Australasian Plant Disease Notes (2019) 14: 16
https://doi.org/10.1007/s13314-019-0347-4

First record of *Phytophthora drechsleri* on *Gynura formosana*

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Received: 15 March 2019 / Accepted: 19 May 2019 / Published online: 27 May 2019

Abstract
In October 2018, collar rots were observed on seedlings of *Gynura formosana* in Taiwan. The causal agent was identified as *Phytophthora drechsleri* based on the morphological and molecular characteristics. Pathogenicity tests demonstrated that the isolate is pathogenic to *G. formosana* and *G. bicolor*. To our knowledge, this is the first report of *G. formosana* as a host of *P. drechsleri*.

Keywords *Phytophthora drechsleri* · Oomycetes · Medicinal plant · Vegetables · DNA barcodes · Taiwan

Gynura formosana, known as bai-feng-cai, is a herbaceous plant belonging to the Compositae (Asteraceae) family that is native to Taiwan. It is consumed as a vegetable and as a folk medicine by Taiwanese indigenous tribes (Yang and Gao 2011) and in East Asia (Chen et al. 2003; Hou et al. 2005; Ma et al. 2017, 2018). The herb has been proved to possess antioxidant, anti-inflammatory, and potent anticancer activities (Chen et al. 2003; Hou et al. 2005; Lin et al. 2014; Ma et al. 2017, 2018).

There are some reports of *Gynura* diseases caused by various groups of pathogens such as viroids (Gross et al. 1982; García-Arenal et al. 1987), fungi, and oomycetes. The plant pathogenic fungi have been reported to cause rusts (Hiratsuka and Hashioka 1937), powdery mildew (Shen et al. 2015), anthracnose (Wehlburg et al. 1975), leaf spots (Wehlburg et al. 1975; Hsieh and Goh 1990; Nishikawa and Nakashima 2015), and root and stem rots (Wehlburg et al. 1975), whereas the *Gynura*-infecting oomycetes have caused downy mildew (Palmateer et al. 2015), *Pythium* root rots (Wehlburg et al. 1975), and *Phytophthora* blights (Shen et al. 2011). However, these studies do not mention any *G. formosana* diseases caused by the pathogens.

In October 2018, *G. formosana* and *G. bicolor* were cultivated under organic practices at the organic experimental farm (24.004934, 120.534028) of Taichung District Agricultural Research and Extension Station (TCDARES) in Dacun, Changhua County, Taiwan. Two weeks after transplanting, wilt and collar rot symptoms were observed on *G. formosana* (Fig. 1) with an incidence of 9% (300 plants surveyed) while 300 plants of *G. bicolor* investigated in the field were healthy. Brown to black lesions extending on the base of *G. formosana* seedlings became dried. Eventually, the plants were toppled and died. Symptomatic basal stems were collected, dissected, surface-sterilized for 10 s in 0.6% sodium hypochlorite solution, rinsed with sterilized water twice, dried on sterile paper tissues and plated on 2% water agar. White colonies with the same traits resembling *Phytophthora* were consistently isolated and the hyphal tips of one representative colony were further sub-cultured. The isolate was routinely transferred onto potato dextrose agar (PDA) and 5% unclarified V8 vegetable juice agar (V8A). The colony growth patterns were rose-shaped and uniform (Safaiefarahani et al. 2015) on PDA and V8A, respectively (Fig. 2a).

To record the growth rates of the isolate under the temperatures between 28 and 40 °C, Petri dishes (9 cm diam.) containing V8A were inoculated with mycelial plugs (5 mm diam.) excised from the edge of a mycelial colony. The plugs were placed upside down in the center of each plate. After incubating in the dark for 3 and 5 days, the radius of each colony under various temperatures was measured in two perpendicular directions. Three replicates were measured for each temperature. The mean radial growth rates were 10.74, 6.86, 5.00 and 0.08 mm d^{-1} at 28, 34, 36 and 38 °C, respectively (the growth rate at 28 °C was calculated by data derived from 3 days of incubation, the other three were calculated by data derived from 5 days of incubation). No mycelial growth was observed at 40 °C.

