(2019) Volumen: 70 (17), Páginas: 4489-4503

Nitric oxide shape plant-fungi interactions

Ainhoa Martínez-Medina[1], Leyre Pescador-Azofra[2,3], Laura Terrón-Camero[2], María J. Pozo[3], María C. Romero-Puertas[2]

[1]Plant-Microorganism Interaction Unit, Institute of Natural Resources and Agrobiology of Salamanca (IRNASA-CSIC), Salamanca, Spain; [2]Department of Biochemistry, Cell and Molecular Plant Biology and [3] Department of Soil Microbiology and Symbiotic Systems, Estación Experimental del Zaidín (CSIC), Granada, Spain;

*Author for correspondence:
Dr. María C. Romero-Puertas,
Departamento de Bioquímica, Biología Celular y Molecular de Plantas,
Estación Experimental del Zaidín, CSIC, Apartado 419, E-18080 Granada, SPAIN.
Tel: +34 958 181600 Ext.175, 299
maria.romero@eez.csic.es

Running title: NO in plant-fungi interactions
Research area: Environmental stress and adaptation
Keywords: Biotrophs, Fungal pathogens, Fungal mutualists, Disease, Mycorrhiza, Necrotrophs, Nitric oxide, Plant immunity, Symbiosis.

Highlights: Nitric oxide is a key signal in plant-fungal interactions and apparently different signatures, both quantitative and spatio-temporal distribution, govern the type of interaction, pathogenic or beneficial.

Abstract

In their complex environments, plants continuously interact with fungi. While many of those interactions are detrimental for plants and challenge plant capability for growth and survival, others are beneficial improving plant growth and stress tolerance. Accordingly, plants have evolved sophisticated mechanisms to restrict pathogenic interactions while promoting mutualistic relationships. Several studies demonstrated the importance of nitric oxide (NO) in the regulation of plant defence mounted against fungal pathogens. NO triggers a reprograming of defence related gene expression, the production of secondary metabolites with antimicrobial properties and the hypersensitive response. More recent evidences have further shown the regulation of NO during the establishment of plant-fungus mutualistic associations from early steps of the interaction. Indeed NO has been recently shown to be produced by the plant after the recognition of root fungal symbionts, and to be required for the optimal control of the mycorrhizal symbiosis. Although studies dealing with NO function in plant-fungus mutualistic associations are still scarce, experimental data support a different regulation patterns and functions for NO in plant interactions with pathogenic and mutualistic fungi. Here we review recent evidences about NO function in plant-fungus interactions, trying to identify common and differential patterns related to the fungus life-style and their impact on plant health.

1. Introduction

Fungi play a major role in natural and agricultural ecosystems. They are important decomposers and recyclers of organic materials and they can interact with plant roots in the rhizosphere or with aboveground plant tissues (Zeilinger *et al.*, 2015). The interactions between plants and their associated fungi are complex and the outcomes are diverse, ranging from parasitism to mutualism. Fungal plant pathogens are of huge economic importance because they threaten the production of crops already when growing in the field, but also they can cause postharvest diseases. Indeed, most of the major economically relevant plant pathogens are fungi such as *Botrytis cinerea*, *Fusarium* spp, *Rhizoctonia* spp, and *Magnaporthe* (Dean *et al.*, 2012). On the other hand mutualistic associations between fungi and plants are common in nature and can improve the productivity of crop plants. For instance, it is estimated that about 90% of the plants present in our planet form mycorrhizal symbioses, in which plant photosynthates are exchanged for mineral resources acquired by the fungus from the soil (Ferlian *et al.*, 2018). To cope with pathogenic fungi, plants are able to activate defence mechanisms, and being generally at least partially resistant to most fungal pathogens. Hence mutualistic and neutral associations dominate and parasitic associations are considered to be the exception (Staskawicz, 2001).

The interactions of plants with fungi are characterized by a series of sequential events including the contact with the host plant, the fungal attachment to the host structures, the entry and colonization of the plant tissues, and the fungal reproduction (Lo Presti *et al.*, 2015). Depending on the nature of the interaction (pathogenic, neutral or mutualistic) and the lifestyle of the fungus (necrotrophic or biotrophic), plants respond to fungal colonization with an immune response in which several plant signalling compounds including intracellular calcium (Ca^{2+}) and other ions, reactive oxygen and nitrogen species (ROS/RNS), phytohormones and small RNAs, play pivotal roles (Mur *et al.*, 2006; Pieterse *et al.*, 2012; Weiberg *et al.*, 2014; Pozo *et al.*, 2015; Waszczak and Carmody, 2018). It is remarkable that the signalling networks and key regulatory elements that are involved in the plant in response to pathogenic and mutualistic fungi overlap (Pozo *et al*., 2015). This indicates that the regulation of the adaptive response of the plant is finely balanced between protection against aggressors and acquisition of benefits from mutualistic associations (Pieterse *et al.*, 2014). Achieving this balance requires the perception of potential invading fungi, followed by the rapid and tight regulation of immune responses to promote or contain the fungal colonization of plant

tissues (Zamioudis and Pieterse, 2012; Zipfel and Oldroyd, 2017; Plett and Martin, 2018).

Nitric oxide is a diffusible free radical reactive gaseous molecule involved in the regulation of a wide range of plant developmental processes such as seed germination (del Castello *et al*., 2019; Gibbs *et al.*, 2014; Albertos *et al.*, 2015), root development (Sanz *et al.*, 2015; Castillo *et al.*, 2018), flowering (Prado *et al.*, 2004; He *et al.*, 2004; Serrano *et al.*, 2012) and fruit development (Manjunatha *et al.*, 2012; Du *et al.*, 2014). NO also regulates plant responses to several abiotic stresses such as hypoxia, salinity and heavy metal (Gupta *et al*., 2016; Romero-Puertas *et al*., 2018); and it is involved in plant defence responses against microbial pathogens, including bacteria and fungi (Trapet *et al*., 2015). Indeed, during plant immune responses against fungal pathogens, NO triggers a global reprograming of gene expression, the production of secondary metabolites with antimicrobial properties and the hypersensitive response (Mur *et al*., 2016). A growing body of literature is further supporting that NO is also produced during the establishment of mutualistic interactions between plants and fungi (Calcagno *et al.*, 2012; Espinosa *et al.*, 2014; Gupta *et al.*, 2014; Martínez-Medina *et al*., 2019). Although the specific role(s) of NO in plant-fungus mutualisms remains obscure, recent evidence suggests that a tight control of the NO levels is required for the control of the mycorrhizal symbiosis (Martínez-Medina *et al*., 2019).

The diverse roles of NO during detrimental and mutualistic plant-fungus interactions might seem contradictory but could be explained by the versatile properties of this molecule. As signalling molecule, NO function depends on the rate and location of its production; and its concentration is critical acting as a signal at low concentrations but displaying toxic effect when present at high concentrations (Hancock and Neill, 2019). Moreover the highly reactive nature of NO facilitates its different regulatory roles as it reacts directly with other free radicals, metals and proteins, leading to posttranslational modifications that regulate protein activity and stability, and gene expression (Abello *et al*., 2009; Martínez-Ruiz *et al*., 2013; Yu *et al*., 2014; Lamotte *et al.*, 2014; Romero-Puertas and Sandalio, 2016).

Here we review and synthesize the recent and relevant information dealing with the role(s) of NO in the interaction of plants with pathogenic and beneficial fungi, highlighting recent advances and identifying the major gaps in our knowledge. We acknowledge that both the plant and the fungal partners are potential sources and regulators of NO during plant-fungi interactions. However, several excellent reviews

have been recently published on fungal NO (Arasimowicz-Jelonek and Floryszak-Wieczorek, 2016; Cánovas *et al*., 2016) so we here focus on the NO produced by plants during their interaction with diverse fungi.

2. Role and metabolism of NO in plant immunity

Plants are unexpectedly healthy despite the enormous number of potential pathogens in their environments (Dangl, 2013) and this is mainly due to the plant immune system. After the recognition of potential aggressors, through the perception of pathogen (or microbe) associated molecular patterns (the so called PAMPs; MAMPs in the case of non-pathogenic microbes) or from self-damage related signals (damage associated molecular patterns, DAMPs), plant activates a defence response called basal or PAMP (pathogen associated molecular pattern)-triggered immunity (PTI). Some pathogens are able to avoid PTI by evading recognition or by blocking defense response through small molecules called effectors, which promote infection (Couto and Zipfel, 2016). Plants can hold however, a second layer of perception involving intracellular receptors with nucleotide-binding domain leucine-rich repeats (NLR or NBS-LRR), by which is able to recognize microbe effectors, inducing the effector-triggered immunity (ETI; Couto and Zipfel, 2016). Although both responses, PTI and ETI, activate similar mechanisms, ETI is stronger and faster and leads to the programmed cell death of the invaded area, restraining pathogen dispersion, a process known as hypersensitive response (HR; Dodds and Rathjen, 2010).

One of the first biological functions assigned for NO in plants was related to plant immunity (Yu *et al*., 2014). The occurrence of a peak of NO has been evidenced during both PTI and ETI responses. However, most studies have dealt with the role of NO in ETI and HR, and less attention has been paid to NO production and function during PTI. Different MAMPs or DAMPs, such as cryptogein, lipopolysaccharides or oligogalacturonides, have been shown also to induce NO production (Trapet *et al*., 2015), showing a feedback interaction with Ca^{2+} (Courtois *et al.*, 2008). In this context, NO is able to arrange a plethora of different plant immune responses (Yu *et al.*, 2012; Bellin *et al.*, 2013). Indeed, it is well known that NO produced after microbe recognition triggers a global reprograming of gene expression, the production of secondary metabolites with antimicrobial properties and finally, the HR and systemic acquired resistance (Bellin *et al.*, 2013; Wendehenne *et al.*, 2014). NO and related RNS perform their bioactivity mainly via chemical reactions with specific target proteins,

leading to NO-dependent post-translational modification (PTMs): *S*-nitrosylation, nitration or nitrosylation. For more details see comprehensive reviews published on this topic (Scheler *et al*., 2013; Yu *et al.*, 2014). In fact, the levels of nitrosothiols are very important in the evolution of plant defence responses, as mutants with altered GSNOR levels showed impaired pathogen resistance (Feechan *et al.*, 2005; Rusterucci *et al.*, 2007). Furthermore, proteomic analysis in plants undergoing HR showed changes in *S*-nitrosylated proteins related with intermediary metabolism, hormone-dependent signalling, ROS-producing enzymes and proteins related to antioxidant defences and programmed cell death (Feechan *et al.*, 2005; Romero-Puertas *et al.*, 2007, 2008). Also, different transcription factors have been shown to be targets of S-nitrosylation. This fact could explain how NO can coordinate gene expression changes. For example, in Arabidopsis NO has been proposed to switch the translocation into the nucleus of NPR1, a transcriptional co-activator involved in the induction of pathogenesis related genes (PR); and to regulate the specific DNA-binding of its transcription factor interactor TGA1 (Tada *et al.*, 2008; Lindermayr *et al.*, 2010). Recently, it has been shown that the zinc finger transcription factor SRG1, which functions as a positive regulator of plant immunity, is a central target of NO bioactivity. The SRG1-SNO establishment may, therefore, contribute to a negative feedback loop that decreases the plant immune responses (Cui *et al.*, 2018). Proteomic analysis have been shown also protein targets of nitration during plant defence response involved in different cellular processes such as photosynthesis, glycolysis and nitrate assimilation (Cecconi *et al.*, 2009). Additional analysis in tobacco suggested that tyrosine nitration may regulate MAPKK signalling and therefore, phosphorylation cascades during the defence response (Vandelle and Delledonne, 2011).

