Untangling the role of ethylene beyond fruit development and ripening: A physiological and molecular perspective focused on the *Monilinia*-peach interaction

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Abstract

It is already well known that ethylene plays a crucial role in peach fruit growth and ripening, by triggering an unset of biochemical and physiological changes that finally make the fruit attractive for consumption. This said, ethylene is not only responsible for fruit ripening but, in conjunction with other hormones, or key compounds (ROS, polyamines, etc.) is involved in the plant response to numerous abiotic stresses (drought, salt and heat tolerance) as well as the plant/fruit response against certain pathogens. Among peaches, one of the most devastating pathogens is the brown rot causing fungus *Monilinia* spp. that can affect the fruit both on the field or postharvest. Nonetheless scarce information exists regarding the *Monilinia*-peach interaction from a physiological and molecular perspective. In this sense, recent studies point out to the importance of ethylene during such interaction, which seems to be dependent on the fruit developmental stage and also on the *Monilinia* species or even the strain's virulence. Why the fruit or the fungus reacts different to distinct *Monilinia* species or strains and why such reaction depends on the fruit physiological stage is, however, still elusive. Accordingly, this review aims to shed light on the role of ethylene, alone or through a complex cross-talk with other compounds, not only during peach development and ripening but also during the *Monilinia*-peach interaction. Based on the available literature, it is clear that not only ethylene biosynthesis but ethylene signaling and the activation of ethylene response factors via ROS may play an essential role during this specific host-pathogen interaction.

Keywords: hormones, virulence, host-defence, brown rot, ROS, polyamines

1. Background

Among the Rosaceae family, peaches are one of the most important crops in Europe and even worldwide. Since official data became available (in 1961), there has been a clear trend towards a higher harvest area and production of peaches all around the world (Fig. 1A). Peaches are also the most important species in terms of cultivated volume among other stone fruit (Fig. 1B). In fact, only in Europe, a total of 233,737 Ha were harvested in 2019, accounting for a production of 4.24 Mt. Worldwide, the harvested area within the same period reached 1,527,052 Ha with a production of *ca.* 25.74 Mt and being Asia by far the largest producer (71.8 %), followed by Europe (16.5 %) (Fig. 1C) (FAO, 2021).

Peaches are highly appreciated by consumers for their unique flavour, texture, juiciness, and nutritional value. Thus said, once harvested and if compared with other fruit, peaches are highly perishable, in part, due to its climacteric nature as well as their high susceptibility to postharvest decays. In this sense, and from a physiological perspective, ethylene (ET) alone or in combination with other key compounds, such as other hormones, reactive oxygen species (ROS) and polyamines plays an essential role orchestrating the developmental, ripening and senescence stages of peaches but also the fruit response to both abiotic (i.e., cold storage) and biotic stresses (i.e., postharvest rots). Among the latter, it is of special interest brown rot, a wide-spread disease caused by *Monilinia* spp., a necrotrophic fungus belonging to the Ascomycota phylum. This disease, mainly caused by the species *M. laxa* and *M. fructicola*, greatly affects pome but especially stone fruits (Oliveira Lino et al., 2016) such as peaches (Fig. 1D), causing substantial losses both on the field and during postharvest handling (Martini and Mari, 2014).

Hence, the aim of this review, with particular interest in peaches and *Monilinia* spp., is to deeply examine: i) the role of ET not only during peach development and ripening but also during the biotic interactions; ii) the role of ET from the point of view of the pathogen;

and iii) the importance of polyamines and ROS as signaling molecules interacting with ET during biotic interactions.

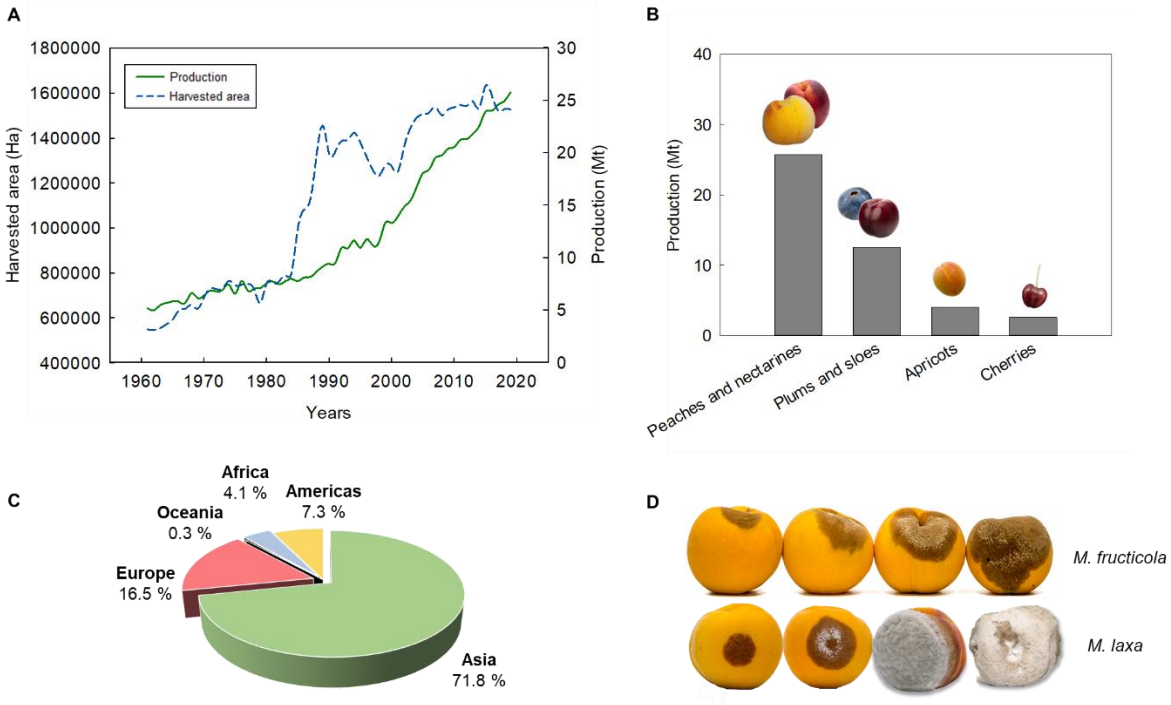


Figure 1. Importance of peach fruit. (A) Total world peach and nectarine harvested area (ha) and production (Mt) from 1961 to 2019 (FAO, 2021). (B) Production of the main stone fruit species in the world during 2019 (FAO, 2021). (C) Peach and nectarine fruit production for each region during 2019 (FAO, 2021). (D) Brown rot disease caused by *M. fructicola* and *M. laxa* in peach fruit.

2. Ethylene as a key element involved in peach ripening

Developmental and ripening processes are tightly dependent on a symphony of internal and external stimuli. Among them, the gaseous hormone ET stands out for controlling from seed germination to plant/fruit growth, development, ripening and senescence.

Peach development is accomplished through four different stages (Tonutti et al., 1991), which include a cell division and elongation phase (S1), a second phase encompassing the stone formation (S2), and a third phase of exponential growth of the pericarp (S3).

Lastly, once the fruit reaches its final size (S4), ripening process understood as changes in fruit colour, enhanced sweetness and the loss of firmness are initiated (Fig. 2A).

Based on the ET production and the respiratory profile during ripening, fruit are classified as climacteric and non-climacteric. The regulation of ET biosynthesis is accomplished within two different systems: system 1 and system 2. The system 1 production is typical from non-climacteric or pre-climacteric fruit, consisting of low or basal ET production, and generally its autoinhibited. System 2 operates during ripening and typically entails an autocatalytic ET burst (reviewed by Pech et al. 2012). Peaches are considered climacteric fruit, like tomatoes, apples, pears, or bananas, since they exhibit a synchronization of a respiration burst accompanied by an autocatalytic increase in ET production during ripening.

The ET profile during peach development has been widely studied. Some years ago, Tonutti et al. (1991) reported how the kinetics of ET production of 'Redhaven' peaches changed depending on the developmental stage. Thus, ET levels were high during the first days after full bloom (DAFB) and decreased thereafter to almost undetectable levels. Finally, levels increased coinciding with the onset of peach ripening. The same ET pattern was later demonstrated for 'Merrill O'Henry' peaches (Baró-Montel et al., 2021), and for 'Diamond Ray' (Vall-llaura and Fernández-Cancelo et al., 2022) or 'Fantasia' nectarines (Rasori et al., 2010).

