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Effect of atmospheric CO_2 on plant defense against leaf and root pathogens of Arabidopsis

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Abstract Climate change and the associated increase in atmospheric CO_2 levels may affect the severity of plant diseases and threaten future crop yields. Here, we compared responses of the model plant *Arabidopsis thaliana* to leaf and root pathogens with hemi-biotrophic or necrotrophic infection strategies under pre-industrial, current, and future atmospheric CO_2 conditions. Defenses against biotrophs are generally regulated by salicylic acid (SA) signaling, whereas jasmonic acid (JA) signaling controls defenses against necrotrophs. Under the CO_2 conditions tested, basal expression of the JA-responsive marker gene *PDF1.2* increased at increasing CO_2 concentrations. The SA-responsive marker genes *ICS1* and *FRK1* showed an opposite behavior, being lower expressed under high CO_2 and higher expressed under low CO_2, respectively. Accordingly, plants showed enhanced resistance to the necrotrophic leaf pathogen *Botrytis cinerea* under high CO_2, while resistance to the hemi-biotrophic leaf pathogen *Pseudomonas syringae* pv. *tomato* was reduced. The opposite was true for plants grown under low CO_2. Disease severity caused by the soil-borne pathogens *Fusarium oxysporum* f.sp. *raphani* and *Rhizoctonia solani* was similar under all CO_2 conditions tested. Collectively, our results stress the notion that atmospheric CO_2 impacts the balance between SA- and JA-dependent defenses and concomitant resistance against foliar (hemi)biotrophic and necrotrophic pathogens. The direction of the CO_2-mediated effects on SA- and JA-mediated defenses varies between reported studies, suggesting that the defense output is influenced by environmental context. These findings highlight that a wider dynamic range of climate change parameters should be studied simultaneously to harness plant traits for the development of future climate-resilient crops.

Keywords Atmospheric CO_2 · Arabidopsis · Disease resistance · Foliar pathogens · Soil-borne pathogens · Defense signaling

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Introduction

Climate change due to increasing CO_2 levels in the Earth's atmosphere may affect interactions between plants and their attackers resulting in significant effects on crop yields in agriculture. In order to secure food

production for the increasing human population it is essential to anticipate on this development. Plants are exposed to a wide array of pathogenic microbes. It is only since one and a half centuries that microbes have been recognized as causal agents of plant diseases. Early pioneers in plant pathology, such as Johanna Westerdijk – who gave her inaugural address in 1917 at Utrecht University entitled "New directions in phytopathological research" (Westerdijk 1917) – laid the foundation of present-day plant pathology (Kerling et al. 1986). Nowadays, plant pathogens are typically distinguished by their infection strategy. Pathogens with a biotrophic lifestyle, like the oomycete pathogen *Hyaloperonospora arabidopsidis*, commonly feed on living host cells, whereas pathogens with a necrotrophic lifestyle, like the fungal pathogen *Botrytis cinerea,* derive nutrients from killed plant tissues (Glazebrook 2005). Many pathogens, for example the bacterial pathogen *Pseudomonas syringae* pv. *tomato* (*Pst*), have both a biotrophic and a subsequent necrotrophic infection stage and are thus referred to as hemi-biotrophs (Glazebrook 2005). To defend themselves against pathogenic invaders, plants have developed a sophisticated defense system that recognizes pathogen-associated molecules and subsequently activate downstream defense cascades. The mutually antagonistic phytohormones salicylic acid (SA) and jasmonic acid (JA) are crucial for the regulation of the plant immune signaling network (Pieterse et al. 2009), in which the SA pathway typically regulates defenses against biotrophic pathogens, whereas JA is effective against necrotrophic pathogens and insect herbivores.

Atmospheric CO_2 concentration can induce changes in hormone levels in many plant species (Noctor and Mhamdi 2017). In general, SA, auxin, and gibberellin levels and signaling increase under elevated CO_2 conditions, whereas ABA and JA levels and signaling decrease, but effects vary between studies and plant species (Arteca et al. 1980; Li et al. 2011a, b; Teng et al. 2006; Zavala et al. 2008; Mhamdi and Noctor 2016; Noctor and Mhamdi 2017; Zhou et al. 2017; Williams et al. 2018a). For example, suppression of JA-related signaling by high CO_2 levels was associated with increased susceptibility of maize to *Fusarium verticillioides* (Vaughan et al. 2014) and of soybean to herbivores (Zavala et al. 2008, 2013). In tomato plants, elevated CO_2 levels induced an increase in SA levels and concomitantly a decrease in JA signaling. This was associated with enhanced resistance against yellow leaf curl virus, tobacco mosaic virus and *Pst*, which are typically sensitive to SA-dependent defenses, and increased susceptibility to *B. cinerea,* which is sensitive to JA-dependent defenses (Huang et al. 2012; Zhang et al. 2015). In *Arabidopsis thaliana* (hereafter Arabidopsis), elevated CO_2 similarly conferred enhanced resistance to the biotrophic pathogens *H. arabidopsidis* and *Pst,* but also to the necrotrophic fungi *Plectosphaerella cucumerina* and *B. cinerea* (Mhamdi and Noctor 2016; Williams et al. 2018a). In another study, the resistance to *Pst* in Arabidopsis was found to be reduced at high CO_2 levels, while low CO_2 levels increased the level of resistance against this pathogen (Zhou et al. 2017). This was correlated with enhanced pre-invasion, stomatal defenses and so far uncharacterized post-invasion defenses.

