Lethal Yellowing of Java Citronella (*Cymbopogon winterianus*) Caused by *Pythium aphanidermatum*

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ABSTRACT

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Pythium aphanidermatum was the predominant fungus recovered from the roots of Java citronella showing lethal yellowing in the northern part of India. The disease occurred most frequently between July and October. Roots of infected plants showed marked discoloration, and the cortical region was completely disintegrated and sloughed from the vascular tissue. Diseased plants were chlorotic and stunted. Rotting was often found to spread from roots to stem, leading to severe chlorosis and death of the infected plants. The pathogenicity of the fungus was established. The disease is a potential constraint to citronella cultivation in nonarid climates where the crop is irrigated extensively.

Java citronella (*Cymbopogon winterianus* Jowitt) is an aromatic grass cultivated in tropical and subtropical regions with moderately high summer rainfall. Its oil is used widely in soaps, perfumes, and cosmetics and also for the manufacture of synthetic menthol. In recent years, commercial plantations in the northern part of India were found to be adversely affected by severe chlorosis and basal rotting, especially in the rainy seasons. Infected plants were characterized by poor growth, which resulted in the death of large populations of plants in irregular patches. The disease has spread at a rapid rate since its first occurrence and has posed a serious threat to the commercial cultivation of Java citronella in the country.

Pythium aphanidermatum (Edson) Fitzp. has been recognized as the cause of the root rot and chlorosis complex of sugarcane (*Saccharum officinarum* L.) (12), root and basal stalk rot of corn (*Zea mays* L.) (1,6,16), cottony blight of turfgrasses (2,11), and root rot of bentgrass (5), safflower (*Carthamus tinctorius* L.) (10), lentil (*Lens culinaris* Medik.) (3), guayule (*Parthenium argentatum* Gray), (8) and sugar beet (*Beta vulgaris* L.) (14), but there is no report available of its association with lethal yellowing of Java citronella. In this communication we report the occurrence of the disease and the establishment of its cause for the first time.

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MATERIALS AND METHODS

Commercial plantations of Java citronella in northern India, especially in Uttar Pradesh (U.P.), were surveyed for the evidence of yellowing disease problems between 1987 and 1991. The survey included sites at the Central Institute of Medicinal and Aromatic Plants (CIMAP) in Lucknow, the plain area of U.P., and the CIMAP regional center in Pantnagar, the *tarai* region of U.P. In Lucknow, five commercial fields per year in different locations in the surrounding of CIMAP were observed for disease incidence at 15-day intervals from July to October during the period of 1987–1991. In Pantnagar, 10 commercial fields per year in the CIMAP regional center were surveyed once each September from 1989 to 1991. Disease incidence was based on the population of infected plants showing characteristic symptoms of the disease from 10 rows of 100 plants in each field. The average percentages of yellowing disease in the living population and of mortality of the total population from each site were determined every year.

Ten sample infected plants showing chlorosis were dug along with root systems from each commercial field of Java citronella, and 55 representative samples from different fields were brought to the laboratory in polyethylene bags. Their roots were washed thoroughly in running tap water and examined under a microscope for colonization by the pathogen. Infected roots were cut into small pieces (1–2 cm long), washed gently in distilled water, surface-disinfected in 1% sodium hypochlorite for 30 sec, rinsed twice in sterile distilled water, blotted dry on sterile filter paper, and then plated onto medium 1 (containing Difco corn meal agar [CMA] plus 30 mg ml^{-1} rifamycin Na salt, 10 μg ml^{-1} benomyl, and 10 μg ml^{-1} pimaricin) and medium 2 (containing CMA plus 10 μg ml^{-1} of benomyl and 100 μg ml^{-1} streptomycin sulfate). After 48 hr at 25 C, hyphal tips of colonies of *Pythium* species growing from the infected root tissues were transferred to CMA. All isolates of the pathogen were maintained on CMA slants under mineral oil at 25 C.