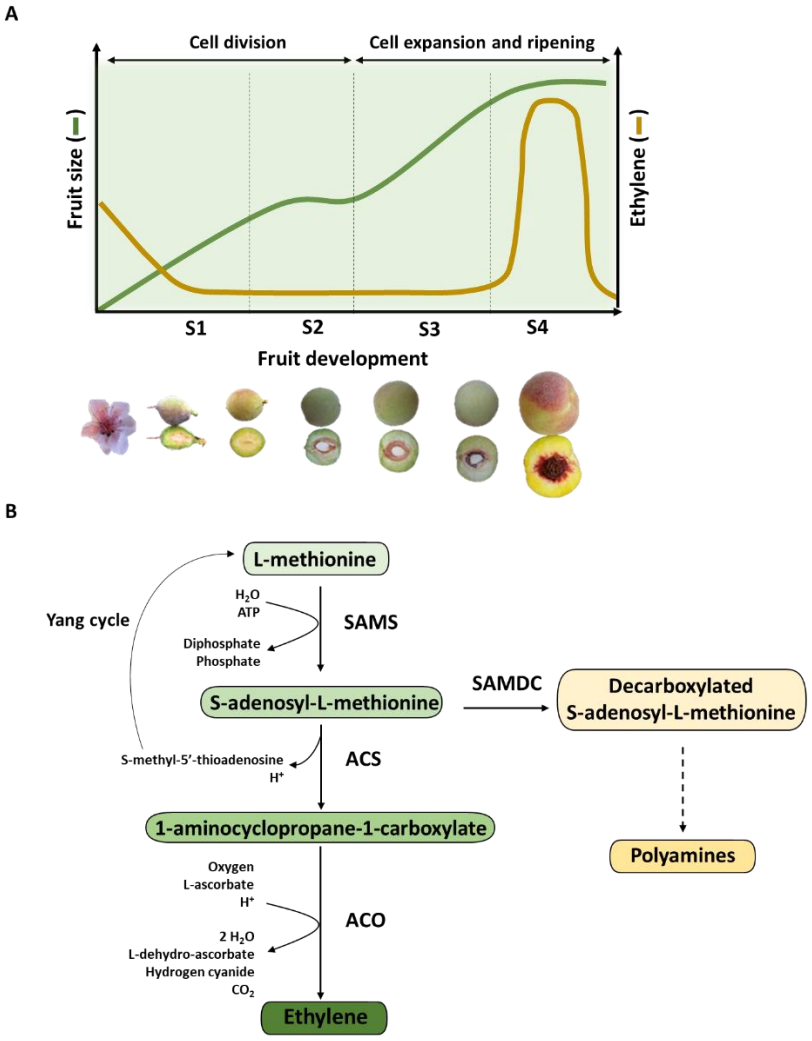


Figure 2. (A) Schematic representation of the main stages of peach fruit development characterized by a double sigmoid growth pattern. A typical ethylene profile based on available literature is given. (B) Ethylene biosynthetic pathway from the precursor methionine and mediated by the action of S-adenosyl-methionine synthase (SAMS), 1-aminocyclopropane-1-carboxylic acid synthase (ACS), and 1-aminocyclopropane-1-carboxylic acid oxidase (ACO). S-adenosyl-L-methionine could also act as a precursor of polyamines by the action of S-adenosyl-L-methionine decarboxylase (SAMDC). Further details about the biosynthesis of polyamines are given in section 3.4 and Figure 8.

The hormone ET is enzymatically synthesized from the amino acid methionine (Fig. 2B), which is first converted to S-adenosyl-methionine (S-AdoMet) by the action of the S-AdoMet synthase (SAMS). The activity of 1-aminocyclopropane-1-carboxylic acid (ACC) synthase generates ACC and 5′-deoxy-5′methylthioadenosine (MTA), which is then recycled to methionine through the Yang cycle, allowing higher rates of ET production without depletion of the endogenous methionine pool (Miyazaki and Yang, 1987). ACC is finally oxidised to form ET by the action of ACC oxidase (ACO), generating cyanide and CO_2 as subproducts. Generally, ACS is thought to act as the rate-limiting enzyme in ET biosynthesis for most fruit (Argueso et al., 2007).

Both ACS and ACO are encoded by a multigene family. In peaches, at least five ACS with reported ACC synthase activity have been described (*PpACS1*, *PpACS2*, *PpACS3*, *PpACS5*, *PpACS7* and *PpACS8*), while a total of four ACO (*PpACO1*, *PpACO2*, *PpACO3* and *PpACO5*) genes are known to be transcribed (Tadiello et al., 2016). Both ACS and ACO genes are differently regulated depending on the developmental stage (Tadiello et al., 2016), and hence allowing for ET levels to fluctuate along fruit development and ripening.

Once generated, ET is perceived by the cells through a series of endoplasmic reticulum-localized receptors (Broekaert et al., 2006) such as ETHYLENE RESISTANT 1 (ETR1), which acts as negative regulator of the ET pathway (Ju and Chang, 2015) (Fig. 3). Specifically, in peach, four receptor genes have been described; *PpETR1*, *PpETR2*, *PpETR3* and *PpERS1* (Tadiello et al., 2016). Once ET binds to ETR1 receptor, the CTR1 (CONSTITUTIVE TRIPLE RESPONSE 1) kinase activity is inactivated and consequently, EIN2 (ETHYLENE INSENSITIVE 2) becomes dephosphorylated and its C-terminal domain (CEND) enters the nucleus to attenuate EBF (EIN3 binding F-box protein) E3 ligase function, thereby preventing EIN3 degradation (Ju et al., 2012; Qiao et

al., 2012). Finally, EIN3 directly activates a range of transcription factors including APETALA2/ETHYLENE RESPONSIVE FACTOR (AP2/ERF) and OCTADECANOID-RESPONSIVE ARABIDOPSIS 59 (ORA59) (Pré et al., 2008; Solano et al., 1998) involved in mediating the ET response.

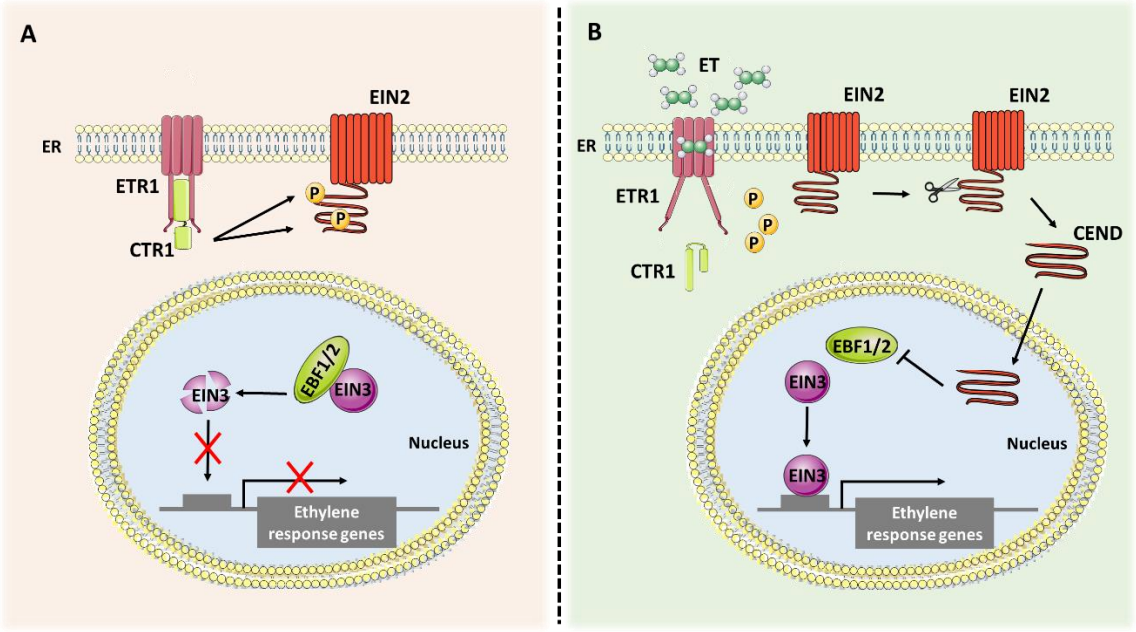


Figure 3. Representation of the ET signaling pathway in (A) the absence or (B) presence of ET (Adapted from Ji and Guo (2013)).

A transcriptomic analysis of the ET signal transduction in peaches revealed that the expression of ETR-like genes increased during ripening concomitantly with ET levels, while those from the six identified CTR-like genes remained constant in a similar way that did EIL and EIN-like gene expression (Wang et al., 2017). Regarding to the AP2/ERF family, a total of fifteen genes were proved to be differentially regulated upon 1-methylcyclopropene (1-MCP) treatment, a well-known inhibitor of ET perception, and during ripening in a species-specific manner (Wang et al., 2017). In fact, a comprehensive study of the peach transcriptome during ripening revealed that at least five ERFs were

induced in melting flesh peaches (those generally presenting a sharp increase of ET production during the ripening process) while not in stony hard ones (those that barely softens and sustains low ET production levels) (Wang et al., 2017). These ERFs, responsible for the final steps of ET signaling, contain a highly conserved DNA-binding domain (AP2 domain) which binds to the GCC box of the promoter regions of a series of genes involved in the ripening process (Zhou et al., 2020). All the genes involved from ET biosynthesis to ET perception/transduction and signaling, and described in this manuscript, are listed in a Supplementary Table S1.

The onset of fruit ripening is characterized by a set of changes that include fruit softening, colour changes, and alterations on the fruit texture, sugar metabolism, and the volatile composition. Extensive literature has somehow related all these changes to ET. For instance, Ziliotto et al. (2008) detected 106 differentially expressed genes, some of which were involved in ripening-related events such as softening, colour development and sugar metabolism, in nectarine upon 1-MCP treatment (Zhang et al., 2020). Specifically, and also upon 1-MCP treatment, Cai et al. (2019) also detected an inhibition of peach-like volatiles and a decrease in their precursors (fatty acids). Earlier studies also demonstrated that ET is required for initiation and progression of peach softening by regulating cell wall-metabolism related genes such as endopolygalacturonase (*PpPG*), alpha-L-arabinofuranosidase/beta-xylosidase (*PpARF/XYL*), or an expansin (*PpExp3*) (Hayama et al., 2006). Nonetheless, recent evidence for other fruit suggest that ET does not act alone, but rather in a tightly coordinated manner with other hormones (Iqbal et al., 2017).

In spite of the action of ET alone or in conjunction with other hormones, it is well accepted that ripening-related changes along fruit development, and especially over ripening, render peach fruit to be more susceptible to *Monilinia* (Guidarelli et al., 2014; Mari et al., 2003). Whether enhanced fruit susceptibility in ripe fruit may be driven by the action of

ET is to date still partially unknown. However, recent studies (Baró-Montel et al. 2021) observed that the ET production, together with the respiratory activity and the content of specific compounds, were correlated with the fruit resistance to *Monilinia* spp. at the different peach developmental stages.

3. The dual role of ethylene in promoting or inhibiting pathogenesis

Ethylene has not only been associated with the development and ripening of fruits but also as an important molecule during the host-pathogen interactions, especially when referring to necrotrophic pathogens. The role of this hormone on inhibiting or promoting fungal progression is, however, still controversial, and some conflicting results exists in the literature depending on the pathosystem. On one hand, fruit increases its ET production once challenged by a pathogen aiming to activate a defence response. However, the action of this hormone on the fruit itself triggers several changes, as detailed above, including cell wall re-structurisation and ripening that can actually lead to an increased susceptibility to pathogens. Besides, the ability of some pathogens to produce ET and its implication during the host-pathogen interactions is still a matter of debate. In this section, we will further discuss such dual role of ET considering both the host and pathogen players with especial emphasis on the *Monilinia*-peach interaction.

