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# Ethylene as a modulator of disease resistance in plants

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**The role of ethylene in the hormonal regulation of plant development has been well established. In addition, it has been implicated in biotic stress, both as a virulence factor of fungal and bacterial pathogens and as a signaling compound in disease resistance. This apparent discrepancy has stimulated research on the effects of various types of pathogens on mutant and transgenic plants that are impaired in ethylene production or perception. It has become clear that ethylene differentially affects resistance against pathogens with different lifestyles and plays an important role in mediating different types of induced resistance.**

## The multiple functions of ethylene

The gaseous hormone ethylene is known to regulate multiple physiological and developmental processes in plants, such as seedling emergence, leaf and flower senescence, ripening, and organ abscission, and is also involved in the reactions of plants to abiotic and biotic stresses [1]. Ever since the discovery that ethylene functions as an endogenous regulator of fruit ripening, investigators have been wondering how this simple hydrocarbon can have such profound effects on plant development. Nowadays, the primary signal-transduction pathway of ethylene is known in substantial detail [2]. A common pathway involving perception by membrane-bound receptor proteins is likely to diverge downstream of the central regulatory protein EIN2, leading to the activation of various transcription factors that mediate the different responses in conjunction with other regulatory factors.

Enhanced ethylene production is an early, active response of plants to perception of pathogen attack and is associated with the induction of defense reactions [3]. It is generally assumed that ethylene production during stress contributes to stress alleviation, but several plant pathogenic fungi and bacteria are capable of producing ethylene as a virulence factor, which improves their ability to colonize plant tissues [4,5]. For instance, the ability of the bacterial leaf pathogen *Pseudomonas syringae* pv. *glycinea* to proliferate in the leaves of its host plant soybean is impaired in mutants that lack the capacity to produce ethylene [6]. Such observations indicate that ethylene produced during infection promotes disease rather than alleviates it. Indeed, ethylene is responsible for the epinasty and defoliation caused by the soilborne fungus *Verticillium dahliae* in cotton [7], and for the stunting and chlorosis of cucumber infected by cucumber mosaic virus [8,9]. Yet, similar to the defense-regulating compounds salicylic acid and jasmonic acid, plant-derived ethylene is generally considered to be involved in resistance [10]. In this review we attempt to explain these contradictory results and to clarify our current knowledge of the complexity of ethylene function in plant defense.

## Effects of ethylene on symptom severity of diseases caused by different pathogens

The discrepancy of the double signaling function of ethylene in disease resistance has been addressed in studies of various plant–pathogen interactions. Some authors gassed plants with ethylene or applied ethephon (2-chloroethylphosphonic acid), a chemical regulator that decomposes into ethylene, hydrochloric acid and phosphonic acid when taken up by plant tissues [1,3]. Other studies made use of inhibitors of ethylene synthesis or action. The committed step in the biosynthesis of ethylene from the amino acid methionine is the conversion of *S*-adenosylmethionine into 1-aminocyclopropane-1-carboxylic acid (ACC) by the enzyme ACC synthase (ACS), which can be blocked by aminoethoxyvinylglycine. ACC is converted to ethylene by ACC oxidase (ACO). This reaction is inhibited by cobalt ions or by the less-specific compound aminooxyacetic acid. Microorganisms produce ethylene from methionine by a different pathway or use α-ketoglutarate as an immediate precursor [4,11], allowing discrimination between pathogen- and plant-derived ethylene. Ethylene perception is abolished by compounds such as silver ions or the competitively acting gasses 2,5-norbornadiene or methylpropene [12].

Depending on the conditions and the plant–pathogen combination, seemingly contradictory results have been obtained [1,3,4]. For example, the causal agent of gray mould, the fungus *Botrytis cinerea*, is able to infect a wide range of vegetables, ornamentals and fruits. Ethylene treatments typically promote disease development (e.g. [13]) but, on carrot, ethylene appears to be involved in resistance [14]. In general, treatments with ethylene promote leaf senescence and fruit ripening, which can

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make tissues either more susceptible to disease or more resistant [15]. Often, ethylene treatment must increase disease development simply through its acceleration of ripening or senescence. In addition, the conditions under which experiments have been carried out are not always clearly specified. Furthermore, various abiotic stresses can inadvertently affect plant susceptibility to disease. However, several observations indicate that when ethylene is applied before inoculation with a pathogen, it reduces or has no effect on disease development, whereas disease development is accelerated when plants are treated with ethylene after infection [1]. Thus, it seems that the timing of the exposure of plants to ethylene can determine whether resistance is stimulated or reduced.

