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Black Streak of Edible Burdock Caused by *Itersonilia perplexans* in Japan

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ABSTRACT

Black streak disease of edible burdock (*Arctium lappa* L.) has been observed periodically in Hokkaido Prefecture, Japan since 1988. Symptoms appeared initially as small, dark brown to black spots on the leaf veins and petioles. The necrotic spots developed longitudinally along the leaf veins or petioles. Diseased leaf veins or petioles occasionally snapped off at the necrotic lesions. An *Itersonilia* sp. was isolated from rotting leaf veins and petioles. Laboratory inoculations of edible burdock seedlings using ballistospore suspensions produced typical symptoms observed in nature. The fungus had a feathery mycelium and developed a white to pale cream colony color. The mycelium was composed primarily of branched hyphae with clamp connections at the septa. Ballistospores, formed at the apex of inflated cells, were lunate, ovoid to pyriform. The fungus occasionally produced appressoria, chlamydospores and yeast cells. Based on the morphological characteristics, the causal agent was identified as *Itersonilia perplexans* Derx. Edible burdock strains were also pathogenic to chrysanthemum and caused petal blight. This report is the first of a foliar disease of edible burdock caused by *I. perplexans* in Japan.

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Key words : edible burdock, black streak, *Itersonilia perplexans*.

INTRODUCTION

Edible burdock (*Arctium lappa* L.), which is a member of the family Asteraceae, is an important vegetable crop in Japan. Edible burdock is grown from April to October in Hokkaido Prefecture and is famous in Japan for its high quality.

Black streak disease of edible burdock was first described in 1988[17], has been observed periodically, and is a serious problem for growers in Hokkaido. The disease generally occurs in vigorously growing edible burdocks with high yield potentials and can result in total yield loss in severely infested fields. Initial symptoms are observed as necrotic spots of leaf veins and/or petioles. These spots develop into necrotic streaks along leaf veins or petioles.

The fungus isolated from black streak lesions produced a white to cream colony in culture and had clamp connections. The fungus was pathogenic to edible burdock and was isolated from inoculated plants[17]. However, the taxonomic status of the causal fungus has thus far not been investigated in detail. From 1994 to 1996, many attempts were made to identify the causal agent of black streak disease, which was similar to the previously described fungus[17] and constantly isolated from black streak lesions.

The objectives of this study were to identify the causal fungus of black streak of edible burdock, describe symptoms of the disease, and determine the pathogenicity and host range of the causal fungus. A preliminary report on black streak of edible burdock has been published[8].

MATERIALS AND METHODS

Isolation of the causal fungus Infected plants of edible burdock with black streak symptoms were collected from commercial fields in Kuriyama-cho and Memuro-cho, Hokkaido from 1994 to 1996. The causal fungus was recovered from symptomatic petioles or veins by the following procedure. Small pieces of diseased tissue were removed from the margins of infected lesions, placed in a solution of 1% sodium hypochlorite for 1 min, rinsed twice in sterile distilled water, and placed on potato dextrose agar (PDA) containing streptomycin sulfate (100 mg per litter). Mycelial blocks from PDA were transferred to PDA and incubated at 15°C for 3 weeks. A small amount of a ballistospore suspension from PDA cultures was used to streak on yeast malt agar

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(YMA) plates and germinating ballistospores were then selected. Single spore isolates were maintained at 10°C on YMA until use.

Cultural and morphological characteristics To determine radial growth rates of the isolates of the causal fungus, a 5-mm plug was taken from the edge of an actively growing colony and transferred to a plate of YMA. Inoculated plates were incubated in the dark at the desired temperatures. The diameter of each colony was measured 3 and 14 days after inoculation. The rate of increase in colony radius was determined as the difference in colony size between 3 and 14 days after inoculation. There were three replicate plates per treatment, and each experiment was repeated twice. Morphological characters, including size of ballistospores and chlamydospores were observed for cultures grown for 2–3 weeks on YMA at 15°C. The size of yeast cells was also measured on cultures grown for 1 week on yeast-peptone-malt agar (YPMA) at 20°C. Forty spores or yeast cells of each isolate were measured.

