 HPI over stomatal apertures or rarely, on epidermal cell walls and bulliform cells. No infection cushions were formed. Penetration occurred by the formation of a fine penetration peg underneath the appressorium. Yellowing reaction was observed in areas beneath and adjacent to appressoria and germ tubes as well as infected cells. Granulation of epidermal cells near germ tubes and appressoria was observed in Norin 21 inoculated with isolate 93-022. Resistance of IR 64 and Norin 21 to infection by *C. tuberculata* and *C. oryzae* was expressed mainly after penetration as slow and restricted fungal growth and lack of sporulation.

5.2. Introduction

Rice (*Oryza sativa* L.) is one of the most important crops in the world, second only to wheat in terms of annual production (IRRI, 1993). Ninety-two percent of the world's rice

is grown and eaten in Asia where millions of people depend on this food. It is estimated that world rice production will have to increase by almost 70% in the next 35 years to keep up with population growth and the income-induced demand for food (IRRI, 1993).

Weeds are a considered a major constraint to world rice production. Weeds in rice commonly cause yield losses of 10 to 40% and occasionally, losses of 100% (Watson, 1994). Current weed management practices using mechanical, cultural, and chemical methods need to be improved in the wake of increasing concerns about economical and environmental sustainability of intensive rice production (Watson, 1994; Moody, 1995).

Biological control of weeds has good potential as a component of an integrated weed management program in rice (Watson, 1994). *Curvularia* isolates were obtained in 1993 from diseased Cyperaceae weeds in the Philippines. Isolates 93-020 from *Cyperus difformis* and 93-022 from *C. iria* were identified as *Curvularia tuberculata* Jain and isolate 93-061 from *Fimbristylis miliacea* as *Curvularia oryzae* Bugnicourt. These pathogens produce leaf spots and leaf blight and cause up to 100% mortality on their primary hosts within two weeks after inoculation (Chapter 2). It has also been demonstrated that these three isolates are cross- pathogenic on two other closely related weeds (Chapter 2). Tests on rice varieties revealed a range of reactions to the isolates, from highly resistant to moderately susceptible (Chapter 3).

Curvularia tuberculata has been reported on citrus (Lele et al., 1968), sorghum (Lambat and Ram, 1969), purple nutsedge (Misra et al., 1973), cosmos (Ghosh and Gupta, 1980), sugarcane (Seshadri et al., 1980), mango (Lele et al., 1981), and guava (Kapoor and Tandon, 1971). *Curvularia oryzae* was reported as causing fruit rot in okra (Lal and Goel, 1989). Both *C. tuberculata* and *C. oryzae* have been implicated in the

black kernel disease of rice, together with 12 other *Curvularia* species (Webster and Gunnell, 1992; Ou, 1985). Early studies using *C. lunata* showed that discolored and infected grains could be produced by inoculating rice plants at flowering stage and by injecting inoculum into the seeds (Martin, 1939; Martin and Altstatt, 1940).

Species of *Curvularia* are regarded as weak pathogens and common saprophytes (Webster and Gunnell, 1992). Studies on turf grasses showed that *C. lunata* could colonize heat stressed and/or old clipped leaves but could not colonize juvenile or mature leaves (Muchovej and Couch, 1987). Apparently, leaf clipping allowed entry of *C. lunata* into the leaf tissue (Muchovej, 1986). Although some species of *Curvularia* such as *C. lunata* (Chu and Chen, 1973; Zhang, 1996), *C. cymbopogonis* (Santamaria et al., 1971), and *C. verruculosa* (Aulakh, 1966) may cause leaf spots under certain conditions (Ou, 1985), these diseases are not considered economically important.

The effect of *C. tuberculata* and *C. oryzae* on rice seedlings needs to be studied if these isolates are to be further evaluated as potential biocontrol agents for sedge weeds in irrigated rice. Information about the resistance mechanisms operating during the pre- and post-penetration stages of the infection process will be useful in the assessment of incompatibility between the weed biological control agent and the crop plant. The aim of this study is to examine the histological responses of high to moderately resistant varieties of rice to the three *Curvularia* isolates.

5.3. Materials and methods

All experiments were conducted at the International Rice Research Institute (IRRI), Los Baños, Laguna, Philippines. Some histology and photomicrography work was done at McGill University.

5.3.1. Fungal isolates

Three isolates belonging to two species of *Curvularia* were used in the experiments. *Curvularia tuberculata* Jain was isolated from diseased *Cyperus difformis* (isolate 93-020) and from diseased *Cyperus iria* (isolate 93-022) from Lian, Batangas, Philippines. *Curvularia oryzae* Bugnicourt (isolate 93-061) was obtained from diseased *Fimbristylis miliacea* from Manicahan, Zamboanga, Philippines. All fungal isolates were cultured on ½ PDA plates or slants for initial observation and later stored on agar-oil slants and/or on soil cultures.

5.3.2. Host plants

Seeds of *Oryza sativa* (rice) were obtained from the Genetic Resources Center, International Rice Research Institute while seeds of *Cyperus difformis*, *C. iria*, and *Fimbristylis miliacea* were obtained from mature plants in rice fields in and around the International Rice Research Institute, Los Baños, Laguna, Philippines. Seeds were germinated in petri dishes lined with moist filter paper. Weeds were included in the experiment to serve as positive checks for the virulence of the three *Curvularia* isolates. Seedlings of approximately the same height and vigor were transplanted when they were

a week old in pots filled with soil (Maahas clay, Suborder Haplustic, Order Alfisol) and maintained in the greenhouse with a 35/25 ± 5° C day/night temperature regime.

5.3.3. Preparation of inoculum

Agar plugs were used to seed ½ PDA plates for the mass production of the inoculum. Seeded plates of isolates 93-020 and 93-022 were incubated in the dark at 28° C while cultures of 93-061 were maintained under lighted conditions (30 $\mu E\ m^{-1}\ s^{-1}$) at 28° C. Spore suspensions were prepared by flooding each dish with distilled water and lightly scraping the surface of the colony with a clean glass slide or camel's hair brush. The suspension was filtered using a piece of cheesecloth to separate mycelial fragments from the spores. Spore concentration was determined using a hemacytometer and the spore suspension was sprayed at the rate of 1×10^8 spores/m^2. Two drops of Tween 20 or oxysorbic (20 POE) (polyoxyethylene sorbitan monolaurate) were added per 100 ml of spore suspension.

5.3.4. Inoculation procedure

Inoculation occurred when the plants were two weeks old by spraying them with the spore suspension using an atomizer connected to a pressure source (A.H. Thomas Co., Scientific Apparatus, Philadelphia, U.S.A.). At this stage, *O. sativa, C. difformis,* and *C. iria* had three to four expanded leaves, *F. miliacea,* five to six leaves and *C. rotundus* had approximately six to eight leaves. Distilled water with Tween 20 was used as the control.

The control and inoculated seedlings were incubated for 24 h in the dew chamber set at 28° C and 100% RH in the dark. Then they were moved to the mist room of the greenhouse. For each of the *Curvularia* isolate, an experiment was performed following

a completely randomized design with six replications. The experiments were repeated twice.

5.3.5. Histopathology of infection

The third and fourth leaves of *O. sativa* from infected and uninfected (control) plants were randomly harvested at 2, 4, 8, 16, 20, 24, 36, 48, 72, 96, 120, 144, and 168 hours after inoculation (HPI). Two sets of leaf samples were obtained, one for whole leaf clearing and the other for the paraffin method. The first set of leaf segments for whole leaf clearing were dropped in vials containing 70 parts absolute ethyl alcohol and 30 parts glacial acetic acid while the second set of samples for cross-sectioning were fixed in formaldehyde-acetic acid-alcohol solution (FAA) (Johansen, 1940) until they could be processed. Spore deposition on leaf segments obtained at two to four HPI from the leaf tip, middle portion of the lamina, and the leaf base were observed under the microscope.