### Effects of pathogens on mutant and transgenic plants

More recently, the availability of plant mutants that are affected in their response to ethylene has enabled the role of ethylene during infection to be studied without possible side effects inherent in the use of chemicals (Figure 1). Moreover, a tomato line expressing the ACC deaminase (*ACD*) gene from *Pseudomonas* sp. strain 6G5 has been constructed that is deficient in ethylene production [16], and a transgenic ethylene-insensitive (Tetr) tobacco line has been generated [17] through transformation with the mutant ethylene receptor gene *etr1-1* from *Arabidopsis* [18]. When the reactions of these mutant and transgenic plants to different types of attackers were compared, either enhanced or reduced disease development was apparent (Table 1). Because of increased symptom severity in non-responsive mutants, ethylene was found to reduce diseases caused by several fungi and bacteria that kill their hosts (necrotrophs), or have a mixed biotrophic–necrotrophic lifestyle (in which they start exploiting the living host before killing it). By contrast, the occurrence of less severe symptoms indicated that ethylene stimulated diseases caused by various other fungi and bacteria with varying lifestyles, as well as infection by a cyst nematode and insect attack (Table 1).

The lack of ethylene perception also weakened the ability of transgenic Tetr tobacco plants to withstand

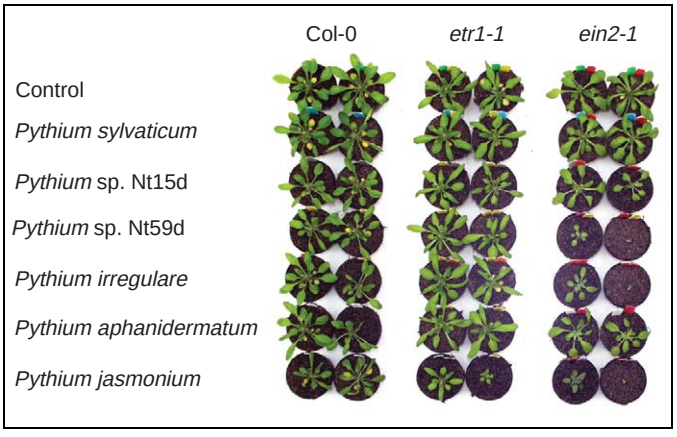


**Figure 1.** Differences in the susceptibility of *Arabidopsis* accession Col-0 wild type and the *etr1-1* and *ein2-1* mutants to 'damping-off' and growth retardation by various *Pythium* spp. Except for the plants inoculated with the isolate of *Pythium sylvaticum*, all *Pythium*-inoculated plants had reduced shoot fresh weights compared with the non-inoculated controls. Ethylene responsiveness is impaired more strongly in the *ein 2-1* than in the *etr1-1* mutant [18].

common, generally non-pathogenic, opportunistic soil-borne fungal organisms [17,19,20]. When grown in ordinary potting soil, Tetr plants gradually start wilting and develop necrosis at the crown, progressing into rotting of the stem and finally plant collapse (Figure 2a). These symptoms could be attributed to a 'spontaneous' infection by rot-causing oomycetous *Pythium* species and the root-infecting fungi *Chalara elegans*, *Fusarium oxysporum*, *Fusarium solani* and *Rhizopus stolonifer*, which were present in the potting soil but not recoverable from non-transformed control plants. Based on the use of a *Pythium*-specific DNA probe, inoculated Tetr seedlings exhibited more pathogen growth in stem and leaf tissue than did the few inoculated control plantlets that developed similar wilting symptoms. These results demonstrate that ethylene perception is also required to limit growth and systemic spread of pathogens in the non-host resistance of tobacco plants.

However, other results (Table 1) seem contradictory and indicate both enhancement of disease and reduction of symptoms upon infection of a single plant host by the same pathogen [e.g. the bacterium *Xanthomonas campestris* pv. *campestris* on *Arabidopsis* accession Columbia-0 (Col-0)]. Andrew Bent and co-workers [21] showed that the strongly ethylene-insensitive *Arabidopsis* mutant *ein2-1* was resistant to *X. campestris* pv. *campestris*, whereas Philip O'Donnell and colleagues [22] reported more severe symptoms in the *etr1* and *etr2* mutants, indicative of enhanced susceptibility. A similar situation appears to apply to infection by *Pseudomonas syringae* pv. *tomato*. Upon vacuum infiltration into the leaves, the pathogenic bacteria caused no symptoms but multiplied to the same level in the *ein2-1* mutant as in wild-type Col-0 plants, indicating that the mutant was not resistant but merely tolerant to the pathogen [21]. Apparently, the lack of ethylene perception prevented the development of yellow chlorotic symptoms resembling leaf senescence, creating the impression that the plants were generally healthy. By contrast, dipping *etr1-1* or *ein2-1* plants into a more concentrated suspension of the bacteria led to increased proliferation of the pathogen, whereas the leaves still displayed the water-soaked lesions characteristic of bacterial infection as in wild-type plants [23,24]. The use of mutants that are more (*ein2-1*) or less strongly (*etr1*, *etr2*) ethylene-insensitive, and inoculation by flooding the intercellular leaf space during vacuum infiltration or entry of the bacteria through the stomata upon dipping must have affected the results obtained. Other seemingly conflicting data make it difficult to judge their significance when only effects on symptom development are described. Furthermore, when ethylene is produced by infecting fungi or bacteria as a virulence factor, a lack of ethylene perception by the plant is likely to impair pathogen activity and symptom development independent from the intrinsic resistance level of the plant.