Pathogenicity and host range Pathogenicity of isolates of the causal fungus was tested on leaves and roots of edible burdock and on flowers of chrysanthemum. Seeds of edible burdock cv. Yanagawarisou were sown in black plastic flats (25×50×4.3 cm) filled with commercial potting soil (Sankyo-pottace, Hokkai-sankyo Inc., Hokkaido, Japan). After sowing, the soil was tamped down and watered until saturated. Seeded trays were placed in a greenhouse. Healthy seedlings were selected 14 days after sowing and transplanted (three plants per pot) into clay pots (15 cm diameter×12 cm high) filled with commercial potting soil. Pots were watered and placed in a greenhouse until inoculation.

Ballistospores of each isolate were harvested by flooding the YMA plates with sterile distilled water, scraping the plates with a sterile paintbrush and filtering the suspension through double layers of sterile cheesecloth. Ballistospores were counted with a hemacytometer and adjusted to approximately 1×10^4 spores/ml.

Seedlings of edible burdock at the 3- or 4-true-leaf stage were inoculated with a ballistospore suspension of each isolate using a sterile glass sprayer. Inoculated plants were put into a plastic bag and placed in a controlled chamber at 15°C. Plants sprayed with sterile distilled water served as controls.

Pathogenicity of representative isolates (IB9602 and Blme951) on edible burdock roots was also tested. Roots, cut transversely into a 10-cm length, were inoculated with mycelial plugs (5-mm diameter, 3 plugs per root) of each isolate growing on YMA plates. Roots inoculated with YMA plugs served as controls. The experiment was conducted twice with three replicates.

To test the pathogenicity of the isolates on flowers of chrysanthemum (*Chrysanthemum morifolium*), three detached, mature flowers of chrysanthemum cv. Start were used for each isolate. Pedicels of three flowers were placed in a 300-ml flask filled with 100 ml sterile distilled water and sprayed with a ballistospore suspension (1×10^4 spore/ml). Inoculated flowers were put into plastic bags and incubated in a controlled environment chamber at 15°C with a 12-h photoperiod. Flowers sprayed with sterile distilled water served as controls.

Twenty-one crops listed in Table 3 were used to test the host range of representative isolates of causal fungus and reference isolates of *Itersonilia perplexans*. The pathogenicity of each fungus was tested by inoculating leaves of each crop, except chrysanthemum. On chrysanthemum, pathogenicity of each fungus was tested by inoculating leaves and detached flowers. A total of 5 or 6 plants of each crop were inoculated with a ballistospore suspension (1×10^4 spore/ml) of the fungus in sterile distilled water. Plants sprayed with sterile distilled water served as controls. Inoculated plants were put into plastic bags and placed in a controlled chamber at 15°C. Disease incidence was evaluated 14 days after inoculation. Each experiment was repeated twice.

Table 1. Strains of *Itersonilia* spp. used in this study

Strain	Host plant	Locality	Year isolated	Other designation
Test isolates				
Blme941-1, Blme944-2, Blme943	Edible burdock	Hokkaido, Japan	1994	
Blme951, Blme952	Edible burdock	Hokkaido, Japan	1995	
IB9601, IB9602, IB9603, IB9604, IB9606 IB9607, IB9608, IB9609, IB9610	Edible burdock	Hokkaido, Japan	1996	
Itersonilia perplexans (reference isolates)				
IFO31350	Air	Netherlands	—	CBS 144.68, ATCC 36404
IFO31655 (*Itersonilia pastinacae*)	Parsnip	U.K.	1960	CBS 356.64, ATCC 36403, IMI 092075
IFO31656 (*Itersonilia pyriformans*)	*Acer macrophyllium*	U.S.A.	1948	CBS 286.50, ATCC 15496

Table 2. Dimensions of ballistospores, chlamydospores and yeast cells of *Itersonilia perplexans*

