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Stem Canker of Cat-Tail Willow Caused by *Botryosphaeria dothidea* in Taiwan

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ABSTRACT

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Cat-tail willow (*Salix gracilistyla*) has become popular as cut flower for house decoration during Chinese new year in Taiwan in recent years. As a consequence, the acreage of cultivation has expanded to more than 200 hectares, primarily in Ilan County. In a field survey, stem canker was found to be a very common disease on cat-tail willow. Infected twigs were dark brown at first and typical symptoms appeared as small, oval reddish brown lesions each with a deep long fissure in the center. Canker developed and the stem died in the later stage of the disease development. Lesions varied in size ranging from 28-30 ×6 mm to 60-65×6 mm. Numerous small black fruiting bodies were present in the canker. They were pycnidia and perithecia of the causal agent, *Botryosphaeria dothidea*. Pycnidia were globose to conical and ostiolate. Conidia were fusiform, elliptical and hyaline, 14.5-26.3 ×6.5-8.5 μm. Each ascus contained eight ellipsoid, hyaline ascospores, 19.2-33.3×8.2-14.8 μm. On PDA plate, pycnidia fully matured 7 days after spore germination. Conidia formed in the pycnidia were infectious upon artificial inoculation. *Botryosphaeria dothidea* is first reported as the causal agent of stem canker of cat-tail willow.

Key words: Cat-tail willow, *Salix gracilistyla*, stem canker, *Botryosphaeria dothidea*, new disease

Cat-tail willow (*Salix gracilistyla*) was introduced to Taiwan for cut flower in 1963. The areas of cultivation were located primarily in San Hsin of Ilan County. In late August, 1996 and 1997, We conducted a field survey at San Hsin, and found stem canker to be very common among cat-tail willow plants. The disease incidence ranged from 34 to 65%. Infected twigs initially appeared as small, oval, reddish brown lesions each with a deep long fissure in the center (Fig. 1 and 2). At the later stage, cankers enlarged and the stem died eventually. Cankers of diseased twigs contained numerous small black fruiting bodies (ascostroma) (Fig. 3). Pycnidia and perithecia were on diseased twigs which were closely resembled those produced by *Botryosphaeria dothidea* which causes gummosis of peach trees (*Prunus persica*), fruit ring rot of pear (*Pyrus pyrifolia* cv. *hensan*) and stem blight of blueberry (*Vaccinium corymbosum* cv. *homebell*) [7.8.9].

For isolation, small pieces of infected tissues (0.3×0.3 cm^2) were surface-sterilized in 0.5% sodium hypochlorite for 1 min then rinsed in sterilized water, and dried on sterilized filter paper, before being placed on acidified Difco potato-dextrose agar plate (APDA) (2.5ml of a 25% solution of lactic acid per liter of medium). All dishes were incubated at 25 ±1 ℃.

Single-spore cultures of *B. dothidea* isolated from diseased cat-tail willow twigs were used for pathogenicity tests, morphological studies and observation of pycnidial development. Mycelial-agar discs and spore suspensions of *B. dothidea* containing approximately 10^5 conidia per ml were used for inoculation. To prepare the mycelial-agar discs, fungal isolates were grown on PDA plates for 3 days, and mycelial discs were cut from the margins of actively growing cultures with a 5-mm sterilized cork borer. Conidial suspensions were made from cultures grown on Difco PDA slants under continuous light for 8 days. Cat-tail willow twigs were wounded by a shallow, tiny cut on epidermis made by a sterilized scalpel and inoculated with a 5-mm mycelial-agar discs or spore suspension. Inoculated areas on twigs were covered with moist cotton. The entire twig was wrapped with a plastic bag immediately after inoculation. Six replications for each treatment were used. Symptoms as described above were observed. Positive results were obtained consistently when wounded twigs were inoculated with mycelial discs or with conidial suspensions 14 days after inoculation. However, all inoculation attempts with mycelium discs or with conidial suspension on nonwounded twigs failed to result in infection.

Control twigs remained healthy throughout the experiment. Reisolation from inoculated diseased twigs consistently yielded pure culture of *B. dothidea*, thus fulfilling the Koch's postulates.

Indentification of *B. dothidea* was based on morphological characteristics of conidia of anamorph phase from disease twigs of cat-tail willow and cultures isolated from peach tree with gummosis [7], pear with ring rot [8], diseased grape cluster [1], and blueberry with stem blight [9]. Ascospores from infected twigs were also examined. The pycnidia on diseased twigs were black or dark brown. Conidia were hyaline, single cell, spindle-shaped (Fig. 4), on naturally infected cat-tail willow twigs, 18.8-29.0×6.2-7.3 μm in size. The size of conidia produced on the PDA plate was 14.5-26.3×6.5-8.5 μm. Ascocarps on diseased twigs were usually black (Fig. 5). Asci, interspersed among filiform paraphyses, were clavate or cylindrical, hyaline, bitunicate, 80.7-126.3×12.0-18.80 μm in size (Fig. 6). Ascospores are hyaline, spindle-shaped or fusoid, 19.2-33.3×8.2-14.8 μm in size (Fig. 6).