In further studies, systematic testing of several pathogenic fungi and bacteria on different accessions and various mutants of *Arabidopsis* led Bart Thomma and co-workers [25] and Jurriaan Ton and colleagues [26] to conclude that, in general, ethylene contributes to resistance against necrotrophic, but not biotrophic pathogens,

**Table 1. Ethylene-related mutant and transgenic plants with altered sensitivity to pathogens**

| Plant species | Mutant or transgenic | Pathogen | Lifestyle | Disease severity[a] | Refs |
|---|---|---|---|---|---|
| *Arabidopsis* | *ein2-1* | *Botrytis cinerea* | Necrotrophic | + | [48] |
| *Arabidopsis* | *ein2-5, ein3-1* | *Botrytis cinerea* | Necrotrophic | + | [41] |
| *Arabidopsis* | *etr1-1, ein2-1* | *Chalara elegans* | Necrotrophic | + | [20] |
| *Arabidopsis* | *ein2-1* | *Erwinia carotovora* pv. *carotovora* | Necrotrophic | + | [69] |
| *Arabidopsis* | *ein2-5* | *Fusarium oxysporum* f.sp. *conglutinans* | Necrotrophic | + | [44] |
| *Arabidopsis* | *ein2-5* | *Fusarium oxysporum* f.sp. *lycopersici* | Necrotrophic | + | [44] |
| *Arabidopsis* | *etr1-1, ein2-1* | *Fusarium oxysporum* f.sp. *matthiolae* | Necrotrophic | + | [20] |
| *Arabidopsis* | *etr1-1, ein2-1* | *Fusarium oxysporum* f.sp. *raphani* | Mixed | − | [20] |
| *Arabidopsis* | *eto1 – eto3* | *Heterodera schachtii* | Biotrophic | + | [70] |
| *Arabidopsis* | *etr1-1, ein2-1* | *Heterodera schachtii* | Biotrophic | − | [70] |
| *Arabidopsis* | *ein3-1, eir1-1, axr2* | *Heterodera schachtii* | Biotrophic | − | [70] |
| *Arabidopsis* | *ein2-5* | *Plectosphaerella cucumerina* | Necrotrophic | + | [41] |
| *Arabidopsis* | *ein2-1* | *Pseudomonas syringae* pv. *maculicola* | Mixed | − (tolerant) | [21] |
| *Arabidopsis* | *ein2-1,-3,-4,-5* | *Pseudomonas syringae* pv. *tomato* | Mixed | − | [21] |
| *Arabidopsis* | *etr1-1, ein2-1* | *Pythium* spp. | Necrotrophic | + | [19] |
| *Arabidopsis* | *ein2-1, eto3* | *Ralstonia solanacearum* | Necrotrophic | − | [71] |
| *Arabidopsis* | *etr1* | *Spodoptera exigua* | Herbivore | − | [72] |
| *Arabidopsis* | *ein2-1, hls1-1* | *Spodoptera littoralis* | Herbivore | − | [73] |
| *Arabidopsis* | *etr1-1* | *Verticillium dahliae* | Necrotrophic | − | [74] |
| *Arabidopsis* | *etr1-1, etr2-1* | *Xanthomonas campestris* pv. *campestris* | Mixed | + | [22] |
| *Arabidopsis* | *ein2-1* | *Xanthomonas campestris* pv. *campestris* | Mixed | − | [21] |
| *Arabidopsis* | *eto1-1* | *Xanthomonas campestris* pv. *campestris* | Mixed | + | [21] |
| Potato | *AtEtr1, AtEtr1*AS | *Phytophthora infestans* | Mixed | + | [75] |
| Soybean | *Gmetr1, Gmetr2* | *Phytophthora sojae* | Mixed | −/= | [50] |
| Soybean | *Gmetr1, Gmetr2* | *Pseudomonas syringae* pv. *glycinea* | Mixed | −/= | [6,50] |
| Soybean | *Gmetr1, Gmetr2* | *Rhizoctonia solani* | Necrotrophic | =/+ | [50] |
| Soybean | *Gmetr1, Gmetr2* | *Septoria glycines* | Necrotrophic | =/+ | [50] |
| Tobacco | *Atetr1-1* (Tetr) | *Botrytis cinerea* | Necrotrophic | + | [20] |
| Tobacco | *Atetr1-1* (Tetr) | *Cercospora nicotianae* | Necrotrophic | + | [20] |
| Tobacco | *Atetr1-1* (Tetr) | *Chalara elegans* | Necrotrophic | + | [20] |
| Tobacco | *Atetr1-1* (Tetr) | *Colletotrichum destructivum* | Mixed | + | [76] |
| Tobacco | *Atetr1-1* (Tetr) | *Erwinia carotovora* pv. *carotovora* | Necrotrophic | + | [20] |
| Tobacco | *Atetr1-1* (Tetr) | *Fusarium oxysporum* | Necrotrophic | + | [20] |
| Tobacco | *Atetr1-1* (Tetr) | *Fusarium solani* | Necrotrophic | + | [20] |
| Tobacco | *Atetr1-1* (Tetr) | *Oidium neolycopersici* | Biotrophic | −/= | [20] |
| Tobacco | *Atetr1-1* (Tetr) | *Peronospora parasitica* | Biotrophic | − | [20] |
| Tobacco | *Atetr1-1* (Tetr) | *Pythium sylvaticum* | Necrotrophic | + | [17] |
| Tobacco | *Atetr1-1* (Tetr) | *Pythium* spp. | Necrotrophic | + | [19] |
| Tobacco | *Atetr1-1* (Tetr) | *Ralstonia solanacearum* | Necrotrophic | =/+ | [20] |
| Tomato | *ACD* | *Botrytis cinerea* | Necrotrophic | + | [77] |
| Tomato | *Epi* | *Botrytis cinerea* | Necrotrophic | − | [77] |
| Tomato | *ACD* | *Verticillium dahliae* | Necrotrophic | − (tolerant) | [78] |
| Tomato | *ACD* | *Xanthomonas campestris* pv. *vesicatoria* | Mixed | − (tolerant) | [79] |
| Tomato | *NR, Nr* | *Xanthomonas campestris* pv. *vesicatoria* | Mixed | − (tolerant) | [51] |
| Tomato | *Nr* | *Fusarium oxysporum* f.sp. *lycopersici* | Necrotrophic | − | [79] |
| Tomato | *Nr* | *Pseudomonas syringae* pv. *tomato* | Mixed | − (tolerant) | [79] |
| Tomato | *Nr* | *Xanthomonas campestris* pv. *vesicatoria* | Mixed | − (tolerant) | [79] |
| Tomato | *Atetr1-1-LeEtr3* | *Xanthomonas campestris* pv. *vesicatoria* | Mixed | − | [79] |

