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Original Article

Jasmonic acid signalling mediates resistance of the wild tobacco *Nicotiana attenuata* to its native *Fusarium*, but not *Alternaria*, fungal pathogens

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ABSTRACT

We recently characterized a highly dynamic fungal disease outbreak in native populations of *Nicotiana attenuata* in the southwestern United States. Here, we explore how phytohormone signalling contributes to the observed disease dynamics. Single inoculation with three native *Fusarium* and *Alternaria* fungal pathogens, isolated from diseased plants growing in native populations, resulted in disease symptoms characteristic for each pathogen species. While *Alternaria* sp.-infected plants displayed fewer symptoms and recovered, *Fusarium* spp.-infected plants became chlorotic and frequently spontaneously wilted. Jasmonic acid (JA) and salicylic acid (SA) levels were differentially induced after *Fusarium* or *Alternaria* infection. Transgenic *N. attenuata* lines silenced in JA production or JA conjugation to isoleucine (JA-Ile), but not in JA perception, were highly susceptible to infection by *F. brachygibbosum* Utah 4, indicating that products derived from the JA-Ile biosynthetic pathway, but not their perception, is associated with increased *Fusarium* resistance. Infection assays using ov-*nahG* plants which were silenced in pathogen-induced SA accumulations revealed that SA may increase *N. attenuata's* resistance to *Fusarium* infection but not to *Alternaria*. Taken together, we propose that the dynamics of fungal disease symptoms among plants in native populations may be explained by a complex interplay of phytohormone responses to attack by multiple pathogens.

Key-words: native fungal pathogens, disease dynamic, phytohormone signalling.

INTRODUCTION

The wild tobacco *Nicotiana attenuata* Torrey ex Watson forms ephemeral populations in the post-fire environment of the Great Basin Desert, United States (Baldwin & Morse 1994). After synchronized germination from a long-lived seed bank, this annual tobacco species faces attack from a variable herbivore community composed of at least 20 different herbivore taxa, which assembles from neighbouring habitats after wild fires (Baldwin 2001). This plant displays a high degree of morphological and chemical plasticity which allows it to respond to these herbivores and the selective pressures of intraspecific competition in order to survive and reproduce in the post-fire niche (Baldwin 2001). In contrast to herbivory, *N. attenuata* plants with disease symptoms are not commonly observed in natural populations. The relative low humidity and high ultraviolet (UV) fluence of the Great Basin Desert may be responsible for the observed low rates of pathogen infections (Wilks & Shen 1991).

In the summer of 2011, a native *N. attenuata* population comprising 873 plants suffered from a fungal disease outbreak with symptoms of leaf chlorosis, necrosis and dark spotted patterns on the abaxial leaf surface (Schuck *et al*. 2014b). The presence or absence of disease symptoms was tracked for each individual plant and the disease severity was categorized into four disease stages based on the presence of the different disease symptoms. Notably, 16 days after the first location and survey, more than half of all previously diseased plants had recovered either partly or completely, whereas many previously asymptomatic plants had developed disease symptoms. Such dynamics in incidence (the development of symptoms in previously asymptomatic plants) and severity (the recovery of diseased plant) of pathogen-mediated diseases are known to occur in crop plants as well as wild plants, but are commonly studied over a large temporal (several seasons or years) and spatial scale (metapopulation) (Ericson *et al*. 1999). In contrast, the dynamics of the disease outbreaks in the native population of *N. attenuata* was recorded over a relatively short period of time (16 days) and in a small area (approximately 1500 m^2). In addition, the spatial effect was not observed as diseased plants were surrounded by completely asymptomatic plants. A potential explanation for the distribution of diseased plants could be the microspatial heterogeneity of spore density in soil. Moreover, this wild tobacco population was likely highly genetically heterogenous in part, a consequence of being derived from of a long-lived seed bank (Baldwin 2001). This led to a hypothesis that the disease dynamics in a relatively small area could result from trade-offs in defence responses within individual plants that differ in their resistance against different fungal pathogens, as proposed by Burdon & Thrall (2009). Testing this hypothesis required an understanding of *N. attenuata's* responses to microbial phytopathogens, which had previously been uncharacterized.

