Leaf Gall of *Torilis japonica* Caused by *Protomyces macrosporus* in Arkansas

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ABSTRACT

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A leaf gall disease of the cool-season umbelliferous weed, hedge parsley (*Torilis japonica*), was found in northwestern Arkansas during surveys for weed pathogens that might be developed as biological herbicides. The causal fungus is *Protomyces macrosporus*, which is common in Europe, South Asia, and North Africa on many genera in the Umbelliferae. Galls on leaves or stems were cream to golden with swellings 1 mm wide and high and up to 3 mm long. Germination of the resting cells, growth of yeastlike endospores (ascospores) in pure culture, and disease development from controlled inoculation were demonstrated. *P. macrosporus* caused galls on *T. japonica* and *Anethum graveolens* but not on 10 other species of Umbelliferae.

Surveys for weed diseases have been initiated by the Southern Regional Research Project S-136 to identify indigenous pathogens that may have potential as biological herbicides. Since autumn of 1981, a leaf gall of hedge parsley (*Torilis japonica* (Houtt) DC.), a common cool-season umbelliferous weed, has been found at the University of Arkansas Experiment Station Farm at Fayetteville. The pathogen was tentatively identified as a species of *Protomyces*. A study was undertaken to confirm its identity and to make a preliminary evaluation of its potential as a mycoherbicide.

DESCRIPTION

Symptoms. Blisterlike galls that average about 1 mm in diameter occur on the leaves and stems but primarily on petioles, midribs, veins, and veinlets of leaves (Fig. 1A). Galls are circular on leaflets. They are most common on the lower leaves, beginning as translucent, cream-colored swellings that become opaque and light yellow to golden with age. Galls are most prominent on petioles and are elongate (3 mm long) along the axis of the petioles. They coalesce in heavily infected tissue, causing distorted petioles and leaf blades that become chlorotic and prematurely senescent.

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Pathogen. Cross sections of galls revealed numerous round, thick-walled resting fungal cells (30–60 μm in diameter) borne intercalarily on intercellular mycelia scattered within hypertrophied and hyperplastic tissues (Fig. 1B). Resting cells were multinucleate and smooth-walled. Walls appeared gold to light brown in transmitted light. Resting cells did not germinate when teased from host tissue and held in distilled water at 20 C for as long as 4 wk. After storage in host tissue at 4 C or at −20 C for 4 mo or more and immersion in distilled water at 20 C, resting cells germinated within 1–5 days by protrusion of a vesicle to form a spore sac (Fig. 1C), which contained multiple spores. These are considered ascospores within an ascus originating from the resting ascogenous cell. Morphology of the pathogen and its occurrence on an umbelliferous host justify its assignment to the class Hemiascomycetes, order Protomycetales, and family Protomycetaceae (2,3). The pathogen from *Torilis* was assigned to the genus *Protomyces*, and by convention (3), to the species *P. macrosporus* Unger because of its occurrence on an umbelliferous host.

The endospores were germinable and produced in a yeastlike state on either solid or liquid media. Colonies were pink on potato-dextrose agar or yeast-malt extract agar at 20 C. Cells increased by unipolar budding and were variable in shape, from ovoid-ellipsoid to cylindrical (Fig. 1D). Their dimensions were 4–10 × 3–6 μm. Endospores were also produced in abundance in Richards' V-8 medium (1) on a rotary shaker (150 rpm) at 18 C within 7–10 days. Cultures on solid media were viable after 1 yr at 4 C.

Disease development. Symptoms developed abundantly on inoculated seedlings of *T. japonica* in a greenhouse. Seedlings were inoculated when 3–5 wk old (5–10 cm high, four- to six-leaf stage) by spraying to runoff with washed suspensions of endospores at a concentration of 5×10^6 spores per milliliter. Inoculated plants were held for 48 hr in a dew chamber at 100% RH at 20 C, then placed in a greenhouse at 16–24 C. Symptoms first appeared within 10–15 days as small white flecks that increased to blisterlike galls within 20–30 days. Affected tissue was distorted and chlorotic, but tissue that emerged from the crown of the plants after inoculation was not noticeably affected in size, shape, or color.

Similar inoculations were made to seedlings of 11 other umbelliferous plant species, with inoculated plants of *T. japonica* as controls. The only other plant to be infected was dill (*Anethum graveolens* L). Plants that did not become infected were celery (*Apium graveolens* L.), caraway (*Carum carvi* L.), coriander (*Coriandrum sativum* L.), *Chervil* sp., carrot (*Daucus carota* L.), fennel (*Foeniculum vulgare* Mill.), pennywort (*Hydrocotyle verticillata* Thunb.), parsnip (*Pastinaca sativa* L.), and anise (*Pimpinella anisum* L.). Cultures of four species of *Protomyces*, three from composites and one from an umbelliferous host, were supplied by C. P. Kurtzman, USDA Northern Regional Research Center, Peoria, IL: *P. inouyei* Hennings from *Crepis japonica*, *P. lactucae-debilis* Sawada from *Lactuca debilis*, *P. pachydermus* Thuemen from *Taraxacum platycarpum*, and *P. inundatus* Dangeard from *Apium* sp. None of these infected *T. japonica* under our conditions.