[a]Disease severity: +, increased; −, decreased; =, no change; tolerant, plants are susceptible and colonized by the pathogen but do not show visible symptoms.

whereas biotrophic, but not necrotrophic pathogens, are resisted primarily through salicylic acid-dependent mechanisms. Ethylene appears to exert its resistance-stimulating action in conjunction with jasmonic acid, suggesting that jasmonic acid and ethylene have to act in concert to reduce infection by necrotrophic fungi and bacteria [25]. Both salicylic acid- and jasmonic acid- or ethylene-dependent defenses appear to provide resistance to pathogens with mixed lifestyles [22,27–29]; however, resistance to some pathogens appears to be through signaling pathways that involve neither of these regulators [30].

## Involvement of ethylene in induced systemic resistance

Ethylene can induce certain types of pathogenesis-related proteins or phytoalexins, and, through stimulation of the phenylpropanoid pathway, can rigidify cell walls in various plant species [1,4]. However, in several studies it has been demonstrated that ethylene is not necessary for these defensive activities to be expressed, or that comparable defenses are regulated differentially by ethylene, jasmonic acid or salicylic acid in different plant species. For example, the salicylic acid-dependent defense pathway is involved in resistance against *Botrytis cinerea* in tomato, but not in tobacco; whereas the salicylic acid-dependent defense pathway is involved in resistance against the powdery mildew fungus *Oidium neolycopersici* in tobacco but not in tomato [31]. In general, ethylene appears to stimulate and enhance defense responses [32–34]. Treatment of *Arabidopsis* seedlings with 1 mM ACC enhances resistance against *P. syringae* pv. *tomato*; this induced resistance response strongly resembles

