

**Title** Mutations of  $\beta$ -tubulin codon 198 or 200 indicate thiabendazole resistance among isolates of *Penicillium digitatum* collected from citrus in Taiwan

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### Abstract

*Penicillium digitatum* causes green mold on citrus, resulting in severe postharvest fruit decay and economic losses in many citrus-producing areas of the world. Forty isolates of *P. digitatum* were cultured from citrus groves, packinghouses, and local markets in Taiwan, and assessed quantitatively for their sensitivity to thiabendazole (TBZ) fungicide. Sensitivity assays using a 96-well microtiter plate revealed that, of 40 isolates examined, only one isolate collected from fruit produced in Taiwan and two isolates from Florida-imported citrus fruit were sensitive to TBZ. The concentration of TBZ causing a 50% growth reduction ( $EC_{50}$ ) was less than 1  $\mu\text{g/mL}$ . The remaining 37 isolates could tolerate high concentrations of TBZ, with an  $EC_{50}$  greater than 80  $\mu\text{g/mL}$ . Overall, more than 97% of *P. digitatum* isolates tested in Taiwan were found to be resistant to TBZ. In vitro assays also revealed the ineffectiveness of TBZ for controlling a TBZ-resistant isolate on sweet oranges. A sequence analysis of  $\beta$ -tubulin genes revealed that all TBZ-resistant isolates displayed a single transversion point mutation, resulting in a change at either amino acid 198 (glutamic acid  $\rightarrow$  glutamine) or 200 (phenylalanine  $\rightarrow$  tyrosine). The repetitive use of a single fungicide over several decades has favored the selection and dominance of TBZ-resistant isolates of *P. digitatum*.