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Fungal pathogens belonging to the genera *Alternaria* (Pleosporaceae, Pleosporales, Ascomycota) and *Fusarium* (Nectriaceae, Hypocreales, Ascomycota) were isolated from leaves of diseased *N. attenuata* (Schuck *et al*. 2014b). *Alternaria* and *Fusarium* fungi are known to be important phytopathogens. For instance, *Alternaria* species are able to cause diseases on a wide variety of economically important crops, including apple, rice, tomato, potato and tobacco (Chung 2012). The genus *Fusarium* contains a number of soil-borne species which cause various blights, root rots and wilts on solanaceous plants (Roncero *et al*. 2003). To defend themselves against fungal pathogens, plants use different defensive responses depending on whether the pathogen is a biotroph (feeding on living plant tissue) or a necrotroph (feeding on dead plant tissue) (Glazebrook 2005). Because biotrophic pathogens require a living host, the hypersensitive response, which forms regions of localized controlled cell death in response to pathogen attack, functions as an effective defence that limits pathogen spread. In contrast, necrotrophic pathogens actively kill host tissues and thus, programmed cell death initiated by the plant is not an effective strategy (Glazebrook 2005; Kliebenstein & Rowe 2008). To generalize, salicylic acid (SA)-mediated resistance is effective against biotrophs, whereas jasmonic acid (JA)-mediated responses elicit resistance against necrotrophs (McDowell & Dangl 2000; Glazebrook 2005). Hemibiotrophic phytopathogens combine both strategies, acting as biotrophs at the early stages of colonization but as necrotrophs at the later stages and can elicit complex defence responses (Ding *et al*. 2011).

In *N. attenuata*, JA is known to be produced after herbivore attack and is further conjugated with isoleucine (Ile) by JASMONATE RESISTANT 4 and 6 (JAR4 and JAR6) to form JA-Ile (Wang *et al*. 2008), which activates the SCF^{COI1}-JAZ complex. As a result, JA-Ile transcriptionally activates biosynthetic genes of defence molecules such as alkaloids (e.g. nicotine), phenolics (e.g. cryptochlorogenic acid) and phenylpropanoid-polyamine conjugates (PPCs) (Keinanen *et al*. 2001; Kessler & Baldwin 2002; Kaur *et al*. 2010). In contrast, SA signalling has been shown in other plants to elicit the synthesis of low molecular weight pathogenesis-related proteins such as PR-1, PR-2 and PR-5 (Park *et al*. 2009) and to antagonize the JA signalling pathway (Stout *et al*. 2006). The JA signalling pathway has been shown to mediate most of *N. attenuata*'s herbivore resistance traits and is activated by fatty acid-amino acid conjugates (FACs) in the oral secretions (OS) of attacking larvae. As OS likely contain microbial elicitors, the plant also has a number of FAC-elicited signalling elements, including ethylene (Barazani *et al*. 2007) and LecRK (Gilardoni *et al*. 2011) signalling which suppresses SA signalling. This inhibition of SA signalling leads to an unfettered and strong defence responses against chewing insects (Meldau *et al*. 2012).

Just as plants are simultaneously attacked by both pathogens and herbivores, they can be attacked either simultaneously or subsequently by multiple pathogens with different lifestyles. For example, in the sudden death syndrome of soybean caused by *Fusarium solani*, usually numerous saprophytic and parasitic fungi are also present in the same necrotic lesion, such as *Alternaria alternata* (Fries) Keissler, *Epicoccum nigrum* and *Cladosporium herbarum* (Melgar *et al*. 1994). Most *Fusarium* species are shown to have a hemibiotrophic lifestyle (Thatcher *et al*. 2009), whereas most *Alternaria* species possess a necrotrophic lifestyle (Thomma 2003). To tailor their defence responses most efficiently, plants need to strongly minimize cross-talk between SA- and JA-dependent defences to avoid antagonistic effects (Stout *et al*. 2006). We hypothesized that the disease dynamics observed in natural *N. attenuata* population could be explained by trade-offs between SA- and JA-mediated defences when the plants are under attack from different pathogens. To unravel this complex interaction between *N. attenuata* plants and several pathogens present in the native habitat, the interaction of this plant with each individual pathogen first needed to be investigated.