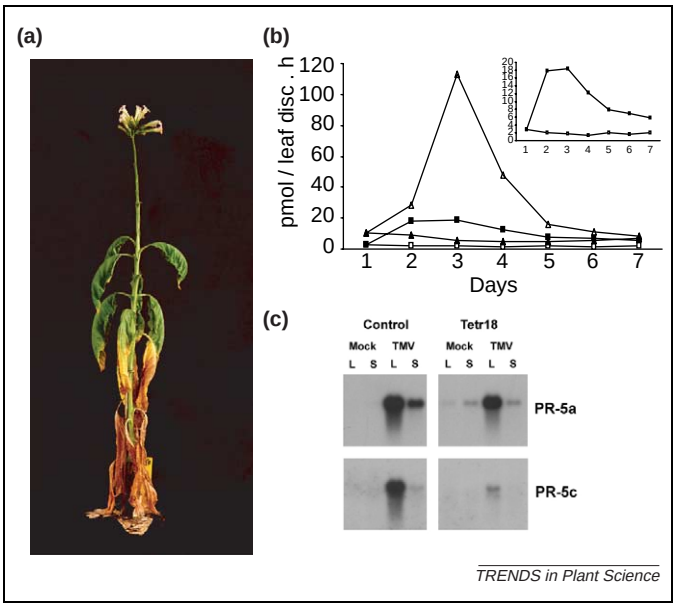


**Figure 2.** **(a)** Phenotype of *Atetr1-1*-transformed, ethylene-insensitive Tetr tobacco growing in non-autoclaved potting soil and showing 'spontaneous' wilting and stem base necrosis. **(b)** Ethylene production of leaves of Tetr plants after inoculation with tobacco mosaic virus (TMV) (unfilled triangle) or mock inoculation (filled triangle), compared with non-transformed control plants that were TMV- (filled rectangle) or mock- (*unfilled rectangle) inoculated. For comparison, the insert shows the TMV-induced ethylene production of control plants at a different scale. Ethylene production was measured on 2.5 cm diameter leaf discs [44]. **(c)** Expression of salicylic acid-inducible acidic *PR-5a* and ethylene-inducible basic *PR-5c* mRNAs in inoculated lower (L) and non-inoculated upper (S) leaves of mock- and TMV-infected Tetr and control plants, 5 days after inoculation.

the induced systemic resistance (ISR) that is elicited by specific strains of non-pathogenic, root-colonizing bacteria, such as *Pseudomonas fluorescens* strain WCS417 [35] (Box 1). ACC activates the PR-4 type hevein (*Hel*) gene, which can be used as a marker of this treatment [36]. However, when triggering ISR, WCS417 bacteria neither increase ethylene production nor activate ethylene-dependent *PR* gene expression [37]. Instead, ethylene responsiveness is required for ISR to be induced and expressed [23]. Hence, the ethylene response mutants *etr1-1*, *ein2-1 – ein7* and *axr1-12* do not express ISR in response to root colonization by strain WCS417 [24], and neither does the enhanced disease susceptibility (*eds*) mutant *eds4-1* nor the accessions RLD and Wassilewskija-0, which all have reduced responsiveness to ethylene and develop more severe symptoms after infection with *P. syringae* pv. *tomato* [37]. These results indicate that in *Arabidopsis* ethylene is required for basal resistance against *P. syringae* pv. *tomato*. Moreover, ethylene perception is required for the plant to react to rhizobacteria by developing ISR.

The *eir1-1* mutant, which is insensitive to ethylene in the roots but not in the leaves, does not express ISR when WCS417 is applied to the roots, but does when the inducing bacteria are infiltrated into the leaves [24], which demonstrates that ethylene responsiveness is required at the site of resistance induction by ISR-eliciting rhizobacteria. Apparently, treatment with ACC can activate the same pathway leading to induced resistance and the ethylene mediates the generation of a mobile signal that enhances resistance systemically [27].

### Box 1. Systemic acquired resistance and induced systemic resistance

Two main mechanisms of induced resistance against pathogens have been characterized [23,35,55]. Systemic acquired resistance (SAR) is induced upon infection by an avirulent pathogen or upon restricted infection by a virulent pathogen. SAR depends on the synthesis of salicylic acid (SA) by the host (Figure I) and is effective against pathogens that are restricted by salicylic acid-dependent basal resistance responses, such as tobacco mosaic virus in tobacco [59]. This type of induced resistance is marked by the local and systemic accumulation of newly induced pathogenesis-related proteins (PRs) that might, or might not, be effective against the pathogen involved. Induced systemic resistance (ISR) is triggered by selected strains of non-pathogenic rhizobacteria and does not require salicylic acid but does depend on the responsiveness of the plant to jasmonic acid (JA) and ethylene. ISR is effective against pathogens that are restricted by jasmonic acid- and ethylene-dependent basal resistance mechanisms, such as the fungus *Alternaria brassicicola* in *Arabidopsis* [26]. This type of induced resistance is not associated with induction of PRs, even though ISR, like SAR, requires the presence of a functional NPR1 protein.

Several pathogens of *Arabidopsis* have been shown to be resisted by a combination of salicylic acid-dependent and jasmonic acid- and ethylene-dependent defenses, for example, *Pseudomonas syringae* pv. *tomato*. Both SAR and ISR are effective against such pathogens; induction of both types of induced resistance in the same plant additively enhances protection against such pathogens [27].