Cultural and morphological characters of the pathogen were studied as described by Middleton (7), Waterhouse (15), and Van Der Plaats-Niterink (13). The isolates of the pathogen were grown on CMA in petri plates for 2 wk at 30 C. The shape, attachment, and size of oogonia, antheridia, and oospores of the fungus were determined from specimens mounted on glass slides in lactophenol cotton blue.

Pathogenicity of *P. aphanidermatum* isolates (five isolates each year from 1987 to 1991) was tested on Java citronella under glasshouse conditions on 1-mo-old plants. Greenhouse plants were grown from healthy slips in sterilized soil in 30-cm earthen pots. Additionally, pathogenicity was tested on axenic citronella plantlets raised in tubes of MS medium (9) through tissue culture techniques from callus and on axenic plants transplanted to 8-cm earthen pots filled with a 1:1 mixture of sterilized soil and vermiculite.

Freshly harvested 2-wk-old cultures grown on CMA were used for the preparation of inoculum. Mycelial mats were rinsed twice with distilled water and homogenized for 10 sec with 20 ml of distilled water in a blender. Inoculum (10 ml per pot) was applied to exposed roots around the base of each plant in pot experiments, whereas plantlets growing in test tubes (25 × 100 mm) were treated with 1 ml of inoculum. Plants treated with distilled water served as a control. In each experiment, ten replicates were used, and experiments were repeated three times. Inoculated and control plants were observed periodically for the appearance of disease symptoms.

RESULTS

Survey of the disease. The surveys carried out during 5 yr (1987–1991) showed widespread occurrence of the yellowing disease in the northern part of India, especially in the areas having excessive rainfall or irrigation (Table 1). The incidence of the disease in commercial Java citronella fields occurred during the month of July, depending on the onset of monsoon, and appeared in epiphytotic form during the months of August and September (Fig. 1). In Lucknow during the last week of July, incidence was 10–18%. Infection spread rapidly, and 78–88% of the plant population in the survey fields showed yellowing disease by the end of the 2nd wk of September during 1988–1991. Thereafter, incidence decreased to 31–42% by the end of October (Fig. 1). In Pantnagar, incidence of the disease was comparatively low, showing 18–21% infection (Table 1) during the 2nd wk of September during 1989–1991.

Percentage of mortality in commercial plantations of Java citronella was low (5–6%) at the onset of epidemics but had increased by the end of October during 1987–1991 (Fig. 1). Thereafter, infected plants survived and recovered from yellowing symptoms.

Isolation of the pathogen. Isolations from the infected roots on medium 1 or 2 consistently yielded a species of *Pythium*. The fungus produced white, cottony, aerial mycelium and abundant oogonia, antheridia, and oospores in culture. Structure and morphology of oospores on agar were identical to those observed in infected roots. The pathogen could not be isolated from healthy plants.

Identification of the pathogen. The pathogen showed a "chrysanthemum" pattern of growth in culture. The hyphae were hyaline and produced strongly inflated sporangia of varying length and width. Zoospores were liberated from sporangia under wet conditions. The production of sporangia or hyphal swelling was stimulated by treatment with 5 mM $Ca(NO_3)_2$ as used for *Phytophthora fragariae* C. J. Hickman by Kennedy et al (4). Protoplasmic streaming was clearly visible in young hyphae. The oogonia were terminal, globose, smooth, 21–24 μm (average 23 μm) in diameter, and attached with a straight stalk. Antheridia (1–2 per oogonia) were mostly intercalary, 10–14 μm long and 10-14 μm wide, and diclinous. Oospores were aplerotic and 19–23 μm (average 21 μm) in diameter and had walls 1–2 μm thick. Given cultural as well as morphological studies, the pathogen was identified as *Pythium aphanidermatum*. The identification was later confirmed by CAB International Mycological Institute (CABIMI), Kew, England. An isolate (89CyPF$_1$ [S]) of the pathogen has been deposited with CABIMI (IMI no. 335084).