3.1. Ethylene production as a host defence response

Once challenged by a pathogen, hosts (i.e., plants in whole or their specific parts (i.e., fruit)) respond rapidly by activating defence responses to alleviate the biotic stress. Such defence activation is mediated by a complex phytohormone signaling network, mainly involving ET, jasmonates (JA) or salycilates (SA) (Schenk et al., 2000). Thus said, other hormones including auxins, cytokinins, abscisic acid, gibberellins and brassinosteroids

are to some extent also involved in mediating the plant defence, either alone or in a well-coordinated manner (Robert-Seilaniantz et al., 2011).

The defence strategy adopted by the host will be ultimately dictated by the pathogen. Thus, the type of pathogen, and more specifically, its lifestyle (necrotrophic or biotrophic) will trigger a different phytohormone signaling cascade (Li et al., 2019). Salicylic acid-mediated response is typically associated with resistance to biotrophic pathogen infections (Gaffney et al., 1993; Wildermuth et al., 2001), while both JA and ET are known to trigger resistance to necrotrophic pathogens (Glazebrook, 2005) (Fig. 4).

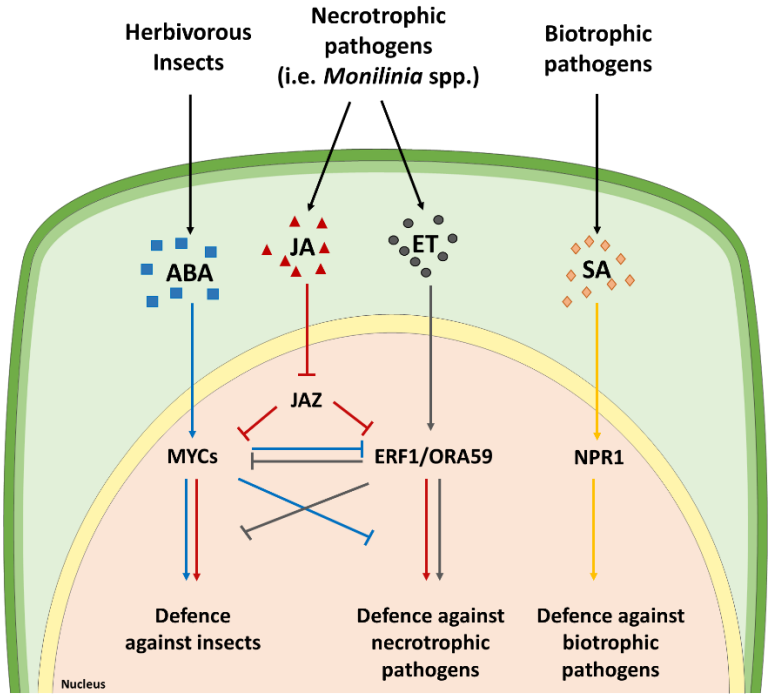


Figure 4. Schematic representation of the different hormonal cascades triggered depending on the biotic stimuli. The interplay among them is also represented.

There are numerous genes/proteins that respond upon a pathogen attack and many of them can be directly or indirectly linked to ET. The ET-mediated defence cascade initiates when host ET accumulates as a response to the pathogen recognition. As afore mentioned, such increase in ET is perceived by the ETR receptors and initiates the ET signaling cascade in

which some ERFs play a pivotal role (Fig. 5). For instance, a study from Berrocal-Lobo et al. (2002) demonstrated that ERF1 is induced in Arabidopsis upon infection, and its overexpression was sufficient to confer resistance to *B. cinerea* and *Plectosphaerella cucumerina*. Peach fruit also displays a similar profile when facing some pathogens, yet no literature is available specifically focused on peach-fungi interactions. For bacterial diseases, Sherif et al. (2012a) described that a resistant peach cultivar to the bacterial spot disease *Xanthomonas campestris* pv. *pruni* presented a major and more rapid expression of several ERF genes. Besides, such expression was found to be triggered by ET and/or JA. When referring to the *Monilinia*-peach interaction, although no specific studies are available, recent data from Balsells-Llauradó et al. (2020) also suggest a similar activation of specific *PpERF1b* paralogues in response to *M. laxa* infection. Besides, Vall-llaura and Fernández-Cancelo et al. (2022) proved that both *PpERF1a* and *PpERF1b* expression levels decreased to some extent as the fruit develops/ripens, paralleling the increased susceptibility to *Monilinia* (Mari et al., 2003).

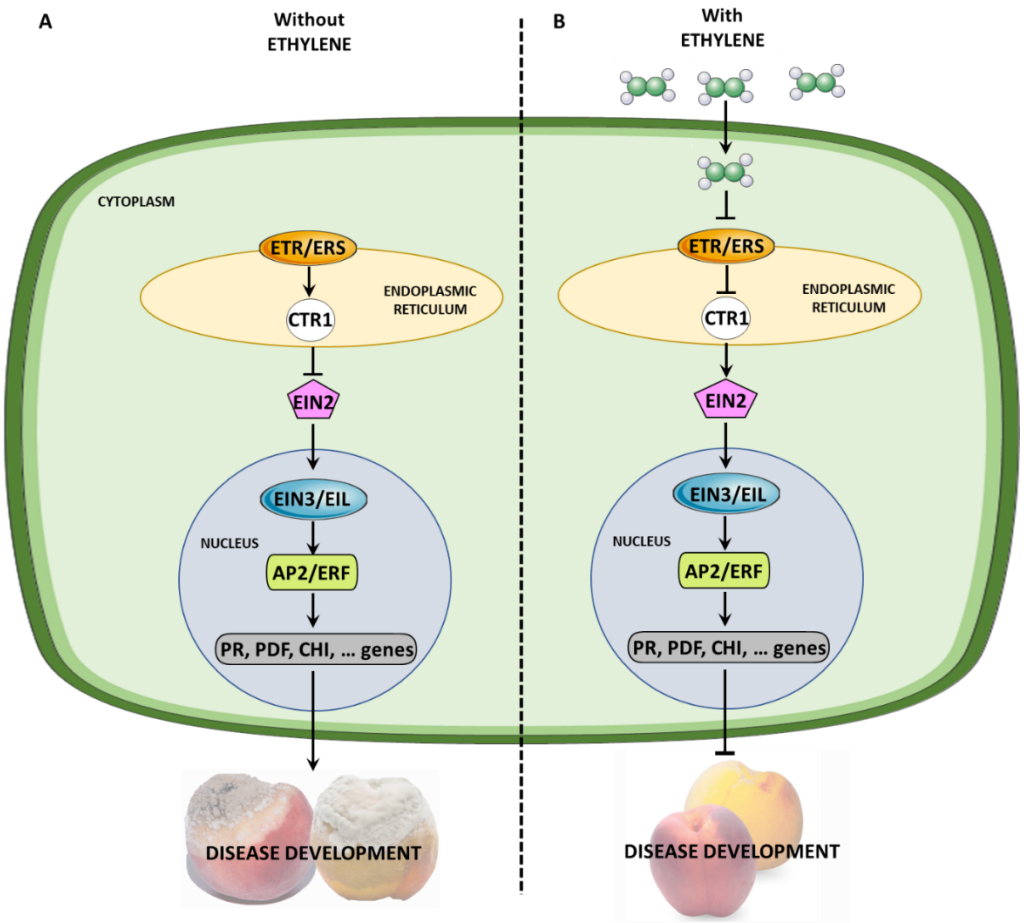


Figure 5. Schematic representation of the ET signaling pathway in (A) the absence or (B) presence of ET mediating plant/fruit resistance to pathogens.

To date, the induction of the ET-induced defence-related effector proteins known as pathogenesis related (PR) proteins (Chakravarthy et al., 2003; Thomma et al., 1999) is among the most well-described defence mechanism against pathogens. Within the huge family of PR proteins (Van Loon and Van Strien, 1999), vacuolar β-1,3-glucanases (PR-2), vacuolar basic-chitinases (PR-3), acidic hevein-like proteins (PR-4), and plant defensins (PDFs; PR-12) are known to respond to ET through the GCC-box located in their promoters. All these PRs act as antimicrobials by targeting β-1,3-1,6-glucans, chitin or even the microorganisms membrane (Broekaert et al., 2000; Van Loon and Van Strien, 1999). Their activation is, however, not only dependent on ET but also on JA (Lorenzo et

al., 2003; Penninckx et al., 1998). For instance, in Arabidopsis, both ET and JA act through several AP2/ERF transcription factors such as ORA59, ERF1, ERF2 and ERF14 to finally activate a set of defence-related genes including PDF1.2, basic chitinase and agmatine coumaryl transferase (Li et al., 2018; McGrath et al., 2005). Specifically, Sherif et al. (2012b) proved that in peaches, PR genes were more induced in a resistant peach cultivar, and that the expression of some PR was dependent on both ET and JA. Hence, these results pointed out the role of the ET-signaling pathway in mediating the resistance to *X. campestris* pv. *pruni*. Likewise, the PR-1 gene from both mature and immature nectarines was induced in response to *M. laxa* in the transcriptomic study recently published by Balsells-Llauradó et al. (2020).

This said, other studies, working with other pathosystems such as Arabidopsis, have described to some extent controversial results by showing that JA can also antagonize ET signaling through the inactivation of EIN3 transcription factor by MYC2 (Song et al., 2014; Zheng et al., 2017). In this regard, Díaz et al. (2002) also proposed that ET and SA have a synergistic effect on defence gene expression of tomato plants to *B. cinerea*, but an antagonistic role when referring to resistance.