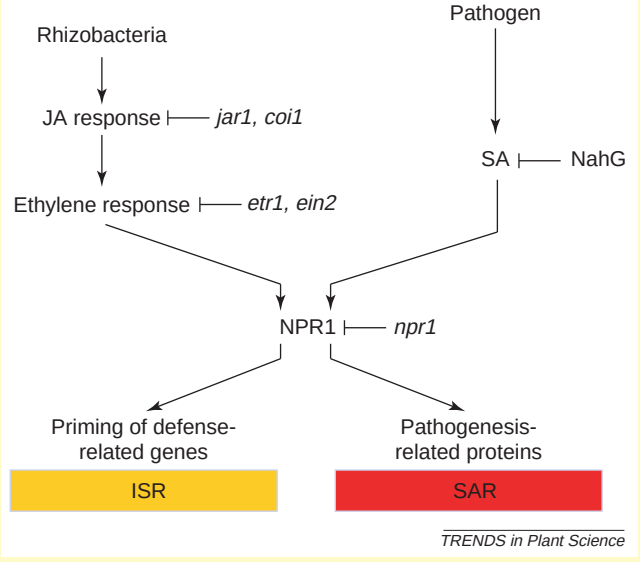


**Figure I.** Signal transduction pathways of SAR and ISR (adapted from [23]). Abbreviation: NPR1, non-expressor of *PR* genes.

ISR is also dependent on jasmonic acid responsiveness: treatment with 0.1 mM methyl-jasmonate enhances resistance and activates the jasmonic acid-responsive genes *Vsp2* and *Pdf1.2*, which encode a vegetative storage protein and plant defensin, respectively. Rhizobacteria-mediated ISR does not involve induction of these genes either but, upon challenge inoculation of induced plants with *P. syringae* pv. *tomato*, expression of jasmonic acid- and ethylene-dependent genes is accelerated and enhanced [38]. Like ethylene non-responsiveness, a deficiency in jasmonic acid perception has been shown to increase the susceptibility of *Arabidopsis* and tomato to a wide range of pathogens with different lifestyles

[25,39,40]. Yet, in the generation of ISR in *Arabidopsis*, eliciting enhanced protection by jasmonic acid is dependent on ethylene responsiveness because treatment with methyl-jasmonate fails to elicit ISR in *etr1* or *ein2* mutants [23]. Ethylene and jasmonic acid cooperate in inducing ethylene response factor 1 (ERF1), which drives the activation of defense-related genes such as *PR-4* and *Pdf1.2* and positively regulates the expression of jasmonic acid-inducible genes involved in defense responses [41–43]. Moreover, constitutive overexpression of ERF1 in *Arabidopsis* or of homologous ethylene-responsive element binding proteins (EREBPs) from tobacco, tomato and pepper confers enhanced resistance to several pathogenic fungi and bacteria [44,45].

### Role of ethylene in incompatible interactions and systemic acquired resistance

In incompatible plant–pathogen interactions, the hypersensitive reaction (HR) is associated with a large burst of ethylene production around the time of necrotic lesion formation (Figure 2b). Typically, in tobacco plants that react hypersensitively to tobacco mosaic virus (TMV), local necrotic lesions become visible ~40 h after inoculation but ethylene production starts increasing 24 h after inoculation. ACS mRNA and enzyme activity, ACC content, ACO mRNA and activity and ethylene production peak in rapid succession between 36 and 60 h after infection. Thereafter, ethylene production decreases but remains substantially elevated compared with water-inoculated control plants [46,47]. It has been debated whether the ethylene is required for necrosis formation. However, ethylene-insensitive Tetr plants produce necrotic lesions at the normal time [17], as do *Arabidopsis etr1-1* and *ein2-1* mutants in response to avirulent fungi, bacteria (e.g. [21,48]) and turnip crinkle virus [49], as well as ethylene-insensitive soybean upon infection with avirulent *P. syringae* pv. *glycinea* [50] and the tomato *Never ripe* (*Nr*) mutant in response to avirulent *X. campestris* pv. *vesicatoria* [51]. In wild-type tomato, infection by *X. campestris* pv. *vesicatoria* increased expression of the ethylene receptor genes *NR* and *LeETR4*, leading to reduced ethylene sensitivity and reduced necrosis [51]. Conversely, *LeETR4* antisense plants displayed a more rapid and extensive cell death during infection, associated with an enhanced defense response [52]. Because *X. campestris* pv. *vesicatoria* is a pathogen with a mixed biotrophic–necrotrophic lifestyle, it is difficult to relate resistance to necrosis in this plant–pathogen interaction.