Whole leaf segments were immersed in several changes of alcohol-acetic acid clearing solution (70 parts absolute ethyl alcohol:30 parts glacial acetic acid) until they appeared translucent. They were stained in lactophenol-acid fuchsin (Dhingra and Sinclair, 1985) for 15 minutes or until a good contrast was achieved between the epidermal cells (colorless) and the fungal pathogen (deep pink). Stained tissues were dipped in plain lactophenol to remove excess stain and mounted on glass slides using 50% glycerine. Microscopic observations were conducted and photographs were taken using a compound microscope mounted with a Nikon 35-mm camera.

Leaves for cross sectioning were prepared following the method of Johansen (1940). Samples were first dehydrated in a graded ethanol-tertiary butyl alcohol series, cleared and then infiltrated with Paraplast (Fischer Co.) in a 56° C oven. After three

changes, they were embedded in the same medium and sectioned using a rotary microtome. The sections (12 μm thick) were affixed to clean glass slides using egg albumin and stained with safranin-fast green. Microscopic examination was performed and photographs taken using a compound microscope mounted with a Nikon 35-mm camera.

5.4. Results

Reactions of IR 64 and Norin 21 to the *Curvularia* isolates ranged from highly to moderately resistant (Table 5.1). The infection process of the three isolates was similar and histopathological reactions of moderately or highly resistant rice varieties did not differ. However, more lesions that were slightly larger in size were found in moderately resistant combinations. Necrotic areas in leaf tips and leaf margins were also observed more frequently.

5.4.1. Spore deposition

Spore deposition was greatest in the middle part of the leaf blade although many were also deposited at the leaf tips. Generally, distribution of spores was even, unlike in sedges where spores tended to accumulate at the leaf bases. Many were caught at the junctions of trichomes and papillae found abundantly on the rice leaf surface.

5.4.2. Responses to Curvularia tuberculata (Isolate 93-020 and 93-022)

The pre-penetration events of two isolates of *C. tuberculata* in IR 64 and Norin 21 were similar to that in sedge weeds reported in Chapter 4. Spores germinated after four hours and germ tubes emerged only from either the hilar or apical end of the spore. By 24 HPI, most of the spores had germinated. The direction of germ tube growth was

mainly random in both varieties (Fig. 5.1A) although some were found to follow the long axis of epidermal cells. Appressoria were formed mainly on the stomata (Fig. 5.1B) beginning 24 HPI. After penetration, infection hyphae were observed within epidermal cells but growth was restricted and slow (Figs. 5.1C and D). Yellowing and browning of the infected cell/cells was pronounced (Figs. 5.1C and D). Small lesions were present on the leaf lamina (Fig. 5.1E) at 168 HPI.

In Norin 21 inoculated with *C. tuberculata*, spores also germinated within 24 hours (Fig. 5.2A). Appressoria developed preferentially over stomata although some were formed over epidermal cells or over bulliform cells (Fig. 5.2C). They were round, oval or clavate and were 3 to 7 μm in diameter. Some epidermal cells in the vicinity of germ tubes or appressoria became granular in appearance (Figs. 5.2B and D).

Small lesions resulted from infection of Norin 21 by *C. tuberculata* isolate 93-022 in epidermal and mesophyll cells (Figs. 5.3A, E, and B). Although this rice variety had a median disease severity score of "0" (immune) when inoculated with 93-022, some plants developed tiny flecks which became more apparent when histological examinations were performed. Cross-sections of lesions revealed that growth of the infection hyphae was sparse (Figs. 5.3C and D) and that the yellowing reaction was present at the border of the lesion (Fig. 5.3D). This indicates that Norin 21 exhibits a high degree of resistance of to isolate 93-022. No sporulation was observed in both rice varieties inoculated with either of the two *C. tuberculata* isolates.

5.4.3. Responses to Curvularia oryzae (Isolate 93-061)

The reactions of IR 64 and Norin 21 to *C. oryzae* were similar. Spores germinated within two hours and germ tube growth was both perpendicular (Fig. 5.5A)

and parallel (Fig. 5.5B) to the epidermal cells. Appressorium formation began 24 hours after inoculation and preferentially developed over the stomatal region (Figs.5.4A and B) although a few were formed over epidermal cells (Fig. 5.4C). Appressoria were round (Fig. 5.4A) or clavate (Fig. 5.4B). Attempted penetration was immediately accompanied by yellowing of the stomata or epidermal cell underneath the infection structure (Fig. 5.4E).

Penetration was accomplished by thin and narrow penetration peg (Figs. 5.4B and 5.5C). When the appressorium was removed, a small hole was found where the penetration peg penetrated the cell (Fig. 5.5D). The upper cuticle where the appressorium is found appeared yellow while the lower cuticle and the rest of the upper cuticle remained red from the safranin stain (Fig. 5.5D). Yellowing and granulation occurred in a stomata directly underneath a branched germ tube (Fig. 5.5E) and after the appressorium was formed, granulation and yellowing of the stomatal region became more pronounced (Fig. 5.5F). The mesophyll cells underneath the invaded stomata also showed the beginnings of the yellowing reaction (Fig. 5.5F). Initially, yellowing was confined to intercellular regions (Fig. 5.5F) but later spread intracellularly even if there was no clear evidence of penetration (Fig. 5.6E).

Immediately after penetration, primary infection hyphae were formed and grew within the epidermal cell (Fig. 5.6A). However, the spread of the fungus to the neighboring cells was slow (Fig. 5.6B and C). By 72 HPI, only a few cells in the layer of mesophyll cells closest to the epidermis had been infected (Fig. 5.6D) and further

colonization of this region was slow (Fig. 5.6E). Small, brown spots were produced at 168 HPI consisting of collapsed epidermal and mesophyll cells (Fig. 5.6F) but no sporulation occurred in these lesions.

5.5. Discussion

Leaves may be considered spore traps (Gregory, 1971) with trapping efficiency dependent on physical characteristics such as leaf shape and the presence of hairs or related structures. Leaf angle also affects spore deposition on leaves (Heather, 1967). Spores were evenly distributed over the rice leaf surface in contrast to the clumped distribution of spores on leaves on the sedge weeds, *Cyperus difformis* or *Fimbristylis miliacea*. This could be due, in part, to the presence of papillae on the rice leaf. These are siliceous projections on the leaf surface that may help trap the spores. In addition, fully expanded rice leaves tend to bend with their tips turned downward which facilitates the deposition of spores on the leaf surface.

Surface development of two isolates of *C. tuberculata* and one isolate of *C. oryzae* on leaves of IR 64 and Norin 21 did not differ and were similar to the events recorded for sedge hosts (Chapter 4). Spore germination was polar and began four HPI for *C. tuberculata* while it was bipolar and started at two HPI for *C. oryzae*. Germination from the middle cells did not occur in both pathogens. Sivanesan (1987), however, reported that *C. tuberculata* exhibited bipolar germination, and sometimes, lateral germination. There were no obvious signs of orientation in the direction of germ tube growth. They developed perpendicular and parallel to the long axis of epidermal cells but there was generally less branching than in sedge hosts.

Only simple appressoria were formed in the rice hosts treated with any one of the three isolates. Compound appressoria or infection cushions as described by Emmett and Parberry (1975) were not observed. In *Botrytis* and *Sclerotinia*, appressorial complexity is directly related to the nutritional status of the inoculum (Garcia-Arsenal and Sagasta, 1980; Abawi et al., 1975). However, a later study by Tariq and Jeffries (1984) showed that compound appressoria are more likely to be formed when the host surface is more physically resistant to penetration. The early stage of infection cushion formation in *Rhizoctonia solani* is primarily a response to the characteristics of the host plant surface and not to plant exudates (Armentrout et al., 1987). It is possible that the different surface architecture of rice plants did not provide the appropriate stimulus for infection cushion formation.

The penetration site may also correlate with the type of infection structure formed. Dodman et al. (1968) reported that direct penetration of hosts by *Rhizoctonia solani* was always associated with the formation of complicated dome-shaped infection cushions while stomatal penetration was often associated with less complicated lobate appressoria. They suggest that stomatal penetration requires less effort on the part of the fungus and thus, only necessitates the formation of simpler infection structures.