In this study, we examined the response of *N. attenuata* to three native fungal pathogens with regard to observable disease symptoms and changes in plant growth and development. Furthermore, we explored the role of induced JA and SA phytohormone signalling in mediating disease resistance using previously characterized near-isogenic transgenic plants silenced in different steps in jasmonate biosynthesis or perception and SA accumulation. The interplay of these phytohormones in response to different species of pathogens is important to describe the dynamics observed during disease outbreaks in natural *N. attenuata* populations.

MATERIALS AND METHODS

Fungal isolates

The three fungal isolates (*Alternaria* sp. Utah 10, *F. brachygibbosum* Padwick Utah 4 and *F. solani* (Mart.) Saccardo Utah 4) used in this study were obtained from diseased leaves (exhibiting curving, chlorosis, necrosis and darkish discoloration) of *N. attenuata* plants grown in native populations in the southwestern Utah, United States (Schuck *et al*. 2014b). These fungal isolates were grown in the dark on potato dextrose agar (PDA) (Fluka Analytical, Steinheim, Germany) at 24 °C. The isolates were reisolated from infected *N. attenuata* plants from time to time and confirmed by morphological comparison to the original cultures by their pattern of mycelium growth, structure, colour and conidia shape.

Plant materials and growth conditions

Seeds of the 31st generation of an inbred *N. attenuata* Torr. ex S. Watson accession originally collected from southwestern Utah in 1988 were used for all experiments, and are referred to as wild-type plants (WT). *N. attenuata* seeds were germinated on agar plates containing Gamborg's B5 medium as previously described by Krugel *et al*. (2002). Seeds were germinated and maintained in a growth chamber (Snijders Scientific, Tilburg, the Netherlands) at 26 °C for 16 h light (155 μmol s^{-1} m^{-2}) and 24 °C for 8 h dark for 10 days.

All transgenic *N. attenuata* plants used in this study were transformed with endogenous and heterologous genes in sense (ov) or antisense (as)/inverted repeat (ir) orientations by *Agrobacterium*-mediated transformation and fully characterized in previous publications. As-*lox3* plants are reduced in the expression of a gene encoding for LIPOXYGENASE 3 (*LOX3*), an enzyme catalyzing an important step for JA production in *N. attenuata* plants [peroxidation of trienoic fatty acids at the C_{13}-position (Halitschke & Baldwin 2003)]. Ir-*gla1* plants are silenced in the expression of *GLA1* which encodes a lipase that cleaves polyunsaturated fatty acids (PUFAs) from chloroplast membrane lipids to provide fatty acid substrates to *LOX3* (Bonaventure *et al*. 2011). Ir-*coi1* plants produce less *COI1* which is a key component of the JA-Ile receptor (Paschold *et al*. 2007). A cross between two *N. attenuata* lines (ir-*jar4/6*) silenced in either *JAR4* or *JAR6* is compromised in the conjugation of JA to amino acids, resulting in low levels of JA-Ile, JA-leucine and JA-valine (Wang *et al*. 2008). Ov-*nahG* plants ectopically overexpress the bacterial salicylate hydroxylase gene (*nahG*) from *Pseudomonas putida* and have reduced pathogen-inducible levels of SA (Gilardoni *et al*. 2011). All transgenic plants were homozygous for a single transgene insertion, diploid as determined by flow cytometry and used in the T_2-T_3 generation, with the exception of the ir-*jar4/6* plants which were hemizygous.

