"Fungicide resistance in postharvest pathogens and its management as a tool to prevent food losses"

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Introduction

In the realm of postharvest crop management, the menace posed by pathogens during transportation, handling, and storage is a critical concern. The primary approach to mitigate this threat involves the application of preharvest and postharvest fungicides, critical for safeguarding crops. Despite substantial efforts to devise innovative management techniques, the emergence and dissemination of resistance to key fungicides among postharvest pathogens have become a formidable challenge. This review delves into the intricate dynamics of fungicide resistance development in pivotal postharvest pathogens, focusing on case histories, while underscores the molecular mechanisms underpinning resistance, encompassing target site alterations and heightened expression of efflux transporters.

Case Histories

P. expansum

- According to FRAC, *P. expansum* is considered as a pathogen of medium risk for resistance **(Table 1&2).**
  - *Penicillium expansum* resistance was initially reported to the benzimidazole fungicide thiabendazole and to the DMI fungicide Imazalil (Baraldi et al., 2003; Sholberg et al., 2005).
  - Resistance to benzimidazoles has been associated with several mutations in *β-tubulin* (Malandrakis et al., 2013). **(Figure 1)**
  - Resistance in *P. expansum* to the DMI difenoconazole was demonstrated to be linked to a mutation in the *PeCYP51* gene (Ali and Amiri, 2018).
- Multidrug resistance (MDR) in *P. expansum* was linked to the overexpression of drug efflux transporters (Samaras et al., 2020) **(Figure 2).**

B. cinerea

- Global resistance in *B. cinerea* populations to all site-specific fungicides has emerged **(Figure 1) (Table 1&2).**
- Characterized by modifications at the target site due to the presence of mutations in genes encoding the fungicides' target site.
- Recent studies revealed the presence of strains possessing more than one target-site mutation conferring resistance to the respective fungicides and leading to multiple resistance (MLR) to fungicides (Naegele et al., 2022)
- In *B. cinerea*, four different types of MDR strains have been recognized (MDR1, MDR1h, MDR2, and MDR3) linked with overexpression of atrB- and mfsM2- encoded efflux transporters (Leroch et al., 2013) **(Figure 2).**

Table 2. Fungicides used to control postharvest decay: Modes of Action and Detected Resistance.

Mode of Action	FRAC Group	Group Name	Common Name	Risk	Comments
Respiration	C3 (11)	Strobilurin (QoI)	Azoxystrobin	H	Target site mutations in cyt b gene (G143A, F129L)
	C2 (2)	Succinate dehydrogenase inhibitors	Fluopyram	M-H	Target site mutations in sdh gene, e.g. H/Y (or H/L) at 257, 267, 272 or P225L, dependent on fungal species
Sterol synthesis	G1 (3)	DeMethylation Inhibitors (SBI: Class I)	Difenoconazole	M	Several resistance mechanisms are known incl. target site mutations in cyp51 (erg 11) gene, e.g. V136A, Y137F, A379G, I381V; cyp51 promotor; ABC transporters and others
			Tebuconazole	M	Several resistance mechanisms are known incl. target site mutations in cyp51 (erg 11) gene, e.g. V136A, Y137F, A379G, I381V; cyp51 promotor; ABC transporters and others
			Propiconazole	M	Several resistance mechanisms are known incl. target site mutations in cyp51 (erg 11) gene, e.g. V136A, Y137F, A379G, I381V; cyp51 promotor; ABC transporters and others
			Imazalil	L-M	Resistance is known in various fungal species. Several resistance mechanisms are known incl. target site mutations in cyp51 (erg 11) gene, e.g. V136A, Y137F, A379G, I381V; cyp51 promotor; ABC transporters and others
Signaling	E2 (12)	PhenylPyrroles	Fludioxonil	L-M	Resistance found sporadically, mechanism speculative.
Lipid synthesis or transport	F8 (48)	Polyene	Natamycin	N/A	Resistance not known
Cell wall biosynthesis	H4 (19)	Polyoxins	Polyoxin-D	M	Resistance known
Amino acids and proteins	D1 (9)	Anilino-Pyrimidines	Pyrimethanil	M	Resistance known in Botrytis and Venturia, sporadically in Oculimacula
Mitosis and cell devision	B1 (1)	Methyl Benzimidazole Carbamates	Thiabendazole	H	Resistance common in many fungal species. Several target site mutations, mostly E198A/G/K, F200Y in β-tubulin gene.

Table 1. Pre- & Post-harvest fungicides used to control postharvest decay of fruit and vegetables.

