

Title A real-time PCR assay for the detection the frequency of imazalil-resistant *Penicillium digitatum*

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Abstract

Citrus green mold caused by *Penicillium digitatum* (pers) Sacc. is the most destructive disease of postharvest citrus. To control this disease, imazalil, belonging to one of fungicides in 14- α -demythlation inhibitors (DMIs), is commonly used since early 1980s. Since the extensive and sequential using, imazalil-resistant *P. digitatum* was first reported in USA, subsequently in European and China. The potential impact of imazalil-resistant biotype on postharvest decay control was demonstrated in many commercial packinghouses. Monitoring the frequency of imazalil-resistant *P. digitatum* in a packinghouse would be a step to ensure the control of green mold. To date, two molecular mechanisms (insert of a 504 bp and 199 bp in promoter region of *CYP51* gene, respectively) were demonstrated to be response for the formation of imazalil-resistance. Based on these mechanisms, two pieces of forward primers specific to resistant type I (504 bp insert) and type II (199 bp insert) were designed, respectively. Combining with a common reverse primer, a method of real-time PCR assessment was subsequently developed to quantify the frequency of imazalil-resistant *P. digitatum* (F_R) in one reaction for a multiple samples collected from a packinghouse. This method was demonstrated to be as accurate as conventional PCR, but have the advantage of high throughput, time and labor-saving.