Despite an increasing body of literature on the roles of NO in plants, there are still "dark boxes" regarding the sources of NO, as well as the proteins/molecules that regulate NO levels in the cell. In brief, several mechanisms have been reported regarding NO production in plants. The best characterized enzymatic pathway of NO production in plants is the nitrate reductase (NR) pathway, in which nitrate is reduced to nitrite. Moreover the oxidative pathway and NOS-like activity has been also involved in NO production during plant defence. Readers are referred to several excellent reviews for additional information in this topic (Mur *et al*., 2013; Baudouin and Hancock, 2014; Yu *et al*., 2014; Jeandroz *et al*., 2016; Astier *et al*., 2018). As for NO plant sources, our knowledge on NO catabolism is also very incomplete. NO can quickly react with GSH

to form GSNO; with O_2 and $O_2^{\cdot-}$ to form nitrogen dioxide (NO_2) and peroxynitrite (ONOO-), involved in NO-dependent PTMs as described above (Neill *et al.*, 2008). On the other hand, phytoglobins (previously known as non-symbiotic haemoglobins), which are able to modulate NO levels through its NO dioxygenase activity, have been also involved in NO modulation in plant immunity (Hebelstrup *et al.*, 2014). Overall, the complex regulation of NO has slowed down the identification of downstream NO-regulated processes, by rendering difficult the generation of null NO-producing mutants (Bruand and Meilhoc, 2019). However, thanks to the use of NO donors and scavengers, and mutants impaired in NO metabolism, it is now well established the regulatory role of NO in numerous plant processes including plant immunity.

Although our knowledge on the molecular mechanisms mediating the role of NO in plant immunity has increased considerably during the last decades, most of the studies were performed on model plants (mostly *Arabidopsis thaliana*) interacting with bacteria. Despite the importance of both, beneficial and pathogenic fungi on plant health, the role of NO in plant-fungi interactions have been far less explored. In the following sections we tried to compile and summarize the available information on these interactions, and to highlight common and differential patterns and functions during interactions with beneficial and pathogenic fungi.

3. NO in plant-fungus pathogenic interactions

Pathogenic fungi can use diverse strategies to colonize plants and cause disease. Necrotrophic fungal pathogens, which often show a broad host range, kill their hosts and take up nutrients released from the dead tissues. Several compounds as cell wall-degrading enzymes, ROS and/or toxins have been implicated in the degradation of host cells by necrotrophic fungi (Wolpert *et al.*, 2002). In contrast, biotrophic fungal pathogens, which show host specificity, do not produce toxins but often secrete effectors to suppress the host immune system (Perfect and Green, 2001). Hemibiotrophic fungal pathogens are intermediate between the necrotrophic and the biotrophic lifestyles, initially growing as biotrophs and later switching to a necrotrophic lifestyle (Koeck *et al.*, 2011). In agreement with the essential role of NO in plant immunity (see section 2 in this review), several studies indicate that NO is an early component of the defence response triggered by plants to combat fungal infections (Table 1, and references therein). However, the specific role(s) of NO during the interaction of plants with pathogenic fungi seems to be influenced by the

234 necrotrophic/biotrophic character of the pathogen, which dictates the concentration and
235 the spatio-temporal patterns of NO accumulation in the plant tissues. Strikingly, in
236 plant-fungus pathogenic interactions, fungi also may participate in the production and
237 metabolism of NO (Arasimowicz-Jelonek and Floryszak-Wieczorek; Cánovas *et al.*,
238 2016). Several studies indicate that NO plays an important role in fungal development
239 (Wang *et al*., 2005; Prats *et al*., 2008; Baidya *et al*., 2011). Moreover, fungal pathogens
240 may use NO to its own benefit to accelerate the spread of infection, especially in plant
241 interactions with necrotrophic and hemi-biotrophic pathogens (Van Baarlen *et al.*, 2004;
242 Sarkar *et al.*, 2014; Arasimowicz-Jelonek and Floryszak-Wieczorek, 2016). Indeed, NO
243 was found to be produced by several necrotrophic pathogens as *B. cinerea, Aspergillus*
244 *nidulans*, *Macrophomina phaseolina*, *Fusarium oxysporum*, and *Colletotrichum*
245 *coccodes* (Conrath *et al*., 2004; Wang and Higgins, 2005; Floryszak-Wieczorek *et al*.,
246 2007; Turrion-Gomez and Benito, 2011; Sarkar *et al*., 2014). Thus, fungus-produced
247 NO can also be considered as a virulence factor, determining the success of the
248 aggressor. As mentioned above, excellent recent reviews focused on fungal-produced
249 NO during pathogenesis are available (Arasimowicz-Jelonek and Floryszak-Wieczorek,
250 2016; Cánovas *et al*., 2016).
251
252 ***3.1. Necrotrophic fungi***
253 The use of the well characterized necrotrophic foliar pathogen *Botrytis cinerea*
254 has evidenced the importance of NO in the onset of the plant immune response mounted
255 against shoot-associated necrotrophic fungi in different plant species. For instance, *B.*
256 *cinerea* infection of tobacco (*Nicotiana benthamiana*) plants triggered an increase in
257 NO levels in adjacent cells of invaded areas, concomitant with the activation of the SA-
258 regulated defence pathway (Asai and Yoshioka, 2009). By using a pharmacological
259 approach, the same authors showed that NO plays a pivotal role in the basal defence
260 against *B. cinerea*, and in pathogen triggered *PR-1* expression. Similarly, an increase in
261 NO was observed in *B. cinerea*-infected cells and surrounding uninfected cells in the
262 model plant Arabidopsis (*Arabidopsis thaliana*; van Baarlen *et al.*, 2007). The critical
263 role of NO in Arabidopsis resistance to *B. cinerea* was later confirmed by manipulation
264 of NO levels through a genetic approach (Mur *et al.*, 2012): Arabidopsis mutant lines
265 displaying increased NO levels (due to a mutation in the *Phytogb1* gene) showed
266 increased levels of the defence-related plant hormones jasmonic acid and ethylene, and
267 increased resistance to *B. cinerea* infection; while decreased NO levels in *Phytogb1*

overexpressing lines resulted in the opposite phenotype (Mur *et al.*, 2012). Pharmacological approaches also revealed the importance of the NO burst in plant resistance against *B. cinerea* in tomato plants (*Solanum lycopersicum;* Sivakumaran *et al.*, 2016). Altogether these studies demonstrate a key role of pathogen-triggered NO in plant immunity against *B. cinerea* in different plant species. Moreover, a similar role for NO has been suggested for the plant immune responses mounted against other leaf-associated necrotrophic fungi as *Colletotrichum orbiculare* (Asai *et al.*, 2008) and *Sclerotinia sclerotiorum* (Perchepied *et al.*, 2010).

Strikingly, the study by Turrion-Gomez and Benito, (2011) indicated that *B. cinerea* may use NO-signalling for spreading within plant cells. Although the authors focused mostly on NO produced by the fungus, they hypothesized that the plant cell death mediated by the NO-triggered HR might favour the growth of the necrotrophic fungus within plant tissues. It is remarkable that we recently found that in tomato leaves, *B. cinerea* triggered the downregulation of the *Phytogb1* gene, most likely to increase NO levels and enhance cell death (Martínez-Medina *et al*., 2019). This offers an apparently contradictory scenario where NO is being used by the host plant for defence and by the pathogenic fungus to promote virulence. Understanding this disparate data may require careful spatiotemporal measurement of NO concentrations (Box 1), as the relative concentration of NO during the different stages of the infection process could play a key role in governing its action. Indeed, Turrion-Gomez and Benito (2011) hypothesized that above a certain threshold, NO triggers plant cell death which would favour the infection; while below this threshold, NO would act as a key signalling molecule in the onset of the plant immune response to the fungus. In line with this hypothesis, Floryszak-Wieczorek and colleages (2007) found an uncontrolled NO generation in *B. cinerea* infected tissues of susceptible *Pelargonium peltatum*. This was accompanied by a very intensive H_2O_2 and ethylene synthesis. Moreover, the pathogen colonizing susceptible cells further produced considerable amounts of NO, which enhanced the nitrosative and oxidative stress in host tissues. By contrasts, a more controlled burst of NO was observed in the incompatible interaction of *B. cinerea* with the resistant *Pelargonium* genotype. In this case, the resistance response was accompanied by a strong first NO burst followed by a controlled secondary wave of NO generation, which was co-expressed with the activation of plant defences. This response triggered a non-cell death-associated resistance with an enhanced pool of antioxidants, which finally favoured the maintenance of homeostasis of surrounding cells. According

to these findings, in susceptible interactions, necrotrophic fungi may exploit the NO-related plant defence system for expanding the infection. However, in incompatible interactions, NO would be mostly acting as a key signal in the onset of the plant immune response.

3.2. Biotrophic fungi

In contrast to necrotrophic pathogens, that feed on dead tissue, and accordingly, are not deterred by the plant cell death, biotrophs feed require compounds from living host cells. Thus, HR-triggered cell death is most likely one of the most important strategies in impeding the growth of biotrophic fungi (Govrin and Levine, 2000). Accordingly, it is a likely hypothesis that NO-triggered HR would restrict the spreading of biotrophic fungi. Indeed, Prats *et al*. (2005) found NO as one of the first responses of barley epidermal cells against *Blumeria graminis*. However, the role of NO in plant interaction with biotrophic fungal pathogens has not been thoroughly studied. The study by Schlicht and Kombrink (2013) suggests an important role for NO in plant resistance to powdery mildew. The authors found that Arabidopsis responded to both compatible (*Golovinomyces orontii*) and incompatible (*Erysiphe pisi*) interactions with powdery mildew with a rapid and transient accumulation of NO. However, there were significant differences in the patterns of the NO accumulation. In leaves infected with *G. orontii*, the NO level rapidly declined after the initial burst. The authors suggested that this was most likely a consequence of the active effector-mediated defence suppression by *G. orontii*. By contrast, NO levels remained high for an extended period of time during the incompatible interaction with *E. pisi*, indicating a correlation between the resistance phenotype and the amount and duration of NO production. In analogy, Piterková *et al.*, (2009) found significant differences in the extent and timing of the increase in NO production triggered by *Oidium neolycopersici* between susceptible and resistant tomato genotypes. In the susceptible genotype, elevated NO production was observed only during the early moments following inoculation. However, a two-phase increase in NO production was detected in the resistant genotypes. Similarly, the study by Qiao *et al.*, (2015) suggests the importance of the intensity and duration of the NO burst in plant immunity against the biotrophic fungus *Puccinia triticina*. In the incompatible wheat-*P. triticina* interaction, a continuous and sustained increase of NO was found in the stomatal guard cells at the *P. triticina* infection site. This NO burst primarily occurred in the cells undergoing a hypersensitive response. Nevertheless, for the compatible

interaction, a smaller and transient NO accumulation was found. These data suggest that the plant ability to rapidly and continuously increase NO production forms part of the molecular basis of plant resistance to biotrophic fungi.