Another defence mechanism, described in carrot roots and triggered by ET, includes the confinement of the pathogen by building-up or reinforcing the cell wall, for instance, by accumulating hydroproline-rich glycoprotein encoding transcripts through an ET-response mechanism (Ecker and Davis, 1987). Lloyd et al. (2011) also demonstrated the importance of the ET-related accumulation of hydroxycinnamates and monolignols at the cell wall of Arabidopsis to confine the disease caused by *Botrytis cinerea*. As peach fruit develops, the accumulation of these compounds decreases, as recently shown by Vall-llaura and Fernández-Cancelo et al. (2022), when analysing ‘Diamond Ray’ nectarines. The lower concentration of those hydroxycinnamates in ripe fruit may not be sufficient to

confine the disease and hence partially explain the enhanced susceptibility to *Monilinia* in mature fruit (Baró-Montel et al., 2021; Mari et al., 2003). Along these lines, Balsells-Llauradó et al. (2020) also found that phenylpropanoid compounds were enriched in nectarine fruit in response to *M. laxa* inoculation and, although this increase was higher in the mature tissue, the pathogen finally succeeded in the infection process. Similar results have been previously observed in other pathosystems such as grape berries-*Botrytis cinerea* (Blanco-Ulate et al., 2015). Hence, it is clear that not specific compounds but rather a coordinated balance among multiple substances accounts for enhanced resistance or susceptibility to pathogens.

Lastly, another way to overcome the pathogen attack is through the biosynthesis of antimicrobial secondary metabolites such as phytoalexins. As for other compounds, it has also been demonstrated in carrot cells that some phenylpropanoid-derived phytoalexins are inducible by ET (Ishigaki et al., 2004) thereby further reinforcing the crucial role that ET may have during host-pathogen interactions.

What is so far clear is that the activation of all these defence fruit responses aims to prevent or at least to delay the onset and the spreading of the infection. However, the pathogen itself has also the capacity to modulate or escape such host changes, finally accomplishing the infection process.

3.2. Pathogens have also the ability to produce ethylene

Not only plants, but also fungi have the ability to produce a wide range of hormones (Chanclud and Morel, 2016), including ET. Just like plants, fungi can biosynthesize ET from methionine by the action of the ACS. However, this pathway is rarely used, and unlike plants, microorganisms have also the ability to produce ET through other pathways (Fig. 6). The first pathway is the 2-keto-4-methylthiobutyric acid (KMBA) pathway, in

which methionine is deaminated to produce KMBA, which, in turn, is spontaneously or enzymatically oxidated to finally give rise to ET (Yang, 1969). In the other pathway, the 2-oxoglutarate is used as a substrate by the ET-forming enzyme (EFE) to produce ET.

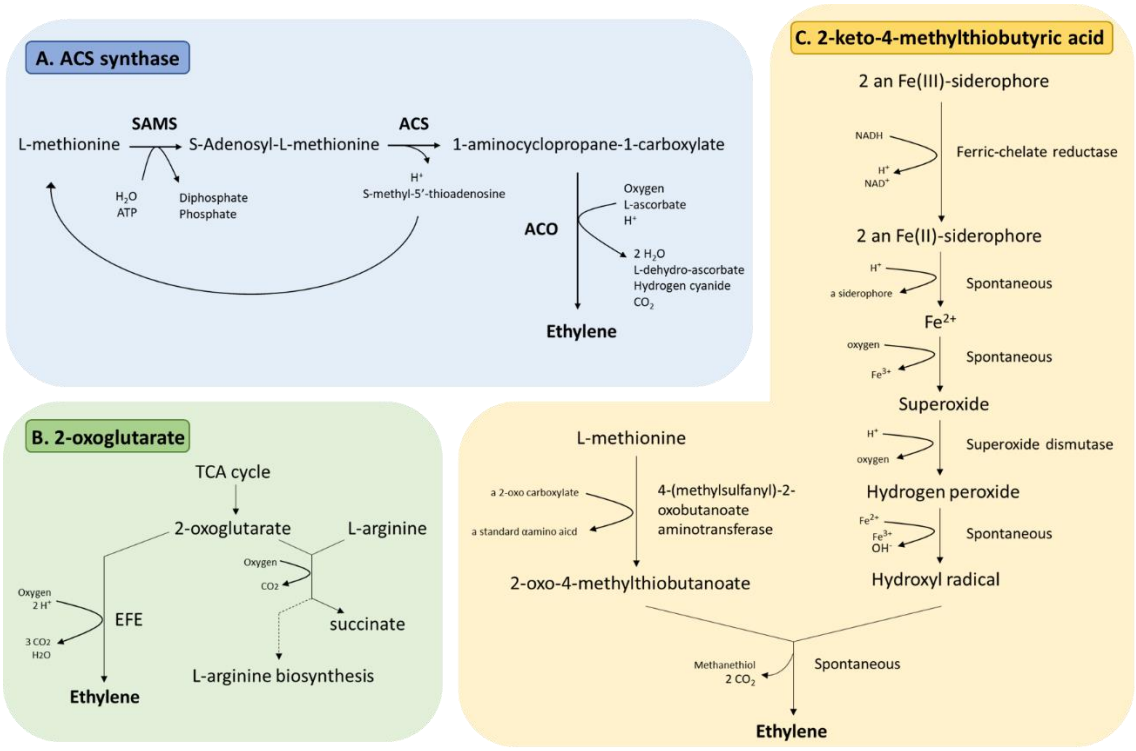


Figure 6. Ethylene biosynthetic pathways. (A) Typical plant ET biosynthesis from methionine through the action of the ACC synthase. (B) ET synthesis from 2-oxoglutarate mediated by the EFE enzyme. (C) Non-enzymatically mediated ET synthesis from methionine through the 2-keto-4-methylthiobutyric acid (KMBA) pathway.

The ability to produce ET has been demonstrated in various fungal species with different lifestyles, including pathogenic but also symbiotic fungi (Chanclud and Morel, 2016). Since the first study demonstrating the ability of *Penicillium digitatum* to produce ET (Fergus, 1954), many reports have described the same capability in other fungi including *Aspergillus* spp., *Botrytis* spp., *Fusarium* spp., *Colletotrichum* spp. or *Mucor* spp., among others (Table 1). For instance, Cristescu et al. (2002) proved that ET generation by *B. cinerea* was dependent on the methionine concentration in which the fungus was grown.

Besides, and by using some specific inhibitors, these authors demonstrated that *B. cinerea* does not produce ET by the ACC pathway but mainly through KMBA. This hypothesis was further reinforced by Qadir et al. (2011) who demonstrated that this fungus does not require ACC as a precursor but rather utilises an enzyme similar to ACO from plants.

Table 1. Example of microorganisms and conditions in which ET production has been detected.

Microorganism	Growth conditions	Substrate/Precursor	Reference
Alternaria alternata	Liquid medium incubated under light or darkness	Methionine or KMBA	Zhu et al., 2017
Aspergillus nidulans	Solid medium incubated under light or darkness	-	Roze et al., 2004
Aspergillus parasiticus	Solid medium incubated under light or darkness	-	Roze et al., 2004
Botrytis cinerea	Static or shaken cultures incubated in the dark	Methionine	Qadir et al., 1997
Botrytis cinerea	Liquid medium incubated under light or darkness	Methionine	Chagué et al., 2002
Botrytis cinerea	Solid medium	Methionine or KMBA	Cristescu et al., 2002
Botrytis cinerea	Liquid medium	Methionine or KMBA	Qadir et al., 2011
Colletotrichum dematium var. truncatum	Liquid cultures incubated under light	Methionine	Tzeng and DeVay 1984
Fusarium oxysporum* f. sp. *pini	Liquid media	Methionine	Graham and Linderman 1980
Fusarium oxysporum f. sp. vasinfectum	Liquid cultures incubated under light	Methionine	Tzeng and DeVay 1984
Monilinia fructicola	Solid or liquid media	Methionine	Vall-llaura et al. unpublished
Monilinia laxa	Solid or liquid media	Methionine	Vall-llaura et al. unpublished
Mucor hiemalis	Liquid media	Methionine	Lynch and Harper 1974
Penicillium digitatum	Shaken or static cultures	Citric, malic, lactic, or succinic acids	Fergus 1954

Penicillium digitatum	Shaken or static liquid cultures	Methionine	Chalutz and Lieberman 1977
Penicillium digitatum	Static or shaken solid or liquid media	Methionine or 2-oxoglutarate	Yang et al., 2017
Penicillium expansum	Shaken cultures	Methionine or KMBA	Yang et al., 2017
***Verticillium dahliae* Kleb.**	Liquid cultures incubated under light	Methionine or KMBA	Tzeng and DeVay 1984

Chagué et al. (2002) also showed that the ET production by *B. cinerea* through the KMBA pathway in liquid media supplemented with methionine was dependent on the light. Similarly, Roze et al. (2004) showed that both *Aspergillus nidulans* and *Aspergillus parasiticus* produce 2-fold higher amounts when growing under light conditions if compared to darkness. In other pathogens such as *P. digitatum* and *Penicillium expansum*, the amounts and the pathway used for ET production *in vitro* seemed to be dependent on both the species and the nutrient availability (Yang et al., 2017). Other fungi including several species of *Verticillium*, *Fusarium oxysporum* f. sp. *vasinfectum* and *Colletotrichum dematium* var. *truncatum* also produce ET either in presence of methionine or in a light-dependent manner (Tzeng and DeVay 1984).

All these specific observations revealed that the ET production capability is strictly dependent on the pathogen and both the conditions and the growth media. To date, however, no studies have been published to demonstrate the ability of *Monilinia* spp. to produce ET under *in vitro* conditions, although sharing some common features and lifestyle with *B. cinerea*. However, studies from our team revealed that both *M. fructicola* and *M. laxa* could produce ET when grown in a peach-based media supplemented with different concentration of methionine and under specific photoperiod conditions (Vall-llaura et al., unpublished). These results together with the identification of putative genes coding for ET biosynthetic genes (namely EFE; Vall-llaura et al., unpublished), by means

of a BLAST analysis on the available genomes of both *M. laxa* and *M. fructicola* species (Naranjo-Ortíz et al., 2018; Vilanova et al., 2021), suggest that ET may have an important role for these fungi under specific growth conditions. Further studies elucidating the capability and the metabolic pathways involved in the biosynthesis of ET by *Monilinia* spp. are warranted.