Structure[a]	Isolate measurements (μm)[b]							
	Edible burdock isolate						*I. perplexans*	
	IB9601	IB9602	IB9604	Blme941-1	Blme943	Blme951	IFO31350	IFO31655
Ballistospore								
Length	10.4-15.4	12.1-19.4	9.9-18.7	10.5-14.7	10.8-18.2	12.0-21.6	9.2-23.6	14.7-22.1
Width	7.2-11.1	7.4-15.0	7.0-11.1	7.0-10.6	7.6-12.0	9.0-13.4	7.4-13.6	8.7-14.1
Chlamydospore								
Diameter	±[c]	—	—	—	9.9-15.1	—	—	11.0-18.5
Yeast cell								
Length	9.7-22.6	—	7.1-12.6	8.2-25.5	—	—	—	—
Width	5.4-9.8	—	3.5-7.1	4.9-8.9	—	—	—	—

a) Structures were taken from 2-3 week old yeast-malt agar plates (ballistospores and chlamydospores) or 1 week old yeast-peptone-malt agar (yeast).
b) For each isolate, 40 spores were measured.
c) ± : rarely production, — : no production.

Disease symptoms on inoculated leaves or roots of edible burdock and flowers of chrysanthemum were evaluated 14 days after inoculation. The causal organism was re-isolated from necrotic lesions on YMA using the spore fall method described by Derx[6]. Diseased fragments were attached with cellophane tape onto the inside of lids of plastic petri dishes and incubated at 15°C over YMA for 4 days.

RESULTS

Symptoms

The symptoms of black streak on edible burdock in the field were observed 2 or 3 months after sowing and characterized initially as small, dark brown to black spots on leaf veins and petioles (Plate I-A). The necrotic spots developed longitudinally along the veins and petioles, giving the appearance of black streaks. Dark brown or black streaks, surrounded by pale yellow halos, were observed on infected leaves. Larger necrotic spots occasionally formed in the centers or on the margins of lamina of young leaves. In severe cases, diseased leaf veins or petioles snapped off at the necrotic lesions (Plate I-B).

Isolation and identification of the causal fungus

Itersonilia sp. was consistently isolated from edible burdock samples with typical black streak symptoms collected from commercial fields in Hokkaido. Fungal isolates used in this study are listed in Table 1. All 14 isolates produced a feathery mycelium and developed a white to pale cream color on YMA. The mycelium was composed primarily of branched hyphae with clamp connections at the septa (Plate I-C). Hyphae were hyaline and 2-5 μm wide and terminated in inflated sporogenous cells with clamp connections at the base. Sporogenous cells were thin-walled, pyriform, ovoid to subglobose,

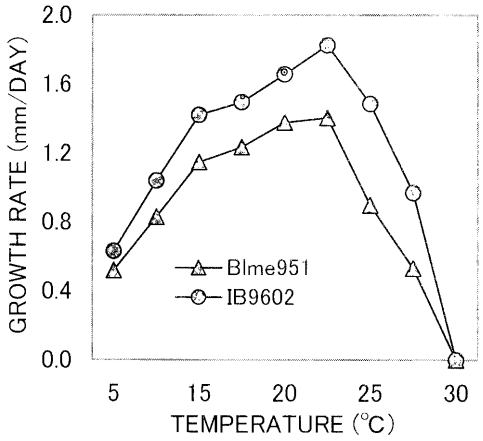

Fig. 1. Growth rates of *Itersonilia perplexans* isolates on yeast malt agar at various temperatures.

14.7-19.1×10.1-13.3 μm. These cells germinated to form hyphae or attenuated germ tubes on which ballistospores formed. Ballistospores were lunate, ovoid to pyriform, and subglobose, 9.9-21.6×7.0-15.0 μm and germinated immediately either with hyphae, secondary ballistospores or appressoria (Table 2, Plate I-D). Appressoria were light brown to brown, irregular, crenate, and lobed, and formed from germinating ballistospores on a hard surface such as a glass slide or plastic petri dish. Edible burdock isolates rarely formed chlamydospores on YMA. When present, chlamydospores were terminal or intercalary, globose to subglobose, and thick walled. Only isolate Blme943 produced abundant chlamydospores, 11.8-15.2×9.9-14.2 μm (Table 2, Plate I-E). Yeast cells spontaneously formed in isolate IB9601, IB9604 and Blme941-1 when grown on YPMA and measured 7.1-25.5×3.5-9.8 μm (Plate I-F). Yeast colonies were smooth, dull to shiny, white to pale cream.

