10th Brazilian Conference on Natural Products
XXXVI RESEM
4-7 November 2025, Belo Horizonte, MG, Brazil

Section: 01

VOLATILE SECONDARY METABOLITES IN POSTHARVEST CITRUS FUNGAL INFECTIONS: FROM PATHOGEN-HOST INTERACTION TO TERPENE BIOSYNTHESIS

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Brazil is the world's largest producer of oranges and one of the main exporters of citrus juice, making citriculture a strategic sector for the global economy. However, postharvest fungal pathogens such as *Penicillium digitatum*, *Penicillium italicum*, and *Geotrichum candidum* cause significant losses due to rapid enzymatic degradation of the pulp and dissemination through wounds. These pathogens produce a variety of secondary metabolites (SMs) with cytotoxic, enzymatic, and phytotoxic functions essential for host colonization. Among these, volatile organic compounds (VOCs) are also known to play central roles in e.g. plant–insect, plant–microbe and microbe–microbe interactions. To better understand these interactions and characterize the volatile profile involved, we analyzed VOC emissions using the solid-phase microextraction (SPME) technique. In order to monitor the VOCs produced during the interaction between these pathogens and the citrus host, analyses using SPME, associated with two-dimensional gas chromatography (GC×GC-TOFMS), were performed on healthy fruits and fruits after 7 days of infection. A total of 240 compounds were identified, including esters, biotransformed monoterpenoids (e.g., carvone, carveol, α-terpineol), and sesquiterpenes such as himachoulene and β-caryophyllene. Biotransformation assays with limonene and terpineol confirmed the conversion of these volatiles by the pathogens, reinforcing their adaptive role in response to citrus defense. *P. italicum*, in particular, produced triquinane-type tricyclic sesquiterpenes both *in vitro* and during host infection. Genome mining revealed 53 biosynthetic gene clusters (BGCs), of which 24.53% were associated with terpene biosynthesis. Within BGC of the *P. italicum* PHI genome, we identified a putative Presilphiperfolan-8-β-ol synthase, an enzyme related to sesquiterpenoids biosynthesis. This enzyme was heterologously expressed in *Escherichia coli* ArcticExpress (DE3), purified — with an approximate size of 60 kDa — and incubated *in vitro* with the natural substrate (2E,6E)-FPP in order to correlate the sesquiterpenoids produced under this condition with those identified during the interaction between *P. italicum* strain PHI and the citrus host. The integration of VOC analysis provided new insights into the chemical mechanism of infection by different phytopathogens through the identification of characteristic biomarkers of each pathogen. In addition, this study delves into the investigation of the biosynthetic machinery employed by *P. italicum* strain PHI in terpene production.

Keywords: *Chemistry Ecology, Penicillium italicum, VOCs, pathogen–host interaction, genome mining, biotransformation*