Viruses are obligate biotrophs and are impeded from spreading during a hypersensitive reaction. On ethylene-insensitive Tetr tobacco plants, TMV lesions enlarged in a similar way to those on non-transformed control plants for about a day, but then started to expand more slowly, resulting in a final size that was substantially smaller than those on the control plants by day 7 [53]. Virus content was similarly decreased. Thus, the ethylene-insensitive plants appeared to be more resistant to TMV, a phenomenon resembling pathogen-induced systemic acquired resistance (SAR) (Box 1). The Tetr plants were hampered in the expression of ethylene- and jasmonic acid-inducible PR-1g [17] and PR-5c (Figure 2c), even though their ethylene production in response to TMV infection was greatly stimulated (Figure 2b). Levels of free and bound salicylic acid were similar in TMV-inoculated leaves of non-transformed and Tetr plants, and expression of the salicylic acid-inducible PR-1a and PR-2a were the same, indicating that the ethylene insensitivity did not influence local TMV-elicited salicylic acid signaling [17,54].

As could be concluded from the small size of the TMV lesions on Tetr plants, in non-transformed plants the ethylene that is produced in response to TMV infection must contribute to further lesion expansion. This seems at variance with observations that applying gaseous ethylene, ethephon or ACC all reduced lesion size when made before or shortly after virus inoculation, before lesions were visible [53]. This discrepancy was addressed by considering that TMV induces substantial SAR against itself and that the ethylene produced in copious amounts by the inoculated leaves during a primary TMV infection might be involved in the development of SAR in upper, non-inoculated leaves. Indeed, compared with non-transformed control plants, Tetr plants showed a substantially reduced SAR response, with levels of free and bound salicylic acid in upper, non-inoculated (systemic) leaves lowered by 88% and 79%, respectively [54]. Moreover, no significant expression of the salicylic acid-inducible *PR-1a*, *PR-2a* [54] and *PR-5a* (Figure 2c) was apparent, in contrast with upper leaves from TMV-induced control plants. These observations indicate that the ethylene-insensitive Tetr tobacco plants are defective, at least in part, in SAR signaling [17,53,54].

Originally, salicylic acid was considered to be the systemically transported signal for SAR [55]. Labeling studies have indicated that at least part of the increase in salicylic acid in SAR-expressing upper leaves can be derived from primary infected lower leaves [56,57]. Yet, salicylic acid does not seem to function as the transported signal, as demonstrated by making use of tobacco plants transformed with the *NahG* gene from *Pseudomonas putida*. The *NahG* gene encodes a salicylate hydroxylase that converts salicylic acid into non-SAR-inducing catechol. As elegantly established by Bernard Vernooij and co-workers [58] using reciprocal graftings, NahG rootstocks, although unable to accumulate salicylic acid in response to TMV infection, did produce SAR in wild-type scions. Conversely, wild-type rootstocks that produce salicylic acid normally in response to TMV, failed to transmit the signal for SAR or PR production to NahG scions (Figure 3), which indicates that salicylic acid must be produced locally for PRs and SAR to be expressed. This situation can explain the absence of PRs in non-infected upper leaves of TMV-inoculated Tetr plants that do not show a significant increase in salicylic acid content and are defective in pathogen-induced SAR.

Reciprocal graftings of non-transformed and Tetr plants have confirmed the dependence of the SAR signal on ethylene (Figure 3). When wild-type rootstocks were inoculated with TMV and scions of Tetr plants were tested for systemic *PR* gene expression, salicylic acid-inducible PR-1a and PR-2a mRNAs were present in the Tetr scions

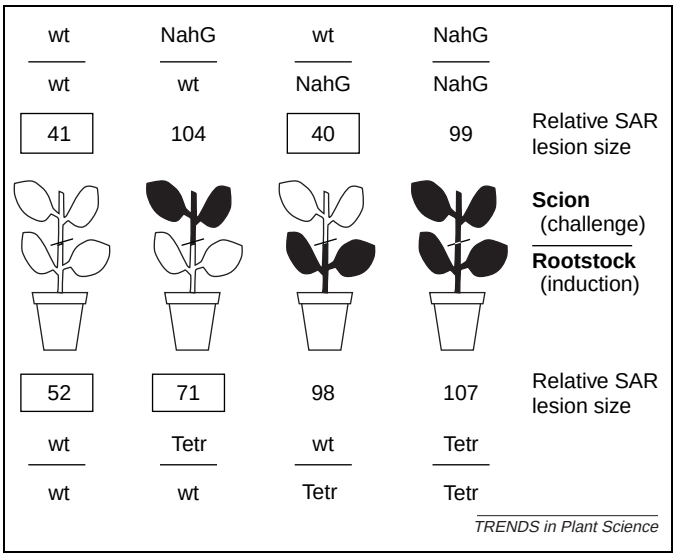


**Figure 3.** Diagrammatic representation of grafting experiments between non-transformed (white) and transgenic salicylic acid-non-accumulating NahG or ethylene-insensitive Tetr (black) tobacco plants. The expression of TMV-induced SAR in scions upon inoculation with the same virus is indicated above (NahG) and below (Tetr) as TMV lesion sizes relative to those on plants where the rootstocks were not induced. Relative SAR lesion sizes that are significantly different from those on non-induced plants are boxed. Recalculated from [54] and [58].