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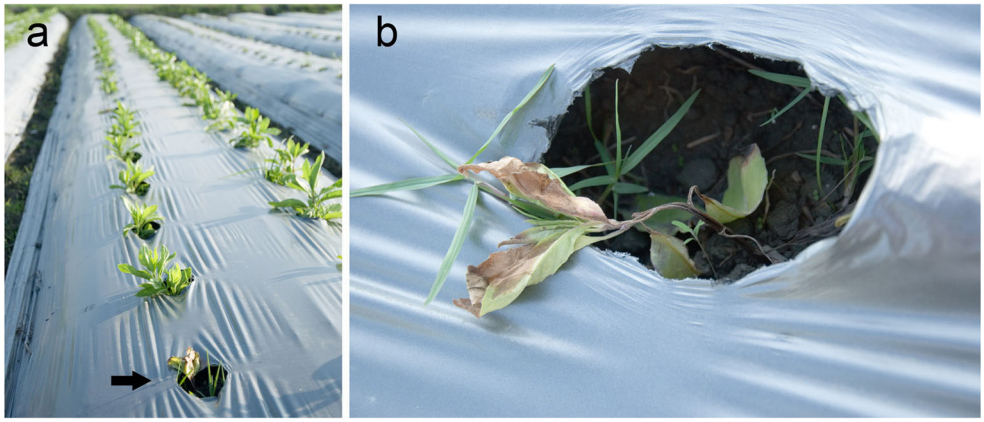

Fig. 1 Symptoms in the field: **a** Bai-feng-cai (*G. formosana*) grown in the organic experimental farm, black arrow indicates a diseased seedling. **b** Wilted seedling with brownish lesions on the dried basal stem

To induce sporangia production and characterize the isolate, the 8-day-old mycelia on V8A were flooded with sterile distilled water under 28 °C for 5 days. The sporangia (Fig. 2b) were noncaducous, nonpapilate, ovoid to obpyriform in shape, with an average of 42.9 μm in length and 27.3 μm in width (ranges from 30.0–62.5 μm long and 20.0–30.0 μm wide; $n = 30$). Hyphal swellings were present but oospores were absent. Oospores were formed in dual cultures with *P. nicotianae* p731 isolate (A2 mating type) (Ann 1996), suggesting that the present isolate belongs to a heterothalic, A1 mating type *Phytophthora* species.

Molecular comparisons were performed for species identification. The genomic DNA was extracted with a Taco Plant DNA/RNA Extraction Kit using Taco™ Nucleic Acid Automatic Extraction System (GeneReach Biotechnology, Taiwan) following the manufacturer's protocol. The internal transcribed spacer (ITS), cytochrome c oxidase subunit 1 (COX) gene fragment, ß-tubulin (TUB) gene fragment, NADH dehydrogenase subunit 1 (NADH1) gene fragment, heat shock protein 90 (HSP90) gene fragment, and a portion of the elicitin (ELI) gene were amplified using primer pairs ITS1/ITS4 (White et al. 1990), COXF4N/COXR4N, TUBUF2/TUBUR1, NADHF1/NADHR1 (Kroon et al. 2004), HSP90_F1int/HSP90_R2 (Blair et al. 2008), and PEX1F/PEX1R (Mostowfizadeh-Ghalamfarsa et al. 2010), respectively. BLAST searches against the GenBank database showed that the sequences of the isolate had close identity with those of *P. drechsleri* Tucker ex-type materials (ATCC46724, CBS 292.35, CH 23J5, and SCRP232) (Kroon et al. 2004; Mostowfizadeh-Ghalamfarsa et al. 2010; Yang and Hong 2018): 99.9% for the ITS, 99.7% for the COX, 99.5% for the TUB, 100% for the NADH1, 99.9% for the HSP90, and 99.0% for the ELI sequences.

Based on the morphological and molecular characteristics, the *Phytophthora* derived from *G. formosana* is identified as *P. drechsleri*. Sequences of the isolate have been submitted to GenBank, accessions MK605947 (ITS), MK609543 (COX), MK609540 (TUB), MK609544 (NADH1), MK609541 (HSP90), and MK609542 (ELI). The isolate has been deposited in Bioresource Collection and Research Center, Taiwan, under the BCRC number CH30305.

