

# Biogenic Volatile Organic Compounds

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Upon pathogen attack, plants very quickly undergo rather complex physico-chemical changes, such as the production of new chemicals or alterations in membrane and cell wall properties, to reduce disease damages. An underestimated threat is represented by root parasitic nematodes. In *Vitis vinifera* L., the nematode *Xiphinema index* is the unique vector of *Grapevine fanleaf virus*, responsible for fanleaf degeneration, one of the most widespread and economically damaging diseases worldwide. The aim of this study was to investigate changes in the emission of biogenic volatile organic compounds (BVOCs) in grapevines attacked by *X. index*. BVOCs play a role in plant defensive mechanisms and are synthesized in response to biotic damages. In our study, the BVOC profile was altered by the nematode feeding process. We found a decrease in  $\beta$ -ocimene and limonene monoterpene emissions, as well as an increase in  $\alpha$ -farnesene and  $\alpha$ -bergamotene sesquiterpene emissions in nematode-treated plants. Moreover, we evaluated the PR1 gene expression. The transcript level of *PR1* gene was higher in the nematode-wounded roots, while in the leaf tissues it showed a lower expression compared to control grapevines.

BVOCs

dagger nematodes

GC-MS

grapevine

monoterpenes

PR1 gene

sesquiterpenes

SPME

Xiphinema index

The European Union is the world's main wine producer, with a share of about 60% <sup>[1]</sup>. Given the economic relevance of *Vitis vinifera* L., grapevine pests are of rising interest to agrochemical companies and plant researchers. Among root parasites, nematodes can go undetected for years, especially in perennial crops, but eventually, they strongly decrease crop productivity.

The phylum Nematoda is largely widespread around the world and occupies a huge range of ecological niches <sup>[2]</sup>. In soil, nematodes play an important role in the decomposition of organic matter and the recycling of nutrients, determining the health of the soil itself. However, several taxa are harmful to many crops of economic importance <sup>[3]</sup>, such as grapevine.

Annual crop losses caused by plant-parasitic nematodes are estimated at 8.8–14.6% of total crop production and 80 billion USD worldwide <sup>[4][5]</sup>. At least 2000 species of plant-parasitic nematodes are characterized by the presence of a stylet used for root tissue penetration. Some species are endoparasitic, others ectoparasitic <sup>[6]</sup>. Worldwide, several grapevine-parasitic nematodes can be mentioned, but root-knot nematodes *Meloidogyne* spp. and dagger nematode *Xiphinema index* are the most diffused. They are representative of the two root-feeding models, endoparasitic and ectoparasitic, respectively. *X. index* is a soil-borne nematode that lives in proximity to the rhizosphere <sup>[7]</sup> and feeds on cell content thanks to its strong stylet <sup>[8]</sup>. *X. index* is per se a harmful pathogen for

viticulture because it causes root necrosis and deformation which considerably reduce productivity [9]. Besides, it specifically transmits the *Grapevine fanleaf virus* (GFLV) [10][11][12], whose symptoms are belatedly visible at the leaf level. Nevertheless, GFLV disease can lead to severe economic losses with a yield decrease up to 80% [13] due to the reduction in fruit quality and the shortening of plant longevity [14].

Preventive application of nematicides, due to their limited efficacy in pest control and negative impact on the environment, is no longer used routinely by farmers [15]. For this reason, it is of fundamental importance to find a way to detect nematode attacks early and prevent their damage.

Plants defend themselves from parasite attacks in different ways, in continuous coevolution with pathogens [16]. Their stationary status makes them vulnerable but plants limit damage using a variety of defense mechanisms [17], so disease is an exceptional condition rather than normality. Defense mechanisms can be both constitutive and inducible, but while the first is pre-established and energetically irrelevant, the second requires a high amount of energy and is stimulated by pathogen attacks. Inducible defenses act at the time of pathogen recognition and rapidly limit possible damages. A typical feature of resistance is the induction of cell death at the site of attempted attack such as the hypersensitive response (HR) [18], a mechanism which highly limits pathogen proliferation in the host organism. Subsequently, a large set of defense-related genes are expressed as resistance develops [19]. HR settlement involves the induction of many defense mechanisms such as the strengthening of cell walls, salicylic acid (SA) pathway, synthesis of phytoalexins organic molecules and HR-related molecules ( $H_2O_2$ ) which are among the main molecules secreted and produced during the plant/pathogen interaction [20]. Among proteins involved in defense mechanisms, the so-called pathogenesis-related proteins (PRs) certainly have deep importance in plant protection.

Besides accumulating locally in the infected tissues, PRs are also induced systemically, associated with the development of systemic acquired resistance (SAR) against other infections [21]. For example, in *Arabidopsis thaliana* there are 17 evolutionarily conserved families of PRs [22] with 22 *PR1*-type genes [23], but only one of them is activated by pathogens whereas other *PR1*-type genes are constitutively expressed [24].

Nematode attack can affect PR gene expression through the injection of substances produced in salivary glands, which can inhibit host response. Root-knot nematodes secrete molecules called “effectors” to facilitate the invasion of the host roots, avoid plant defense responses and reprogram root cells to form specialized feeding cells [25]. Various PRs have been identified as direct targets of nematode effectors, but nevertheless, their precise mode of action remains largely unknown and only a few of their direct targets in plants have been identified [25].

Plants can either act directly on pathogen feeding and reproduction, for example through trichomes or thorns or indirectly, through the emission of phytochemicals. In particular, the production of secondary metabolites is a defense strategy to cope with several pests [26]. Among secondary metabolites, plants produce root-specific volatile organic compounds (VOCs) [27], which can influence the rhizosphere and plant-pathogen interaction [28][29][30][31].

There is growing evidence that both the quantity and type of volatiles produced by roots are dramatically altered by the presence of different biotic and abiotic stresses [32][33]. It was also reported that VOC changes in response to pathogens or symbionts are species-specific [34]. For example, it has been demonstrated that plants produce chemical signals to ward off herbivorous insects by attracting their natural enemies [35]. Biogenic VOCs (BVOCs) are the major secondary metabolites in plants involved in communications between plants and the external environment, in a mechanism known as “talking plants” [36].

The term BVOCs defines organic atmospheric gases different from carbon dioxide and monoxide [37]. BVOCs include a wide range of different compounds, among which isoprene and monoterpenes are the most prominent [37]. BVOC emission can be stimulated in response to insect feeding [38] and it is largely demonstrated that plants vary the emission of organic compounds in different plant-parasite interactions [16][38][39][40][41]. Moreover, BVOC emission seems to be stimulated by the presence of elicitors present in parasite oral secretions [39].

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