Pathogenicity tests. Plants inoculated with five isolates of *P. aphanidermatum* showed interveinal chlorosis 7–10 days after inoculation. Growth was arrested, followed by complete yellowing of the leaves in the later stage of infection (Fig. 2). Root tips and roots of most of the chlorotic plants were found to be severely damaged, showing marked discoloration with decay and sloughing-off of the cortical tissues (Fig. 3). In severe stages of infection, rotting spread from roots to the stem, resulting in the death of infected plants. All control plants were free of chlorosis (Fig. 2) and root rot (Fig. 3). Reproductive organs such as oogonia, antheridia, and oospores, characteristic of *P. aphanidermatum*, were abundantly associated with infected roots. The pathogen was reisolated from the inoculated plants.

Axenic plants inoculated with the pathogen produced the symptoms of the disease after 3–4 days of incubation, and infection was pronounced on the roots. The roots turned markedly discolored and became pulpy. The cortical region was completely dissolved and sloughed off from the vascular region. Roots, when macerated, yielded a large number of oospores. Roots of all control plants were free of infection.

Table 1. Incidence of yellowing disease and mortality of Java citronella caused by *Pythium aphanidermatum* in two locations (Lucknow and Pantnagar) in northern India during the 2nd wk of September, 1987–1991

	CIMAP,[a] Lucknow		CIMAP-RC,[b] Pantnagar	
Year	Yellowing (%)	Mortality (%)	Yellowing (%)	Mortality (%)
1987	40.0 ± 1.7[c]	10.2 ± 0.7	...	...
1988	79.0 ± 1.2	23.0 ± 1.4	...	...
1989	78.0 ± 1.2	21.0 ± 1.0	18.0 ± 0.7	10.0 ± 1.8
1990	85.5 ± 1.7	16.0 ± 1.7	19.0 ± 1.6	13.0 ± 0.7
1991	88.0 ± 1.6	12.0 ± 1.1	21.0 ± 0.8	9.0 ± 0.6

[a] Central Institute of Medicinal and Aromatic Plants.
[b] CIMAP regional center.
[c] Values are the means (± standard error) of the disease incidence occurring in five (Lucknow) and 10 (Pantnagar) commercial Java citronella fields.