Fungicide-active ingredient	Class	Crop	Decay
Azoxystrobin	QoIs	Citrus, potato	Penicillium decays
Difenoconazole	DMI-triazoles	Pome fruits, potato, sweetpotato	Bull's eye rot, Fusarium rot, Penicillium decays, and Rhizopus rot
Fludioxonil	Phenylpyrrole	Citrus, stone fruits, pome fruits, pomegranate, pineapple, kiwi, tomato, potato, and sweet potato	Alternaria decay, brown rot, gray mold, Rhizopus rot, and Penicillium decays
Imazalil	DMIs-imidazoles	Citrus	Penicillium decays
Natamycin	Polyene	Citrus, pome fruits, and stone fruits	Alternaria decay, brown rot, gray mold, Rhizopus rot, Penicillium decays, and sour rot
Polyoxin-D	Polyoxins	Stone fruits	Alternaria decay, brown rot, and gray mold
Potassium phosphite	Phosphonate	Citrus	Phytophthora brown rot
Propiconazole	DMIs-triazoles	Citrus, stone fruits, tomato, and pepper	Brown rot, gray mold, Rhizopus rot, sour rot, and Penicillium decays
Pyrimethanil	Anilinopyrimidines	Citrus, stone fruits, pome and fruits	Brown rot, gray mold, Rhizopus rot, and Penicillium decays
Thiabendazole	Methyl benzimidazole carbamates	Citrus, pome fruits	Penicillium decays, stem end rots
Tebuconazole	DMIs-triazoles	Sweet cherry, plum	Alternaria decay, Cladosporium decay, gray mold, Rhizopus rot, and Mucor rot
Fluopyram	SDHIs	Stone fruits, pome fruits	Brown rot, gray mold, Alternaria decay, Rhizopus rot, and Mucor rot

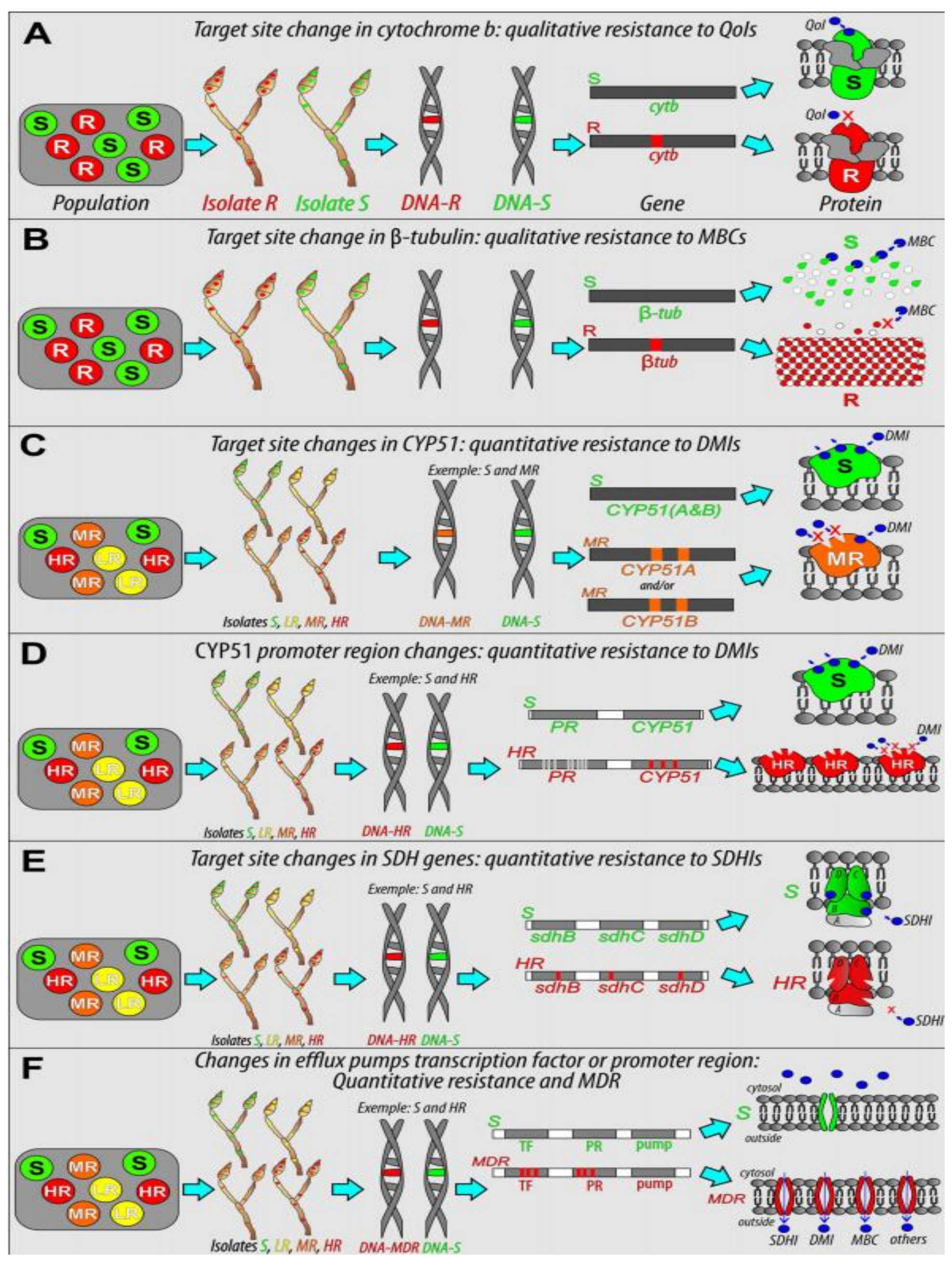
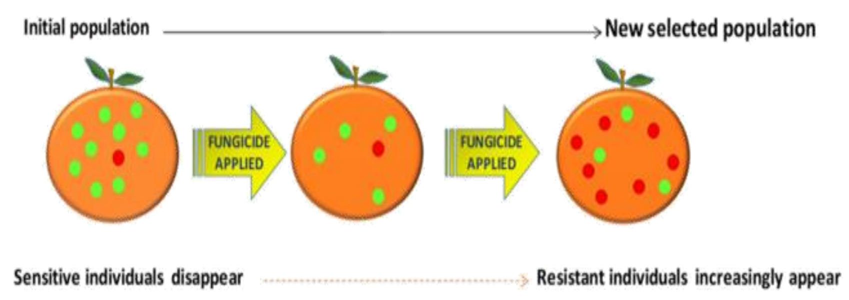