Most appressoria were formed over the stomatal aperture and penetration of epidermal cell walls occurred infrequently. This is in contrast to what occurs in sedge hosts where epidermal cell wall junctions are the preferred penetration sites. Penetration of host cells via stomata has been reported for *Cladosporium macrocarpum* in barley (Christianssen and Smedegaard, 1990), *Uromyces appendiculatus* in bean (Wynn, 1976) and *Cladosporium cladosporioides* (Fres.) de Vries, *C. herbarum* (Pers.) Link and

Alternaria alternata in bean (O'Donnell and Dickinson, 1980). In the rust fungi, it appears that appressorium formation is triggered mainly by leaf surface topography although the exact nature of the inductive signal provided by the stoma has not yet been resolved (Allen et al., 1991). Recent results suggest that a combination of chemical and physical signals which allow the stomata to be accurately recognized by cereal rusts could be the reason for the higher differentiation in host tissues compared to polystyrene leaf replicas (Collins and Read, 1997).

Since there was no indication of oriented germ tube growth in both *C. tuberculata* and *C. oryzae* in rice, it is possible that a substance present in the area of the stomatal aperture could be the stimulus for appressorium formation. Evidence of the involvement of chemical stimuli in appressorium formation has been identified for the rust fungi and include K^+ and Ca^+ ions (Kaminsky and Day, 1984), sucrose (Hoch and Staples, 1987), and a volatile compound from host plants (Grambow, 1977; Grambow and Riedel, 1977).

The occasional penetration of epidermal cells could be due to leaching of the stimulatory substance(s) from cuticular breaks resulting from opening and closing of the stomata (Wheeler, 1977). Few appressoria were formed over bulliform cells. These are special epidermal cells in the Poaceae that cause the leaf to fold up during conditions of water stress. Bulliform cells have been reported as the preferred penetration sites for *Pyricularia oryzae* (Hau and Rush, 1982). Hau and Rush (1982) attributed this preference to a lower resistance to penetration since the radial walls of bulliform cells are thin (Esau, 1965) and they remain longer in a pectic-cellulosic state compared to other epidermal cells which become lignified (Clark and Lorbeer, 1976). Lower

concentrations of chlorogenic acid, a fungitoxin, was found in the bulliform cells compared to other epidermal cells (Whitney, 1977).

Stomata occur on the adaxial and abaxial sides of the rice leaf in files between veins (Hoshikawa, 1989) while in the sedges, they are found only on the abaxial side (Metcalfe, 1971). Huge hydathodes or hydropores occur along the leaf margins at the edges of the rice leaf blade. These structures have pore diameters more than twice as large as those of ordinary stomata and are involved in the guttation of water from the leaf when active water uptake is occurring in the plants (Hoshikawa, 1989). Since lesions observed along leaf margins developed from the edge to the inner part of the leaf lamina (Fig 2G), this suggests that hydathodes are also sites of penetration by *C. tuberculata* and *C. oryzae* in rice.

Whether the preferred penetration site is related to the percentage leaf area occupied by stomata in rice remains to be determined. Work done with *Helminthosporium victoriae, H. sorokiana*, and other foliar pathogens showed that they have a tendency to penetrate cells adjacent to stomata such as guard cells and subsidiary cells (Wood 1967; Wheeler, 1977). However, it was not clearly established if the choice of penetration site was a function of topography or other factors. It is likely that, in rice, stomatal penetration is due to the greater ease at which the pathogens can enter the plant.

There was a delay in the initiation of infection structures by about 12 h. In sedge hosts, infection structures were observed between eight to12 HPI but in rice, appressoria did not form until about 20 to 24 HPI. The repression of the ability of pathogens to form infection structures can be viewed as a defense mechanism to disease (Marshall and Rush, 1980).

The mode of penetration of rice by the three isolates followed the same pattern as that observed in the sedge hosts. A fine penetration peg that emerged from the appressorium (Fig. 5.4C) accomplished penetration. Both mechanical force and enzymatic action was apparently required for penetration since a hole was present (Fig. 5.4D) and yellowing of the cuticle beneath and adjacent to the appressorium was observed (Fig. 5.4F). Alterations of the cuticle under and around infection structures and the imprints left by these structures led Garcia-Arsenal and Sagasta (1980) to suggest that both mechanical force and enzymatic factors are involved in the penetration of *Phaseolus vulgaris* by *B. cinerea.* In this study, a yellowing reaction occurred in the cuticle beneath and immediately adjacent to the appressorium. Differential staining of the area beneath, adjacent, or just ahead of hyphal tips and infection structures have been previously reported. Skoropad and Amy (1956) found that the area just ahead of swollen hyphal tips of *Helminthosporium gramineum* stained red temporarily in the susceptible barley hosts but this area was intensely red in the resistant hosts. O'Connell et al. (1985) attributed this change in staining properties of the cell wall to chemical modifications, probably due to enzymatic activity.

After penetration, primary infection hyphae were observed but their growth was restricted and slow (Figs. 5.2B, C, and 5.5C). Restricted colony and lesion formation is considered a component of resistance since it reflects the growth rate of the pathogen in the host and therefore its spore production (Parlevliet, 1979). Johnson (1975 cited in Bassi et al., 1979) studied resistance to soil rot caused by *Rhizoctonia solani* in tomatoes and found that infection was delayed and lesion development was slow in partially resistant fruits. In *Malvaceae* weeds inoculated by *Colletotrichum gloeosporioides* f. sp.

malvae, the infection hyphae progressed more slowly in the moderately resistant hosts than in highly susceptible hosts (Morin et al., 1996). Milholland (1973) attributed the symptoms of blueberry resistant to *Gloeosporium minus* to a hypersensitive reaction where the host tissue immediately accumulates a dark-staining material after penetration and growth of the fungus is restricted to a very few cells in the vicinity of the infection court. Sridhar et al. (1972) studied lesion development of Philippine races of *Pyricularia oryzae* on the rice cultivar Peta and found that in the resistant type of host-pathogen combination, browning of the tissues occurred at a faster rate, inhibiting lesion formation which resulted in a resistant type of spot. In a subsequent study, Peng and Shishiyama (1988) reported that expression of race-specific resistance to *P. oryzae* results in inhibition of fungal hyphae accompanied by light browning of epidermal cells in rice leaves.

Sporulation is often the most sensitive indicator of vertical or horizontal resistance (Johnson and Taylor, 1976), and inhibited infection and colonization often accompany it (Cohen and Rotem, 1987). Resistance of *Cucumis melo* to *Psuedoperonospora cubensis* and to *Sphaerotheca fuliginea* race 2 was characterized by small lesions with few spores (Cohen et al., 1984; Cohen and Cohen, 1986). In tobacco, resistance to powdery mildew does not result from a failure of the pathogen to infect but from inhibition of both mycelial growth and sporulation (Cohen, 1982). This study showed that in rice cultivars IR 64 and Norin 21, partial resistance to *Curvularia tuberculata* and *C. oryzae* was operative during the pre-penetration phase expressed as the delay in initiation of infection structure formation. Post-penetration resistance

mechanisms were expressed as inhibition of fungal growth after penetration and lack of sporulation.

5.6. Literature cited

Abawi, G.S., F.J. Polach, and W.T. Molin. 1975. Infection of bean by ascospores of *Whetzelinia sclerotiorum*. Phytopathology 65:673-678.

Allen, E.A., B.E. Hazen, H.C. Hoch, Y. Kwon, G.M.E. Leinhos, R.C. Staples, M.A. Stumpf, and B.T. Terhune. 1991. Appressorium formation in response to topographical signals by 27 rust species. Phytopathology 81:323-331.

Armentrout, V.N., A.J. Downer, D.L. Grasmick, and A.R. Weinhold. 1987. Factors affecting infection cushion development by *Rhizoctonia solani* on cotton. Phytopathology 77:623-630.

Aulakh, K.S. 1966. Rice, a new host of *Curvularia verruculosa*. Plant Dis. Reptr. 50:314-316.

