Dual silencing of fungicide target genes *sdhB* and *cytB* via SIGS enhances control of *Botrytis cinerea* and improves fungicide performance

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Abstract

The widespread emergence of fungicide-resistant Botrytis cinerea populations, together with increasingly strict regulatory constraints, has intensified the need for alternative and environmentally sustainable strategies for gray mold management. This study evaluates spray-induced gene silencing (SIGS) targeting mitochondrial respiration through double-stranded RNAs (dsRNAs) directed against sdhB and cytB genes. These genes encode key subunits of respiratory complexes II and III, key targets and determinants of resistance to SDHI and QoI fungicides, respectively. Exogenous application of these dsRNAs significantly reduced B. cinerea conidial germination (~50%), lesion development in tomato leaves and apple fruits (~45%), fungal biomass (~50%), and transcript levels of sdhB (3.2-fold) and cytB (2.0-fold). When combined with sublethal doses of boscalid (SDHI) or azoxystrobin (QoI), dsRNA treatments markedly decreased lesion severity in fungicide-sensitive isolates, achieving substantial but not full equivalent to field-dose fungicide efficacy. In resistant isolates, dsRNA alone consistently reduced disease symptoms, and its combination with sublethal-dose fungicides further enhanced control. When dsRNA was applied together with full-rate fungicide, lesion development declined sharply across all resistant isolates, and in some cases gray mold symptoms were completely eradicated, indicating the strong biological effect achieved by the RNAi-fungicide combination. Sequence identity analyses revealed strong cross-species activity among closely related Botrytis and Sclerotiniaceae species, with no predicted effects on unrelated fungi, plants, or humans. This work provides the first demonstration of a dual-target SIGS strategy acting on mitochondrial respiration, highlighting its potential as a precise, eco-compatible, and effective tool for managing B. cinerea resistance within integrated pest management frameworks.