3.3. Root fungal pathogens

The role of NO in plant interactions with root fungal pathogens has been far less explored, most likely because of the challenge of studying interactions in the belowground realm (Shelef *et al*., 2019). By using an *in vitro* system, we recently found that the compatible interaction of tomato with the necrotrophic pathogen *F. oxysporum* was associated with an early strong and transient burst of NO in tomato roots. This first burst was followed by a sustained and uncontrolled NO accumulation that was concomitant with cell death (Martínez-Medina *et al*., 2019). Moreover, with the progress of the infection a downregulation of the *Phytogb1* gene in *F. oxysporum* infected tomato roots occurred, most likely to further increase NO levels and promote cell death. By manipulating NO levels through a genetic approach, we demonstrated the important role of NO in tomato susceptibility to *F. oxysporum*. Higher biomass of *F. oxysporum* and host cell death was observed in tomato lines displaying increased NO levels. By contrast, a decreased susceptibility of the pathogen was found in *Phytogb1* overexpressing plants, displaying decreased NO levels (Martínez-Medina *et al*., 2019). An increase in NO levels was also found within the first hour after *F. oxysporum* infection of Arabidopsis roots (Gupta *et al*., 2014). Furthermore, Espinosa and coworkers (2014) found a strong increase in NO in roots of olive seedlings 1 hour after contact with the necrotrophic fungus *Verticillium dahliae*. NO was spread across cell walls and in the cytoplasm of epidermal and cortical cells, and a concomitant increase in phenolic compounds was observed. Although the authors did not study the temporal dynamics of the NO burst and of the infection, they suggested that the NO burst was related to the activation of the plant immune response to the pathogen. Moreover, the application of the NO donor sodium nitroprusside (SNP) reduced the disease caused by *Rhizoctonia solani* in resistant and susceptible tomato cultivars *via* involvement of both the octadecanoid and phenylpropanoid pathways (Noorbakhsh and Taheri, 2016). These studies may suggest that similarly to the observations of aboveground plant parts NO might play a dual role in root interactions with necrotrophic fungi. NO might act as a signal to initiate a defence response in incompatible interactions, while NO-signal might also be exploited by the pathogen to spread the lesions in compatible interactions.

The rapid induction kinetics of the first NO burst and the lack of specificity of this early response during the plant-pathogenic fungi interaction may indicate that NO accumulation is part of the plant response to fungal PAMPs. Indeed, the application of chitosan, a mycelial fungal elicitor of cell walls from *F. oxysporum* triggered a rapid burst of NO (Wang and Wu, 2004; Srivastava *et al.*, 2009; Martínez-Medina *et al.*, 2019). According to this, we propose the following model: the interaction of the plant with necrotrophic pathogenic fungi triggers a rapid and unspecific PAMP-triggered NO burst, which activates plant response at early stages and NO is massively produced after the first NO peak, with the advance of the infection, and the associated cell death would be exploited by the pathogen to further expand the lesions at later stages (Figure 1A). In the case of plant interaction with biotrophic fungal pathogens, it seems that there is a correlation between the concentration and duration of the NO burst with plant resistance (Figure 1B) although the experimental data are scarce.

4. NO in plant-fungi mutualistic interactions

Interactions between plants and mutualistic fungi are ubiquitous and diverse, and often result in the improvement of plant growth and stress tolerance. In return, plants deliver carbohydrates and an ecological niche to their fungal associates contributing to a stable association between the interacting partners (Zeilinger *et al.*, 2015). Intimate mutualistic plant-fungi interactions include the plant interaction with foliar and root mutualistic endophytes and the mycorrhizal symbiosis. The establishment and maintenance of intimate mutualistic interactions require mutual recognition and substantial coordination of the plant and fungal responses. This coordination is based on a finely regulated molecular dialogue between the partners in which the host immune responses are tightly regulated to enable successful colonization and to maintain the balance of mutual benefits (Zipfel and Oldroyd, 2017; Plett and Martin, 2018). According to the crucial role of NO in plant immunity (see section 2 in this review), one might speculate that NO operates in the establishment and maintenance of mutualistic plant-fungi interactions. Remarkably, we could not find any report related to NO signalling during plant interaction with fungal endophytes in leaves, despite their well-recognized benefits in plant health (Porras-Alfaro and Bayman, 2011). We found however several studies on the specific roles of NO in endophyte-induced secondary metabolites in plants (Ren and Dai, 2013; Fan *et al.*, 2014; Cui *et al.*, 2017). In contrast to the NO studies in plant-pathogen interactions that are better known in aboveground tissues, the only reports

regarding plant-produced NO during beneficial plant-fungus interactions deal with root colonizers. Indeed, few recent studies report the occurrence of a burst of NO during the early steps of the arbuscular mycorrhizal (AM) symbiosis and during the early interaction of roots with mutualistic fungal endophytes (Calcagno *et al.*, 2012; Espinosa *et al.*, 2014; Gupta *et al.*, 2014; Zou *et al.*, 2017; Martínez-Medina *et al*., 2019). However, the specific role(s) of NO in plant-fungi mutualistic interactions remains particularly uncovered.

The first experimental data demonstrating the occurrence of a NO burst in the mycorrhizal symbiosis was reported by Calcagno *et al.* (2012). The authors found that NO increased in the roots of *Medicago truncatula* within minutes following the treatment with exudates of germinating spores of the AM fungus *Gigaspora margarita*. The authors suggested that this increase was mediated by the activity of the nitrate reductase, and that was associated to the activation of the symbiotic regulatory (SYM) pathway. In accordance with these findings we recently found a similar response in roots of tomato after the treatment with exudates from germinating spores of the AM fungus *Rhizoglomus irregularis*. This response was specific for the AM fungus, as exudates from germinating spores of the pathogenic fungus *F. oxysporum* did not trigger NO accumulation (Martínez-Medina *et al*., 2019). These findings indicate that the perception by the plant of bioactive molecules present in the exudates of AM fungi germinating spores triggers a NO-related response. It is remarkable that the chemical communication between the host plant and the AM fungus is initiated prior to the physical contact between the symbionts (Buee *et al.*, 2000; Chabaud *et al.*, 2011). Plant perception of fungal diffusible signals, the MYC factors, is translated into a transcriptional response that prepares the plant for the following fungal colonization (Maillet *et al.*, 2011; Genre *et al.*, 2013). In accordance, it seems that NO is a component of the SYM that is triggered in the host plants after the perception of MYC factors during the pre-symbiotic stage of the AM symbiosis.

Besides the pre-symbiotic stage, NO also accumulates in root cells shortly after contacting with the mycelium of AM fungi. For instance, NO increased in roots of olive seedlings (Espinosa *et al.*, 2014) and tomato plants (Martínez-Medina *et al*., 2019) within hours following the contact with the mycelium of *R. irregularis*. The authors suggested that NO may function as a signalling component regulating some key processes in the early stages of the AM interaction, as cell wall remodelling, lateral root development and host defence regulation. Moreover, an increased NO level was

observed in roots of trifoliate orange (*Citrus trifoliata*) seedlings 21 days after the inoculation with the AM fungus *Diversispora versiformis* (Zou *et al.*, 2017), suggesting that NO might further function as a regulatory component in the maintenance of a well-established AM symbiosis (Figure 1C). Indeed, by manipulating the levels of NO in tomato roots through a genetic approach we showed that NO appears to be a regulatory component of the AM symbiosis establishment (Martínez-Medina *et al*., 2019). Tomato roots displaying increased NO levels (through the silencing of the *Phytogb1* gene) or decreased NO levels (through the overexpression of the *Phytogb1* gene) displayed an increased mycorrhizal colonization, suggesting a role for NO in the tight regulation of the mycorrhizal symbiosis.

In analogy to the mycorrhizal symbiosis, an increase of NO was observed in roots of *Arabidopsis* within minutes following the contact with the mycelium of the mutualistic endosymbiotic fungus *Trichoderma asperelloides* (Gupta *et al.*, 2014). The increase of NO was mediated by the activity of the nitrate reductase, and was restricted to discrete root cells. These findings might suggest that NO is a common component of the plant signalling pathways regulating the establishment of different plant-fungus mutualistic symbiosis. It is remarkable, that in the case of the *Trichoderma* symbiosis, the increase of NO triggered by the fungus was limited to the first 30 minutes of the interaction (Gupta *et al.*, 2014). This result contrasts with the temporal organization displayed by the NO accumulation during the AM interaction. In the AM interaction, NO levels spiked in the host roots during the first days following the contact with the AM fungal mycelium (Martínez-Medina *et al*., 2019). These differences in the patterns of NO accumulation might highlight the different colonization strategies followed by these different mutualistic fungal symbionts. In the case of the AM symbiosis, the plant actively accommodates the fungal partner in specialized host-membrane compartments in root cortical cells, forming arbuscules (Bonfante and Genre, 2010). This relies in a continual signalling between the symbiont and in the activation of an extensive genetic and developmental program in both partners during the entire colonization process (Maclean *et al.*, 2017). In contrast, the strategy followed by *T. asperelloides* to colonize roots is mostly based on the early repression of plant immune responses to scape plant defences (Brotman *et al.*, 2013). These findings suggest that although NO is a common component of the plant signalling pathways regulating the establishment of different plant-fungus mutualistic interactions, the NO patterns and possibly its particular role(s) might be specific for every type of mutualistic association. Yet the experimental data on

NO signalling during mutualistic plant-fungi interactions are still scarce to develop accurate models.