Spore suspension of *B. dothidea* at a concentration of 10^5 spores/ml were evenly spread on the PDA plate. Upon spore germination, mycelium mass was collected daily from the plate surface and immediately mounted with cryomatrix and frozen sectioned with a cryo-microtome (Bright-model OTF, England). Anatomical details of pycnidium and conidial development were observed and photographed through 5 μm thickness of serial cryo-sections.

After spore germination, rapid hyphae growth occurred during the first 16-20 hours, hyphae growth rate slowed down thereafter and hyphae increased in branching subsequently. The aerial hyphae aggregated and then formed a pycnidium initial on the second day after the spore germination (Fig. 7). On the 3rd day, the pycnidium initial aggregated tightly, and mycelia at the periphery of pycnidium initial began to appear brown in color (Fig. 8). On the 4th day, young conidia developed from the locule located within the pycnidium initial (Fig. 9). Ostiole was not visible on the 5th (Fig. 10) and 6th day (Fig. 11). Pycnidium matured, ostiole developed fully and outward release of conidial mass began on the 7th day (Fig. 12).

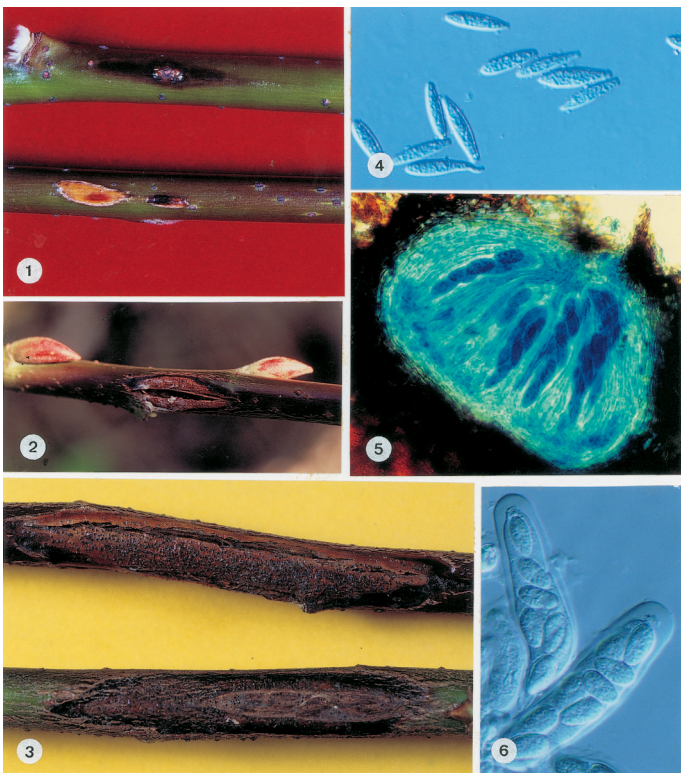

Fig. 1-3. Symptoms of stem canker on cat-tail willow caused by *Botryosphaeria dothidea* : (1) stem canker on infected twigs ; (2) lesion of stem canker with a deep long fissure ; (3) fruiting bodies on diseased twigs.
Fig. 4. Conidia of *Botryosphaeria dothidea*.
Fig. 5. Perithecium of *Botryosphaeria dothidea* on a diseased twig.
Fig. 6. Asci and ascospores of *Botryosphaeria dothidea*.