Bassi, A., Jr., E.L. Moore, and W.E. Batson, Jr. 1979. Histopathology of resistant and susceptible tomato fruit infected with *Rhizoctonia solani*. Phytopathology 69:556-559.

Christianssen, S.K. and V. Smedegaard. 1990. Microscopic studies of the interaction between barley and the saprophytic fungus, *Cladosporium macrocarpum*. J. Phytopathology 128:209-219.

Chu, C.L., and C.C. Chen. 1973. Physiological study of *Curvularia lunata* and its pathogenicity to rice plant. J. Taiwan Agric. Res. 22:213-220.

Clark, C.A. and L. Lorbeer. 1976. Comparative histopathology of *Botrytis squamosa* and *B. cinerea* in onion leaves. Phytopathology 66:1279-1289.

Cohen, Y. 1982. Cultivar resistance and species immunity in *Nicotiana* spp. against tobacco powdery mildew. Colloq. INRA 11, 143-155.

Cohen, S. and Cohen, Y. 1986. Nature and genetics of resistance to powdery mildew race 2 in *Cucumis melo* PI 124111. Phytopathology 76:1165-1167.

Cohen, Y. and J. Rotem. 1987. Sporulation of foliar pathogens. Pages 315-333 *in* Fungal Infection of Plants. British Mycological Society Symposium Series 13. G.F. Pegg and P.G. Ayres (Eds.). Cambridge University Press. Cambridge, England.

Cohen, Y., H. Eyal, A. Cohen, and C.E. Thomas. 1984. Evaluating downy mildew resistance in *Cucumis melo* L. Cucurbits Genetics Cooperative Report 7:38-40.

Collins, T.J. and N.D. Read. 1997. Appressorium induction by topographical signals in six cereal rusts. Physiol. Molec. Plant Pathol. 51:169-179.

Dhingra, O.D. and J.B. Sinclair, 1985. Basic Plant Pathological Methods. CRC Press, Inc., Boca Raton, FL, USA. 355 pp.

Dodman, R.L., K.R. Barker, and J.C. Walker. 1968. A detailed study of the different modes of penetration by *Rhizoctonia solani*. Phytopathology 58:1271-1276.

Emmett, R.W. and D.G. Parberry, 1975. Appressoria. Annu. Rev. Phytopathol. 13:147-167.

Esau, K. 1965. Plant Anatomy. John Wiley & Sons. New York, NY, USA. 767 pp.

Garcia-Arsenal, F. and E.M. Sagasta. 1980. Scanning electron microscopy of *Botrytis cinerea* penetration of bean (*Phaseolus vulgaris*) hypocotyls. Phytopath. Z. 99:37-42.

Ghosh, R.N. and S. Gupta. 1980. Two new host records form India. Indian Phytopath. 33:498-500.

Grambow, H.J. 1977. The influence of leaf volatile constituents on the *in vitro* differentiation and growth of *Puccinia graminis* f. sp. *tritici*. Phytopath. Z. 85:361-372.

Grambow, H.J. and S. Riedel. 1977. The effect of morphogenically active factors from host and nonhost plants on the *in vitro* differentiation of infection structures of *Puccinia garminis* f. sp. *tritici*. Physiol. Plant Pathol. 11:213-224.

Gregory, P.H. 1971. The leaf as a spore trap. Pages 239-243 *in* Ecology of Leaf Surface Microorganisms. T.F. Preece and C.H. Dickinson (Eds.). Academic Press. London, Englanad.

Hau, F.C. and M.C. Rush. 1982. Preinfectional interactions between *Helminthosporium oryzae* and resistant and susceptible rice plants. Phytopathology 72:285-292.

Heather, W.A. 1967. Susceptibility of juvenile leaves of *Eucalyptus bicostata* Maiden *et al.* to infection by *Phaeoseptoria eucalypti* (Hansf.) Walker. Aust. J. Biol. Sci. 20:769-775.

Hoch, H.C. and R.C. Staples. 1987. Structural and chemical changes among the rust fungi during appressorium development. Annu. Rev. Phytopathol. 25:231-247.

Hoshikawa, K. 1989. The Growing Rice Plant: An Anatomical Monograph. Nosan Gyosan Bunka Kyokai (Nobunkyo). Tokyo, Japan. 310 pp.

International Rice Research Institute. 1993. IRRI Rice Almanac. International Rice Research Institute, Manila, Philippines.142 pp.

Johansen, D.A. 1940. Plant Microtechnique. McGraw -Hill Book Co. New York, NY, USA. 523 pp.

Johnson, R. and A.J. Taylor. 1976. Spore yield of pathogens in investigations of the race specificity of host resistance. Annu. Rev. Phytopathol. 14:97-119.

Kaminsky, S.G.W. and A.W. Day. 1984. Chemical induction of infection structures in rust fungi. II. Inorganic ions. Exp. Mycol. 8:193-201.

Kapoor, I.J. and R.N. Tandon. 1971. Occurrence of *Curvularia tuberculata* Jain on stored fruits of *Psidium guajava* L. Sydowia 24(1-6):201-202.

Lal, B. and D. Goel. 1989. A new rot of *Abelmoschus esculentus*. Indian Phytopath. 42:482.

Lambat, A.K. and Ram, A. 1969. Seed-borne infection of *Curvularia* causing a new blight disease of Jowar. Indian Phytopath. 22:282-284.

Lele, V.C., J. Singh, S.N. Rai, and J. Kandhari. 1981. Occurrence of a new blight disease of mango caused by *Curvularia*. Curr. Sci. 50:464-465.

Lele, V.C., S.P. Raychaudhuri, R.B. Bhalla, and A. Ram. 1968. *Curvularia tuberculata*, a new fungus causing die-back disease of citrus in India. Indian Phytopath. 21:66-72.

Martin, A.L. 1939. Possible cause of black kernels in rice. Plant Dis. Reptr. 23:247-249.

Martin, A.L. and G.E. Altstatt. 1940. Black kernel and white tip of rice. Bulletin. Texas Agricultural Experiment Station No. 584. 14 pp.

Marshall, D.S. and M.C. Rush. 1980. Relation between infection by *Rhizoctonia solani* and *R. oryzae* and disease severity in rice. Phytopathology 70:941-946.

Metcalfe, C.R. 1971. Anatomy of the Monocotyledons. V. Cyperaceae. Clarendon Press. Oxford, England. 597 pp.

Milholland, R.D. 1973. Histopathology of fleck and lesion symptoms on blueberry infected with *Gloeosporium minus*. Phytopathology 63:320-323.

Misra, A.P., O. Prakash, B. Mishra, and K.K. Dutta. 1973. A new leaf spot disease of motha (*Cyperus rotundus* L.) caused by *Curvularia tuberculata*. Indian Phytopath. 26:165-167.

Moody, K. 1995. Sustainability in rice weed management. Pages 93-103 *in* Proc. 15th Asian Pacific Weed Science Society Conference. Vol. IA. July 24-28, 1995, Tsukuba, Japan.

Morin, L., J.L. Derby, and E.G. Kokko. 1996. Infection process of *Colletotrichum gloeosporioides* f. sp. *malvae* on Malvaceae weeds. Mycol. Res. 100:165-172.

Muchovej, J.J. 1986. Definition of leaf health in *Agrostis palustris* at the time of infection and colonization by *Curvularia lunata*. Ann. Appl. Biol. 109:249-258.

Muchovej, J.J. and H.B. Couch. 1987. Colonization of bentgrass turf by *Curvularia lunata* after leaf clipping and heat stress. Plant Dis. 71:873-875.

O'Connell, R.J., J.A. Bailey, and D.V. Richmond. 1985. Cytology and physiology of infection of *Phaseolus vulgaris* by *Colletotrichum lindemuthianum*. Physiol. Plant Pathol. 27:75-98.

O"Donnell, J. and C.H. Dickinson. 1980. Pathogenicity of *Alternaria* and *Cladosporium* isolates on *Phaseolus*. Trans. Br. Mycol. Soc. 74:335-342.

Ou, S.H. 1985. Rice Diseases. 2nd Edition. Great Britain: C.A.B. International. Wallingford, UK. 380 pp.