5. Differential NO role in pathogenic and mutualistic plant-fungi interactions

According to the above findings it seems that NO is a common component of the plant signalling pathways controlling both immunity against fungal pathogens and symbiosis establishment with fungal mutualists. However, the spatiotemporal kinetics of NO accumulation in pathogenic and mutualistic scenarios seems to differ widely. When comparing the NO accumulation triggered in tomato roots by the AM fungus *R. irregularis* and the one triggered by the necrotrophic pathogen *F. oxysporum* we found remarkable differences (Martínez-Medina *et al*., 2019). After a first rapid and unspecific burst of NO, the pathogen triggered a massive accumulation of NO through the complete root, which was concomitant with a strong downregulation of the *Phytogb1* gene and cell death progression. In contrast, the AM mutualistic interaction triggered a series of more controlled oscillations of NO accumulation, which overlap with the regulation of the *Phytogb1* gene. In the case of the mutualistic association, the accumulation of NO was further restricted to the outer cell layers and root hairs. It is remarkable that this specific root zones are associated with Ca^{2+} signalling during early stages of the mycorrhization process (Genre *et al.*, 2013) maybe suggesting an interplay between Ca^{2+} and NO in the onset of the AM symbiosis. In analogy, Espinosa and coworkers (2014) found that *R. irregularis* triggered a controlled burst of NO that was localized in the external cell layers. By contrast, the NO burst triggered by the pathogen *V. dahliae* was stronger and spread not only to external cell layers, but also to cortical cells. A similar pattern was observed when comparing the NO accumulation triggered by *T. asperelloides* and *F. oxysporum* in Arabidopsis roots (Gupta *et al.*, 2014). While NO accumulation triggered during the mutualistic interaction was weak and restricted to discrete root cells, NO accumulation triggered by the pathogen was stronger and spread over wide portions of the roots (Gupta *et al.*, 2014). Accordingly, it seems that although NO-related signalling is a common regulatory component in mutualistic and pathogenic plant-fungi interactions, the NO-related signature triggered in both interactions, and most likely the specific NO functions differ widely. We envisage that future studies including the comparison between pathogenic and mutualistic plant-fungus interactions within the same plant system will allow deciphering the specific role(s) of NO as regulator in pathogenic and mutualistic plant-fungus relationships.

6. Concluding remarks

The information available on NO regulation during plant-fungi interactions allows to conclude that NO is a key signal in the establishment and the fine-tuning of mutualistic and pathogenic plant-fungi interactions. Although NO production is a common feature to both types of interactions, the NO-related signature triggered seems to differ quantitatively and in its spatio-temporal distribution in both types of interactions. These differences most likely determine the specific NO functions that may shape the final outcome of the interaction. Based in the current knowledge, we propose a model for NO regulation and function in the different types of interactions (Fig.1), but important information gaps have been identified. Comparative studies among different mutualistic and pathogenic interactions, using similar methodologies and across multiple plant systems are required in order to identify common patterns and major regulatory nodes. Moreover, studies devoted to integrate NO as a cue in the plant defence signalling network are required to explore the specific functions of NO in mutualistic and pathogenic plant-fungi interactions. This review highlights the importance of the spatiotemporal dynamics in NO production, and the need of precise and sensitive methods to measure it and to determine its sources and metabolism. Thus, important technical challenges remain ahead, as described in Box1, but careful designing of the new experiments, together with the technical progress already taking place will offer great advances in the field in the coming years. This research would boost our knowledge on NO functions and the regulation of plant-fungi interactions, and the potential biotechnological applications of this knowledge for plant health in agricultural systems.

BOX 1: Future challenges for NO studies in plant-fungi interactions

The role of NO in plant-fungi interactions is of outmost complexity, having a regulatory role in both, plant defence responses and in the pathogenicity process and/or the proper establishment of beneficial interactions. Accordingly, we need a more accurate understanding of NO dynamics, distribution and function in particular plant-fungi interactions. This knowledge should contribute to the improvement of biotechnological applications for crop resistance through the identification of key regulation points determining pathogenicity or beneficial effects of microbial inoculants.

For that, we propose that the following technical and experimental challenges need to be addressed:

- Development of appropriates NO sensors to allow monitoring NO levels *in vivo* in order to follow the spatial and temporal dynamics and source of NO production during plant-fungi interactions.
- We need to conduct functional studies through the manipulation of plant or fungal NO levels at specific sites or time points, and studying the impact of such manipulation in the interaction and on plant health (for example, overexpression of phytoglobins in an inducible way, with specific tissue or responsive promoters...)
- Identification of targets of NO bioactivity during plant-fungus interaction would help to unravel molecular mechanisms underlying NO function in these interactions.
- Further studies are required including plant species from diverse plant families in order to identify possible general patterns in NO regulation and potential family or species-specific aspects of the plant responses and their impact on deleterious or beneficial interactions.

Acknowledgments

We apologize to any colleagues whose studies have not been cited due to space limitations. This research was supported by grants EX12-BIO296 and AGL2015-64990-C2-1-R from Junta de Andalucía and the Spanish Ministry of Economy and Competitiveness, respectively. L P-A and LC T-C were supported by University Staff Training (FPU) fellowships from the Spanish Ministry of Education, Culture and Sports. AMM further acknowledges funding from the program for attracting talent to Salamanca from Fundación Salamanca Ciudad de Cultura y Saberes.

References

Abello N, Kerstjens HAM, Postma DS, Bischoff R. 2009. Protein Tyrosine Nitration_Proteomics Methods for the Identification of Tyrosine Nitrated Proteins.Journal of Proteome Research **8**, 3222–3238.

Albertos P, Romero-Puertas MC, Tatematsu K, Mateos I, Sánchez-Vicente I, Nambara E, Lorenzo O. 2015. S-nitrosylation triggers ABI5 degradation to promote seed germination and seedling growth. Nature Communications **6**, 8669.

Arasimowicz-Jelonek M, Floryszak-Wieczorek J. 2016. Nitric oxide in the offensive strategy of fungal and oomycete plant pathogens. Frontiers in Plant Science **7**, 1–8.

Asai S, Ohta K, Yoshioka H. 2008. MAPK signaling regulates nitric oxide and NADPH Oxidase-dependent oxidative bursts in *Nicotiana benthamiana*. Plant Cell Online **20**, 1390–1406.

Asai S, Yoshioka H. 2009. Nitric oxide as a partner of reactive oxygen species participates in disease resistance to necrotrophic pathogen *Botrytis cinerea* in *Nicotiana benthamiana*. Molecular Plant-Microbe Interactions **22**, 619–629.

Astier J, Gross I, Durner J. 2018. Nitric oxide production in plants: an update. Journal of Experimental Botany **69**, 3401–3411.

Baidya S, Cary JW, Grayburn WS, Calvo AM. 2011. Role of Nitric Oxide and Flavohemoglobin Homolog Genes in Aspergillus nidulans Sexual Development and Mycotoxin Production. Applied and Environmental Microbiology **77**, 5524–5528.

Baudouin E, Hancock JT. 2014. Nitric oxide signaling in plants. Frontiers in Plant Science **4**, 553.

Bellin D, Asai S, Delledonne M, Yoshioka H. 2013. Nitric oxide as a mediator for defense responses. Molecular Plant-Microbe Interactions **26**, 271–277.

Bonfante P, Genre A. 2010. Mechanisms underlying beneficial plant–fungus interactions in mycorrhizal symbiosis. Nature Communications **1**, 1–11.

Brotman Y, Landau U, Cuadros-Inostroza Á, Takayuki T, Fernie AR, Chet I, Viterbo A, Willmitzer L. 2013. *Trichoderma*-plant root colonization: escaping early plant defense responses and activation of the antioxidant machinery for saline stress tolerance. PLoS Pathogens **9**, e1003221.

Bruand C, Meilhoc E. 2019. NO in plants: pro or anti senescence. Journal of Experimental Botany erz117, https://doi.org/10.1093/jxb/erz117

Buee M, Rossignol M, Jauneau A, Ranjeva R, Bécard G. 2000. The pre-symbiotic growth of arbuscular mycorrhizal fungi is induced by a branching factor partially

purified from plant root exudates. Molecular Plant-Microbe Interactions **13**, 693–698.

Calcagno C, Novero M, Genre A, Bonfante P, Lanfranco L. 2012. The exudate from an arbuscular mycorrhizal fungus induces nitric oxide accumulation in *Medicago truncatula* roots. Mycorrhiza **22**, 259–269.

Cánovas D, Marcos JF, Marcos AT, Strauss J. 2016. Nitric oxide in fungi: is there NO light at the end of the tunnel? Current Genetics **62**, 513–518.

Castillo M-C, Coego A, Costa-Broseta Á, León J. 2018. Nitric oxide responses in *Arabidopsis* hypocotyls are mediated by diverse phytohormone pathways. Journal of Experimental Botany **69**, 5265–5278.

Cecconi D, Orzetti S, Vandelle E, Rinalducci S, Zolla L, Delledonne M. 2009. Protein nitration during defense response in *Arabidopsis thaliana*. Electrophoresis **30**, 2460–2468.

Chabaud M, Genre A, Sieberer BJ, Faccio A, Fournier J, Novero M, Barker DG, Bonfante P. 2011. Arbuscular mycorrhizal hyphopodia and germinated spore exudates trigger Ca^{2+} spiking in the legume and nonlegume root epidermis. New Phytologist **189**, 347–355.

Chakraborty N, Chandra S, Acharya K. 2017. Biochemical basis of improvement of defense in tomato plant against Fusarium wilt by CaCl2. Physiology and Molecular Biology of Plants **23**, 581–596.

Conrath U, Amoroso G, Köhle H, Sültemeyer DF. 2004. Non-invasive online detection of nitric oxide from plants and some other organisms by mass spectrometry. Plant Journal **38**, 1015–1022.

Courtois C, Besson A, Dahan J, Bourque S, Dobrowolska G, Pugin A, Wendehenne D. 2008. Nitric oxide signalling in plants: interplays with Ca^{2+} and protein kinases. Journal of Experimental Botany **59**, 155–163.

Couto D, Zipfel C. 2016. Regulation of pattern recognition receptor signalling in plants. Nature Reviews Immunology **16**, 537–552.

Cui B, Pan Q, Clarke D, Villarreal MO, Umbreen S, Yuan B, Shan W, Jiang J, Loake GJ. 2018. S-nitrosylation of the zinc finger protein SRG1 regulates plant immunity. Nature Communications **9**, 4226.

Cui JL, Wang YN, Jiao J, Gong Y, Wang JH, Wang ML. 2017. Fungal endophyte-induced salidroside and tyrosol biosynthesis combined with signal cross-talk and the mechanism of enzyme gene expression in Rhodiola crenulata. Scientific Reports **7**, 1–9.

Dangl JL. 2013. Pivoting the Plant Immune System. Science **563**, 746–751.

Dean R, Van Kan JAL, Pretorius ZA, *et al.* 2012. The Top 10 fungal pathogens in molecular plant pathology. Molecular Plant Pathology **13**, 414–430.