Most studies on the effects of different CO_2 levels on plant diseases and pests have focused on foliar pathogens and herbivores (Braga et al. 2006; Guo et al. 2014; Jwa and Walling 2001; Li et al. 2015; Mhamdi and Noctor 2016; Williams et al. 2018a). Compared to the detailed knowledge generated for interactions between plants and leaf pathogens, relatively little is known about the interactions with soil-borne pathogens, both for necrotrophs (Okubara and Paulitz 2005) and hemi-biotrophs (Chen et al. 2014; Yadeta and Thomma 2013). The few studies on the effects of enhanced atmospheric CO_2 levels on soil-borne diseases show ambiguous results. For example, the incidence of sheath blight in rice caused by the necrotrophic fungus *Rhizoctonia solani* was increased under elevated CO_2 (Kobayashi et al. 2006). Similarly, disease incidence of *Fusarium pseudograminearum* was increased in wheat plants grown under elevated CO_2 (Melloy et al. 2014). By contrast, the tolerance of tomato plants to infection by the hemi-biotroph *Phytophthora parasitica* increased at elevated CO_2 (Jwa and Walling 2001). In other studies, elevated CO_2 levels did not significantly influence disease incidence. In lettuce, disease caused by *Fusarium oxysporum* f.sp. *lactucae* was not affected by elevated atmospheric CO_2 (Ferrocino et al. 2013), and in soybean elevated CO_2 did not affect the severity of the sudden death syndrome caused by *Fusarium virguliforme* (Eastburn et al. 2010).

CO_2 concentrations in soils with active microbial communities are 10–50 times higher than the atmospheric CO_2 levels (Drigo et al. 2008). Thus, it is unlikely that the increase in atmospheric CO_2 levels foreseen for the future have an effect on the CO_2

concentration in the soil. Nonetheless, increases in atmospheric CO_2 concentration can alter plant photosynthetic rate, stimulate plant growth, and lead to increased carbon allocation to the belowground plant tissue, resulting in changes in the release of organic compounds in the rhizosphere (Drigo et al. 2008, 2010). It has been postulated that the composition of these rhizodeposits plays an essential role in shaping the rhizosphere microbiome (Berendsen et al. 2012). Indeed, Williams et al. (2018b) showed a profound effect of the interaction between atmospheric CO_2 and soil nutritional status on the impact of beneficial rhizobacteria on plant growth and induced systemic resistance. Changes in atmospheric CO_2 levels may affect the richness, composition and structure of soil microbial communities through changes in carbon allocation and root exudation (Drigo et al. 2009), which in turn can affect the impact of the root microbiome on plant performance (Bakker et al. 2018). Such effects may also influence growth and virulence of soil-borne plant pathogens.

In this study, we examined the effect of pre-industrial, current, and future levels of CO_2 in the atmosphere on resistance of Arabidopsis to the foliar pathogens *Pst* and *B. cinerea*, and to the soil-borne pathogens *F. oxysporum* f.sp. *raphani* and *R. solani*. *Pst* and *F. oxysporum* are considered as hemibiotrophic pathogens (Glazebrook 2005; Kidd et al. 2011; Ma et al. 2013), while *B. cinerea* and *R. solani* are nectrotrophic pathogens that cause severe damage on a wide range of host plants (Foley et al. 2013; Van Kan 2006). We observed contrasting effects of high and low CO_2 levels on SA- and JA-dependent defense responses in Arabidopsis leaves and associated resistances against the hemibiotrophic and necrotrophic leaf pathogens. This is partly in line with previous findings (Mhamdi and Noctor 2016; Williams et al. 2018a), showing shifts in the expression of SA- and JA-dependent defenses. This points to the notion that other environmental factors may influence the defense output of a plant and that effects of climate change on plant immunity should be studied by taking into account a more dynamic range of environmental parameters. This is in line with what the first Dutch female professor Johanna Westerdijk already stressed during her inaugural address at Utrecht University (Westerdijk 1917): it is important to study the physiology of both host and parasite in their environmental context (Kerling et al. 1986).

Material and methods

Plant material and cultivation

Seeds of *Arabidopsis thaliana* accession Col-0 and mutant line *ein2–1* (Alonso et al. 1999) were sown on sand under ambient CO_2 conditions (450 ppm). Two weeks later, seedlings were transferred to 60-ml pots containing a sand/potting soil mixture (*v*/v, 5:12) that was autoclaved twice for 20 min, after which they were transferred to a growth chamber with continuous high (800 ppm), ambient (450 ppm) or low (150 ppm) atmospheric CO_2 conditions as described (Zhou et al. 2017). Plants were cultivated with a 10-h day at 20 °C and 14-h night at 18 °C cycle (350 μmol/m^2/s) with 70% relative humidity for 4 weeks. The technical specifications of the growth chambers used in this study have been described in detail by Temme et al. (2015).