Harvesting of fungal spores and plant infection

The fungal spores were harvested from the surface of 14-day-old fungal cultures. Spores were filtered through miracloth (Calbiochem, Nottingham, UK) to remove mycelial fragments and centrifuged at 800 *g* for 20 min. The pellets were washed twice with 10 mM $MgSO_4$ and the number of spores was counted under a light microscope using a Neubauer hemocytometer. The spore concentration was adjusted using 10 mM $MgSO_4$ solution.

For *Alternaria* sp. Utah 10 infection, 10-day-old *N. attenuata* seedlings germinated on GB5 medium were transplanted into 3 cm diameter pots filled with sterile sand and placed into a climate chamber (20 °C, 16 h light from Master Son-T PIA: Philips Lighting, Ontario, Canada (www.lighting.philips.com) Agro 400 and 20 °C, 8 h dark, 60% humidity) for 2 d prior to infection. Infection was performed by dipping roots in a spore suspension ('root-dip' method) with 10^5 spores mL^{-1}. For the root-dip method, plants were removed from substrate, dipped in spore suspension for 30 s and afterwards replanted into the sandy substrate. At 8 d after infection (dai), 20-day-old plants were transplanted into pots of 10 cm in diameter. Plants dipped in 10 mM $MgSO_4$ solution were used as controls (non-infected plants). Twenty-five biological replicates were used for each treatment.

F. brachygibbosum Utah 4 was inoculated to 8-day-old *N. attenuata* seedlings germinated on GB5 plates which were transplanted into 3 cm diameter pots and placed into a climate chamber (20 °C, 16 h light from Master Son-T PIA Agro 400 and 20 °C, 8 h dark, 60% humidity) for 2 d prior to the infection. Infection was carried out by root-dip method with 10^7 spores mL^{-1}. At 10 dai, 20-day-old plants were transplanted into 10 cm pots. Non-infected controls and replicate numbers were the same as in the *Alternaria* sp. Utah 10 infections.

For infection with *F. solani* Utah 4, 10-day-old *N. attenuata* seedlings germinated on GB5 plates were transplanted into 3 cm diameter pots and placed in the glasshouse for 10 d prior to infection under high-pressure sodium lamps (200–300 μmol s^{-1} m^{-2} light) with a day/night cycle of 16 h (26–28 °C)/8 h (22–24 °C) and 45 to 55% humidity. Infection was carried out by the root-dip method with 10^5 spores mL^{-1}. After the infection, plants were grown in 10 cm diameter pots in a climate chamber (20 °C, 16 h light from Master Son-T PIA Agro 400 and 20 °C 8 h dark, 60% humidity). Plants dipped in 10 mM $MgSO_4$ solution were used as controls. Twenty biological replicates were used for each treatment.

Symptom quantification and plant morphology

Disease symptoms were recorded every 2 dai. Disease severity was assessed based on the number of dead plants and the percentage of visible disease symptoms (chlorosis, necrosis and wilting). The percentage of chlorotic, necrotic and wilted leaf area was estimated relative to the overall leaf area of each diseased plant. Fungal inoculated but asymptomatic plants were scored with 0% of chlorosis, necrosis and wilting. Average percentage of disease symptoms was calculated and plotted in either line or bar graph, taken in account both diseased and asymptomatic plants. Error bars in these graphs represent standard errors from 20 to 25 biological replicates.

Rosette diameter was measured every 3 dai. About 1 month after germination, when plants reached the elongation stage, stalk length was measured every third to fourth day and the time point of bolting (appearance of the floral meristem) and flowering (appearance of the first fully opened flower) was recorded for each plant. When plants finished the elongation stage (about 60 d after germination), final stalk length was measured. Flower morphology was also characterized. The differences in rosette diameter and stalk height between infected and control plants were calculated for each of the recorded time points.