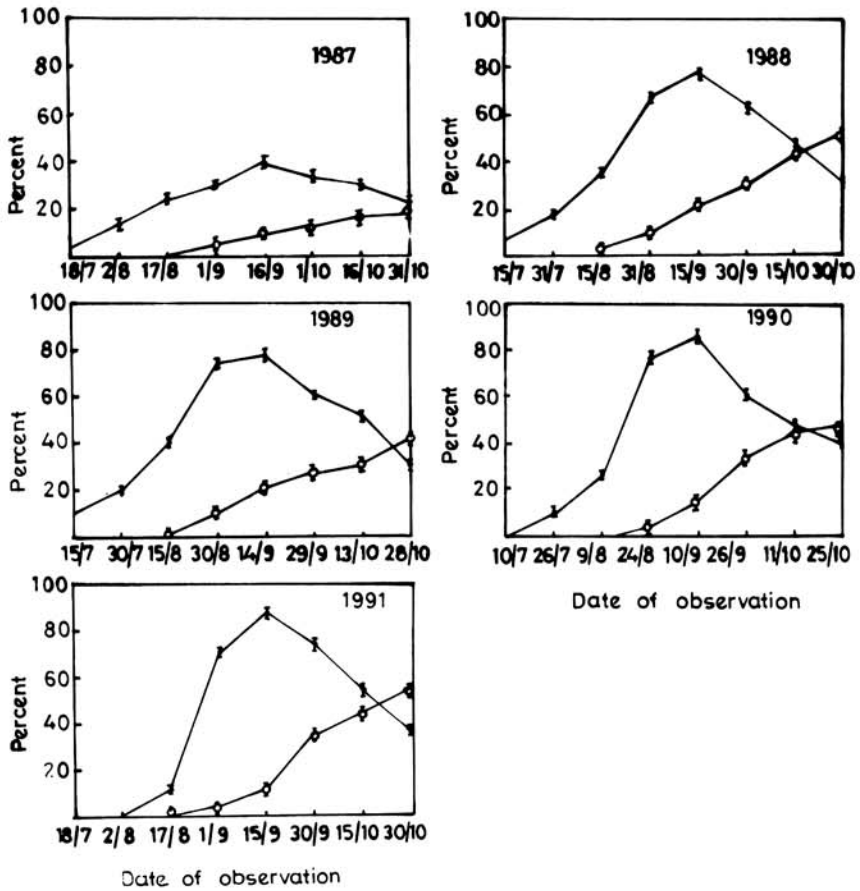

Fig. 1. Mean percentage of yellowing (•) and mortality (○) of Java citronella caused by *Pythium aphanidermatum* in five commercial fields at 15-day intervals from July to October during 1987–1991 in Lucknow, India. Bars represent ± standard error of the mean.

Fig. 2. Pathogenicity of *Pythium aphanidermatum* on greenhouse-grown Java citronella plants. Healthy control plants (left) and diseased plants (right) showing symptoms of chlorosis 15 days after inoculation with *P. aphanidermatum*.

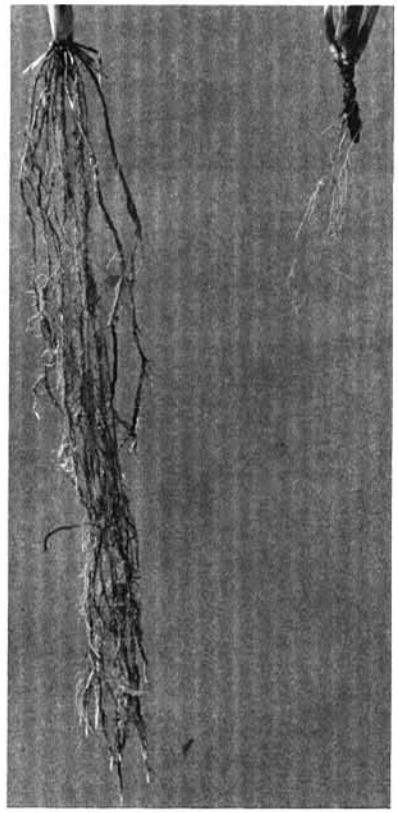

Fig. 3. Roots of uninoculated (left) and *Pythium aphanidermatum*-inoculated (right) Java citronella plants.

DISCUSSION

Commercial plantations of Java citronella were severely affected by the lethal yellowing disease. Although incidence of the disease was 40% in 1987, it increased to affect 85–88% of the plants during the last 2 yr. *P. aphanidermatum* was consistently associated with the roots of infected plants showing chlorotic symptoms. Pathogenicity tests on greenhouse as well as on axenic Java citronella plants show that lethal yellowing disease of Java citronella is caused by *P. aphanidermatum*. The disease is widespread in northern India because it has appeared in epiphytotic form in the plains (Lucknow) and *tarai* regions (Pantnagar) of Uttar Pradesh. The yellowing symptom occurs in July, depending on the onset of monsoon, and reaches a maximum by the end of the 2nd wk of September. Thereafter, disease appearance decreases considerably because plants with mild infection recover from the chlorosis. At the time of high incidence of the yellowing, percentage of mortality was comparatively low, but it gradually increased, and 31–42% of the total population died by the end of October. *P. aphanidermatum* probably spread from roots to stems, leading to death of infected plants. The high incidence of the disease in low areas and poorly drained soil during the rainy season indicate that abundant moisture favors infection. In initial stages, the pathogen attacks the young succulent roots, causing severe damage to the root system. As a result, nutrient uptake is probably hampered, leading to chlorosis. This is the first report implicating *P. aphanidermatum* as the causal organism of the lethal yellowing of Java citronella.

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