RT-qPCR analysis

For gene expression analysis, total RNA was isolated as described (Oñate-Sánchez and Vicente-Carbajosa 2008). RNA was pretreated with DNAse I (Fermentas, St. Leon-Rot, Germany) and RevertAid H minus Reverse Transcriptase (Fermentas) was used to convert DNA-free RNA into cDNA using an oligo-dT primer. PCR reactions were performed in optical 384-well plates (Applied Biosystem) with a ViiA 7 realtime PCR system (Applied Biosystems, Carlsbad, CA, USA), using SYBR® Green to monitor the synthesis of double-stranded DNA. Transcript levels were calculated relative to the constitutively expressed reference gene *PP2A-A3* (*At1g13320*), encoding the protein phosphatase 2A subunit A3, as described previously (Schmittgen and Livak 2008). Primers used for RT-qPCR were as follows. At1g13320 _F, 5′-TAA CGT GGC CAA AAT GAT GC-3′; At1g13320_R, 5′-GTT CTC CAC AAC CGC TTG GT-3′. PDF1.2_F, 5’-CAC CCT TAT CTT CGC TGC TCT T-3′; PDF1.2_R, 5’-GCC GGT GCG TCG AAA G-3′. PR1_F, 5’-CTC GGA GCT ACG CAG AAC AAC T-3′; PR1_R, 5’-TTC TCG CTA ACC CAC ATG TTC A-3′. FRK1_F, 5′- TTT CAA CAG TTG TCG CTG GA-3′; FRK1_R, 5’-AGC TTG CAA TAG CAG GTT GG-3′. ICS1_F, 5′-GGC AGG GAG ACT TAC G-3′; ICS1_R, 5′-AGG TCC CGC ATA CAT T-3′.

B. cinerea bioassay

Botrytis cinerea cultivation and disease resistance bioassays were performed essentially as described previously (Van Wees et al. 2013). *B. cinerea* strain B05.10 (Van Kan et al. 1997) was cultivated on half-strength potato dextrose agar (PDA) plates for 10 days at 22 °C. *B. cinerea* spores were collected and resuspended in half-strength potato dextrose broth to a final density of 5×10^5 spores/ml. Four-week-old plants were inoculated by applying 10-μl droplets to six leaves per plant. Symptoms were scored 4 days later. Disease severity was expressed as the percentage of leaves showing symptoms in the following three classes of disease severity: restricted lesion (<2 mm; class I), spreading lesion (>2 mm; class II), and spreading lesion with accompanying sporulation (class III).

P. syringae bioassay

Pseudomonas syringae pv. *tomato* DC3000 (*Pst*; Whalen et al. 1991) was cultured at 28 °C on King's medium B (King et al. 1954) agar plates supplemented with 50 μg/ml rifampicin. Bacteria were transferred and cultivated overnight in liquid KB medium at 28 °C in an orbital shaker at 220 rpm. Subsequently, bacteria were collected by centrifugation for 10 min at 14,500 g and resuspended in 10 mM $MgSO_4$. The suspension was adjusted to $OD_{600} = 1.0$ (= 10^9 cfu/ml). For dip inoculation, the bacterial inoculum was diluted to a final density of 5×10^7 cfu/ml of 10 mM $MgSO_4$ containing 0.015% (*v*/v) Silwet L-77 (Van Meeuwen Chemicals, Weesp, Netherlands). For disease assessment, leaf discs from treated plants were harvested, surface sterilized in 70% ethanol for 8 s, and subsequently washed with water. Eight biological replicates were included for each data point. Each leaf disc was ground thoroughly in 200 μl of 10 mM $MgSO_4$ and 10 μl aliquots of different dilutions were plated onto KB plates containing 25 μg/ml rifampicin. After 48 h incubation at room temperature, bacterial colonies were counted and the growth of *Pst* was calculated.

R. solani bioassay

Rhizoctonia solani isolate AG2-2IIIB (Chapelle et al. 2016; Mendes et al. 2011) was grown on half-strength PDA at room temperature for 1 week. The PDA plates were chopped up and blended into a sand/potting soil mixture (0.5 Petri dish/kg soil). Two-week-old Arabidopsis seedlings were transferred to the *R. solani*-inoculated soil/sand mixture and from then on, the plants were cultivated in the growth rooms under the three different CO_2 conditions. For the *R. solani* disease assessment, at least thirty plants were tested under each CO_2 condition at 3 weeks after inoculation. Chlorotic symptoms, stunted growth and death of the plant was recorded as "diseased". Additionally, fresh weight of the aboveground plant parts was determined.

F. oxysporum bioassay

Fusarium oxysporum f. sp. *raphani* strain WCS600 causes fusarium wilt disease in Arabidopsis (Pieterse et al. 1996). Before use, the fungus was cultivated on half-strength PDA at 28 °C for 3 days. The inoculum was prepared by transferring two PDA plugs to sucrose sodium nitrate liquid medium (Sinha and Wood 1968) and culturing while shaking for 3 days at 28 °C. Subsequently the culture was filtered over sterile glasswool to remove mycelium fragments and the suspension was adjusted to 10^7 conidia/ml sterile distilled water. Roots of two-week-old seedlings that were grown in sand were dipped in the conidial suspension. The seedlings were then transferred to soil mixed with sand (12:5) and grown under the three different CO_2 conditions.

