Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Aspergillus flavus* Causing Fruit Rot on Kiwifruit in China

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Funding: Funding was provided by National Natural Science Foundation of China (31801697, 3201101306, and 3217170148). Plant Dis. 106:1990, 2022; published online as <https://doi.org/10.1094/PDIS-08-21-1771-PDN>. Accepted for publication 4 December 2021.

In October 2020, fruit rot symptoms were detected on kiwifruit (*Actinidia chinensis* var. *deliciosa* 'Xuxiang') in southwestern Shaanxi (Hanzhong municipality, 107.27° E, 33.23° N) in China. During the harvest period, mature kiwifruit exhibited soft rot and brown lesions. The symptoms were similar to those reported for *Alternaria alternata*, *Colletotrichum* spp., *Fusarium avenaceum*, and *Rhizopus oryzae*, which cause fruit rot on kiwifruit (Feng et al. 2019; Kim et al. 2018; Li et al. 2017; Zhao et al. 2020). The symptoms were observed in approximately 15% of the fruit in six kiwifruit orchards (31 ha in total). Ten samples of symptomatic tissue, approximately 1 cm² in size, were sterilized in 2% NaOCl for 30 s and washed twice with sterilized water. The pathogen was isolated from all collected samples via culturing on PDA medium, which contained 50 µg/ml of chloramphenicol, at 28°C. Green powdery colonies were detected after 5 days. A total of 12 isolates were obtained via single-spore isolation. The internal transcribed spacer (*ITS*), elongation factor 1- α (*EF1- α*), and RNA polymerase II subunit (*RPB2*) genes were amplified using *ITS5/ITS4*, *A_EF1_F/A_EF1_R*, and *RPB2-5F/ RPB2-7cR* (NJC03) or *RPB2-7cF/ RPB2-11aR* (NJC04) primers, respectively. Eleven isolates shared the same sequences (NJC03), MZ801787 (*ITS*), MZ701709 (*EF1- α*), and MZ701707 (*RPB2*), while one of the isolates provided different sequences (NJC04), OK618459 (*ITS*), OK634020 (*EF1- α*), and OL331017 (*RPB2*). The obtained *ITS* sequences shared >99% homology to the *ITS* gene from *Aspergillus flavus* KU20018.4 (MT487825), the *EF1- α* sequences shared 100% homology to the *EF1- α* gene from *A. flavus* clinical2342

(KP054370), and the *RPB2* sequences shared >99% homology to the *RPB2* genes from *A. flavus* PW3170 (LC000581) and *A. flavus* NRRL3357 (XM_041293948). A molecular phylogenetic tree was constructed using MEGA7 with reference to *Aspergillus* strains. Microscope observations of all the isolates showed the presence of septate mycelium and circular unicellular conidia (2 to 4 µm in diameter) and conidiophores and agreed with the morphology of *A. flavus* (Horn 2005). The pathogenicity of all isolates was screened using intact and wounded Xuxiang kiwifruits (10 kiwifruits were used for each combination with three replicates), which were purchased from a local market. An isolate solution of 1×10^6 spores/ml (10 µl) was used for the inoculation. Sterilized water was used in the control experiment. Inoculated kiwifruits were stored at 26°C and 60% relative humidity for 10 days. Rot lesions in the wounded kiwifruits were totally covered by green mycelia, while the lesions on the intact kiwifruits were similar to the symptoms observed in the field. The pathogen was recovered, and its identity was confirmed by sequence analysis of *ITS*, *EF1- α* , and *RPB2*, fulfilling Koch's postulates. *A. flavus* is known to be an important fungal pathogen of corn, cotton, and peanuts (Zhang et al. 2020). During recent years, *A. flavus* was reported to cause fruit rot on grapes (Ghuffar et al. 2020) and was identified on almond, fig, organic spelt, and pistachio (Kruj et al. 2017; Ortega-Beltran et al. 2019). The presence of *A. flavus* in food products is an issue of global concern due to *A. flavus* being able to produce carcinogenic aflatoxin (Maxwell et al. 2021). As far as we know, this is the first report of *A. flavus* causing fruit rot on kiwifruit. This report will help to understand the distribution of *A. flavus* in crops and the food safety hazards that are present in China.

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The author(s) declare no conflict of interest.

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Keywords: *Actinidia deliciosa*, aflatoxin, *Aspergillus flavus*, fungal pathogens, kiwifruit

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