Del Castello F, Nejamkin A, Cassia R, Correa-Aragunde N, Fernández B, Foresi N, Lombardo C, Ramirez L, Lamattina L. 2019. The era of nitric oxide in plant biology: Twenty years tying up loose ends. Nitric Oxide - Biology and Chemistry **85**, 17–27.

Dodds PN, Rathjen JP. 2010. Plant immunity: towards an integrated view of plant–pathogen interactions. Nature Reviews Genetics **11**, 539–548.

Du J, Li M, Kong D, Wang L, Lv Q, Wang J, Bao F, Gong Q, Xia J, He Y. 2014. Nitric oxide induces cotyledon senescence involving co-operation of the *NES1/MAD1* and *EIN2*-associated *ORE1* signalling pathways in *Arabidopsis*. Journal of Experimental Botany **65**, 4051–4063.

Espinosa F, Garrido I, Ortega A, Casimiro I, Álvarez-Tinaut MC. 2014. Redox activities and ROS, NO and phenylpropanoids production by axenically cultured intact olive seedling roots after interaction with a mycorrhizal or a pathogenic fungus. Plos One **9**, 1–12.

Fan G, Liu Y, Wang X, Zhan Y. 2014. Cross-talk of polyamines and nitric oxide in endophytic fungus-induced betulin production in Betula platyphylla plantlets. Trees - Structure and Function **28**, 635–641.

Feechan A, Kwon E, Yun BW, Wang Y, Pallas JA, Loake GJ. 2005. A central role for S-nitrosothiols in plant disease resistance. Proceedings of the National Academy of Sciences of the United States of America **102**, 8054–8059.

Ferlian O, Biere A, Bonfante P, *et al.* 2018. Growing research networks on mycorrhizae for mutual benefits. Trends in Plant Science **23**, 975–984.

Floryszak-Wieczorek J, Arasimowicz M, Milczarek G, Jelen H, Jackowiak H. 2007. Only an early nitric oxide burst and the following wave of secondary nitric oxide generation enhanced effective defence responses of pelargonium to a necrotrophic pathogen. New Phytologist **175**, 718–730.

Floryszak-Wieczorek J, Arasimowicz-Jelonek M. 2016. Contrasting regulation of NO and ROS in potato defense-associated metabolism in response to pathogens of different lifestyles. PLoS ONE **11**, 1–24.

Genre A, Chabaud M, Balzergue C, Puech-pag V, Novero M, Rey T, Rochange S, Guillaume B, Bonfante P, Barker DG. 2013. Short-chain chitin oligomers from arbuscular mycorrhizal fungi trigger nuclear Ca^{2+} spiking in *Medicago truncatul*a roots

and their production is enhanced by strigolactone. , 179–189.

Gibbs DJ, Md Isa N, Movahedi M, *et al.* 2014. Nitric oxide sensing in plants is mediated by proteolytic control of group VII ERF transcription factors. Molecular Cell **53**, 369–379.

Govrin EM, Levine A. 2000. The hypersensitive response facilitates plant infection by the necrotrophic pathogen *Botrytis cinerea*. Current biology : CB **10**, 751–7.

Guo P, Cao Y, Li Z, Zhao B. 2004. Role of an endogenous nitric oxide burst in the resistance of wheat to stripe rust. Plant, Cell and Environment **27**, 473–477.

Gupta KJ, Igamberdiev AU. 2016. Reactive Nitrogen Species in Mitochondria and Their Implications in Plant Energy Status and Hypoxic Stress Tolerance. Frontiers in Plant Science **7**, 1–6.

Gupta KJ, Mur LAJ, Brotman Y. 2014. *Trichoderma asperelloides* suppresses nitric oxide generation elicited by *Fusarium oxysporum* in *Arabidopsis* roots. Molecular Plant-Microbe Interactions **27**, 307–314.

Hancock J, Neill S. 2019. Nitric Oxide: Its Generation and Interactions with Other Reactive Signaling Compounds. Plants **8**, 41.

He Y, Tang R-H, Hao Y, *et al.* 2004. Nitric oxide represses the *Arabidopsis* floral transition. Science **305**, 1968–1971.

Hebelstrup KH, Shah JK, Simpson C, Schjoerring JK, Mandon J, Cristescu SM, Harren FJM, Christiansen MW, Mur LAJ, Igamberdiev AU. 2014. An assessment of the biotechnological use of hemoglobin modulation in cereals. Physiologia Plantarum **150**, 593–603.

Hu X. 2003. NO-mediated hypersensitive responses of rice suspension cultures induced by incompatible elicitor. Chinese Science Bulletin **48**, 358.

Jeandroz S, Wipf D, Stuehr DJ, Lamattina L, Melkonian M, Tian Z, Zhu Y, Carpenter EJ, Wong GK-S, Wendehenne D. 2016. Occurrence, structure, and evolution of nitric oxide synthase–like proteins in the plant kingdom. Science Signaling **9**, re2-re2.

Jiao J, Zhou B, Zhu X, Gao Z, Liang Y. 2013. Fusaric acid induction of programmed cell death modulated through nitric oxide signalling in tobacco suspension cells. Planta **238**, 727–737.

Koeck M, Hardham AR, Dodds PN. 2011. The role of effectors of biotrophic and hemibiotrophic fungi in infection. Cellular microbiology **13**, 1849–57.

Lamotte O, Bertoldo JB, Besson-Bard A, Rosnoblet C, Aimé S, Hichami S, Terenzi

H, Wendehenne D. 2014. Protein S-nitrosylation: specificity and identification strategies in plants. Frontiers in chemistry **2**, 114.

Lindermayr C, Sell S, Müller B, Leister D, Durner J. 2010. Redox regulation of the NPR1-TGA1 system of *Arabidopsis thaliana* by nitric oxide. The Plant cell **22**, 2894–907.

Lo Presti L, Lanver D, Schweizer G, Tanaka S, Liang L, Tollot M, Zuccaro A, Reissmann S, Kahmann R. 2015. Fungal effectors and plant susceptibility. Annual Review of Plant Biology **66**, 513–545.

MacLean AM, Bravo A, Harrison MJ. 2017. Plant signaling and metabolic pathways enabling arbuscular mycorrhizal symbiosis. The Plant Cell **29**, 2319–2335.

Maillet F, Poinsot V, André O, *et al.* 2011. Fungal lipochitooligosaccharide symbiotic signals in arbuscular mycorrhiza. Nature **469**, 58–63.

Manjunatha G, Gupta KJ, Lokesh V, Mur LA, Neelwarne B. 2012. Nitric oxide counters ethylene effects on ripening fruits. Plant Signaling & Behavior **7**, 476–483.

Martínez-Medina A, Pescador-Azofra L, Fernandez I, Rodríguez-Serrano M, García J, Romero-Puertas M, Pozo M. 2019. Nitric oxide and nonsymbiotic hemoglobin 1 are regulatory elements in the *Solanum lycopersicum-Rhizophagus irregularis* mycorrhizal symbiosis. **New Phytologist:** doi.org/10.1111/nph.15898

Martínez-Ruiz A, Araújo IM, Izquierdo-Álvarez A, Hernansanz-Agustín P, Lamas S, Serrador JM. 2013. Specificity in S-Nitrosylation: A Short-Range Mechanism for NO Signaling? Antioxidants & Redox Signaling **19**, 1220–1235.

Méndez-Bravo A, Calderón-Vázquez C, Ibarra-Laclette E, Raya-González J, Ramírez-Chávez E, Molina-Torres J, Guevara-García AA, López-Bucio J, Herrera-Estrella L. 2011. Alkamides activate jasmonic acid biosynthesis and signaling pathways and confer resistance to *Botrytis cinerea* in *Arabidopsis thaliana*. PLoS ONE **6**.

Monzón GC, Regente M, Pinedo M, Lamattina L, de la Canal L. 2015. Effects of nitric oxide on sunflower seedlings: A balance between defense and development. Plant Signaling and Behavior **10**, 9–11.

Mur LAJ, Carver TLW, Prats E. 2006. NO way to live; the various roles of nitric oxide in plant-pathogen interactions. Journal of Experimental Botany **57**, 489–505.

Mur LAJ, Mandon J, Persijn S, Cristescu SM, Moshkov IE, Novikova G V, Hall MA, Harren FJM, Hebelstrup KH, Gupta KJ. 2013. Nitric oxide in plants: an assessment of the current state of knowledge. AoB Plants **5**, pls052.

738 **Mur LAJ, Simpson C, Kumari A, Gupta AK, Gupta KJ**. 2016. Moving nitrogen to
739 the centre of plant defence against pathogens. Annals of Botany **119**, 703–709.
740 **Mur LAJ, Sivakumaran A, Mandon J, Cristescu SM, Harren FJM, Hebelstrup**
741 **KH**. 2012. Haemoglobin modulates salicylate and jasmonate/ethylene-mediated
742 resistance mechanisms against pathogens. Journal of experimental botany **63**, 4375–87.
743 **Neill S, Barros R, Bright J, Desikan R, Hancock J, Harrison J, Morris P, Ribeiro**
744 **D, Wilson I**. 2008. Nitric oxide, stomatal closure, and abiotic stress. Journal of
745 Experimental Botany **59**, 165–176.
746 **Noorbakhsh Z, Taheri P**. 2016. Nitric oxide: a signaling molecule which activates cell
747 wall-associated defense of tomato against *Rhizoctonia solani*. European Journal of Plant
748 Pathology **144**, 551–568.
749 **Perchepied L, Balagué C, Riou C, Claudel-Renard C, Rivière N, Grezes-Besset B,**
750 **Roby D**. 2010. Nitric oxide participates in the complex interplay of defense-related
751 signaling pathways controlling disease resistance to *Sclerotinia sclerotiorum* in
752 *Arabidopsis thaliana*. Molecular plant-microbe interactions : MPMI **23**, 846–60.
753 **Perfect SE, Green JR**. 2001. Infection structures of biotrophic and hemibiotrophic
754 fungal plant pathogens. Molecular Plant Pathology **2**, 101–108.
755 **Pieterse CMJ, Van der Does D, Zamioudis C, Leon-Reyes A, Van Wees SCM**.
756 2012. Hormonal modulation of plant immunity. Annual Review of Cell and
757 Developmental Biology **28**, 489–521.
758 **Pieterse CMJ, Zamioudis C, Berendsen RL, Weller DM, Van Wees SCM, Bakker**
759 **PAHM**. 2014. Induced systemic resistance by beneficial microbes. Annual Review of
760 Phytopathology **52**, 347–375.
761 **Piterková J, Hofman J, Mieslerová B, Sedlářová M, Luhová L, Lebeda A,**
762 **Petřivalský M**. 2011. Dual role of nitric oxide in *Solanum* spp.-*Oidium neolycopersici*
763 interactions. Environmental and Experimental Botany **74**, 37–44.
764 **Piterková J, Petivalský M, Luhová L, Mieslerová B, Sedláová M, Lebeda A**. 2009.
765 Local and systemic production of nitric oxide in tomato responses to powdery mildew
766 infection. Molecular Plant Pathology **10**, 501–513.
767 **Plett JM, Martin FM**. 2018. Know your enemy, embrace your friend: using omics to
768 understand how plants respond differently to pathogenic and mutualistic
769 microorganisms. The Plant Journal **93**, 729–746.
770 **Porras-Alfaro A, Bayman P**. 2011. Hidden fungi, emergent properties: endophytes
771 and microbiomes. Annual Review Phytopathology **49**, 291-315.