For the *F. oxysporum* f. sp. *raphani* disease assessment, twenty plants per CO_2 treatment were analyzed and 3 weeks after inoculation disease symptoms were scored by determining the percentage of plants with Fusarium wilt symptoms in the leaves. The disease index (DI) was calculated from the percentage of leaves in four different disease severity classes as described (Van Wees et al. 1997). Class I. no symptoms; Class II. 1–2 wilted leaves; Class III. 3–5 wilted leaves; Class IV. >6 wilted leaves/dead plants. The aboveground plant parts were harvested and fresh weight was recorded. Relative growth (RG) was calculated as the weight of infected plants/the weight of control plants.

Three weeks after *F. oxysporum* inoculation, the aboveground plant parts were harvested. Per plant, 100–200 mg of leaf material was ground in 1 ml of 10 mM $MgSO_4$. A dilution series of this suspension was made and 100 μl of each dilution (0, 10 and 100 times diluted) was plated in duplicate on Fusarium selective Komada medium (Komada 1975). Komada plates were incubated at 28 °C for 3–4 days. The numbers of colonies were counted to assess the population density of

F. oxysporum in each plant. This experiment was repeated twice, with at least 12 biological replicates for each CO_2 treatment.

Results

Atmospheric CO_2 affects JA- and SA-dependent gene expression in Arabidopsis

To investigate whether the activity of the JA or SA pathways is altered under different atmospheric CO_2 conditions in Arabidopsis plants, we analyzed the expression of genes that are responsive to these two hormones in plants that had grown at three atmospheric CO_2 concentrations. Two-week-old seedlings that had been cultivated at ambient CO_2 were subsequently transferred to low (150 ppm), ambient (450 ppm), and high (800 ppm) CO_2 conditions and gene expression was analyzed when plants were 4 weeks old. Previous studies identified *PDF1.2* as a marker gene for JA-dependent defenses (Penninckx et al. 1996) and *PR1*, *FLG22-INDUCED RECEPTOR-LIKE KINASE1* (*FRK1*), and *ISOCHORISMATE SYNTHASE1* (*ICS1*) as marker genes for SA-dependent defenses (Asai et al. 2002; Wildermuth et al. 2001; Zhang et al. 1999). Analysis of the expression of these JA- and SA-responsive marker genes revealed that the various atmospheric CO_2 concentrations differentially affected their basal expression levels (Fig. 1a–d). More specifically, expression of *PDF1.2* was significantly higher in plants grown under the high atmospheric CO_2 condition and reduced under the low CO_2 conditions (Fig. 1a). Oppositely, the *FRK1* gene was higher expressed at low atmospheric CO_2 levels (Fig. 1c) and *ICS1* expression was reduced under the high CO_2 condition (Fig. 1d).

Atmospheric CO_2 alters Arabidopsis resistance to the foliar pathogens *B. cinerea* and *Pst*

In order to investigate whether differences in atmospheric CO_2 levels affect resistance to a foliar necrotrophic pathogen, we tested the resistance of Arabidopsis plants to *B. cinerea* at three atmospheric CO_2 levels. Four days after inoculation, leaves of plants grown at high CO_2 developed fewer spreading lesions, whereas at low CO_2 significantly more spreading lesions developed (Fig. 2a and b). Thus, with increasing atmospheric CO_2 levels the resistance of Arabidopsis plants to *B. cinerea* also increased.

We also tested the resistance of Arabidopsis plants to the hemi-biotrophic leaf pathogen *Pst* at the different atmospheric CO_2 levels. At 5 days after inoculation, the bacterial titer of *Pst* was significantly higher in high CO_2-grown plants and significantly lower in low CO_2-grown plants compared with bacterial densities in the ambient CO_2-grown plants (Fig. 2c), confirming previously reported results (Zhou et al. 2017), showing that opposite to the increasing resistance level to *B. cinerea*, *Pst* resistance decreases at increasing CO_2 levels under the conditions used in our experimental setup.

Atmospheric CO_2 does not affect disease resistance against the soil-borne pathogens *R. solani* and *F. oxysporum*

To study effects of different atmospheric CO_2 levels on the interaction between Arabidopsis and the necrotrophic soil-borne pathogen *R. solani*, two-week-old seedlings, grown at ambient CO_2, were transplanted into *R. solani*-inoculated soil and subsequently grown for 3 weeks at the three different atmospheric CO_2 conditions. Under ambient CO_2 conditions the percentage of diseased plants was nearly 50% and neither high nor low CO_2 conditions affected the disease incidence (Fig. 3a). *R. solani* infection significantly reduced shoot fresh weight of Arabidopsis plants, but also for this parameter no significant differences between high, ambient and low CO_2 levels were observed (Fig. 3b). These results show that varying atmospheric CO_2 conditions from 150 ppm to 800 ppm has no significant impact on the disease severity caused by *R. solani* infection of Arabidopsis.