Parlevliet, J.E. 1979. Components of resistance that reduce the rate of epidemic development. Annu. Rev. Phytopathol. 17:203-222.

Peng, Y.-L. and J. Shishiyama. 1988. Temporal sequence of cytological events in rice leaves infected with *Pyricularia oryzae*. Can. J. Bot. 66:730-735.

Santamaria, P.A., A. Benoit, and S. B. Mathur. 1971. *Curvularia cymbopogonis*: a hitherto unreported species pathogenic to rice in the Philippines. Plant Dis. Reptr. 55:349-350.

Seshadri, K., P. Padmanaban, and K.C. Alexander. 1980. A new leaf blight disease of sugarcane. Indian Phytopath. 33:325-326.

Sivanesan, A. 1987. Graminicolous species of *Bipolaris, Curvularia, Drechslera* and *Exserohilum* and their teleomorphs. Mycol. Pap. 158:1-261.

Skoropad, W.R. and D.C. Arny. 1956. Histologic expression of susceptibility and resistance in barley to strains of *Helminthosporium gramineum*. Phytopathology 46:289-292.

Sridhar, R., S.H. Ou, and S.P. Ebron. 1972. Lesion development on a rice cultivar by different races of the blast pathogen. Plant Dis. Reptr. 56:961-963.

Tariq, V.N. and P. Jeffries. 1984. Appressorium formation by *Sclerotinia sclerotiorum*: scanning electron microscopy. Trans. Br. Mycol. Soc. 82: 645-651.

Watson, A.K. 1994. Current status of bioherbicide development and prospects for rice in Asia. Pages 195-201 *in* Integrated Management of Paddy and Aquatic Weeds in Asia. Proc. International Seminar " Biological Control and Integrated Management of Paddy and Aquatic Weeds in Asia". H. Shibayama, K. Kiritani, and J. Bay-Petersen (Eds.) Oct. 19-25, 1992. Tsukuba, Japan.

Webster, R.K. and P.S. Gunnell (Eds.). 1992. Compendium of Rice Diseases. APS Press. St. Paul, MN, USA. 62 pp.

Wheeler, H. 1977. Ultrastructure of penetration by *Helminthosporium maydis*. Physiol. Plant. Pathol. 11:171-178.

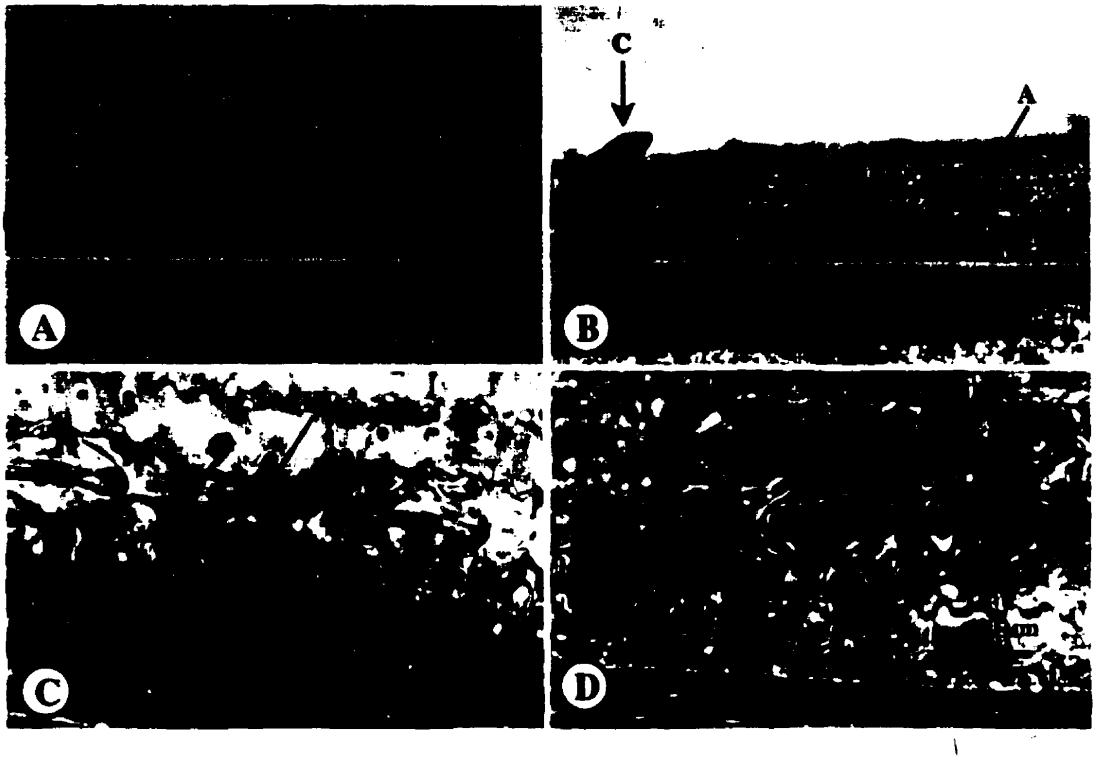
Whitney, P.J. 1977. Microbial Plant Pathology. Pica Press. New York, NY, USA. 160 pp.

Wood, R.K.S. 1967. Physiological Plant Pathology. Blackwell Scientific Publications. Oxford, England. 570 pp.

Wynn, W.K. 1976. Appressorium formation over stomates by the bean rust fungus: response to a surface contact stimulus. Phytopathology 66:136-146.

Zhang, W.M. 1996. Responses of *Echinochloa* species and rice (*Oryza sativa* L.) to indigenous pathogenic fungi. Plant Dis. 80:1053-1058.

Figure 5.1. Infection of *Oryza sativa* cv. IR 64 by *Curvularia tuberculata* (93-020; C,D,E and 93-022; A and B), 24 to 72 hours post inoculation (HPI). **A.** Germinated spores on the leaf surface at 24 HPI. C, conidium. **B.** Appressorium (A) formation on stomata, 24 HPI. **C.** Restricted growth of infection hyphae (IH) within epidermal cells. **D.** Mesophyll cells (MC) showing the yellowing reaction in the vicinity of infection hyphae (IH) visible beneath the stomatal guard cells. **E.** Transverse section of a small lesion (L) on the leaf blade, 72 HPI.


C
A
A
B
C
D


L
E

Figure 5.2. Infection of *Oryza sativa* cv. Norin 21 by *Curvularia tuberculata* (93-020, A and C; 93-022, B and D), 24 to 72 hours post inoculation (HPI). **A.** Longitudinal section of the leaf with a germinated spore on the surface, 24 HPI. C, conidium; GT, germ tube; EC, epidermal cell. **B.** An epidermal cell (EC) beneath a mass of germ tubes (GT) exhibited a granular appearance at 72 HPI. **C.** Transverse section of a conidium (C) and appressorium (A) over bulliform cells. **D.** Granular appearance of mesophyll cells (MC) beneath a stomata (ST) with an appressorium (A), 72 HPI.