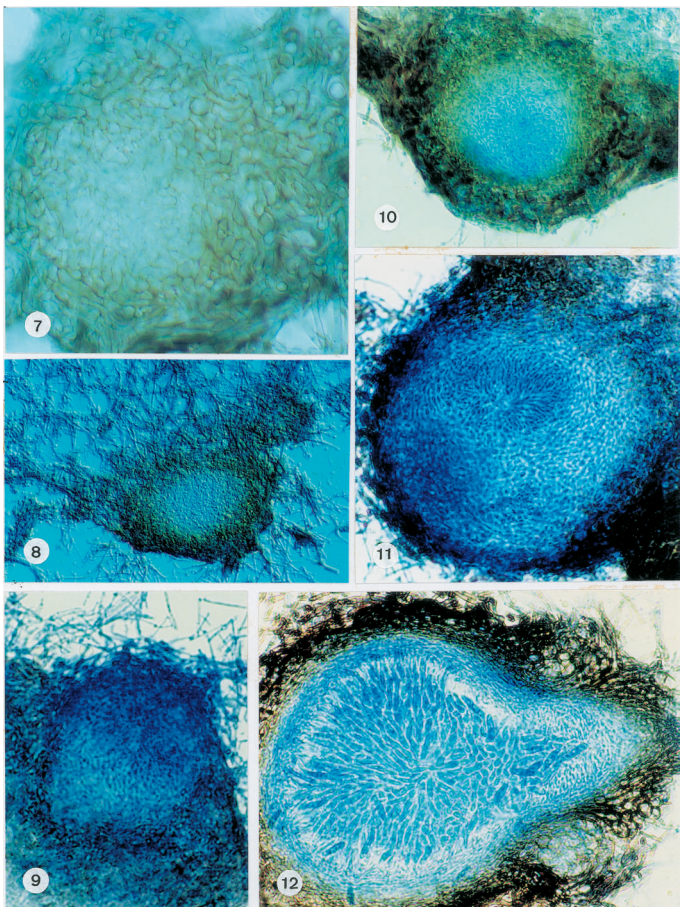

Fig. 7-12. The development of pycnidium of *Botryosphaeria dothidea* : (7) aerial hyphae aggregated and formed pycnidial initial 2 days after spore germination ; (8) Mycelia at the periphery of the pycnidial initial began to appear brown in color 3 days after spore germination ; (9) young conidia developed in a locule within pycnidial initial 4 days after spore germination ; (10) and (11) young pycnidium 5 and 6 days after spore germination , respectively ; (12) pycnidium matured 7 days after spore germination.

Botryosphaeria dothidea was previously reported as the causal agent of gummosis disease on peach, fruit ring rot on pear and stem blight on blueberry in Taiwan [7,8,9]. Both teleomorph and anamorph phases of *B. dothidea* have been described on peach, pear and blueberry in Taiwan [7,8,9].

The genus *Botryosphaeria* was established by de Cesati and de Notaris in 1863. They listed 9 species, including *B. dothidea* [3]. In 1911, Grossenbacher and Duggar [6] gave a detailed description of a fungus causing cane blight of currant, and named it *Botryosphaeria ribis*. In 1954, von Arx and Muller [2] reclassified the amerosporous Pyrenomycetes, and considered *B. ribis* synonymous with *B. dothidea*. In 1966, *B. dothidea* was found on almond (*Prunus dulcis*) in California causing a bandlike canker on the trunk or scaffolds of vigorous young trees [4]. The anamorph and teleomorph phases of *B. dothidea* have been reported on giant sequoia (*Sequoiadendron giganteum*) and coast red woods (*Sequoia sempervirens*) in California [18].

Botryosphaeria dothidea has been reported to cause fruit rot of several plants including apple, pear, citrus, grape, avocado, tung and pistachio [1,5,8,10,11,13,14]. The fungus also has been reported to cause stem blight of many plants in the temperate and tropical regions throughout the world [1,5,10,12,13,15,16,17]. To our best knowledge, this is the first report of *B. dothidea* as the causal agent of stem canker of cat-tail willow.

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摘 要

柯勇[1,3]、孫守恭[1]、藍啓倩[2]. 2003.台灣栽培銀柳之枝潰瘍.植病會刊12:269-272. ([1.] 台中市國光路250號國立中興大學植物病理學系，[2.] 屏東市行政院農業委員會高雄區農業改良場；[3.] 聯絡作者，電子郵件：yeko@dragon.nchu.edu.tw；傳眞：+886-4-22853007)。

銀柳 (*Salix gracilistyla*) 又稱貓柳，1963 年引入台灣栽培作爲切花材料，頗受歡迎，由於栽培面積漸增，病害問題相繼發生，其中枝潰瘍普遍發生且嚴重。本病害主要危害枝條，枝條上早期呈現赤褐色圓形小病斑，略爲凹陷，漸發展成紡綞形，病斑大小不一，從28-30×6 mm 到60-65×6 mm，病斑上密生凸起小黑點，爲病原菌之柄子殼和子囊殼。後期病斑出現縱裂，四周皮層與病斑組織有剝離現象。從罹病枝條潰瘍處進行病原菌之分離、培養，並依柯霍氏法則測定病原菌之病原性後，證明係*Botryosphaeria dothidea* 引起之枝潰瘍病。病菌之柄子殼扁球形，黑色，柄孢子無色，單孢紡綞形，大小14.5-26.3×6.5-8.5 μm，子囊殼內著生棍棒狀子囊，每個子囊內含 8 個無色、單胞的子囊孢子。單一胞子在PDA 平板培養基上培養7 天後，可形成柄子殼，分生孢子供接種後也呈現相同的病徵。本報告爲首次報導在台灣由*B. dothidea* 引起之銀柳枝潰瘍。

關鍵詞：銀柳、*Salix gracilistyla*、枝潰瘍、*Botryosphaeria dothidea*、新病害