We also investigated effects of atmospheric CO_2 on the performance of Arabidopsis plants inoculated with the hemi-biotrophic soil-borne pathogen *F. oxysporum*. Two-week-old seedlings were inoculated by root dipping in a conidial suspension of *F. oxysporum* f.sp. *raphani*, and transferred to high, ambient, or low CO_2 conditions. Disease symptoms were scored 3 weeks after inoculation. Throughout the course of the study, the disease severity caused by *F. oxysporum* infection in Arabidopsis Col-0 plants varied among experiments, and the different atmospheric CO_2 levels had inconsistent effects on disease caused by *F. oxysporum* among the five independent experiments that were performed

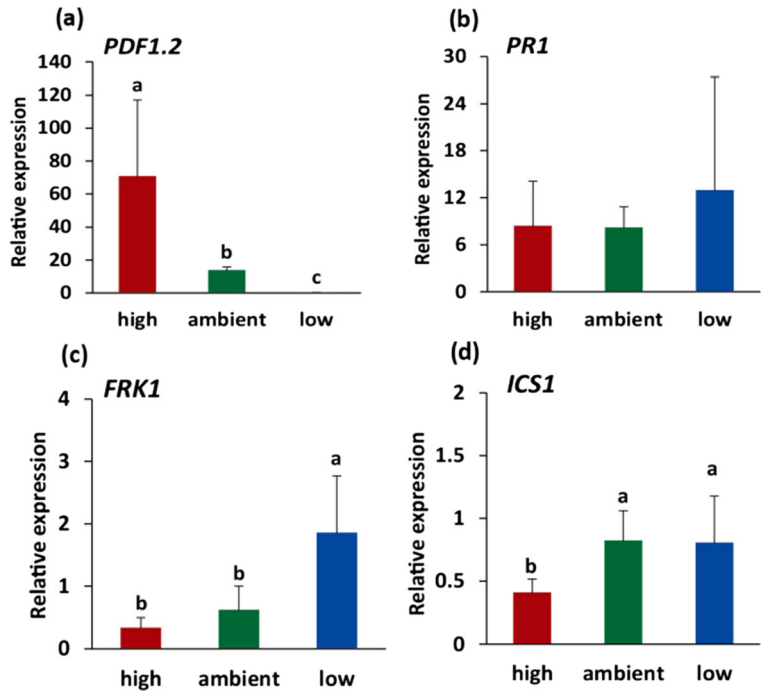

Fig. 1 RT-qPCR analysis of *PDF1.2* (**a**), *PR1* (**b**), *FRK1* (**c**), and *ICS1* (**d**) gene expression in 4-week-old Arabidopsis Col-0 plants grown under high (800 ppm), ambient (450 ppm) and low (150 ppm) levels of atmospheric CO_2. Indicated are the expression levels relative to the reference gene *PP2A-A3* (*At1g13320*). Different letters indicate significant differences between CO_2 treatments (one-way ANOVA, Duncan's multiple range test, $P < 0.05$). Error bars represent SD, $n = 3$ plants. Experiments were repeated with similar results

(Table 1). For example, in experiment 2, plants grown at either high or low CO_2 levels both showed a significantly lower disease index than the plant grown at ambient CO_2 levels, whereas in experiment 3, the disease index was significantly lower under the high CO_2 and higher under the low CO_2 condition than under ambient CO_2 conditions, while in experiment 4 no significant effects were observed at all (Table 1). Since the disease development in these experiments was relatively low, we investigated if atmospheric CO_2 might affect disease in a more susceptible Arabidopsis line. Mutations that disrupt the ethylene signaling pathway increase plant