Pozo MJ, López-Ráez JA, Azcón-Aguilar C, García-Garrido JM. 2015. Phytohormones as integrators of environmental signals in the regulation of mycorrhizal symbioses. New Phytologist **205**, 1431–1436.

Prado AM, Porterfield DM, Feijó JA. 2004. Nitric oxide is involved in growth regulation and re-orientation of pollen tubes. Development **131**, 2707–2714.

Prats E, Carver TLW, Mur LAJ. 2008. Pathogen-derived nitric oxide influences formation of the appressorium infection structure in the phytopathogenic fungus Blumeria graminis. Research in Microbiology **159**, 476–480.

Prats E, Mur LAJ, Sanderson R, Carver TLW. 2005. Nitric oxide contributes both to papilla-based resistance and the hypersensitive response in barley attacked by Blumeria graminis f. sp. hordei. Molecular Plant Pathology **6**, 65–78.

Qiao M, Sun J, Liu N, Sun T, Liu G, Han S, Hou C, Wang D. 2015. Changes of nitric oxide and its relationship with H_2O_2 and Ca^{2+} in defense interactions between wheat and *Puccinia triticina*. PLoS ONE **10**, 1–19.

Qu ZL, Zhong NQ, Wang HY, Chen AP, Jian GL, Xia GX. 2006. Ectopic expression of the cotton non-symbiotic hemoglobin gene GhHbd1 triggers defense responses and increases disease tolerance in *Arabidopsis*. Plant and Cell Physiology **47**, 1058–1068.

Rasul S, Dubreuil-Maurizi C, Lamotte O, Koen E, Poinssot B, Alcaraz G, Wendehenne D, Jeandroz S. 2012. Nitric oxide production mediates oligogalacturonide-triggered immunity and resistance to *Botrytis cinerea* in *Arabidopsis thaliana*. Plant, Cell and Environment **35**, 1483–1499.

Ren CG, Dai CC. 2013. Nitric Oxide and Brassinosteroids Mediated Fungal Endophyte-Induced Volatile Oil Production Through Protein Phosphorylation Pathways in Atractylodes lancea Plantlets. Journal of Integrative Plant Biology **55**, 1136–1146.

Rippa S, Adenier H, Derbaly M, Béven L. 2007. The peptaibol alamethicin induces an rRNA-cleavage-associated death in *Arabidopsis thaliana*. Chemistry and Biodiversity **4**, 1360–1373.

Romero-Puertas MC, Campostrini N, Mattè A, Righetti PG, Perazzolli M, Zolla L, Roepstorff P, Delledonne M. 2008. Proteomic analysis of S-nitrosylated proteins in *Arabidopsis thaliana* undergoing hypersensitive response. Proteomics **8**, 1459–1469.

Romero-Puertas MC, Laxa M, Matte A, Zaninotto F, Finkemeier I, Jones AME, Perazzolli M, Vandelle E, Dietz K-J, Delledonne M. 2007. S-Nitrosylation of Peroxiredoxin II E Promotes Peroxynitrite-Mediated Tyrosine Nitration. The Plant Cell

Online **19**, 4120–4130.

Romero-Puertas MC, Sandalio LM. 2016. Role of NO-dependent posttranslational modifications in switching metabolic pathways. Advances in Botanical Research **77**, 123–144.

Romero-Puertas MC, Terrón-Camero LC, Peláez-Vico MÁ, Olmedilla A, Sandalio LM. 2018. Reactive oxygen and nitrogen species as key indicators of plant responses to Cd stress. Environmental and Experimental Botany.

Rusterucci C, Espunya MC, Diaz M, Chabannes M, Martinez MC. 2007. S-nitrosoglutathione reductase affords protection against pathogens in *Arabidopsis*, both locally and systemically. Plant Physiology **143**, 1282–1292.

Samalova M, Johnson J, Illes M, Kelly S, Fricker M, Gurr S. 2013. Nitric oxide generated by the rice blast fungus *Magnaporthe oryzae* drives plant infection. New Phytologist **197**, 207–222.

Sanz L, Albertos P, Mateos I, Sánchez-Vicente I, Lechón T, Fernández-Marcos M, Lorenzo O. 2015. Nitric oxide (NO) and phytohormones crosstalk during early plant development. Journal of Experimental Botany **66**, 2857–2868.

Sarkar TS, Biswas P, Ghosh SK, Ghosh S. 2014. Nitric oxide production by necrotrophic pathogen *Macrophomina phaseolina* and the host plant in charcoal rot disease of jute: complexity of the interplay between necrotroph–host plant interactions. PLoS ONE **9**, e107348.

Scheler C, Durner J, Astier J. 2013. Nitric oxide and reactive oxygen species in plant biotic interactions. Current Opinion in Plant Biology **16**, 534–539.

Schlicht M, Kombrink E. 2013. The role of nitric oxide in the interaction of *Arabidopsis thaliana* with the biotrophic fungi , *Golovinomyces orontii* and *Erysiphe pisi*. **4**, 1–12.

Shelef O, Hahn PG, Getman-Pickering Z, Martinez Medina A. 2019. Coming to Common Ground: The Challenges of Applying Ecological Theory Developed Aboveground to Rhizosphere Interactions. Frontiers in Ecology and Evolution **7**.

Serrano I, Romero-Puertas MC, Rodríguez-Serrano M, Sandalio LM, Olmedilla A. 2012. Peroxynitrite mediates programmed cell death both in papillar cells and in self-incompatible pollen in the olive (*Olea europaea* L.). Journal of experimental botany **63**, 1479–93.

Shafiei R, Hang C, Kang JG, Loake GJ. 2007. Identification of loci controlling non-host disease resistance in *Arabidopsis* against the leaf rust pathogen *Puccinia triticina*.

Molecular Plant Pathology **8**, 773–784.

Shi F-M, Li Y-Z. 2008. Verticillium dahliae toxins-induced nitric oxide production in *Arabidopsis* is major dependent on nitrate reductase. BMB reports **41**, 79–85.

Shi FM, Yao LL, Pei BL, Zhou Q, Li XL, Li Y, Li YZ. 2009. Cortical microtubule as a sensor and target of nitric oxide signal during the defence responses to *Verticillium dahliae* toxins in *Arabidopsis*. Plant, Cell and Environment **32**, 428–438.

Sivakumaran A, Akinyemi A, Mandon J, Cristescu SM, Hall MA, Harren FJM, Mur LAJ. 2016. ABA Suppresses *Botrytis cinerea* elicited NO production in tomato to influence H_2O_2 generation and increase host susceptibility. Frontiers in Plant Science **7**, 1–12.

Srivastava N, Gonugunta VK, Puli MR, Raghavendra AS. 2009. Nitric oxide production occurs downstream of reactive oxygen species in guard cells during stomatal closure induced by chitosan in abaxial epidermis of *Pisum sativum*. Planta **229**, 757–65.

Staskawicz BJ. 2001. Genetics of plant-pathogen interactions specifying plant disease resistance. Plant Physiology **125**, 73–76.

Tada Y, Mori T, Shinogi T, *et al.* 2004. Nitric Oxide and Reactive Oxygen Species Do Not Elicit Hypersensitive Cell Death but Induce Apoptosis in the Adjacent Cells During the Defense Response of Oat. Molecular Plant-Microbe Interactions **17**, 245–253.

Tada Y, Spoel SH, Pajerowska-Mukhtar K, Mou Z, Song J, Wang C, Zuo J, Dong X. 2008. Plant immunity requires conformational charges of NPR1 via S-Nitrosylation and thioredoxins. Science **321**, 952–956.

Teng W, Zhang H, Wang W, Li D, Wang M, Liu J, Zhang H, Zheng X, Zhang Z. 2014. ALY proteins participate in multifaceted Nep1Mo-triggered responses in *Nicotiana benthamiana*. Journal of Experimental Botany **65**, 2483–2494.

Tischner R, Koltermann M, Hesse H, Plath M. 2010. Early responses of *Arabidopsis thaliana* to infection by *Verticillium longisporum*. Physiological and Molecular Plant Pathology **74**, 419–427.

Trapet P, Kulik A, Lamotte O, Jeandroz S, Bourque S, Nicolas-Francès V, Rosnoblet C, Besson-Bard A, Wendehenne D. 2015. NO signaling in plant immunity: A tale of messengers. Phytochemistry **112**, 72–79.

Turrion-Gomez JL, Benito EP. 2011. Flux of nitric oxide between the necrotrophic pathogen *Botrytis cinerea* and the host plant. Molecular Plant Pathology **12**, 606–616.

Van Baarlen P, Staats M, Van Kan JAL. 2004. Induction of programmed cell death in lily by the fungal pathogen *Botrytis elliptica*. Molecular Plant Pathology **5**, 559–574.

van Baarlen P, van Belkum A, Summerbell RC, Crous PW, Thomma BPHJ. 2007. Molecular mechanisms of pathogenicity: how do pathogenic microorganisms develop cross-kingdom host jumps? FEMS Microbiology Reviews **31**, 239–277.

Vandelle E, Delledonne M. 2011. Peroxynitrite formation and function in plants. Plant science : an international journal of experimental plant biology **181**, 534–9.

Wang J, Higgins VJ. 2005. Nitric oxide has a regulatory effect in the germination of conidia of *Colletotrichum coccodes*. Fungal Genetics and Biology **42**, 284–292.

Wang JW, Wu JY. 2004. Involvement of nitric oxide in elicitor-induced defense responses and secondary metabolism of *Taxus chinensis* cells. Nitric Oxide - Biology and Chemistry **11**, 298–306.

Waszczak C, Carmody M, Kangasjärvi J. 2018. Reactive oxygen species in plant signaling. Annual Review of Plant Biology **69**, 209–236.

Weiberg A, Wang M, Bellinger M, Jin H. 2014. Small RNAs: A new paradigm in plant-microbe interactions. Annual Review of Phytopathology **52**, 495–516.

Wendehenne D, Gao Q, Kachroo A, Kachroo P. 2014. Free radical-mediated systemic immunity in plants. Current Opinion in Plant Biology **20**, 127–134.