DISCUSSION

Identification of species in the Protomycetaceae is complicated by their simple morphology. Historically, they have been identified to genus on the basis of size of resting cells and their arrangement in the host tissues. Assignment to species has been based on presumed host specialization. The *Protomyces* from *Torilis* was found to be specific for two unreported hosts of *P. macrosporus*. It is not possible to obtain cultures of *P. macrosporus* in this country for inoculation to *Torilis*. The fungus isolated from *Torilis* was assigned to *P. macrosporus* because it fit all the

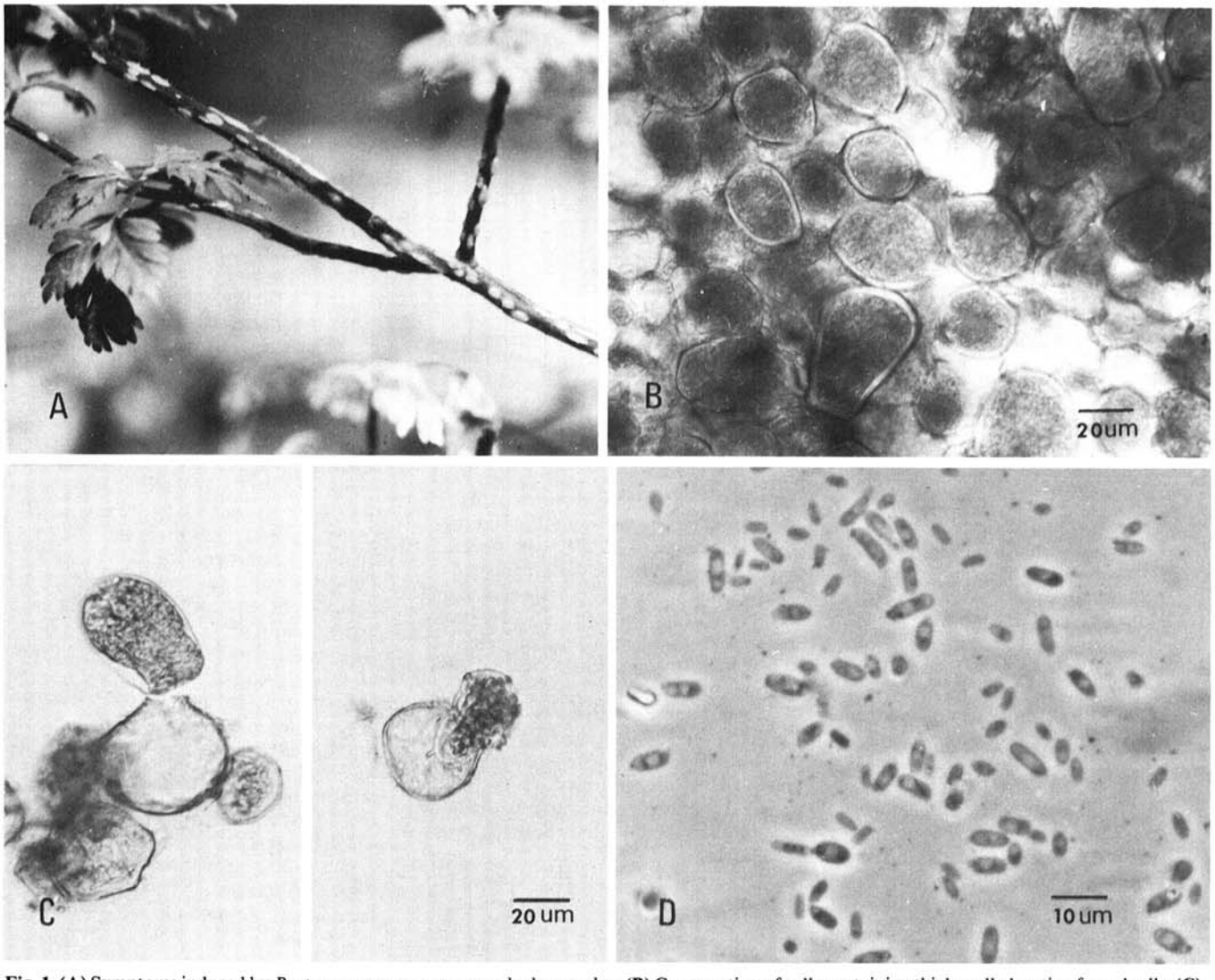

Fig. 1. (A) Symptoms induced by *Protomyces macrosporus* on hedge parsley. **(B)** Cross section of galls containing thick-walled resting fungal cells. **(C)** Germinating resting cell. **(D)** Budding ascospores.

morphological requirements, including germination of the resting cell and unipolar budding (2,5) and because strains of *P. macrosporus* are known that are specific for certain hosts (3). Until a more definitive study of host specialization in this group is conducted, designation of a forma specialis does not seem warranted. One previous record of *P. macrosporus* in North America is the report of it on spotted cowbane (*Cicuta maculata* L.) in Wisconsin (4).

The potential for development of *P. macrosporus* as a mycoherbicide is not promising. A heavy spore dose of the pathogen did not damage the host severely enough to kill it or to seriously suppress emergence of new host tissues. Furthermore, the fungus did not spread to emerging healthy tissue. The latter constraint could perhaps be overcome by repeated applications if the economic importance of the weed warranted it and the pathogen could infect and damage the host over a sufficiently wide environmental range. Selection of more virulent strains from nature would not seem likely from this limited experience, but the yeastlike growth and simple nuclear cycle may make it a good prospect for improving strain virulence by genetic engineering.

Cultures have been deposited in the Northern Regional Research Laboratory, Peoria, IL (NRRL-Y-12, 879), the American Type Culture Collection, Rockville, MD (ATCC-5, 6196), and the University of Georgia Culture Collection (GA). Specimens were deposited in the Millar Herbarium of the University of Georgia, Athens (GA).

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