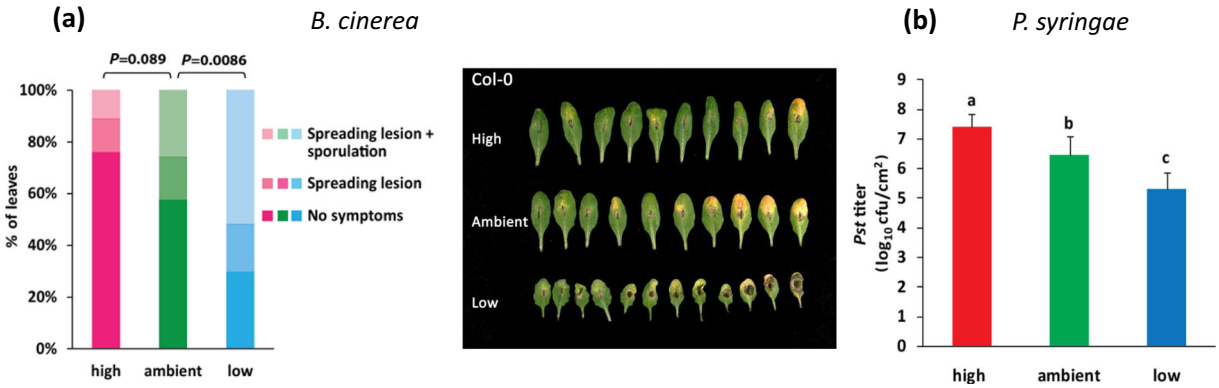

Fig. 2 **a** Quantification of *B. cinerea* disease symptoms on leaves of Arabidopsis Col-0 plants grown under three atmospheric CO_2 conditions. Disease severity of the inoculated leaves was scored in three classes at 4 days after inoculation. Percentage of leaves in each class was calculated per plant. Indicated above the brackets are the *P* values of comparisons between different atmospheric CO_2 conditions (X^2-test; $n = 9$ plants). **b** Representative leaves showing disease symptoms of plants grown under the three CO_2 conditions 4 days after inoculation with *B. cinerea*. **c** The bacterial titers of *Pst* in leaves of Arabidopsis plants grown under the three atmospheric CO_2 conditions. Bacterial population densities were determined at 5 days after inoculation. Indicated is the average of the $\log_{10}$-transformed bacterial titer per leaf area. Different letters indicate significant differences between CO_2 treatments (one-way ANOVA, Fisher's LSD test, $P < 0.05$). Error bars represent SD, $n = 8$ plants. Experiments were repeated with similar results

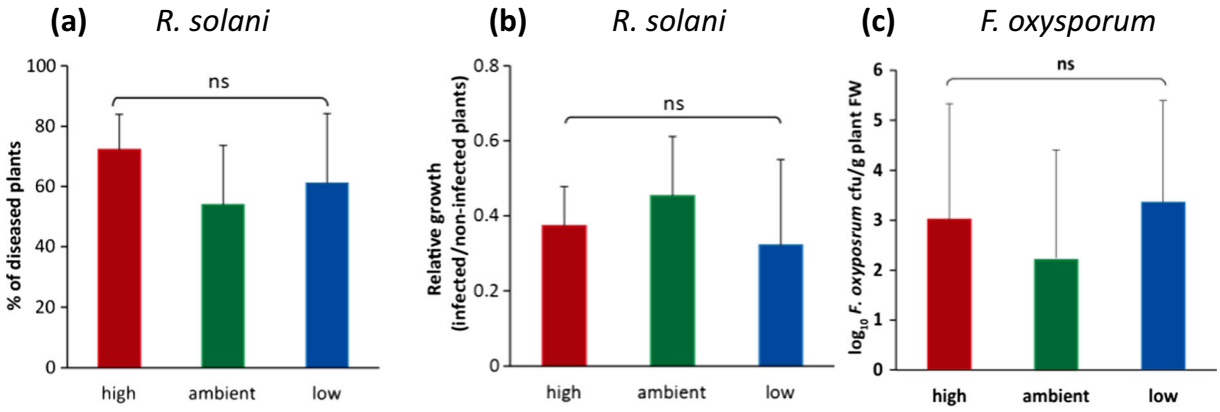

Fig. 3 Effect of atmospheric CO_2 conditions on disease caused by the soil-borne pathogens *R. solani* and *F. oxysporum* in Arabidopsis grown under high (800 ppm), ambient (450 ppm), or low (150 ppm) CO_2 conditions. **a** Disease incidence at 24 days after transplantation of Arabidopsis accession Col-0 seedlings to soil mixed with *R. solani* inoculum. Chlorotic, stunted or dead plants were considered as diseased. **b** Inhibition of plant growth by *R. solani*. At 24 days after inoculation, the aboveground plant parts were harvested and fresh weight was recorded. The weight of *R. solani*-infected plants relative to non-inoculated control plants is depicted. **c** The density of *F. oxysporum* in leaves of Arabidopsis. Arabidopsis accession Col-0 seedlings were inoculated with *F. oxysporum* by root dipping and grown in soil. Arabidopsis leaves were harvested 3 weeks later. The density of *F. oxysporum* was determined by dilution plating on Komada agar medium. Statistical analysis was performed using one-way ANOVA (Duncan's multiple range test), treatments did not differ significantly. Error bars represent SD, $n = 6$ replicated blocks, existing of 9 plants per block for *R. solani* bioassays, and $n = 12$ plants for *F. oxysporum* bioassays. Experiments were repeated with similar results

susceptibility to *F. oxysporum* (Berrocal-Lobo and Molina 2004; Pantelides et al. 2013) and therefore, we included a mutant impaired in ethylene responsiveness (*ein2–1*) in the experiments. Indeed, in four out of the five experiments, the *ein2–1* mutant exhibited a higher disease index (Table 1, experiment 1, 2, 3, and 5). However, also in *ein2–1* the different atmospheric CO_2 levels did not influence the disease severity consistently (Table 1). In addition to disease symptoms, the effect of *F. oxysporum* infection on the growth of Arabidopsis was determined. The shoot fresh weight of infected plants relative to non-infected control plants was calculated. The effects of CO_2 levels were again not consistent when comparing the five independent experiments that were performed (Table 2).