Pathogenicity tests were conducted by inoculating *P. drechsleri* BCRC CH30305 on potted *G. formosana* seedlings in a greenhouse in TCDARES. Each of the seedlings (propagated from 8 to 10 cm stem cuttings, with more than 3 leaves) was grown in a 3-in. plastic nursery pot filled with Tref No. 003B horticultural substrate. Three dishes (9 cm diam.) of the mycelia (8-day-old culture, flooded with sterile distilled water for another 2 days) on V8A were scraped, mixed with an additional 10 g of the substrate, and evenly placed at the base of 3 potted plants on the day of propagation. Three plants treated with sterile V8A processed in the same way served as controls. The inoculated and control seedlings were separately placed into a closed transparent plastic box

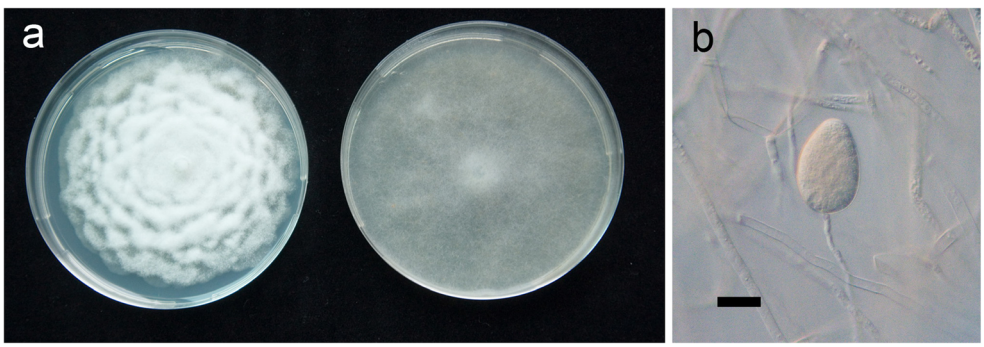

Fig. 2 *Phytophthora drechsleri* BCRC CH30305 isolate **a** Colonies grown on PDA (left) and V8A (right) in 9-cm Petri dishes for 12 days under 28 °C in the dark. **b** A sporangium

and the lid was opened 2 days post inoculation (dpi). Initial symptoms began 5 dpi and all inoculated seedlings showed collar rot and leaf blight 1 week after inoculation (Fig. 3). At the end of the trial (2 weeks), the brownish lesions had extended and the whole plants were deteriorated. The controls remained symptomless. The average temperature was 21.2 °C during the time periods. The pathogen reisolated from the edge of the lesion was identified as *P. drechsleri* based on the morphological and ITS sequence characteristics, fulfilling Koch's postulates. The pathogenicity test was again performed by inoculated the pathogen on *G. formosana* and *G. bicolor* seedlings with approximately the same procedures. All the inoculated plants were symptomatic at 8 dpi while the control plants remained healthy. In the same way, the pathogen was successfully reisolated from artificially inoculated *G. formosana* and *G. bicolor* diseased tissues. The result indicated that the *P. drechsleri* isolated from diseased *G. formosana* was also pathogenic to *G. bicolor*.

This study reports the first occurrence of a *Phytophthora* disease on *G. formosana* in Taiwan. It is demonstrated that the isolated *P. drechsleri* is virulent towards both *G. formosana* and *G. bicolor*. Probably the healthiness conditions of the vegetative propagation materials can give rise to different levels of the disease infection between the two plant species at the early growing stage since the pathogen is able to be introduced through the movement of seedlings (Lamour et al. 2003) or nonsymptomatic plants (Olson and Benson 2013). *Phytophthora drechsleri*, a worldwide distributed pathogen with over 200 reported hosts in 113 genera of 40 plant families (Farr and Rossman 2019), could be specific or nonspecific to the host plants (Olson and Benson 2013). The pathogen can affect different plant parts and may be aggressive to *Gynura* (Shen et al. 2011) and various economic crops (Olson and Benson 2013; Sosa et al. 2015; Farr and Rossman 2019) under favorable conditions for the disease development. To our knowledge, this is the first record of *G. formosana* as a host of *P. drechsleri*.

Fig. 3 A pathogenicity test result at 8 dpi: healthy control plant (left) and inoculated plant (right)

Acknowledgements We thank He, Mei-Ling (TCDARES) for the assistance of laboratory works. We also thank Lin, Chu-Ping and Ann, Pao-Jen (Taiwan Agricultural Research Institute) for providing the *P. nicotianae* isolate.

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