Phytohormone extraction and quantification by Liquid chromatography-electrospray ionization-mass spectrometry (LC-ESI-MS/MS)

For the analysis of phytohormones in fungal infected and non-infected *N. attenuata* seedlings, five biological replicates of three pooled seedlings for each replicate were harvested at three time points. Plants were harvested for the first time point at the time of infection (0 dai) and for the second time point 2 d after infection but before the plants showed any visible disease symptoms. Finally, plants were harvested when 20–30% of plants showed visible disease symptoms (4 or 8 dai).

Phytohormone extraction and quantification were carried out as described in Gilardoni *et al*. (2011) using an LC-ESI-MS/MS instrument. A negative electrospray ionization mode was used for detection. An ion with a specific mass-to-charge ratio generated from each endogenous phytohormone or

internal standard (the parent ion) was selected and fragmented to obtain its daughter ions; a specific daughter ion was used for generating the corresponding compound's chromatogram. Each phytohormone [SA, JA, abscisic acid (ABA), JA-Ile] was quantified by comparing its peak area with the peak area of its respective internal standard as described in Wu *et al*. (2007). Divinyl ethers were quantified using the method described by Bonaventure *et al*. (2011). Phytohormone levels were quantified per seedling fresh mass ($nmol \cdot gFM^{-1}$).

Statistical analysis

Statistical analysis was performed using the SPSS software, version 17.0 (SPSS, Chicago, IL, USA). Statistical significance was evaluated using one-way analysis of variance (ANOVA) with a significance level of 0.05 and means were compared with the Least significant difference (LSD) *post hoc* test. For analysis of differences in plant performance, Student's *t*-tests were used with the two-tailed distribution of two sets of samples with equal variance. To compare the difference in the number of dead plants among different genotypes, the G-test of independence was used (http://udel.edu/~mcdonald/statgtestgof.html). The number of replicates (n) used in each experiment is described in the figure legends.

RESULTS

Diseased plants are able to recover after infection by *Alternaria* but not *Fusarium*

To find out how *N. attenuata* responds the fungal pathogens, single infections using *Alternaria* sp. Utah 10, *F. brachygibbosum* Utah 4 and *F. solani* Utah 4 isolates were performed based on a recently established method that involves dipping roots of seedlings into fungal spore suspension (Schuck *et al*. 2014b). The development of visible disease symptoms (including chlorosis, necrosis and wilting) was recorded (Fig. 1a). Rosette diameter and stalk height as well as time of bolting and flowering were recorded as proxies for the fitness consequences of infection. Flower morphology was also examined.

Leaf chlorosis was the first disease symptom in *N. attenuata* seedlings inoculated with *Alternaria* sp. Utah 10 at 4 to 5 dai (Fig. 1b). A small portion of the chlorotic lesions later developed into necrotic lesions. The severity of chlorosis increased until 8 dai, but started to decrease at 10 to 15 dai. At 24 dai, infected plants had completely recovered from the chlorosis and necrosis symptoms.

Similarly, *N. attenuata* plants infected by *F. solani* Utah 4 showed chlorosis at 4 dai as the first visible disease symptom (Fig. 1b). The chlorotic lesions expanded quickly, reaching their highest intensity (26% of leaf area) at 9 dai. Necrotic lesions appeared later with low abundance (less than 5% of leaf area). Some of the heavily diseased plants had curly leaves (Supporting Information Fig. S1c) and shortened, bent flowers with protruding pistils at 42 dai (Supporting Information Fig. S1d). This abnormal flower shape and curly leaf symptom had also been observed for some of the plants during the disease outbreak in the natural *N. attenuata* population (Supporting Information Fig. S1a,b).

F. brachygibbosum Utah 4-infected plants frequently spontaneously wilted, beginning 14 dai and continuing until plants reached their final developmental stage (Fig. 1b). Plants remained green but wilted nightly. Chlorosis started after wilting and was followed by the appearance necrotic lesions (Fig. 1b). Other infected plants exhibited more subtle phenotypes, such as root crown canker (Supporting Information Fig. S1e) and reduced stability of the root-shoot junction, causing infected plants to topple over.