Wolpert TJ, Dunkle LD, Ciuffetti LM. 2002. Host-selective toxins and avirulence determinants: what's in a name? Annual Review of Phytopathology **40**, 251–285.

Yao L-L, Pei B-L, Zhou Q, Li Y-Z. 2014. NO serves as a signaling intermediate downstream of H_2O_2 to modulate dynamic microtubule cytoskeleton during responses to VD-toxins in *Arabidopsis*. Plant Signaling & Behavior **7**, 174–177.

Yu M, Lamattina L, Spoel SH, Loake GJ. 2014. Nitric oxide function in plant biology: A redox cue in deconvolution. New Phytologist **202**, 1142–1156.

Yu M, Yun B-W, Spoel SH, Loake GJ. 2012. A sleigh ride through the SNO: regulation of plant immune function by protein S-nitrosylation. Current Opinion in Plant Biology **15**, 424–430.

Zamioudis C, Pieterse CMJ. 2012. Modulation of host immunity by beneficial microbes. Molecular Plant-Microbe Interactions **25**, 139–150.

Zeilinger S, Gupta VK, Dahms TES, Silva RN, Singh HB, Upadhyay RS, Gomes EV, Tsui CK-M, Nayak S C. 2016. Friends or foes? Emerging insights from fungal interactions with plants (JR van der Meer, Ed.). FEMS Microbiology Reviews **40**, 182–207.

Zhang RQ, Zhu HH, Zhao HQ, Yao Q. 2013. Arbuscular mycorrhizal fungal inoculation increases phenolic synthesis in clover roots via hydrogen peroxide, salicylic

acid and nitric oxide signaling pathways. Journal of Plant Physiology **170**, 74–79.

Zhang Y, Yang X, Zeng H, Guo L, Yuan J, Qiu D. 2014. Fungal elicitor protein PebC1 from *Botrytis cinerea* improves disease resistance in *Arabidopsis thaliana*. Biotechnology Letters **36**, 1069–1078.

Zhu H, Zhang R, Chen W, Gu Z, Xie X, Zhao H, Yao Q. 2015. The possible involvement of salicylic acid and hydrogen peroxide in the systemic promotion of phenolic biosynthesis in clover roots colonized by arbuscular mycorrhizal fungus. Journal of Plant Physiology **178**, 27–34.

Zipfel C, Oldroyd GED. 2017. Plant signalling in symbiosis and immunity. Nature **543**, 328–336.

Zou YN, Wang P, Liu CY, Ni QD, Zhang DJ, Wu QS. 2017. Mycorrhizal trifoliate orange has greater root adaptation of morphology and phytohormones in response to drought stress. Scientific Reports **7**, 1–10.

925 **Table 1:** A summary of the studies where NO production in plants-fungi interactions have been shown and its proposed role.

Fungus	Plant	Type interac	NO levels (technique)	Time scale	Sour	Gene expression	Pharmacological approach	Genetic approach	Suggested function	Ref
B. graminis	*H. vulgare* (leaf)	Path	DAF-2DA	6-24h	-	-	cPTIO (0.25mM) SNP (0.05mM) L-NAME (1mM)	-	NO contributes to HR and cell death, leading to the stop of the infection. NO also contributes to papilla formation.	(Prats *et al*., 2005)
B. cinerea	*A. thaliana* (leaf)	Path	DAF-2DA	6d	-	*PR1/ LOX2/ LOX3/ AOS/ OPR3/ VSP2/ GDSL/ ERF2* + array	N-isobutyl decanamide (60µM)	*Jar1/ Coi1/ Eds16/ Mpk6*	Alkamides are involved in plant immunity induction and change NO levels.	(Méndez-Bravo *et al.*, 2011)
B. cinerea	*A. thaliana* (leaf)	Path	DAF-2DA	30min-6h	NR Arg	-	OG L-NAME (5mM) cPTIO (500µM) Tungstate (µM)	*nia1nia2/ cngc2/ per4-1/ per4-2/ glu/ RBOH-D*	NO participates in the regulation of OG-responsive genes (*PER4*/ a b-1,3-glucanase). Plants treated with cPTIO, were more susceptible to *B. cinerea.*	(Rasul *et al.*, 2012)
B. cinerea (PebC1)	*A. thaliana* (leaf/ cells)	Path	Griess reagent	3-6h	-	*PR1/ BGL-2/ PR4/ PDF1.2/ This2.1*	-	*Ein2/ Coi1/ Npr1/ NahG*	PebC1 protein promotes *Arabidopsis* resistance to infection by rapid increase of NO.	(Zhang *et al.*, 2014)
B. cinerea	*N. bentham.* (leaf)	Path	DAF-2DA	2-12d	NOS NR	*NbPR-1/ NbLOX/ NbGST/ NbCAT1*	DPI (50µM) L-NAME (5mM) D-NAME (50µM) cPTIO (500µM)	*NbNOA1/ NbRBOHB* VIGS	NO contributes to disease resistance against *B. cinerea.*	(Asai and Yoshioka, 2009)
B. cinerea	*P. peltatum* (leaf)	Path	DAF-2DA/ PGSTAT 30	5min-3d	-	-	-	-	An early NO burst and a later wave of NO generation enhance the resistance of *P. peltatum* to *B. cinerea.*	(Floryszak-Wieczorek *et al.*, 2007)
B. cinerea	*S. lycoper. N. tabacum, A. thaliana* (leaf)	Path	DAF-2DA	1-4d	-	-	c-PTIO (0.25mM) L-NAME (5mM)	-	A NO concentration threshold will trigger plant cell death. Below this threshold, NO acts as a signalling molecule to activate diverse plant defence systems against the fungus.	(Turrion-Gomez and Benito, 2011)
B. cinerea	*S. lycoper* (leaf)	Path	Quantum cascade laser	30min-24h	NR	-	L-NAME (5mM) SNP (0.1mM)	ABA mutant *sitiens*	ABA can decreases resistance to *B. cinerea* via reduction of NO production.	(Sivakumaran *et al.*, 2016)
B. cinerea	*S. tuberos* cv. Bintje/ Bzura (leaf)	Path	Electrochemical method	0-24h	-	*PR-1/ PR-2/ PR-3*	-	-	*B. cinerea* triggered huge NO overproduction.	(Floryszak-Wieczorek and Arasimowicz-Jelonek, 2016)
C. orbiculare	*N. bentham* (leaf)	Path	DAF-2DA	4-6d	NR NOS Non enz.	-	Tungstate (100mM)	*NOA1*-silenced plants (VIGS)	NO helps to defend the plant against *C. orbiculare*. Posttranscriptional control of *NOA1*-influenced NO production and is affected through the *MEK2 SIPK/ NTF4* cascade.	(Asai and Yoshioka, 2008)
Chitiosan (fungal elicitor)	*P. sativum* (leaf)	Path	DAF-2DA	10-20 min	NR NOS	-	cPTIO (0.2mM) L-NAME (0.1mM) Tungstate (0.1mM)	-	NO production may be responsive to fungal PAMPs.	(Srivastava *et al.*, 2009)
F. mosseae (AMF)	*T. repense* (root)	Benef	DAF-FM DA	5-9 weeks	-	*PAL/ CHS*	-	-	AMF increases NO levels in roots, independently of the mycorrhization week.	(Zhang *et al.*, 2013)