Table 1 Disease index (DI) of Arabidopsis wild-type Col-0 plants and a mutant defective in ethylene signaling (*ein2–1*) 3 weeks after root inoculation with *F. oxysporum* and grown under high (800 ppm), ambient (450 ppm), or low (150 ppm) CO_2 conditions in five independent experiments

Genotype	CO_2 level	Disease Index (DI)				
		exp.1	exp.2	exp.3	exp.4	exp.5
Col-0	high	1.33 a	1.67 b	1.07 c	1.72 a	1.24 a
	ambient	1.25 ab	2.15 a	1.47 b	1.64 a	1.10 b
	low	1.14 b	1.85 b	2.11 a	1.61 a	1.29 a
ein2–1	high	1.58 b	2.85 a	1.65 a	1.24 b	2.93 a
	ambient	1.79 a	2.43 b	1.82 a	1.24 b	2.90 a
	low	1.45 b	3.07 a	1.33 b	1.71 a	2.70 a

Indicated are averages of the disease index calculated from the percentage of leaves in four different disease severity classes. Class I. no symptoms; Class II. 1–2 wilted leaves; Class III. 3–5 wilted leaves; Class IV. >6 wilted leaves/dead plants. For each experiment and per genotype different letters indicate statistically significant differences between CO_2 levels (two-way ANOVA, Duncan's multiple-range test; $n = 20$; $P < 0.05$)

Table 2 Growth of *F. oxy*sporum-inoculated Arabidopsis wild-type Col-0 plants and a mutant defective in ethylene signaling (*ein2–1*) relative to non-inoculated plants under high (800 ppm), ambient (450 ppm), or low (150 ppm) CO_2 conditions in five independent experiments

Genotype	CO_2 level	Relative growth (RG)				
		exp.1	exp.2	exp.3	exp.4	exp.5
Col-0	high	0.76 c	0.87 ab	0.98 a	0.97 a	0.63 b
	ambient	1.06 a	0.71 b	0.80 b	0.69 b	1.00 a
	low	0.89 b	0.91 a	0.69 b	0.75 b	0.88 a
ein2–1	high	0.66 b	0.59 ab	0.77 b	1.04 a	
	ambient	0.80 b	0.81 a	0.82 ab	0.86 bc	
	low	1.19 a	0.50 b	0.94 a	0.72 c	

The aboveground plant tissue was harvested 3 weeks after inoculation and the fresh weight was recorded. Indicated are averages of the growth of *F. oxysporum*-infected plants relative to control treated plants. For each experiment and per genotype different letters indicate statistically significant differences between CO_2 levels (two-way ANOVA, Duncan's multiple-range test; $n = 20$; $P < 0.05$)

Finally, the colonization of Arabidopsis shoots by *F. oxysporum* was determined under the different CO_2 conditions at 3 weeks after inoculation of the roots. As shown in Fig. 3c, fungal titer in the leaves was not affected by the atmospheric CO_2 conditions under which the plants were grown. Overall, the results on the three parameters suggest that there is no significant impact of atmospheric CO_2 levels on the interaction between Arabidopsis and the soil-borne pathogen *F. oxysporum*.

Discussion

Various climate change models predict elevation of atmospheric CO_2 levels in the future and this has boosted research on plant-pathogen interactions under high atmospheric CO_2 conditions (Chakraborty and Datta 2003; Huang et al. 2012; Lake and Wade 2009; Pangga et al. 2011; Zhang et al. 2015; Noctor and Mhamdi 2017; Velásquez et al. 2018; Williams et al. 2018a). It has been shown that altered atmospheric CO_2 levels have only a limited direct influence on the overall growth of plant-associated microbes, including pathogenic ones (Drigo et al. 2008; Wells 1974). Nevertheless, altered levels of atmospheric CO_2 are likely to affect infection by plant pathogens through interference with host plant defense responses. Metabolic and transcriptional analyses of plants grown under ambient and high CO_2 conditions revealed distinct alterations in different hormone signaling pathways in different plant species (Casteel et al. 2008; Matros et al. 2006; Teng et al. 2006; Mhamdi and Noctor 2016; Williams et al. 2018a). Plants grown under high CO_2 are slow in photorespiration, but are induced in defense, which may be related to enhancement of reactive oxygen species (ROS) signaling (Noctor and Mhamdi 2017). High CO_2 can affect disease resistance to foliar pathogens. In both tomato and Arabidopsis plants this was shown to be associated with altered activation of SA- and JA-dependent defense pathways, resulting in reduced resistance against the (hemi)biotrophs *Pst* and *H. arabidopsidis,* and enhanced resistance against the necrotrophs *B. cinerea* and *P. cucumerina* (Zhang et al. 2015; Mhamdi and Noctor 2016; Williams et al. 2018a).