Alternaria sp. Utah 10-infected plants had smaller rosette diameters than those of control plants until 30 dai (Student's *t*-test, $P < 0.05$) (Fig. 2a). Stalk heights of control plants were significantly larger than those of *Alternaria* sp. Utah 10-infected plants during the elongation stage (Student's *t*-test, $P < 0.03$) but not at the late stage of flowering (40 dai) (Fig. 2b). The flowering time of infected plants was therefore delayed by almost 2 days (Student's *t*-test, $P = 0.01$) (Supporting Information Table S1). No change in flower morphology was found in the infected plants compared with control plants.

N. attenuata infected by *F. solani* Utah 4 were grown in the glasshouse for 10 d before infection, which resulted in a different pattern of growth of control plants compared with the other experiments with *Alternaria* sp. Utah 10 and *F. brachygibbosum* Utah 4. Regardless, infected plants were retarded in their growth at the rosette stage compared with control plants (Student's *t*-test, $P < 0.04$) (Fig. 2c). Stalk heights were significantly smaller in infected plants compared with control plants at 27 dai (Student's *t*-test, $P < 0.001$) (Fig. 2d), but flowering time did not differ between infected and control plants (Supporting Information Table S1).

F. brachygibbosum Utah 4-infected plants grew at a rate comparable with that of control plants through 32 dai (Fig. 2e). As a result, there was no significant difference between bolting times of infected and control plants (Supporting Information Table S1). Even though stalk heights of control and infected plants did not differ (Fig. 2f), rosette diameters of control plants at the late stage of flowering (35 dai) were significantly larger than those of infected plants (Student's *t*-test, $P < 0.05$) (Fig. 2e). Similar to *Alternaria* sp. Utah 10 infection, *F. brachygibbosum* Utah 4-infected plants showed no difference in their flower morphology compared with control plants.

Both *Alternaria* and *Fusarium* infections increase SA accumulation, but JA accumulation is only induced by *Fusarium* infection

We analysed SA and JA levels in *N. attenuata* seedlings induced by infection with the three fungal species. Entire infected seedlings including roots and shoots were harvested to measure JA and SA levels by LC-MS at three time points. Plants were harvested for the first time point at the time of infection (0 dai) and for the second time point 2 d after