Table 3. Host range of edible burdock and reference isolates of *Itersonilia perplexans*

Test plants	Cultivar	Edible burdock isolate		*I. perplexans*			Control
		IB9602	Blme951	IFO31650	IFO31656	IFO31655	
Seedlings							
Asteraceae							
Arctium lappa	Yanagawarisou	+(BS)[a)]	+(BS)	−	−	−	−
Bellis perennis	Super Sibelius	−	−	−	−	−	−
Calendula officinalis	Orange delight	−	−	−	−	−	−
Carthamus tinctorius	Maruba-syu	−	−	−	−	−	−
Centaurea moschata	—	−	−	−	−	−	−
Chrysathemum coronarium	Chyuyou-syungiku	−	−	−	−	−	−
Chrysanthemum morifolium	Start	−	−	−	−	−	−
Cosmos bipinnatus	Versailles	−	−	−	−	−	−
Cynara scolymus	Green globe	±(BS)	±(BS)	−	−	−	−
Dahlia variabilis	Dapper	−	−	−	−	−	−
Gaillardia pulchella	Red plume	−	−	−	−	−	−
Helianthus annuus	Luna	−	−	−	−	−	−
Lactuca sativa	M-Wrap-231	−	−	−	−	−	−
Rudbeckia sp.	Indian summer	−	−	−	−	−	−
Zinnia elegans	Royal purple	−	−	−	−	−	−
Umbelliferae							
Apium graveolens	Cornel 619	−	−	−	−	−	−
Daucus carota	Kouyou no.2	−	−	−	−	−	−
Coriandrum sativum	—	−	−	−	−	−	−
Cryptotaenia japonica	Kanto-mituba	−	−	−	−	−	−
Pastinaca sativa	Hollow crown	−	−	−	−	−	−
Petroselinum crispum	Paramount	−	−	−	−	−	−
Bloom							
Asteraceae							
Chrysanthemum morifolium	Start	+(PB)	+(PB)	+(PB)	−	+(PB)	−

a) Disease severity was assessed 14 days after inoculation. + : plant diseased, ± : slightly diseased, − : no diseased, BS : black streak symptoms, PB : petal blight symptoms.

Yeast cells occasionally developed monokaryotic hyphae with pseudoclamps. The morphology of ballistospores and chlamydospores of the isolates from edible burdock coincided with that of the reference isolates of *I. perplexans* examined.

Average values for growth rates of representative isolates used in temperature tests are indicated in Fig. 1. Both isolates had maximum growth at 22.5°C and no growth at 30°C.

Pathogenicity and host range

Fourteen edible burdock isolates (Table 1) were used to inoculate seedlings of edible burdock. All isolates tested caused typical symptoms—numerous black streak lesions similar to those on diseased plants observed in the field (Plate I-G). Water-inoculated control plants did not have any symptoms. Nor were disease symptoms observed on edible burdock roots inoculated with mycelium disks of the isolates. In tests of pathogenicity on chrysanthemum petals, all edible burdock isolates also caused petal blight. These symptoms developed 5 days after inoculation as small, brown to red brown spots (Plate I-H). Water-inoculated control blossoms did not have any symptoms of disease.

In tests on host range, edible burdock leaves and chrysanthemum petals were infected by edible burdock isolates (Table 3). Artichoke (*Cynara scolymus*) leaves were slightly infected (approximately 25%) and had symptoms similar to black streak on edible burdock leaves. Other plants had no disease symptoms. Reference isolates (IFO31650 and IFO31656) infected chrysanthemum petals, but no symptoms were observed on other plants. IFO31655 isolate was not pathogenic to any tested plants.