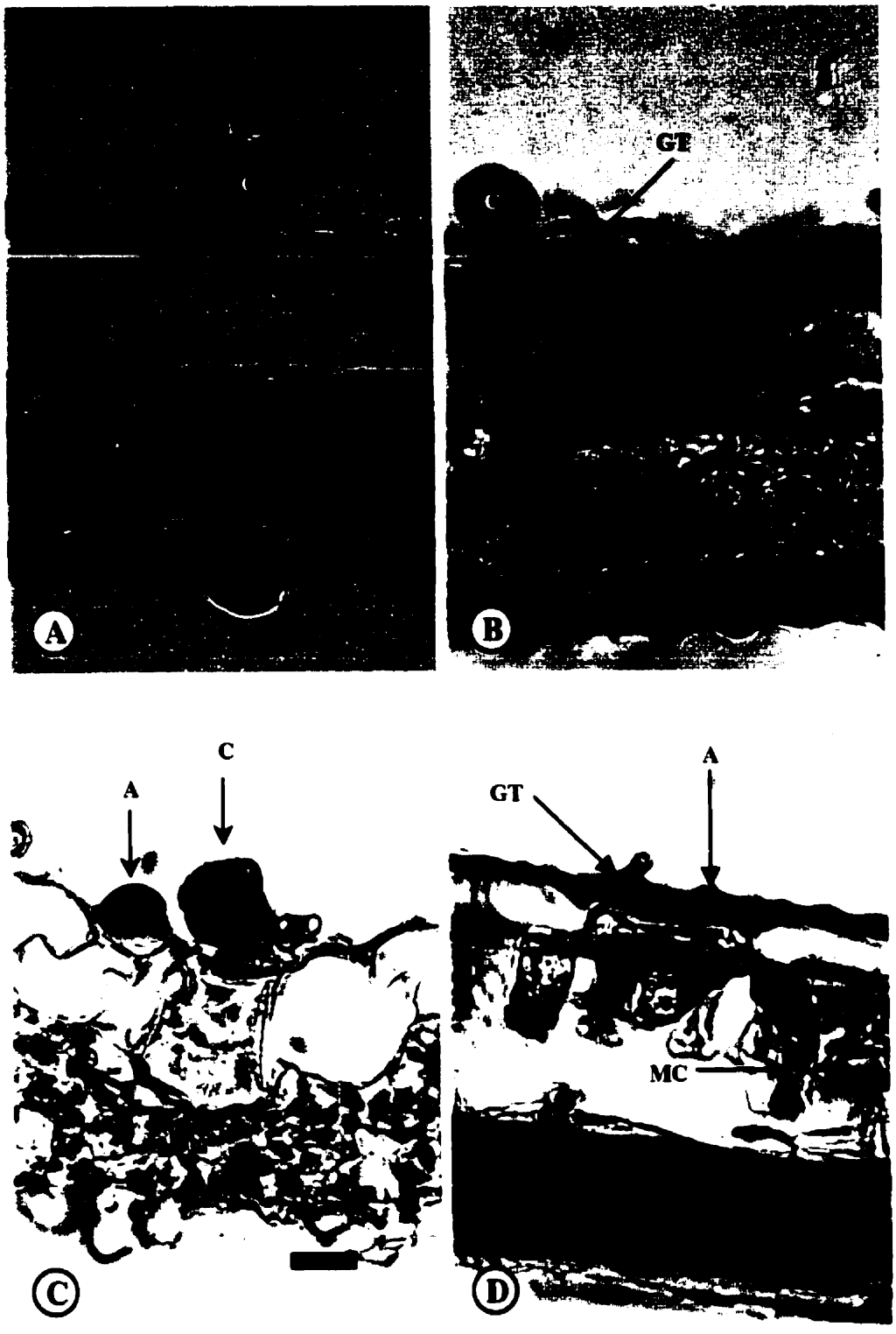
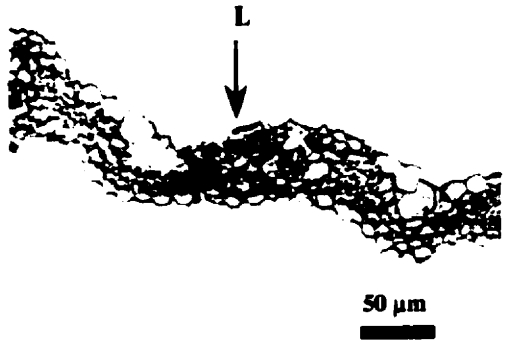
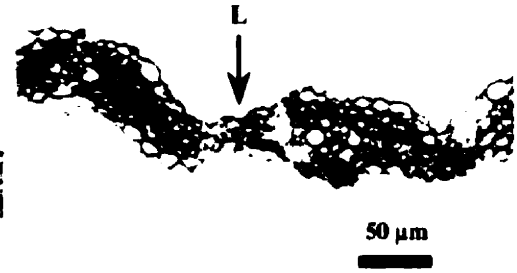

A
GT
B
C
A
A
GT
MC
C
D

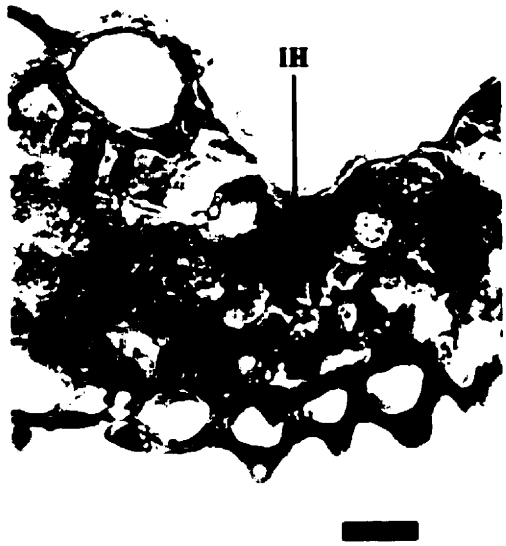
Figure 5.3. Lesion formation in *Oryza sativa* cv. Norin 21 by *Curvularia tuberculata* (93-020, A; and 93-022, B, C, and D), 168 hours post inoculation (HPI). **A, B.** Restricted lesion (L) formation on the leaf blade at 168 HPI. **C.** High magnification view of leaf spot in C showing sparse growth of infection hyphae (IH). **D.** Fungal growth (IH) in C was bordered by light yellow to yellow-stained areas in the leaf. Bar = 10 μm except A and B.