Under our experimental conditions, we also see contrasting effects of the level of CO_2 on SA- and JA-dependent defenses and biotroph and necrotroph resistance, respectively, but these effects are partly opposite from the effects observed in some other studies (Mhamdi and Noctor 2016; Williams et al. 2018a). Hence, the effect of different CO_2 levels on disease resistance may be dependent on the environmental context. Under our experimental conditions, we found a generally enhanced expression of SA-responsive genes in Arabidopsis plants grown under low atmospheric CO_2 conditions, in comparison to plants grown under high CO_2 conditions (Fig. 1). These results match with the observed enhanced resistance against the hemibiotroph *Pst* under low CO_2 and the reduced resistance under high CO_2 conditions (Fig. 2c). Previously, we showed that the effects of atmospheric CO_2 likely impact both pre- and post-invasive immune responses to *Pst* infection (Zhou et al. 2017). The recorded changes

in the level of SA-responsive gene expression as shown in Fig. 1 are in line with these observations and suggest that altered intensities of SA-dependent defenses under low and high CO_2 may contribute to the observed differences in *Pst* resistance. However, our observations contrast those reported by Mhamdi and Noctor (2016), who found that high CO_2 (1000 and 3000 ppm) stimulated SA responses and *Pst* resistance in Arabidopsis, rather than reducing it. These contrasting outcomes are not unrealistic, because antagonism between the SA and the JA defense pathways is known to be dependent on the environmental context (Pieterse et al. 2012). Whether differences in environmental parameters, such as day length (10-h short day versus 16-h long day), and light intensity (350 versus 200 $\mu M/m^2/s$) have influenced the defense outputs in both studies is not known. Notwithstanding, both studies show that different levels of CO_2 antagonistically impact the outcome of plant defenses against pathogens with contrasting infection strategies.

The expression of the JA-responsive marker gene *PDF1.2* was significantly enhanced under the high atmospheric CO_2 condition and reduced under the low CO_2 condition used in our study (Fig. 1a), suggesting that increasing atmospheric CO_2 levels stimulated the JA response pathway in Arabidopsis plants. This is in line with the enhanced resistance to the necrotrophic leaf pathogen *B. cinerea* in high CO_2-grown plants and the reduced resistance to this pathogen in low CO_2-grown plants (Fig. 2a and b). Similar results have been reported by Williams et al. (2018a) who found elevated levels of the JA marker gene *VSP2* and increased resistance to the necrotrophic fungus *P. cucumerina*. In contrast, other studies with Arabidopsis, tomato and soybean showed that elevated CO_2 levels induced the SA signaling pathway and concomitantly repressed the JA signaling pathway, leading to enhanced resistance to (hemi)biotrophs and increased susceptibility to diverse necrotrophic pathogens and herbivores (Huang et al. 2012; Zavala et al. 2008; Zhang et al. 2015; Mhamdi and Noctor 2016). As mentioned above, these differential effects of altered atmospheric CO_2 levels on hormonal signaling between the studies might be attributed to the plant species examined, concentration and duration of CO_2 treatments, or differences in other environmental conditions that may have shifted the balance between SA- and JA-dependent defenses in contrasting directions. Nevertheless, these studies highlight that changes in the level of atmospheric CO_2 can differentially affect the level of resistance against plant pathogens with contrasting infection strategies.

Additionally, we studied to what extent atmospheric CO_2 levels affect the disease severity caused by soil-borne pathogens with different infection strategies. Previous studies on the influence of CO_2 levels on disease caused by the necrotroph *R. solani* showed that elevated CO_2 concentrations reduced the disease in radish and sugar beet seedlings (Papavizas and Davey 1962), but in rice the disease incidence of *R. solani* was increased (Kobayashi et al. 2006). In our study with Arabidopsis, we observed no effect of different levels of atmospheric CO_2 on the level of disease caused by *R. solani* (Fig. 3a and b). Atmospheric CO_2 levels also did not consistently affect development of disease caused by the hemibiotrophic pathogen *F. oxysporum* f.sp. *raphani* (Fig. 3c; Tables 1 and 2). This was not only observed for the moderately susceptible wild-type accession line Col-0, but also for the more susceptible *ein2–1* mutant. In lettuce, elevated CO_2 also did not influence the disease severity caused by *F. oxysporum* (Ferrocino et al. 2013). But in rice, wheat, and maize elevated CO_2 caused an increased in the susceptibility to *Fusarium* (Kobayashi et al. 2006; Melloy et al. 2014; Vaughan et al. 2014). Hence, it appears that atmospheric CO_2 can affect soil-borne diseases, but the effects depend on the host-pathogen system and environmental context.

In summary, our study confirms that alterations in atmospheric CO_2 levels differentially affect SA and JA defense-related pathways, resulting in contrasting effects on pathogens with a (hemi)biotrophic and necrotrophic lifestyle. The study-dependent outcome of the defense outputs suggests that the environmental context is likely to be decisive in the CO_2-mediated shift in the balance between SA- and JA-dependent defenses and concomitant effects on resistance to biotrophic and necrotrophic pathogens. We found no significant effects of high and low CO_2 on the level of disease caused by the soil-borne pathogens *R. solani* and *F. oxysporum*. Like in most other studies, we only varied the level of CO_2 to monitor the effect of this climate change parameter on disease resistance. However, during the anticipated global environmental changes, disease resistance will also be influenced by other parameters, such as elevated O_3, humidity, drought, and increased temperature (Eastburn et al. 2011; Velásquez et al. 2018). Hence, to fully understand the consequences of climate change for the outcome of above- and below-ground plant-pathogen

interactions, future research should capture a wider dynamic range of environmental conditions in order to identify plant traits that allow for the development of future climate-resilient crops.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Human and animal rights No human and/or animal participants were involved in this research.

Informed consent All authors consent to this submission.

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