DISCUSSION

Inoculation of greenhouse-grown edible burdock with edible burdock isolates resulted in symptoms typical of black streak in the field. The inoculated plants developed small necrotic spots on leaf veins or petioles that pro-

gressed to necrotic streaks. The fungus was re-isolated consistently from the diseased leaves. The fungus was identified as *Itersonilia perplexans* Derx based on the morphology of hyphae with clamp connections, ballistospores, chlamydospores and yeast cells. These results fulfill Koch's postulates for *I. perplexans* as a pathogen of mature leaves of edible burdock for the first time in Japan. We propose that this new disease should be called black streak of edible burdock ('Kurosuzibyo' in Japanese).

This report is the first of black streak of edible burdock caused by *I. perplexans*. The disease was limited in location to Hokkaido Prefecture in northern Japan. In retrospect, the disease was observed in edible burdock fields at the Tokachi district in eastern Hokkaido approximately 15 years before and each year thereafter. However, the causal agent was not positively identified during that time, although the isolates having hyphae with clamp connection and producing lunate conidia were observed[17].

I. pyriformans and *I. pastinacae* were listed as different species from *I. perplexans* based on morphological studies[3,12]. However, many mycologists considered these species as synonyms of *I. perplexans* based on further observation of morphology, nutritional physiology, mating between *Itersonilia* species and DNA homology[1,2,13,16,18]. The fungus, isolated from edible burdock in Hokkaido, closely matches the description of *I. perplexans* in cultural and morphological characteristics[2,7]. However, formation of yeast cells and chlamydospores differed greatly among the isolates studied. Three isolates (IB9601, IB9604 and Blme941-1) spontaneously formed yeast colonies when grown on YPMA. Other isolates rarely developed a yeast phase. Two isolates (IB9601 and Blme943) formed chlamydospores on YMA. The Blme-943 isolate in particular formed abundant chlamydospores.

I. perplexans is reported as a petal blight pathogen on various plants, including: artichoke[5], china aster (*Callistephus chinensis*)[9], chrysanthemum[10,11], dahlia (*Dahlia pinnata*)[11] and sunflower (*Helianthus annuus*)[15]. *I. perplexans* has also been reported to cause lesions on cotyledons and first true leaves and seedling rot in sunflowers[14]. While *I. perplexans* can be considered a saprobe or weak pathogen, this study demonstrated that the edible burdock isolates were primary pathogens and can cause significant damage.

When edible burdock isolates of *I. perplexans* were used to inoculate seedlings of other crops, symptoms on mature leaves were not observed on other hosts except artichoke. Typical black streaks similar to edible burdock symptoms were observed on artichoke after inoculation with edible burdock isolates, but they occurred at low frequency. Infection of artichoke may thus be restricted to laboratory conditions. Thus, on the basis of these inoculation experiments, the edible burdock pathogen seems to have a narrow host range.

Host-specific isolates of *I. perplexans* were also reported on parsnip to cause root canker[3]. The parsnip isolates were pathogenic to roots, leaves, petioles and inflorescences of parsnip[3,4]. From the viewpoint of pathogenicity, edible burdock strains and parsnip strains are unique strains of *I. perplexans*. Both strains may be distinct subgroups within *I. perplexans*, although additional investigations would be necessary to confirm this conclusion.

The disease is initiated when edible burdocks begin to produce a dense canopy. Differences in disease development over the years may be due to planting date and various environmental factors. Although no study has yet examined the epidemiology of black streak of edible burdock, outbreaks of the disease tend to develop at low temperatures during the rainy season. Splashing water and free moisture likely assist in the dissemination of *I. perplexans* ballistospores. Further investigations are needed to answer these questions.