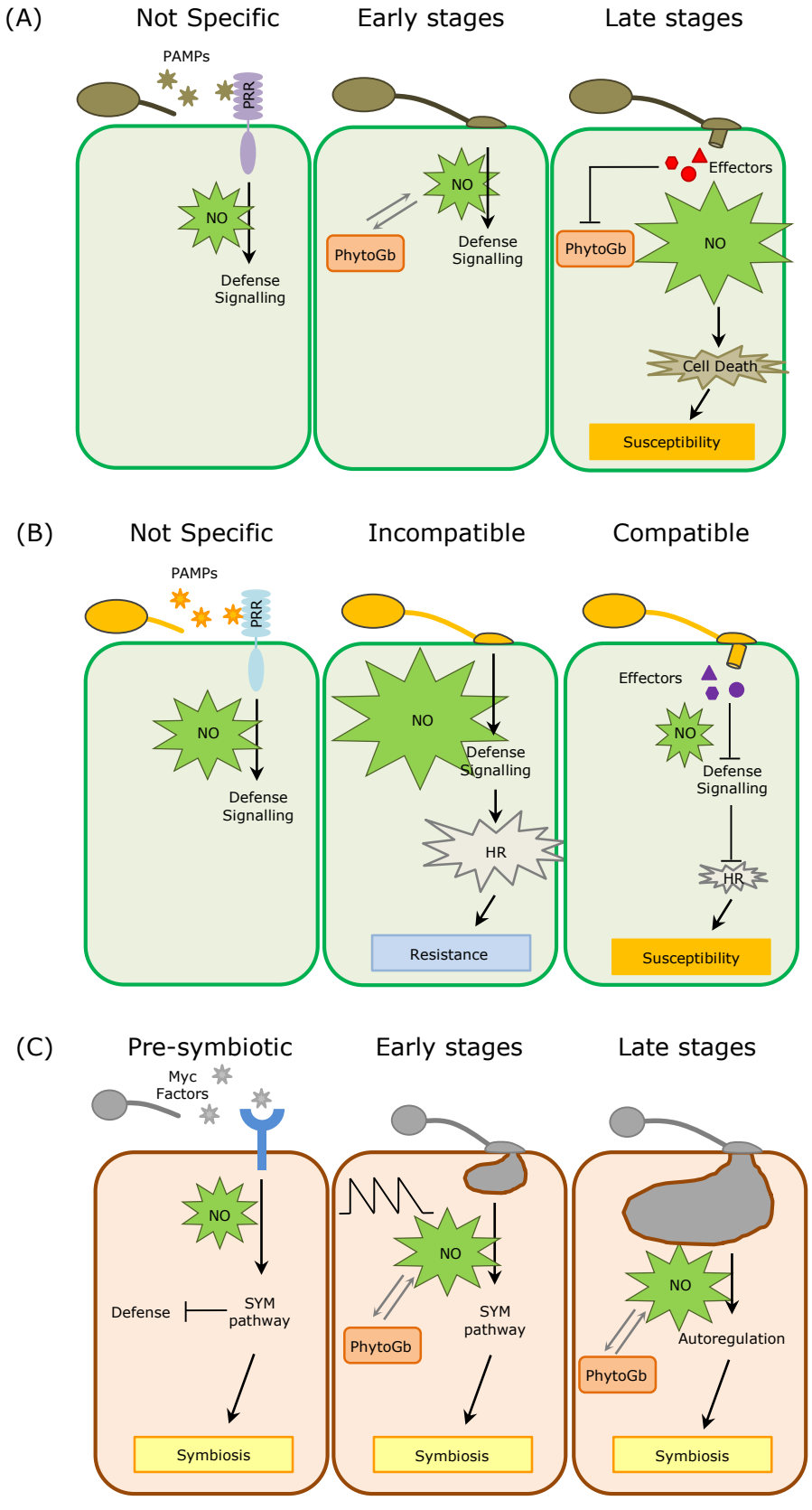
Fungus	Plant	Type interac	NO levels (technique)	Time scale	Sour	Gene expression	Pharmacological approach	Genetic approach	Suggested function	Ref
F. mosseae (AMF)	*T. repense* (root)	Benef	DAF-FM DA	5-9 weeks	-	*PAL/ CHS*	-	-	AMF increases NO in roots, but not systemically to non-mycorrhizal roots in the split root system.	(Zhu *et al.*, 2015)
F. oxysporum (*Fox*) *T. asperelloides*	*A. thaliana* (root)	Path Benef	DAF-2DA	10-120 min	-	78 NO-modulated genes	cPTIO (100µM) L-NAME (2.5mM)	*nia1nia2*	*T. asperelloides* suppresses NO generation elicited by *Fox.*	(Gupta *et al.*, 2014)
Fox (Fusaric acid)	*N. tabacum* (cells)	Path	DAF-2 DAF-FM DA	15-90 min	-	*PAL/ Hsr203J*	cPTIO (100mM) L-NMMA (100mM)	-	FA can induce PCD in tobacco suspension cells in a NO-dependent way.	(Jiao *et al.*, 2013)
Fox	*S. lycoper* (root)	Path	DAF-2DA Haemoglobin assay	48h	NR	*PRs/ PAL/ ProtIn/ PO/ GST/ CAM/ NR*	SNP (100µM) cPTIO (100µM) L-NAME (10µM)	-	Ca-treated plants showed higher NO production *vs* control. Disease incidence was reduced in Ca treated plants, may be due to the higher NO concentration.	(Chakraborty *et al.*, 2017)
Fox (fungal elicitor)	*T. chinensis* (cells)	Path	DAF-2 DA	0-12h	NOS	*PAL*	SNP (10µM) L-NNA (100µM) PTIO (100µM)	-	NO activates fungal elicitor-induced responses involving secondary metabolism.	(Wang and Wu, 2004)
G. margarita (exudates)	*M. truncatula* (root)	Benef (symb)	DAF-2DA	0-15min	NR	*NR/ NiR*	cPTIO (1mM)	Trans. roots (*DMI1-1*, *DMI2*-2, and *DMI3-1*)	There is a NO specific signature related to AM-interactions and a different NO signature when plants were exposed to a general elicitor like bacterial LPS extract.	(Calcagno *et al.*, 2012)
M. grisea (cell wall)	*O. sativa* (leaf/ cells)	Path	Spectrophotometry	30min; 12h	NOS	*PAL/ PR-1/ CHI*			NO acts as a signal mediating the HR induced by the fungus and it is also necessary for the induction of cell death in combination with H_2O_2.	(Hu *et al.*, 2003)
M. oryzae (Nep1Mo)	*A. thaliana* (leaf)	Path	DAF-2DA	3h	-	*AtERF1/ AtLOX3*	SNP (25mM) cPTIO (400µM)	At*ALY4*	*AtALY4*–H_2O_2–NO pathway mediates multiple Nep1Mo-triggered responses, including stomatal closure, HCD, and defence-related gene expression.	(Teng *et al.*, 2014)
M. oryzae	*H. vulgare* *O. sativa* (leaf)	Path	-	-	-	-	PTIO (250-500µM)	-	Removal of NO delays germination development and reduces disease lesion numbers.	(Samalova *et al.*, 2013)
M. phaseolina and xylanase	*C. capsularis* (leaf)	Path	DAF-FM DA	8h	-	-	cPTIO (200mM)	-	Low NO concentration functions as a signalling molecule. High NO concentrations facilitate fungal infection by triggering PCD. *M. phaseolina* could enhance the infection of plant cells through its own production of NO.	(Sarkar *et al.*, 2014)
O. neolycopersici	*S. lycoper* cv. Amateur/ *chmielewskii* / *hirsutum* f. *glabratum* (leaf)	Path	Oxyhemoglobin method DAF-FM DA	0-216h	NOS	-	cPTIO (0.1mM) L-NAME (10mM) AMG (10mM)	-	NO levels are higher in resistant varieties leading to plant resistance.	(Piterkova *et al.*, 2009)
O. neolycopersici	*S. lycoper*/ *chmielewskii* / *habrochaites* f. *glabratum* (leaf/disc)	Path	DAF-FM DA	8-72h	NOS	-	SNP (0.1mM) L-NAME (1mM) PTIO (0.1mM)	-	In moderate susceptible genotype the disease rate is diminished if NO production by NOS is reduced. NO activates defences in resistant genotype. With cPTIO, the fungus germinates better on the leaves.	(Piterková *et al.*, 2011)
P. striiformis CY22-2/ CY29-1	*T. aestivum* cv. Lovrin10 (leaf)	Path	Electron spin resonance	0-120h	-	-	SNP (0.5; 2.5mM)	-	There is a general correlation of NO formation and race-specific resistance.	(Guo *et al.*, 2004)

Fungus	Plant	Type interac	NO levels (technique)	Time scale	Sour	Gene expression	Pharmacological approach	Genetic approach	Suggested function	Ref
P. coronata f.sp. *avenae*	*A. sativa* (leaf)	Path	DAF	12-60h	-	-	cPTIO (500µM)	-	The simultaneous generation of NO and H_2O_2 might be associated with the death of adjacent cells of those infected by an avirulent isolate of *P. coronata*.	(Tada *et al.*, 2004)
P. triticina	*A. thaliana* *T. aestivum* (leaf)	Path	DAF-DA	24h	-	-	-	*atrbohD/ atrbohF/ atrbohD+F/ A. thaliana* (natural variation)	Identification of loci controlling non-host disease resistance and changes in NO levels.	(Shafiei *et al.*, 2007)
P. triticina	*T. aestivum* (leaf)	Path	DAF-FM DA	4-72h	NR NOS	-	Na_2WO_4 (100µM) c-PTIO (200µM) L-NAME (100µM)	-	In the incompatible combination NO acts as an important signalling molecule and mediates HR.	(Qiao *et al.*, 2015)
T. brevicompactum	*A. thaliana* (leaf)	Path	DAF-DA	2h	-	-	Alamethicin (50µM)	-	rRNA cleavage was suppressed by NO.	(Rippa *et al.*, 2007)
V. dahlia (VD-toxins)	*A. thaliana* (leaf)	Path	DAF-2-DA	45min	-	*PR-1*	Tungstate (100µM) cPTIO (100µM)	*Atnoa1*	Cortical microtubule dynamics are mediated by NO-dependent signalling.	(Shi *et al.*, 2009)
V. dahlia (VD-toxins)	*A. thaliana* (leaf)	Path	DAF-2-DA	60min	NR	-	Tungstate cPTIO	*nia1nia2*	VD-toxin-induced NO accumulation H_2O_2-dependent and that H_2O_2 acted synergistically with NO to modulate the dynamic microtubule cytoskeleton responses to VD-toxins.	(Yao *et al.*, 2014)
V. dahliae/ R. irregularis	*O. europaea* (root)	Path Benef	DAF-2DA	1-24h	-	-	PTIO (400mM)	-	NO may be a key in the symbiosis establishment and the defence response to pathogens.	(Espinosa *et al.*, 2014)
V. dahliae	*A. thaliana* (leaf)	Path	DAF2-DA	60min	-	-	SNP (400µM)	GhHb1-trans. *Arabidopsis*	GhHb1 proteins play a role in the defence responses against pathogenic invasions, possibly by modulating the NO level and the ratio of H_2O_2/NO in the defence process.	(Qu *et al.*, 2006)
V. dahliae	*A. thaliana* (leaf)	Path	DAF-2-DA	50-60 min	NR	*NIA1*	Tungstate (100µM) L-NNA (100µM) cPTIO (100µM)	*Atnoa1/ nia1/ nia2*	NO was induced in response to VD-toxins in *Arabidopsis.*	(Shi and Li, 2008)
V. dahliae	*H. annuus* (root)	Path	-	-	-	-	SNP (20µM) GSNO (50µM)	-	NO pre-treatments could not reduce *Verticillium* wilt. NO donors appear to promote fungal infection.	(Monzón *et al.*, 2015)
V. longisporum	*A. thaliana* (root/leaf)	Path	DAF-2	50-80 min	-	Genes analysis at NO peak	-	-	732 genes in the roots and 474 genes in the shoot may be regulated by NO.	(Tischner *et al.*, 2010)

926

Figure Legends

Figure 1: Model of NO function in plant-fungi interactions. (A) During plant interaction with necrotrophic fungi, plant perception of fungal PAMPs by plant PRR receptors triggers a rapid and unspecific NO burst, which activates plant response at early stages. At later stages, NO is massively produced with the advance of the infection, and the associated cell death would be exploited by the pathogen to further expand the lesions (Floryszak-Wieczorek *et al*., 2007; Turrion-Gomez and Benito, 2011; Martínez-Medina *et al*., 2019). (B) In plant interaction with biotrophic pathogens, plant perception of fungal PAMPs also triggers a rapid and unspecific NO burst activating plant response. During incompatible interactions a second NO burst lead to HR response, which prevents the pathogen to spread along the tissue, since biotrophs thrive only in living cells. By contrast, in compatible interactions, NO levels rapidly descend after the initial burst, most likely due to active effector-mediated defence suppression by the fungus, leading to susceptibility (Piterková *et al*., 2009; Schlicht and Kombrink, 2013; Qiao *et al.*, 2015). (C) During the pre-symbiotic stages of the mycorrhizal symbiosis MYC factors released by the fungus are perceived by plant receptors, triggering a NO burst which is linked with the activation of the SYM pathway. The activation of this pathway partially suppresses host immune responses and prepares the plant for the following fungal colonization. After the hyphal contact, NO spikes in root cells in a controlled manner thanks to the action of the phytoglobins. This specific NO pattern may function as a regulatory element in the establishment of the symbiosis. In later stages, when the symbiosis is well established, NO is further controlled by the action of the phytoglobins, and is involved in the autoregulation of the symbiosis (Calcagno *et al.*, 2012; Espinosa *et al.*, 2014; Zou *et al.*, 2017; Martínez-Medina *et al*., 2019).


(A)
Not Specific
Early stages
Late stages
PAMPs
PRR
NO
Defense Signalling
PhytoGb
Effectors
Cell Death
Susceptibility
(B)
Not Specific
Incompatible
Compatible
HR
Resistance
(C)
Pre-symbiotic
Early stages
Late stages
Myc Factors
Defense
SYM pathway
Symbiosis
Autoregulation