at the same levels as those present in wild-type scions on wild-type rootstocks. Conversely, when Tetr plants were used as rootstocks and the wild type as scions, only a minute amount of the *PR* mRNAs was detectable, as was the case in Tetr scions on Tetr rootstocks [54]. These differing levels of the *PR* mRNAs in the scions corresponded perfectly with the levels of salicylic acid in the scions: on wild-type rootstocks Tetr scions contained as much free and bound salicylic acid as wild-type scions, whereas on Tetr rootstocks, both wild-type and Tetr scions contained much smaller amounts. This lack of salicylic acid accumulation in the scions cannot be because the Tetr rootstocks are unable to synthesize sufficient salicylic acid, and, locally, infection with TMV led to normal accumulation of salicylic acid and acidic *PR* gene expression (Figure 2c). Hence, the lack of ethylene perception in the rootstock must have interfered with the synthesis, release or transport of the mobile signal that is transported systemically into the scion and sets off the accumulation of salicylic acid and the expression of *PR* genes in the non-inoculated leaves. As a result, little or no SAR is expressed, as evidenced by the lack of a reduction in lesion size upon challenge inoculation with TMV (Figure 3). On wild-type rootstocks that generate the signal, Tetr scions showed substantially smaller lesions, whereas wild-type scions on Tetr rootstocks did not [54]. It is likely that the burst of ethylene production during a hypersensitive reaction [46] contributes substantially to the local induction of a subset of PRs [17], while also enabling the plant to react systemically and develop SAR [54].

These results indicate an important role of ethylene in establishing SAR in tobacco against TMV, even though salicylic acid by itself has been described as being sufficient to induce SAR [55]. Strong induction of resistance and *PR* gene expression by exogenous application of salicylic acid requires far higher doses of the compound than are present in infected tissues. Most published reports indicate that salicylic acid was sprayed on the plants, and the effects seen were in tissues that had received a dose of salicylic acid. Hence, the 'SAR' induced was only local. Upon carefully applying high doses of salicylic acid to single leaves of a tobacco plant, strong local resistance, but not systemic resistance, and accumulation of PRs was observed [59]. Only when the compound was able to enter the vascular system through uptake by roots or piercing of leaf veins, were systemic effects evident, probably as a result of salicylic acid transportation throughout the plant. In contrast with tobacco, in *Arabidopsis*, pathogen-induced SAR against the downy mildew oomycete *Hyaloperonospora parasitica* and against *P. syringae* pv. *tomato* was maintained in all ethylene-insensitive mutants tested [53,60], but it was observed that ethylene enhanced the sensitivity of wild-type plants to express *PR-1* in response to salicylic acid application [32].

## Conclusions and perspectives

Plant defenses are regulated by complex signaling pathways involving salicylic acid, jasmonic acid and ethylene. Both synergistic and antagonistic interactions have been observed [27,61,62]. The mechanisms of this cross-talk are now the subject of extensive investigations to elucidate the significance for resistance against particular attackers. It is difficult to understand that, in at least some plant–pathogen combinations, ethylene induces resistance when applied before infection but, when generated during infection or applied after symptoms have become manifest, stimulates disease progress. In part, these differing effects might be related to the dual action of ethylene in that it sometimes acts as a virulence factor of the pathogen but, other times, the activity of other pathogens is affected negatively. Furthermore, the speed with which the pathogen is able to colonize infected tissues and the mechanisms that the pathogen uses to overcome the effects of ethylene action might play a role. Cells in front of an advancing pathogen are likely to react differently from cells in the process of succumbing, and possibilities for discriminating in time and space between the effects of ethylene in cells at different stages of infection would be highly desirable.

Unlike salicylic acid and jasmonic acid, which have a relatively restricted range of effects on plants, ethylene affects almost all stages of plant development but does not normally induce resistance or activate defense-related genes. As a gas, ethylene can diffuse rapidly in plant tissues but also escapes readily into the atmosphere. High levels of ethylene can be produced locally at infection sites and gradients could be sensed by surrounding cells, which then activate appropriate defense programs in response to the local concentrations of signaling compounds. This suggests that ethylene-regulated resistance responses depend on the spatial interactions of multiple signals interacting in a network-type fashion. Evidence for the functioning of such a network is becoming available through transcriptome analyses [38,63–67], which indicate that in response to

environmental cues, multiple genes are regulated in complex ways by the interplay of several regulatory factors. Spatial analysis could benefit from the application of laser-assisted microdissection [68] of tissues at varying distances from the infection front. Such temporal and spatial analyses should aid in further delineating the involvement of ethylene in plant defense responses and the elucidation of the underlying mechanisms.

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