Many possibilities exist regarding the rationale for ET production by fungi. Firstly, this hormone has been shown to be necessary for germination and appressoria formation of *Colletotrichum* sp. (Flaishman and Kolattukudy, 1994). In other pathogens such as *B. cinerea* and *Alternaria alternata* (Chagué et al., 2006; Kępczyńska, 1994) it has also been associated to development, including spore germination and hyphal growth. Kępczyńska (1989) already demonstrated, many years back, that the treatment with the ET donor ethephon stimulated the germination of *B. cinerea* while treatments with the ET inhibitor 2,5-norbornadiene (NBD) inhibited the germination. Similarly, Kępczyńska (1994) demonstrated a similar effect in *A. alternata* in which application of NBD inhibited the mycelial growth of the fungus while the compound arninoethoxyvinylglycine (AVG), greatly inhibited spore germination. Although ethephon and the inhibitor AVG have other effects rather than releasing ET or inhibiting the ACC pathway, respectively, the ET involvement on the development of these fungi seems evident. In this line, Zhu et al., 2017 revealed that the ET produced by *A. alternaria* through the KMBA pathway was positively associated with an increased virulence when infecting grapes. The question arises on whether the effects induced by exogenous ET-related treatment benefit or not the fungus, since the effects are apparently dependent on the fungus itself, the development stage, and on the pathosystem being considered.

Despite the abundant studies available for other fungi, limited information is, once again, available regarding *Monilinia* ssp. El-Kazzaz (1983) proved that exposure of *M. fructicola* to ET stimulated spore germination and the germ tube elongation but had little influence on its growth and only under certain concentrations. Similarly, in a more recent study, Vall-llaura et al. (2020) described that the exogenous application of pure ET and 1-MCP had no effect on both *M. laxa* and *M. fructicola* neither on the mycelial growth nor on the colony morphology (Fig. 7).

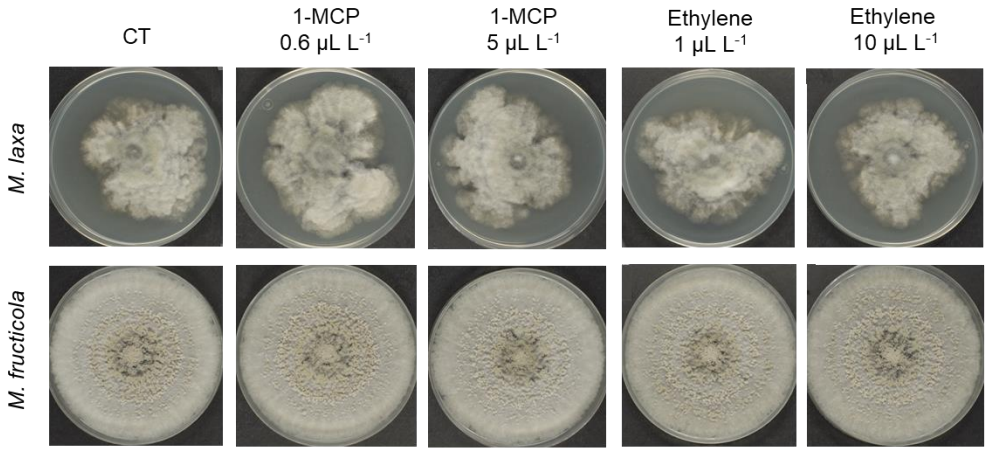


Figure 7. Colony morphology of *M. laxa* and *M. fructicola* submitted to different 1-MCP and exogenous ET treatments (Images are retrieved, with the permission of Elsevier, from Vall-llaura et al., 2020).

More in-depth studies are still encouraged to unravel the role of ET on the development and virulence of *Monilinia* spp. Besides, while the mechanisms of ET perception have been deeply studied in plants (the reader is referred to Section 2), no studies on the cascades mediating such effect in fungal species are yet available.

3.3. The specific role of ethylene during the host-pathogen interaction

Enhanced ET production is typically observed during plant-microbe interactions, including fungi, bacteria, viruses, and nematodes. As described above, it is clear that both

387 the hosts and fungi can produce ET, although for different purposes. While the host
388 hormone seems to be exclusively synthesized as a defence response, especially when
389 referring to necrotrophic pathogens, the pathogen may synthesize ET in an attempt to
390 make the fruit more susceptible to the infection and/or to modulate the host ET
391 biosynthesis, and thus, the defence response. Biotrophic pathogens are characterized for
392 requiring living cells to accomplish their lifestyle. On the other hand, necrotrophic
393 pathogens kill host cells and feed on them and hence, will manipulate the hormonal
394 crosstalk favouring their lifestyle.
395 What it is well accepted is that during the host-pathogen interaction, ET levels tend to
396 increase (Broekaert et al., 2006), yet not being able to discriminate on whether the
397 hormone is being produced by the fruit or the pathogen itself. Cristescu et al. (2002)
398 demonstrated that the infection of tomato cultivars with *B. cinerea* released 100-fold
399 higher ET levels that when measuring the production by the fungi alone, and that the
400 production was not triggered by the fungal ET but rather synchronized with the growth
401 rate of the fungus.
402 The ET profile has also been examined in other pathosystems. For instance, in apples,
403 Ballester et al. (2017) demonstrated by a transcriptomic analysis that a resistant genotype
404 to *P. expansum* over-expressed ET and JA-related genes if compared to a susceptible
405 genotype when infected with *P. expansum*. Silencing of the *MdACS* gene in apples lead
406 to greater susceptibility to *B. cinerea*, further demonstrating the importance of ET
407 production on resistance to infection (Akagi et al., 2011). Some years earlier, Ellis and
408 Turner (2001) already demonstrated that the Arabidopsis *cev1* mutant, producing higher
409 amounts of ET and JA, was more resistant to several species of powdery mildew. In other
410 hosts (i.e. rice), ET is also required for the resistance to *Magnaporthe oryzae*, and both

411 ET-insensitive or deficient mutants displayed a reduced expression of defence-related
412 genes (Helliwell et al., 2016).
413 In the case of tomato plants, Díaz et al. (2002) showed that plants treated with exogenous
414 ET lead to a major expression of several pathogenesis-related genes and a decreased
415 susceptibility to *B. cinerea*. On the other hand, 1-MCP treatment increased the
416 susceptibility of the tomato plants to the fungus. In citrus fruit, referred to as non-
417 climacteric, ET perception was also shown to be important to prevent *P. digitatum* decay
418 (Marcos et al., 2005). The following section is especially focused on the *Monilinia*-peach
419 interaction for which the available literature is rather scarce.
420

421 **3.3.1 A focus on the peach-*Monilinia* spp. interaction**

422 As described earlier, the debate remains on whether and how *Monilinia* spp. can produce
423 ET, and which is its role during the stone fruit-*Monilinia* spp. interaction. Recently, a
424 transcriptomic analysis from Balsells-Llauradó et al. (2020) highlighted cysteine and
425 methionine, as one of the prevailing metabolisms on nectarine fruits infected with *M. laxa*.
426 Besides, *ACS* and *ACO* genes as well as *ERF1/2* were also upregulated in the infected
427 tissue if compared to the control fruit. Once again, these results further suggest that ET
428 may play a predominant role in explaining the peach resistance or susceptibility to
429 *Monilinia* spp.
430 In this sense, other recent studies have also aimed to unravel the specific ET mechanism
431 underlying the infection process by either *M. laxa* or *M. fructicola* on peach/nectarine.
432 Baró-Montel et al. (2019) demonstrated that the fruit ET biosynthetic pathway and the ET
433 production of the peach-*Monilinia* spp. pathosystem was differently regulated depending
434 on the fruit development stage, the *Monilinia* species inoculated, and even the virulence
435 of the inoculated strain. For instance, the synthesis of ET produced by the fruit at 49 DAFB

(when fruit has 35.50 % of the final fruit size) was inhibited once inoculated with the less virulent strain of *M. laxa*, probably as an effort of *Monilinia* to impair the defence response triggered by this hormone. However, the fruit infected with either the more virulent *M. laxa* strain or the *M. fructicola* species displayed a reduced ET production during the first hours post-inoculation to peak thereafter once the fruit was completely colonised. Interestingly, a completely opposite tendency was observed when referring to a more mature fruit (126 DAFB; 67.92 % of the final fruit size and at a pre-climacteric stage fruit). In the latter scenario, the interaction of peach fruit with *Monilinia* species displayed an increased ET emission. Specifically, *M. fructicola* or the more virulent *M. laxa* strain rapidly increase the ET production, while the less virulent strain also did it although in a more sluggish manner. Accordingly, it was hypothesized that, at this specific phenological stage, the fungus was promoting the ET emission leading to a cell wall weakening and thus, facilitating the fungal infection. Importantly, these authors described how such effect was strictly species-dependent since the fruit inoculated with one of the *M. laxa* strains (the less virulent) tested was completely unable or had difficulty, depending on the development stage, to alter the fruit ET production. These interesting results were further reinforced by the work from Vall-llaura et al. (2020) on peach petals. Again, the analysis of the ET metabolism of peach petals infected with *Monilinia* spp. revealed that while the *M. fructicola*-peach petals interaction displayed a huge increase on the ET levels, the ET produced by the *M. laxa*-peach petals interaction was completely inhibited at the initial stages of the interaction. Besides and irrespective of the ET origin, *ERF* gene expression from peach petals, and more specifically *PpERF1b*, *PpERF2a*, and *PpERF2b*, were differentially modulated depending on the *Monilinia* species inoculated. Further evidence of this distinct modulation was obtained by Balsells-Llauradó et al. (2020) who showed

that mature nectarines inoculated with *M. laxa* displayed an ET inhibition at the beginning of the interaction.

One explanation for such results could be provided by the proteomic study performed by Papavasileiou et al. (2020), who demonstrated that *M. laxa* inoculated nectarines displayed higher levels of 1-aminocyclopropane-1-carboxylate deaminase. This enzyme is responsible for the degradation of the ET precursor ACC, and hence, higher levels of this enzyme would result in a reduction of the fruit ET biosynthesis.