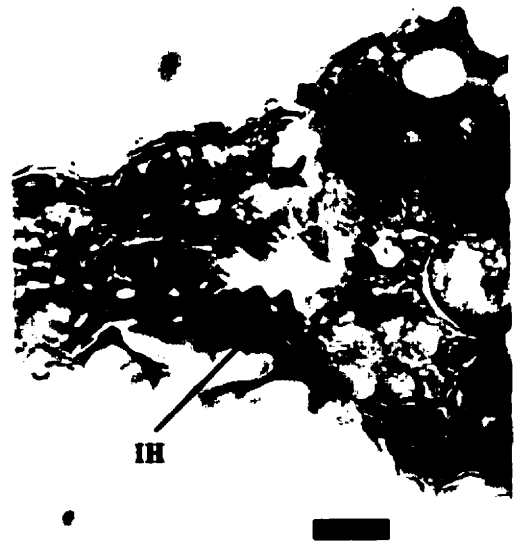
A



B

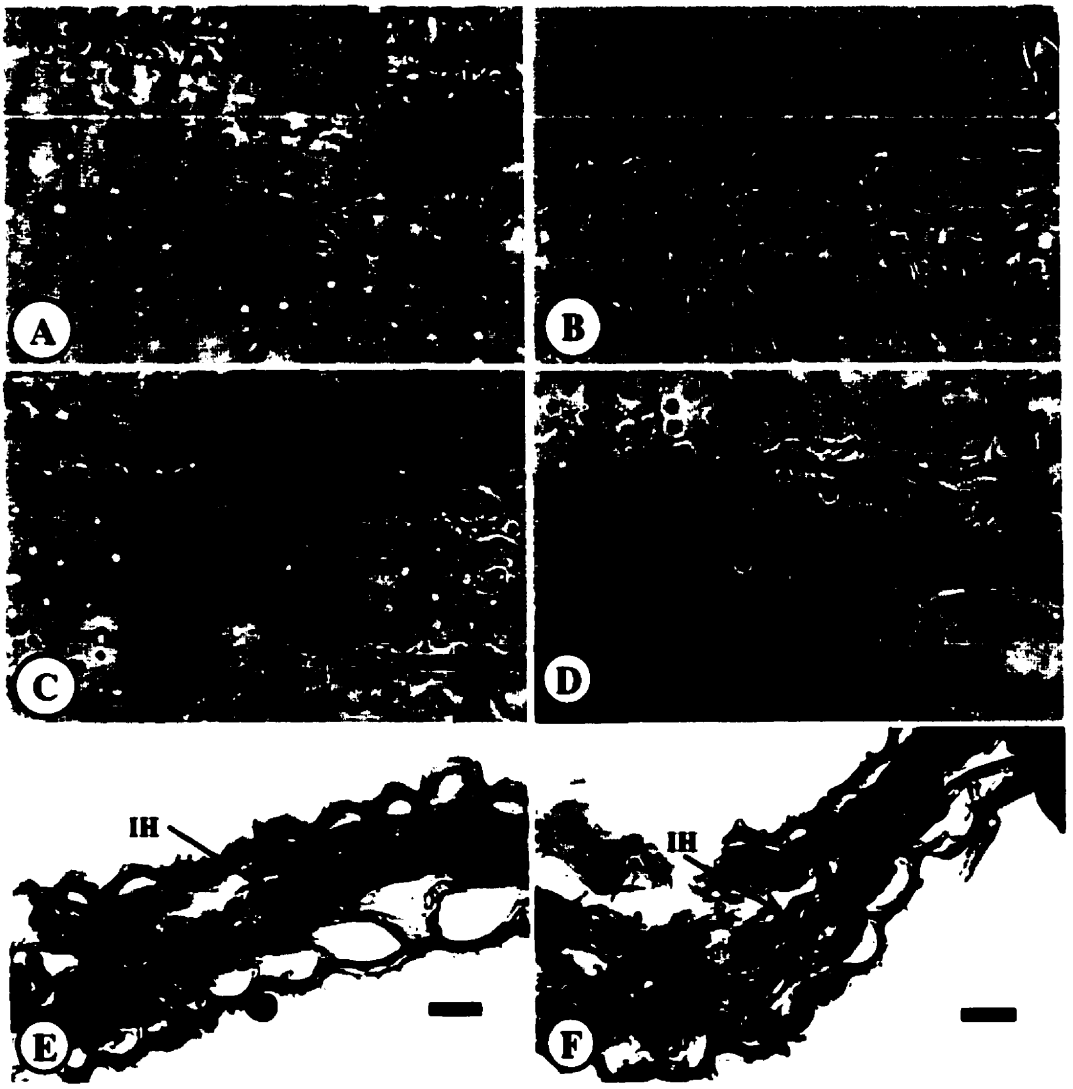


C



D

Figure 5.4. Infection of *Oryza sativa* cv. IR 64 by *Curvularia oryzae* (93-061), 24 to 144 hours post inoculation (HPI). **A.** Formation of an appressorium (A) over a stomata (ST), 24 HPI. C, conidium; GT, germ tube. **B.** Penetration of a stomata (ST) by a penetration peg (PP) arising from an appressorium (A), 36 HPI. **C.** Appressorium (A) formed on an epidermal wall junction (EWJ). Note yellowing reaction near the appressorium. **D.** Infection hyphae (IH) visible under an epidermal cell exhibiting the yellow reaction, 72 HPI. **E** and **F.** Infection hyphae (IH) in transverse leaf sections 144 HPI. Bar = 10 μm.


A
B
C
D
IH
E
IH
F

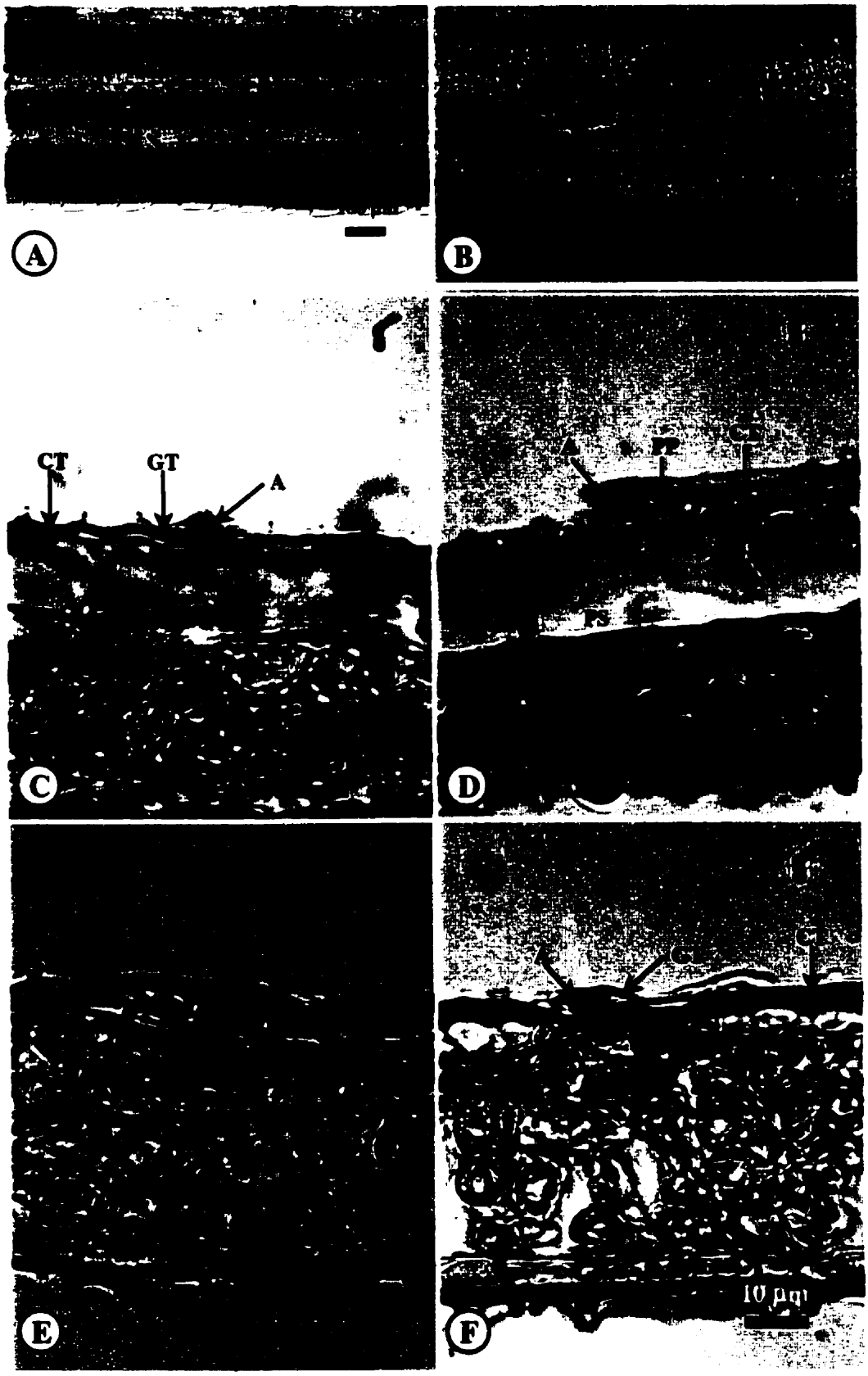

A
B
CT
GT
A
C
D
E
F
10 μm

Figure 5.6. Infection of *Oryza sativa* cv. Norin 21 by *Curvularia oryzae* (93-061), 48 to 168 hours post inoculation (HPI). **A.** Primary infection hyphae (PIH) within an epidermal cell, 48 HPI. **B** and **C.** Infection hyphae (IH) are restricted to a few adjacent cells. **D.** Yellowing reaction (YR) is evident in the infection site (IS) involving adjacent epidermal cells and some mesophyll cells (MC), 72 HPI. **E.** Infected mesophyll cells (MC) underneath conidia (C) and germ tubes (GT), 72 HPI. **F.** Small lesion on the leaf blade, 168 HPI. Bar = 10 μm except F.

