Diseases Caused by Oomycetes

First Report of Root Rot Caused by *Pythium dissotocum* on Hydroponically Grown Collard Greens (*Brassica oleracea* var. *acephala*)

N. C. Luecke,^{1,2,†} K. M. Crawford,¹ S. T. Koike,³ H. Stanghellini,³ and A. Burkhardt³

¹ Department of Biology & Biochemistry, University of Houston, Houston, TX 77204

² Dream Harvest Farming Company LLC, Houston, TX 77071

³ TriCal Diagnostics, Hollister, CA 95023

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Collards (*Brassica oleracea* var. *acephala*) are grown throughout the United States. Hydroponic greens are more common now due to technological advances lowering the cost and increasing the efficacy of production. In January 2021, a 325 m² indoor hydroponic farm opened to provide fresh produce for a school in Los Angeles County, CA. Three-week-old collard seedlings were purchased from a local nursery, rinsed of their rooting media, and transplanted into deep water culture beds (1.2 m × 2.5 m × 0.3 m). Two weeks later, symptoms including plant stunting, chlorosis, leaf curling and wilting, and brown necrotic roots appeared. By and by, 80 to 100% of usable plants were lost to disease. Symptomatic roots were plated on corn meal agar (CMA) amended with 2 ml of 25% lactic acid and CMA amended with pimaricin, ampicillin, rifampicin, and pentachloronitrobenzene (PARP) (Kannwischer et al. 1978). After 2 days, a single colony type emerged on PARP but no growth occurred on acidified CMA. Representative isolates were transferred to CMA and to filtered (0.02 µm) soil extract solution with boiled grass blades (Martin 1992), both of which were incubated at 22°C and ambient light conditions. On CMA, isolates produced coenocytic mycelium with minimal aerial hyphae. After 24 h in soil extract, isolates developed filamentous sporangia, elongated discharge tubes with slightly inflated tips, and zoospores. Oospores were not observed. Pathogenicity was confirmed by soaking the roots of 5-day-old collard seedlings in beakers containing zoospores (1 × 10² zoospores/ml) in filtered soil extract. Four isolates were tested on 15 seedlings each. After 24 h at 22°C in ambient light conditions, plants

were transferred to new beakers with roots placed on filter paper at the bottom and saturated with sterile distilled water. Three days after this transfer, leaves on all plants turned chlorotic and roots developed brown lesions from which morphologically identical colonies were isolated. Control plants, soaked in filtered soil extract, developed no root or foliar symptoms. To molecularly identify the collard isolates, DNA was extracted from mycelial original and reisolated isolates and was amplified by PCR using mitochondrial primers for the cytochrome oxidase I (COI) gene (Robideau et al. 2011) and the cytochrome oxidase II (COX2) gene (Martin 2000). The only species that matched both loci from the original and reisolated isolates with a high percent identity was *Pythium dissotocum*. The COI locus from the original isolate (MZ027311) matched *P. dissotocum* with 99% identity and with 332/334 bp matching the isolate with sequence ID MT981134.1. From the reisolated isolate (MZ027313), the COI sequence perfectly matched 657/657 bp of *P. dissotocum* (sequence ID MT981147.1). The COX2 locus from the original isolate (MZ027312) matched *P. dissotocum* (sequence ID MG719859.1) with a 99% identity and 517/518 matching bp and the reisolated isolate (MZ027314) perfectly matched *P. dissotocum* (sequence ID MG719859.1) with 515/515 matching bp. Based on these molecular and morphological data, the isolates were identified as *P. dissotocum*. To our knowledge, this is the first report of *P. dissotocum* causing root rot on collards. At this same facility, *P. dissotocum* was also confirmed as the cause of declining bean (*Phaseolus vulgaris*) plants. As hydroponics will be necessary to feed a growing population, especially in urban areas, and because leafy greens are a main crop of the hydroponics industry, we anticipate this issue may become common. Hydroponic systems are highly conducive to the persistence of oomycetes and a record of infection and plan of action will be necessary to preserve crop health.

References:

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[†]Indicates the corresponding author.

N. C. Luecke; ncluecke@central.uh.edu