Other possible explanations could rely on the ability that some pathogens may have to overcome the defence signaling, either by evading, hijacking, or disrupting the hormonal signaling. As an example, El-Oirdi et al. (2011) described that *B. cinerea* takes advantage of the antagonism between ET/JA and SA to accomplish the infection process in tomato plants. Hence, through the production of an exopolysaccharide, the fungus induces the SA pathway that in turn antagonizes the JA pathway, responsible for controlling the disease triggered by necrotrophic pathogens. Other examples include the secretion of the PsAvh238 effector of *Phytophthora sojae*, which abolishes ET by binding to the soybean ACS and destabilizing its activity (Yang et al., 2019). Excellent reviews already exist describing the many strategies used by different pathogens to mediate with the host hormonal signaling (Han and Kahmann, 2019; Kazan and Lyons, 2014; Robert-Seilaniantz et al., 2011), and some of them may ease to further shed light on the possible role that ET, in conjunction with other hormones, may play during peach-*Monilinia* interaction.

3.4 Ethylene does not act alone but in combination with other key metabolites during host-pathogen interaction

Besides the complex interaction between ET and other hormones (mentioned in previous sections), the biosynthesis and signaling mediated by this hormone can also be altered by other key metabolites, including polyamines and ROS.

Polyamines are aliphatic polycationic compounds, often conjugated with phenolic compounds and molecules such as DNA and RNA, that actively participate in plant-microbe interactions (Jiménez Bremont et al., 2014). They could be synthesized through arginine or through the decarboxylation of SAM, a common precursor with the ET biosynthetic pathway (Fig. 8).

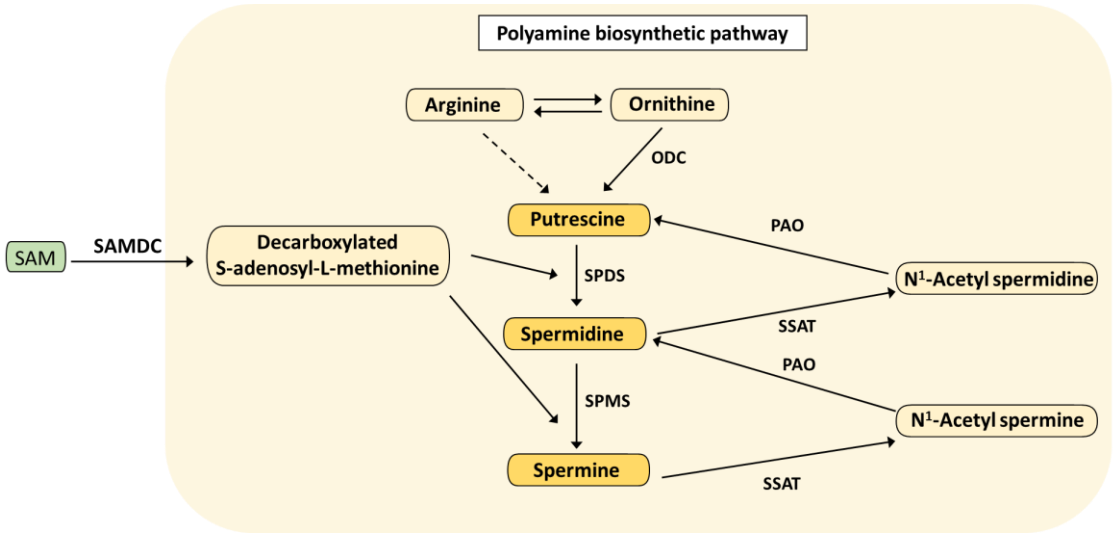


Figure 8. Polyamine biosynthetic pathway from the ethylene precursor SAM and from the amino acids arginine and ornithine. S-adenosyl-L-methionine decarboxylase (SAMDC), ornithine decarboxylase (ODC), spermidine synthase (SPDS), spermine synthase (SPMS), polyamine oxidase (PAO) and spermidine/spermine N^1-acetyltransferase (SSAT).

Hence, the tight relation between ET and polyamines implies that promoting one of these metabolisms impairs the other one (Ziosi et al., 2006). In fact, a transgenic tomato overexpressing the yeast spermidine synthase presented a down-regulation of genes

involved in ET biosynthesis and signaling, which ultimately lead to an increased susceptibility to *B. cinerea* (Nambeesan et al., 2012). Besides, the ability of fungal cells to produce polyamines should not be underrated (Rocha and Wilson, 2019).

Accordingly, the alteration of the ET biosynthetic or signaling pathway during peach-*Monilinia* interaction may be also caused, to some extent, by the action of polyamines. However, and to the best of our knowledge, no information exists about the capability of *Monilinia* spp. to synthesize polyamines. This said, a BLAST analysis on the *M. laxa* and *M. fructicola* genomes using polyamine biosynthetic genes (ornithine decarboxylase, spermidine and spermine synthase and polyamine oxidase) from *S. cerevisiae* reveals the presence of these biosynthetic genes in both *Monilinia* spp. (Table 2).

Table 2. Identification of *M. laxa* and *M. fructicola* polyamine biosynthetic genes using *S. cerevisiae* gene templates for *in silico* searches. Gene ID, coverage, and identity of the BLAST analysis are indicated.

Gene name	***S. cerevisiae* gene ID**	***M. laxa***			***M. fructicola***		
		Gene ID	Coverage (%)	Identity (%)	Gene ID	Coverage (%)	Identity (%)
Ornithine decarboxylase	853651	Monilinia__017910	99.27	84.38	MFRU_037g00770.1	99.64	90.51
Spermidine synthase	856182	Monilinia__058590	94.20	79.42	MFRU_008g01000.1	96.93	79.30
Spermine synthase	850838	Monilinia__057060	36.67	25.6	MFRU_021g00430.1	36.67	25.6
Polyamine oxidase	855034	Monilinia__050860	86.81	28.57	MFRU_012g01390.1	86.81	28.38

Although polyamines are described to be essential on growth, development and differentiation of fungi (Valdés-Santiago et al., 2012), the controversy exists on the role of polyamines during the host-pathogen interactions, especially given recent evidence showing that exogenous application of polyamines reduce fungal rots caused by *A. alternata* in apricots (Li et al., 2019).

Other molecules known to interact with ET during host-pathogen interactions are ROS. Reactive oxygen species encompass those molecules generated from a partly reduced or excited forms of oxygen. They include hydrogen peroxide (H_2O_2), the singlet oxygen ($^1O_2$), the superoxide anion ($O_2^{\cdot-}$) and the radical hydroxyl ($^{\cdot}OH$). The fruit/plant development and ripening processes are mediated by numerous metabolic pathways that generate ROS due to their aerobic and photosynthetic nature. In fact, well-known examples of ROS-producing metabolisms are the mitochondrial respiration, the peroxisomal photorespiration cycle and the chloroplastic photosynthetic processes (Tripathy and Oelmüller, 2012). In non-photosynthetic tissues such as fruits, the main ROS generation occurs in the mitochondria. However, ROS are also produced in other subcellular locations such as the vacuoles, and the cell wall. The plasma membrane-localized NADPH oxidases (also known as respiratory burst oxidase homologs, RBOHs) are one of the primary enzymes for autonomous ROS production (Figure 9). Due to its charged, uncharged or lipophilic nature, ROS can travel through membranes depending on their stability or remain confined (or scavenged) to specific subcellular compartments. For a detailed description of ROS production and signaling within plants cells, the reader is referred to recent reviews from Waszczak et al. (2018) and Kohli et al. (2019).

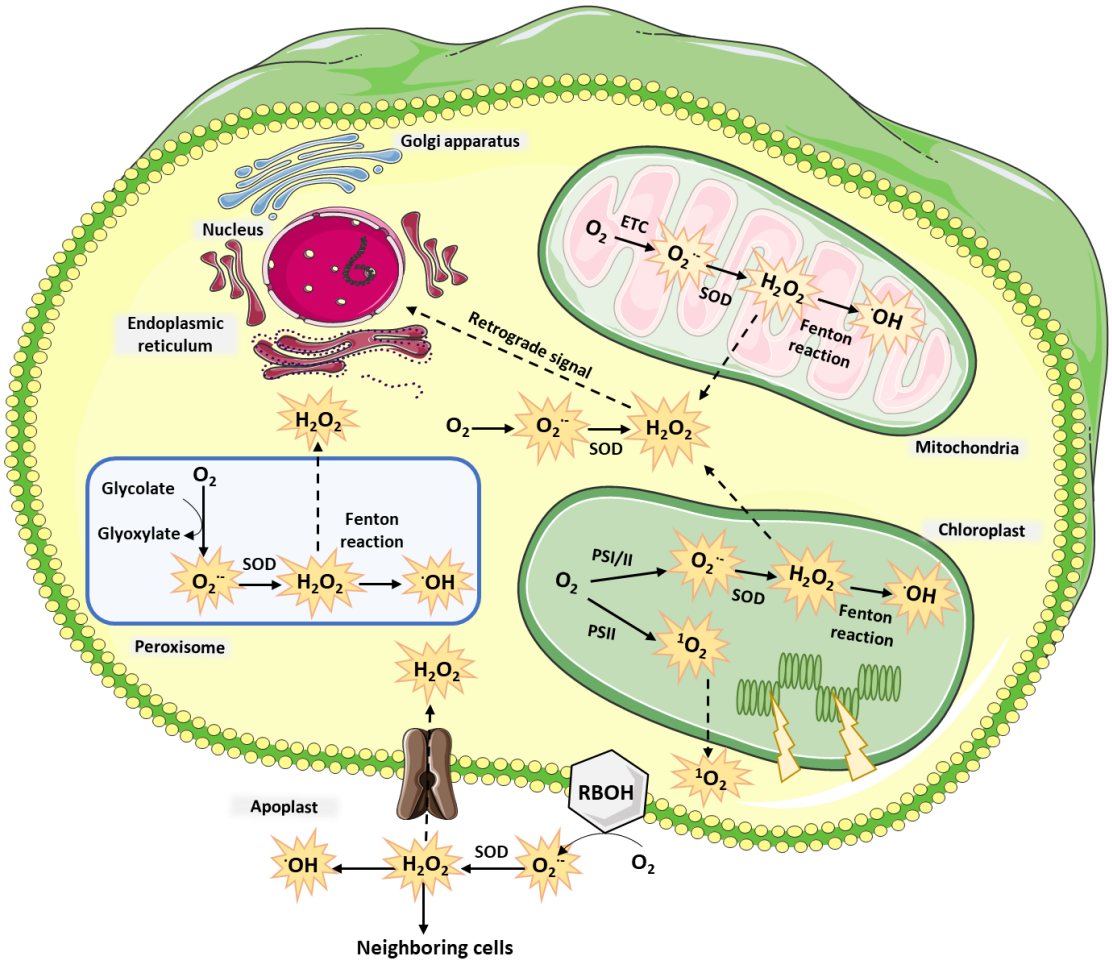


Figure 9. Schematic representation of the main sources of ROS in plant cells. To simplify the figure, some cell structures such as the cell wall have not been represented.