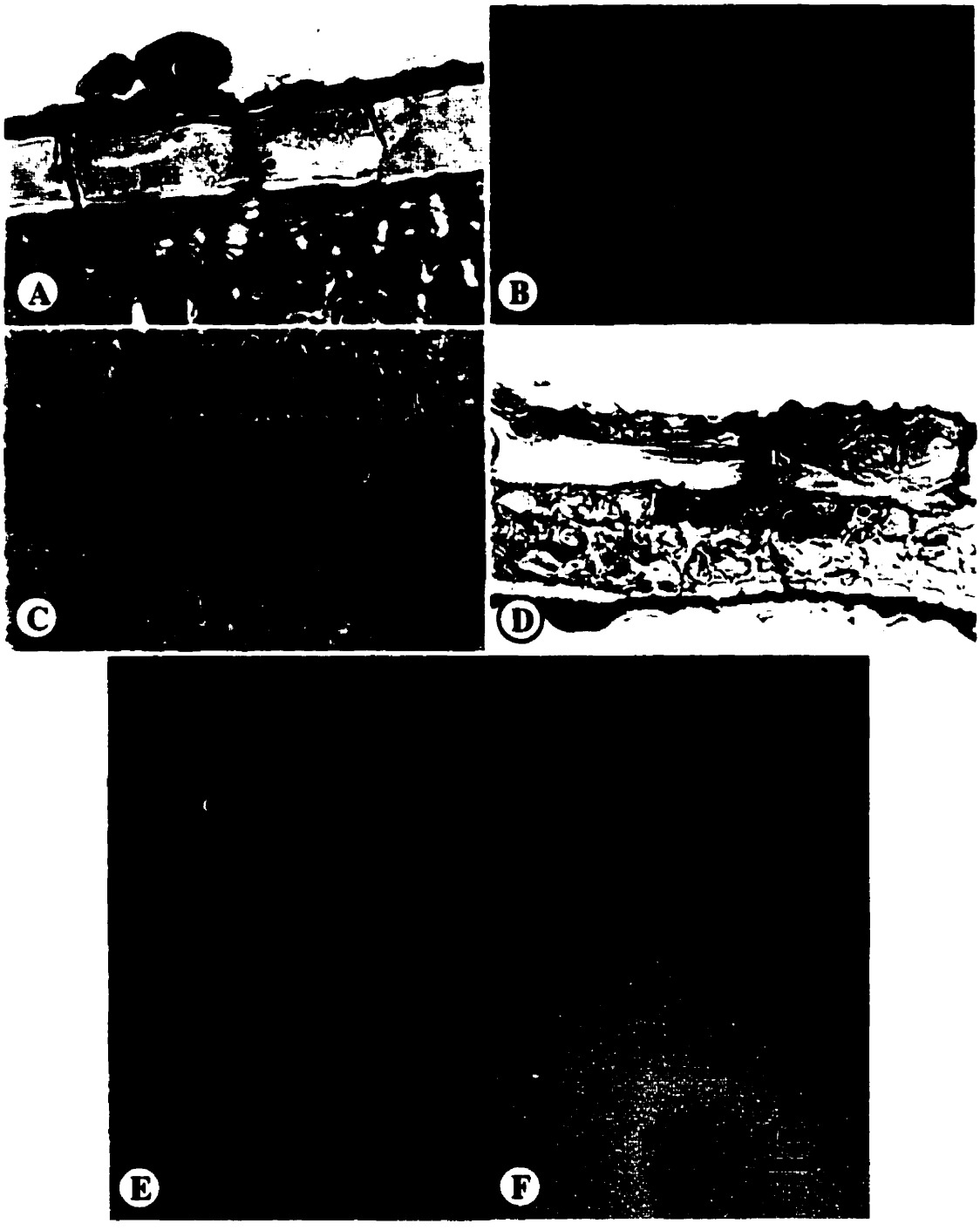

A
B
C
D
E
F

Table 5.1. Reactions of two rice varieties to three *Curvularia* isolates at seven days after inoculation (DAI).*

Isolate	Rice Variety	
	IR 64	**Norin 21**
93-020 (*Curvularia tuberculata*)	MR[2]	HR[1]
93-022 (*Curvularia tuberculata*)	HR	HR
93-061 (*Curvularia oryzae*)	MR	MR

* Seedlings were inoculated with spore suspension at the rate of 1 x 10^8 spores/m^2 and scored for disease severity at seven DAI.

[1] Highly resistant; few to many brown specks, 0.5 to 1.0 mm in diameter, grayish-green to light brown leaf tips and margins

[2] Moderately resistant; discrete brown lesions with or without chlorotic halo, 1.0 to 2.0 mm long, brown leaf tips and margins.

Chapter 6. General Conclusions

This study investigated the pathogenicity of three isolates of *Curvularia* from the Philippines on sedge weeds and rice (*Oryza sativa*). *Cyperus difformis*, *C. iria*, *C. rotundus*, and *Fimbristylis miliacea* are serious weeds of rice in Southeast Asia that have been targeted as candidates for biological control.

Two isolates of *Curvularia tuberculata* (isolate 93-020 and 93-022) and one isolate of *C. oryzae* (isolate 93-061) were virulent on their sedge hosts and were shown to have potential to control these weeds based on high mortality and dry weight reduction. Inoculations using spore suspensions at the rate of 1×10^8 spores/m^2 produced leaf spots and coalescing lesions that developed into a rapid blighting reaction. Weed seedlings were killed within one to two weeks after spraying.

This study also demonstrated that the three fungal isolates are cross-pathogenic to three closely related weeds. *C. difformis* and *C. iria* were highly susceptible to three fungal isolates while *F. miliacea* was moderately susceptible to the *C. tuberculata* isolates and highly susceptible to *C. oryzae*. A biocontrol agent that is virulent on these three weeds is desirable since they occur together in irrigated rice fields. *C. rotundus*, however, was resistant to both *C. tuberculata* and *C. oryzae*. Although brown flecks and dried leaf tips and margins developed, no plants died as a result of the infection. Perhaps two or more applications of the fungal pathogen might be needed to increase leaf area damage to the point where tuber production is also reduced. Integration of the fungal pathogen with insects or herbicides to control *C. rotundus* also warrants further study.

The thirteen rice varieties included in this study were mostly resistant to the *Curvularia* isolates. Flecking and brown leaf tips and margins occurred but damage was minimal. *C. tuberculata* isolate 93-022 was the least pathogenic to rice.

Compatibility between the fungal isolates and their sedge hosts was clearly established. Histological studies showed that spore germination was high and occurred between two to four hours post inoculation (HPI) under optimum dew period and temperature conditions. Appressoria at tips of germ tubes and/or their branches were initiated on epidermal cells eight hours after inoculation. Thus, a single conidium could be capable of penetrating more than one cell. Complex infection cushions were also formed. Infection hyphae grew intercellularly and intracellularly, attacking epidermal and mesophyll cells but not the vascular bundle. Colonization of host tissues was rapid and conidiophores emerged from stomatal apertures by 96 h.

Both *C. tuberculata* and *C. oryzae* are capable of infecting *C. rotundus* since appressoria and infection cushions were formed over epidermal cells and penetration occurred through penetration pegs. However, there was a delay in infection structure initiation and fungal growth was restricted and slow, producing small fungal colonies. No sporulation was observed in *C. rotundus*.

Rice varieties were also penetrated by appressoria of *C. tuberculata* and *C. oryzae* but they were formed over the stomatal aperture. No infection cushions were formed. Resistance was expressed mainly at the post-penetration stage indicated by slow fungal growth and lack of sporulation.

Chapter 7. Contributions to Knowledge

The following are considered to be key contributions to knowledge arising from the research described in this thesis:

1. Results from this research indicate two isolates of *Curvularia tuberculata* and one isolate of *Curvularia oryzae* have potential to control *Cyperus difformis*, *Cyperus iria*, and *Fimbristylis miliacea* in rice.
2. Cross-pathogenicity of the three *Curvularia* isolates to three other sedge weeds was demonstrated. However, *Cyperus rotundus* was resistant to infection.
3. Most of the rice seedling varieties included in this study were resistant to *C. tuberculata* and *C. oryzae*. Flecking on older, inoculated leaves occurred but damage was minor and sporulation was not observed.
4. Findings in this study indicate that *C. tuberculata* isolate 93-022 was safe to rice. A broad host range study is needed to accurately delimit host specificity of this candidate biocontrol agent.
5. This is the first study to describe, in detail, the infection process of *C. tuberculata* and *C. oryzae*.