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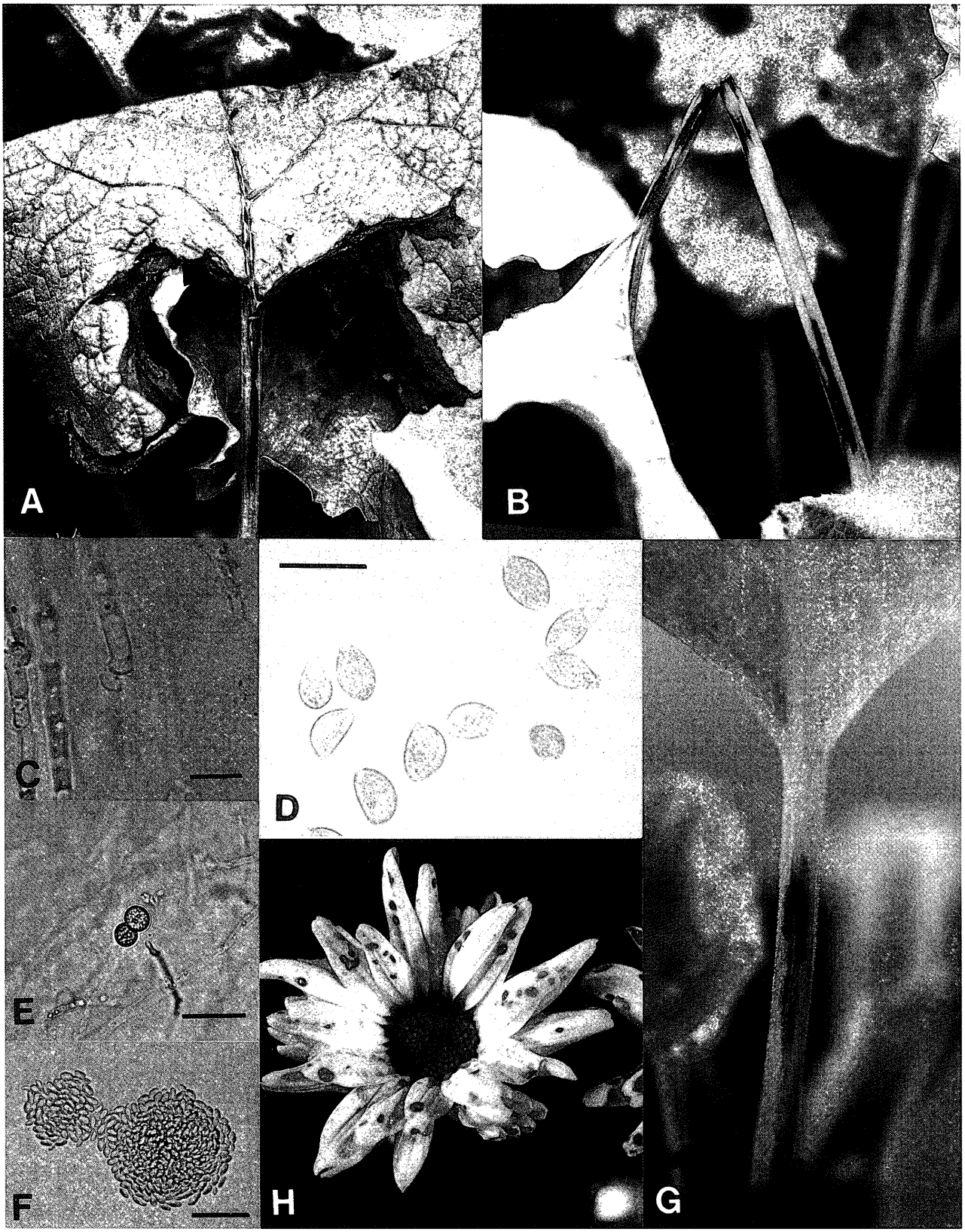
Plate I



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Explanation of plate

Plate I

A. Black streak lesions on edible burdock in natural infection.
B. Diseased petiole of edible burdock snapped off at the necrotic lesions in natural infection.
C. Hyphae with clamp connections at the septa (scale is 10 μm).
D. Ballistospores of *I. perplexans* edible burdock isolate (IB9602) on YMA (scale is 20 μm).
E. Chlamydospores of *I. perplexans* edible burdock isolate (Blme943) on YMA (scale is 30 μm).
F. Yeast cells of *I. perplexans* edible burdock isolate (IB9604) on YPMA (scale is 40 μm).
G. Black streak lesions on petiole of edible burdock after spraying with a ballistospore suspension of *I. perplexans*.
H. Petal blight on flowers of chrysanthemum after spraying with a ballistospore suspension of the edible burdock strain.