To avoid an oxidative stress situation, hence impairing the correct functioning of the cells, ROS levels are tightly regulated by a plethora of either enzymatic (e.g., CAT, SOD) or non-enzymatic (e.g., GSH, carotenoids) antioxidants, both functioning in a tightly regulated manner during all the development/ripening of nectarine fruit (Vall-llaura and Fernández-Cancelo et al., 2022).

However, it is already well-known that ROS are not only considered undesirable products responsible of generating cellular damage but also involved in many signaling processes either alone or in conjunction with other molecules such as ET. Specifically, ROS are also produced by both plants/fruits and pathogens and play a crucial role during biotic

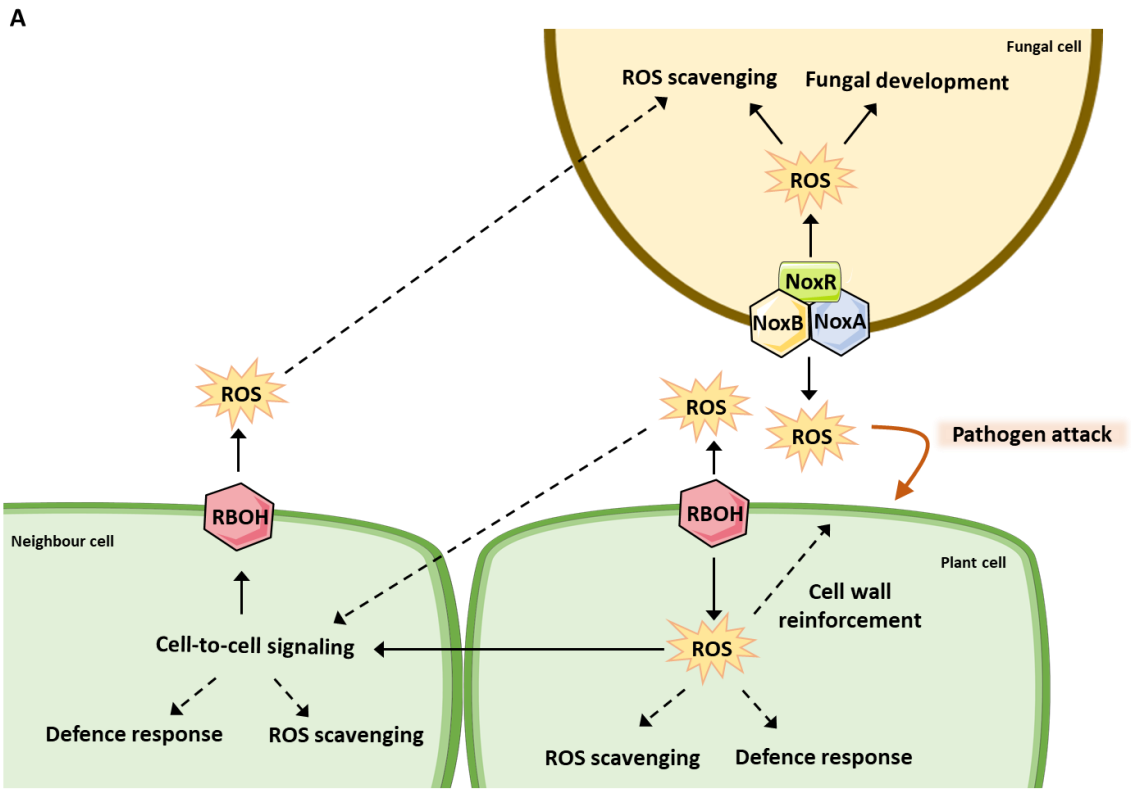
554 interactions. In fact, one of the initial responses of both the fruit and the pathogen resulting
555 from their interaction is the generation of ROS (Fig. 10A). This so-called “oxidative
556 burst”, mainly triggered by H_2O_2 and $O_2^{\cdot-}$ production, is a hallmark of a successful
557 recognition. The main goal of this burst is the protection of the cells, the cross-linking of
558 the cell wall to block pathogen entry, and also to act as local and systemic secondary
559 messengers to trigger stomatal closure or activation of protectant and defence genes
560 (Lamb and Dixon, 1997).
561 Interestingly, ROS and hormones tightly cooperate to ensure a correct and efficient
562 response to both abiotic and biotic stimuli. On one hand, hormones activate ROS
563 production through the NADPH oxidases, while ROS act as secondary messengers in the
564 hormonal signaling (Xia et al., 2015). As an example, ROS and ET act jointly to respond
565 to salinity stress (Shin and Schachtman, 2004) but also during fruit/plant development
566 (Steffens and Sauter, 2009). Regarding to biotic stimuli, Arabidopsis mutants impaired in
567 ET sensing presented a significant reduction in the flg22-triggered oxidative burst, thus
568 demonstrating the requirement of ET for the early stage plant immune response
569 (Mersmann et al., 2010). However, the specific mechanisms underlying the ET-mediated
570 enhancement of ROS production remains to some extent still elusive.
571 Rapid systemic responses are crucial for the acclimatation of plants to abiotic/biotic
572 stresses. This cell-to-cell communication is basically performed by the interplay of
573 second messengers such as calcium (Ca^{2+}) and ROS (Marcec et al., 2019)(Fig. 10B).
574 Hence, either the perception of abiotic stresses or the recognition of pathogens rapidly
575 activates a ROS burst mediated by RBOHs. In *A. thaliana*, a total of ten RBOHs have
576 been described, being *AtRBOHD* completely essential for ROS production (Wang et al.,
577 2020). RBOHs are synergically activated by Ca^{2+}, phosphorylation and modification of
578 their cysteine (Cys) residues (Kärkönen and Kuchitsu, 2015; Marcec et al., 2019). In fact,

such ROS production depends on the receptor-like kinases (RLKs), such as cysteine-rich receptor-like kinases (CRKs) and *BOTRYTIS*-INDUCED KINASE 1 (BIK1), which can interact with RBOHs. Upon ROS perception, they can be directly activated (i.e., direct oxidation of RLK ectodomains through Cys residues) or indirectly (i.e., a ROS sensor conveys the signal to RLKs), triggering phosphorylation events, and finally, the signal transduction (Kimura et al., 2017). In fact, it has been described that upon pathogen perception, BIK1 mediates the phosphorylation of RBOH (Kadota et al., 2014). In parallel, such ROS accumulation can induce calcium channels in a process known as ROS-induced calcium release (RICR), resulting in altered Ca^{2+} levels. Cytosolic Ca^{2+} accumulation can, in turn, enhance ROS production through the modulation of RBOH proteins (Gilroy et al., 2014) leading to what is currently known as ROS wave. The ROS wave can then activate the mitogen-activated protein kinase (MAPK or MPK) cascade.

In plants, the activation of the MAPK cascade could occur by a H_2O_2-mediated activation (either directly or through a redox sensor) as described for instance in Arabidopsis, tobacco or rice, or by inactivating the MAPK repressors (Liu and He, 2017). In fact, recent evidence suggest that MAPK can sense ROS (i.e., H_2O_2), likely through the oxido-reduction of their thiol groups. Arabidopsis AtMPK2, AtMPK4, and AtMPK7 are sulfenylated by the action of H_2O_2 (Waszczak et al., 2014), while in *Brassica napus*, BnMPK4 is H_2O_2-dependent aggregated through one of its Cys residues (Zhang et al., 2015). Finally, another possibility of MAPK signaling activation relies on the redox-dependent activation of cell wall integrity (CWI) sensors (Vainonen and Kangasjärvi, 2015). Thus, one hypothesis could be that the OOMYCETE SUSCEPTIBILITY1 (IOS1) sense the damage caused by both ROS and pathogens to the cell wall, and consequently, triggers the MAPK activation (Kimura et al., 2017). Alternatively, H_2O_2 could also be imported directly to the cytoplasm through aquaporins (Tian et al., 2016). Once activated,

this cascade consists of the mitogen-activated protein kinase kinase kinase (MAPKKK) MEKK1, the mitogen-activated protein kinase kinase (MAPKK) MKK4/5 and the mitogen-activated protein kinase (MAPK) MPK3/6 (Asai et al., 2002). In fact, the kinetics of AtMPK3/AtMPK6 activation parallels those from AtRBOHD-dependent ROS production, although activation of such MAPK could also occur independently of the PAMP-triggered AtRBOHD-dependent ROS production (Liu and He, 2017). Since this is not the main objective of this review, for a detailed information regarding the specific signaling events occurring from stress perception to MAPK activation, the reader is refer to excellent reviews focused on this topic (Kimura et al., 2017; Marcec et al., 2019).

Interestingly, ET biosynthesis is also controlled, to some extent, by MAPK signaling through the regulation of the ACS expression (Li et al., 2018) hence reinforcing the link between ROS and ET in fruit. Not only ET biosynthesis, but also ET signaling its mediated by MAPK (Ouaked et al., 2003; Yoo et al., 2008). MPK6 activates the ET biosynthesis pathway (within few minutes after the perception) through phosphorylation of ACS6 (Liu and Zhang, 2004; Meng et al., 2013) and hence, results in the activation of ERFs. Specifically, MPK6-ERF6 regulate ROS-responsive genes through the GCC box, and for such ERF6 expression, RBOHD is essential in Arabidopsis (Sewelam et al., 2013). An excellent work from Müller and Munné-Bosch (2015) exhaustively reviews the role of ERFs in the hormonal crosstalk as well as in mediating the redox signaling.