Figure 1. Presentation of target and non-target site mechanisms of fungicide resistance adapted from Dorigan *et al.*, 2023



Scheme of the evolution of resistance to fungicides presenting how selection pressure may take place. Initial population with little resistance evolves until resistance becomes widespread due to repeated fungicide applications. Adapted from Deising *et al* 2008.

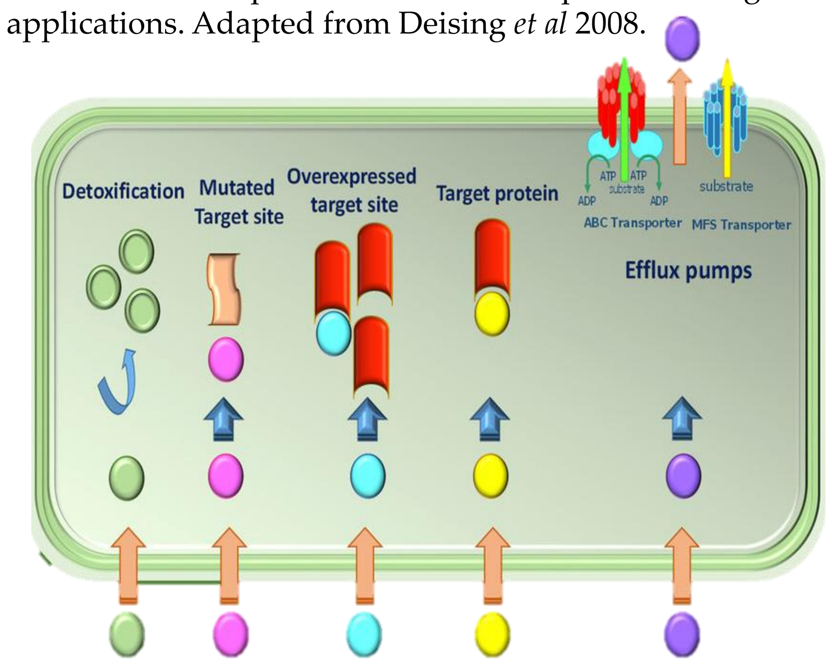


Figure 2. Primary mechanisms involved in developing resistance to fungicides in *P. digitatum*. Resistance mechanisms specific to single-site fungicides include: (a) detoxifying the fungicide via metabolic enzymes; (b) modifying the target protein to decrease fungicide binding; (c) increasing the expression of the target protein; (d) utilizing efflux pumps to expel the fungicide from the cell. Adapted from Lucas *et al.*, 2015

Strategies for Fungicide Resistance management in post harvest pathogens

Crop Management Techniques
- Management of postharvest diseases starts in the field

Good Packinghouse Practices:
- Use of sanitizers to reduce inoculum

Fungicide Application Strategies:
- Avoid excessive use of fungicides
- Implement rotation between fungicides of different classes

Integrated Disease Management:
- Emphasize the importance of integrated disease management.
- Reduce selection pressure by applying alternative control methods.

Efflux Pump Inhibition (EPI) Activity:
- Consider blocking efflux pump activity to restore drug efficacy against resistant microorganisms.

Future Research Directions:
- Explore novel disease management strategies, including epigenetics- RNA-based fungicides and SynComs.

Indicative References
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