

Title Imazalil resistance linked to a unique insertion sequence in the PdCYP51 promoter region of *Penicillium digitatum*

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Abstract

Two mechanisms of resistance to the fungicide imazalil (IMZ) existed among California strains of *Penicillium digitatum*, cause of citrus green mold. Sensitive (S; $n = 50$) strains did not grow on IMZ above $0.1 \mu\text{g mL}^{-1}$, while those resistant (R; $n = 59$) grew $\geq 0.5 \text{ mg L}^{-1}$. After amplification of the promoter region of the *CYP51* gene, fragments 250, 450, and 750 bp in size were generated. All S strains had a 250 bp product, while among R strains, 47 had a 450 bp product and 12 had a 750 bp product. The 450 bp unit was common among R strains, while the 750 bp unit, reported previously by others, was not. The promoter region of all was identical; variations occurred in the region's transcriptional enhancer unit. S strains with a 250 bp product and R strains with a 750 bp product had one and five copies, respectively, of a 126 bp transcriptional enhancer unit. R strains with a 450 bp product had a unique 199 bp insert within the 126 bp transcriptional enhancer unit with no known sequence correlations (GenBank). Both types of R strains exhibited significantly elevated expression, approximately 10-fold, of the target site *CYP51* gene, indicating its overexpression was the mechanism of resistance.