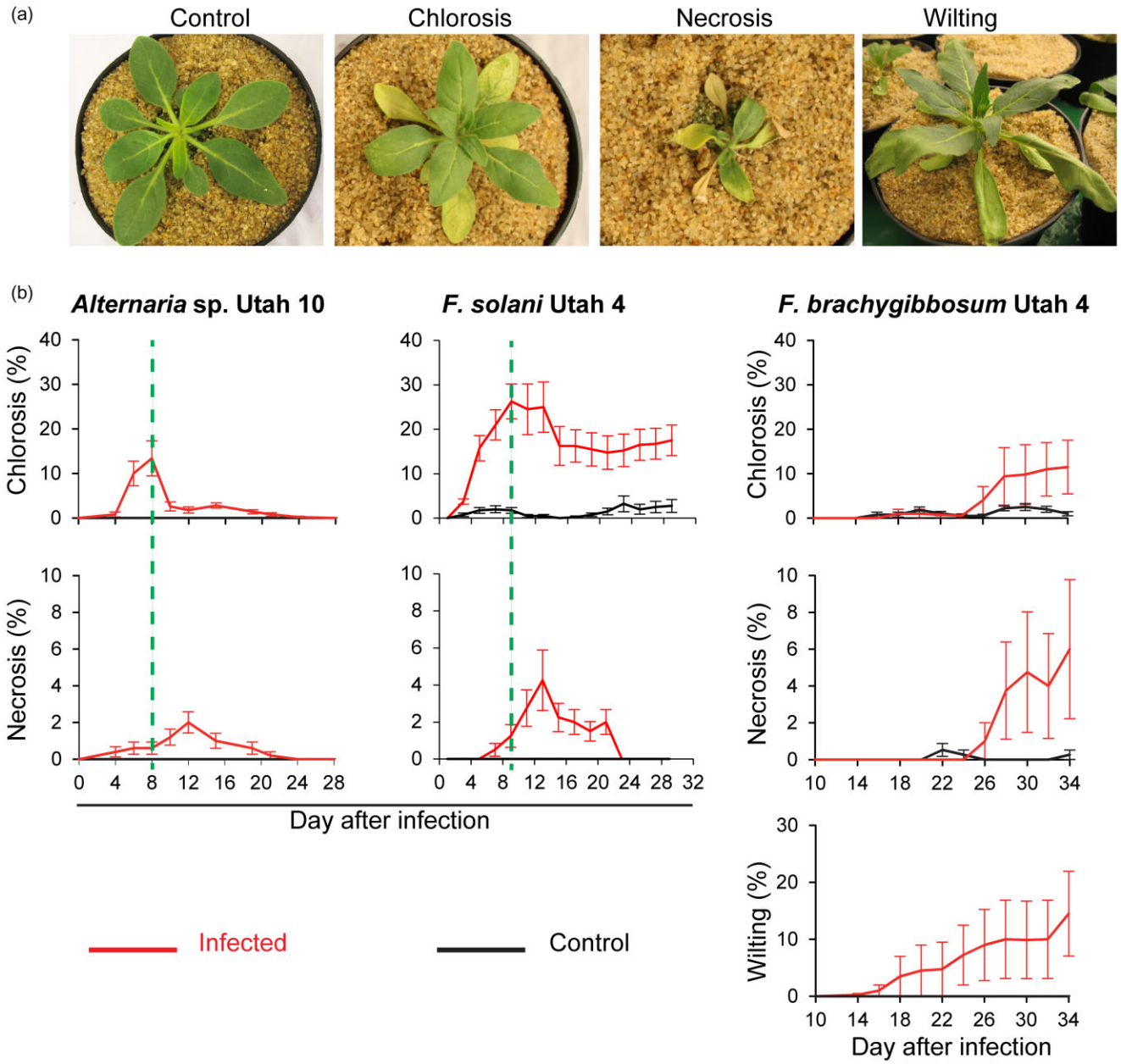

Figure 1. Infection by *Alternaria* and *Fusarium* causes chlorosis and necrosis on *N. attenuata* plants. (a) Disease symptoms (chlorosis, necrosis and wilting) of *Alternaria* and *Fusarium* infected *N. attenuata* plants. (b) Development of chlorosis, necrosis and wilting in plants infected with *Alternaria* sp. Utah 10, *F. solani* Utah 4 and *F. brachygibbosum* Utah 4 (red lines) compared with non-infected controls (black lines). Green dashed lines indicate peaks of chlorosis at 8 and 9 dai for *Alternaria* sp. Utah 10 and *F. solani* Utah 4, respectively. The error bars represent standard errors from 20, 25 and 20 biological replicates for *Alternaria* sp. Utah 10 ($n = 20$), *F. solani* Utah 4 ($n = 25$) and *F. brachygibbosum* Utah 4 ($n = 20$) infection, respectively.

infection but before the plants showed any visible disease symptoms. Finally, plants were harvested when 20–30% of plants showed visible disease symptoms (4 or 8 dai).

After infection by *Alternaria* sp. Utah 10, no significant differences in JA levels were detected compared with non-infected plants at either 2 or 8 dai (Fig. 3a). In contrast, the levels of SA started to increase at 2 dai and were significantly higher in infected plants compared with non-infected control plants at 8 dai (ANOVA, $F_{4;20} = 25.433$, $P < 0.001$) (Fig. 3b).

Infection by *F. solani* Utah 4 led to a similar induction of SA, starting at 3 dai and reaching significantly higher levels compared with control plants at 8 dai (ANOVA, $F_{4;10} = 3.278$, $P = 0.03$) (Fig. 3d). JA concentrations in infected plants were significantly higher at 8 dai compared with control plants (ANOVA, $F_{4;10} = 3.827$, $P = 0.049$) (Fig. 3c).