A
Fungal cell
ROS scavenging
Fungal development
ROS
NoxR
NoxB
NoxA
ROS
ROS
ROS
Pathogen attack
RBOH
RBOH
Neighbour cell
Plant cell
Cell wall reinforcement
Cell-to-cell signaling
ROS
Defence response
ROS scavenging
ROS scavenging
Defence response

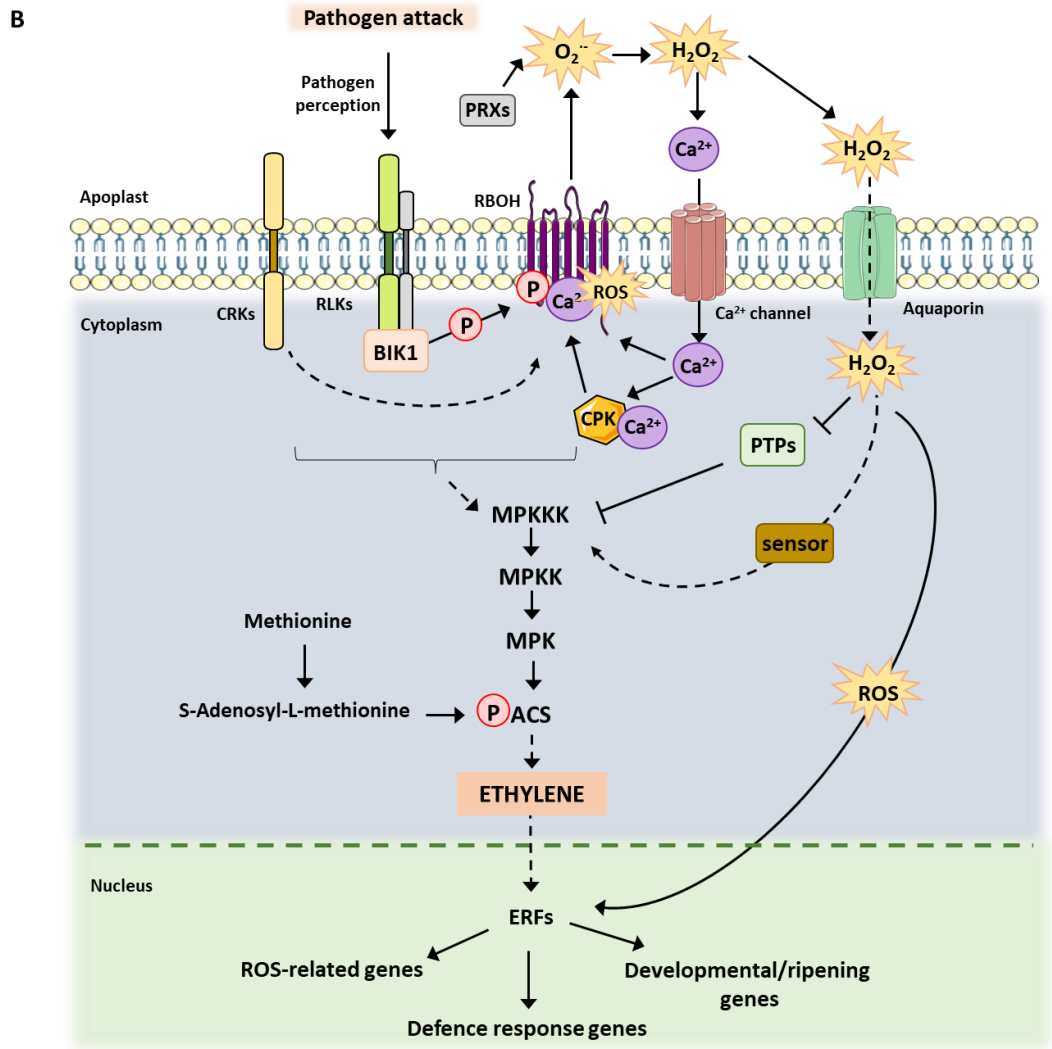

B
Pathogen attack
Pathogen perception
PRXs
$O_2^{\cdot-}$
H_2O_2
Ca^{2+}
H_2O_2
Apoplast
RBOH
P
Ca^{2+}
ROS
Ca^{2+} channel
Aquaporin
Cytoplasm
CRKs
RLKs
BIK1
P
Ca^{2+}
H_2O_2
CPK
Ca^{2+}
PTPs
MPKKK
sensor
MPKK
Methionine
MPK
S-Adenosyl-L-methionine
P
ACS
ROS
ETHYLENE
Nucleus
ERFs
ROS-related genes
Developmental/ripening genes
Defence response genes

Figure 10. (A) Plant and pathogen-derived ROS production during the plant-pathogen interaction and their role as signaling molecules. (B) ROS-mediated ET signaling pathway. The interplay between ROS, Ca^{2+} and phosphorylation events in response to an external stimulus (e. g. pathogen attack) triggers the MAPK cascade. In turn, ACS results activated through phosphorylation and enhances the ET biosynthesis. Finally, ERF activation would lead to the expression of several response genes. BIK1, BOTRYTIS-INDUCED KINASE1, CRKs, cysteine-rich receptor-like kinases; PRXs, peroxidases; PTPs, protein tyrosine phosphatase; RLKs, receptor-like kinases.

Scarce literature, if not null, exists regarding the cross-talk ET-ROS in fruit but especially in peach. Recently, our group demonstrated the tight relation of ROS and ERF signaling during peach fruit development and ripening (Vall-llaura and Fernández-Cancelo et al., 2022) suggesting that enhanced ROS was associated to greater fruit respiration at specific fruit developmental stages. Enhanced respiration could result in an increase in $^1O_2$ levels which could, in turn, mediate either directly or through the activation of ET biosynthesis, the ERF signaling cascade.

Based on the information detailed above, the debate is still active on which are the specific strategies used by pathogens, and specifically by *Monilinia* spp., to evade the peach defence response. Does *M. laxa* deploy a different mechanism from that of *M. fructicola* that could explain the differences on the ET biosynthesis and signaling during the interaction? Besides, further research concerning the complex regulation of ET and ROS in peach fruit and probably also in *Monilinia* spp. is also needed to better understand the host response and the virulence strategy of this brown rot causing agent.

4. Concluding remarks and prospects

Peach production is increasing around the world, as does the incidence of some major peach pathogens such as brown rot caused by *Monilinia* spp. However, the studies regarding *Monilinia* -peach interaction are still very scarce and further research is needed to achieve a correct control of the disease. The irruption of new technologies, firstly to obtain the fruit genome (*Prunus persica*) and fungal plant pathogen genomes (*Monilinia* spp.) and, subsequently, to have global transcriptomic analyses, has significantly changed the vision of the studies on the plant-pathogen interactions. The information gathered from these technologies may unravel key genes/metabolisms with a putative role in pathogen virulence and host resistance. Based on the information so far available, ethylene, acting in tight coordination with critical molecules such as polyamines and ROS, seems to be essential in mediating host-pathogen interactions.

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Conflict of interests

All authors declare no conflict of interest.

Author statement

Núria Vall-llaura: Conceptualization, Writing original draft, Writing- Reviewing and Editing.

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Josep Usall: Funding acquisition, Reviewing and Editing.

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SUPPLEMENTARY TABLE

Supplementary Table S1. Peach genes involved in ethylene biosynthesis, perception/transduction, and signaling, and their corresponding accession numbers.

Ethylene biosynthesis		Ethylene perception/transduction		Ethylene signaling	
Gene	**Gene ID**	**Gene**	**Gene ID**	**Gene**	**Gene ID**
PpSAMS1	Prupe.3G004000.1	***PpETR1***	Prupe.1G556000.1	***PpERF***	Prupe.5G061800.1
PpSAMS2	Prupe.6G306200.1	***PpETR2***	Prupe.1G034300.1	***PpERF***	Prupe.7G194400.1
PpSAMS3	Prupe.7G128500.1	***PpETR3***	Prupe.6G348000.1	***PpERF***	Prupe.3G240000.1
PpSAMS5	Prupe.1G107000.1	***PpERS1***	Prupe.8G265200.1	***PpERF***	Prupe.1G037700.1
PpACS1	Prupe.2G176900.1	***PpCTR1***	Prupe.7G117700.1	***PpERF***	Prupe.2G289500.1
PpACS2	Prupe.5G106200.1	***PpCTR1***	Prupe.5G231800.1	***PpERF***	Prupe.1G545700.1
PpACS3	Prupe.7G213900.1	***PpCTR1***	Prupe.6G337600.1	***PpERF***	Prupe.3G062800.1
PpACS5	Prupe.1G417800.1	***PpCTR1***	Prupe.1G412200.1	***PpERF***	Prupe.5G090800.1
PpACS6	Prupe.2G283100.1	***PpCTR1***	Prupe.1G533900.1	***PpERF***	Prupe.3G096000.1
PpACS7	Prupe.6G214400.1	***PpCTR1***	Prupe.3G117600.1	***PpERF***	Prupe.4G051200.1
PpACS8	Prupe.7G213800.1	***PpCTR1***	Prupe.1G022600.1	***PpERF***	Prupe.4G176200.1
PpACO1	Prupe.3G209900.1	***PpEIN2***	Prupe.6G235600.1	***PpERF***	Prupe.5G062000.1
PpACO2	Prupe.4G013800.1	***PpEIN3/EIL1***	Prupe.6G018200.1	***PpERF***	Prupe.6G165700.1
PpACO3	Prupe.7G212000.1	***PpEIN3/EIL2***	Prupe.2G058400.1	***PpERF***	Prupe.1G418600.1
PpACO5	Prupe.1G490000.1	***PpEIN3/EIL3***	Prupe.2G058500.1	***PpERF***	Prupe.4G051400.1
		PpEIN3/EIL4	Prupe.6G181600.1		
		PpEIN3/EIL5	Prupe.2G070300.1		
		PpEBF1/2	Prupe.1G480700.1		
		PpEBF1/2	Prupe.7G244300.1		