First Report of Phytophthora hydropathica Causing Wilting and Shoot Blight on Bixa orellana Linn. in China

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Annatto (Bixa orellana Linn.) is a shrub or small tree which is best known as the source of achiote, an organic, red-orange extract from the seeds that is commonly used as condiment, food colorant, and cosmetics dye. The extract is also used for its medicinal properties. In summer 2017, a severe wilting of leaves, shoot blight and root rot on all four growing annatto trees was observed in a private garden in Foshan, Guangdong Province. About 30 to 40% of the branches were infected on each tree. Soil and root samples were collected and baited with annatto leaf disks (Ferguson & Jeffers, 1999). Baits were blot dry on paper towels and placed onto PARP-V8 medium (Hong et al. 2010). Three single-zoospore isolates (YZ-1, YZ-2, YZ-3) were randomly selected, and sub-cultured on V8 agar in the dark at 25°C. All isolates had a petaloid colony pattern of growth. All isolates grew very well at the examined temperature ranging from 25 to 35°C, and the optimum temperature was 30°C with growth rates ranging from 7.0 to 8.3 mm per day. The sporangia were almost spherical, ovoid or obpyriform and 45.4-59.5×36.8-42.4 μm (average 53.2×37.6 μm, n=30) in diameter. Chlamydospores with thin walls were terminal and approximately 39.5 μm in diameter. All tested isolates were the A1 mating type. These morphological characteristics were consistent with those described for Phytophthora hydropathica (Hong et al. 2010). Morphological identification was confirmed by sequencing the internal transcribed spacer region (ITS) with the primers ITS1/ITS4 (Vitale et al. 2014); and both the translation elongation factor 1α (EF-1α) with the primers ELONGF1/ELONGR1 and NADH dehydrogenase subunit 1 (NAD1) with the primers NADHF1/NADHR1 (Kroon et al. 2004). BLAST analysis revealed that consensus sequences (GenBank accessions MH321577 for ITS, MH460822 for EF-1α, and MH460823 for NAD1) shared 98 to 99% identity with sequences of P. hydropathica (KF444067, KX252303 and GQ260065, respectively). Pathogenicity assessment was performed by inoculating one representative isolate (YZ-1) of P. hydropathica on ten surface-sterilized leaves which
were detached from a 4-year-old annatto plant. Mycelial plugs of a 4-day-old YZ-1 culture were placed at a distance of 3 cm on the upper part of the same leaf, one plug on the intact epidermis, the other on epidermis previously wounded with a sterile needle. Sterile V8 plugs were used as controls. Inoculated leaves were kept at 28 ± 2°C with high (~95%) humidity and in the dark. After 3 days, all the inoculated points showed blight and rot symptoms consistent with those observed in field infection, whereas controls remained disease-free. An oomycete was re-isolated from symptomatic leaves and exhibited identical morphological characteristics described for *P. hydropathica* fulfilling Koch’s postulates. *P. hydropathica* can also attack several other plants, such as azaleas, mountain laurel, cucumber (Hong et al. 2010), alder (Pintos et al. 2016), Laurustinus (Vitale et al. 2014). To best of our knowledge, this is the first report of *P. hydropathica* causing disease on *B. orellana* in China. Our findings draw the attention to developing effective management strategies before it causes widespread damage to annatto in China.

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**References:**


Figure 1 Morphological characteristics of Phytophthora hydropathica from Bixa orellana.

- a. non-papillated sporangia, oval-shaped and with a conical base
- b. nested sporangia
- c. a hyphal swelling
- d. terminal thin-walled chlamydospore
- e. zoospore, globate and with a flagellum
- f. germination of a cystospore
- g and h. the up and reverse sides of colony on V8 four days after incubation, respectively.

a-f. Bar=20 μm. g-h. Bar=1 cm.
Figure 2 Symptoms of leaf blight and rot after artificial inoculation with isolates of Phytophthora hydropathica

1. Inoculation with sterile V8 plugs (Control), 2. wounded inoculation with mycelial plugs of P. hydropathica, 3. non-wounded inoculation with mycelial plugs of P. hydropathica. The representative photograph was taken at 3 days post-inoculation (dpi) and the mycelial plugs were removed in order to show the complete lesions.

190x254mm (300 x 300 DPI)