F. brachygibbosum Utah 4-infected plants had higher concentrations of both SA and JA compared with non-infected plants (Fig. 3e,f). Notably, levels of SA increased threefold in

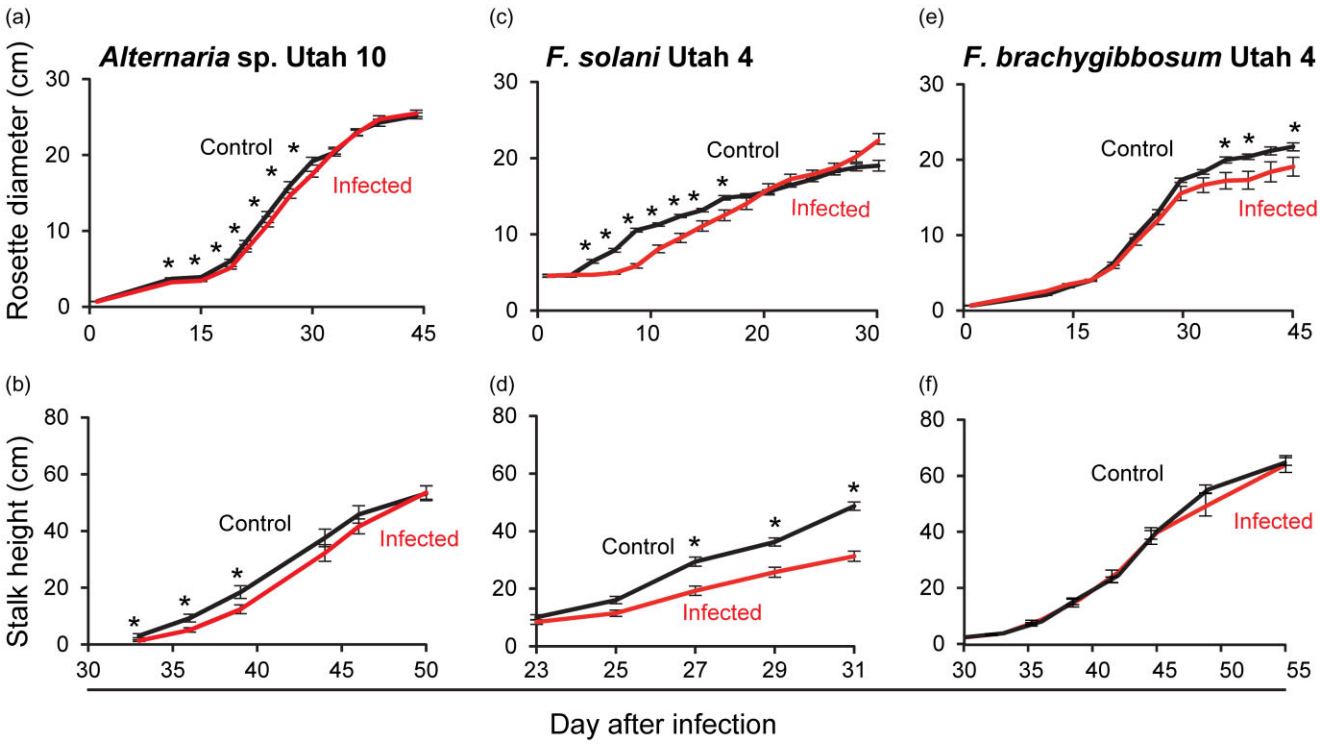

Figure 2. Infection by *Alternaria* and *Fusarium* strongly affects the growth and development of *N. attenuata* plants. Rosette diameter and stalk height of plants infected with *Alternaria* sp. Utah 10 (a, b), *F. solani* Utah 4 (c, d) and *F. brachygibbosum* Utah 4 (e, f) (red lines) compared with control plants (black lines). Asterisks indicate significant differences between infected and non-infected plants at a given time point (Student's *t*-test, $P \leq 0.05$). The error bars represent standard errors from *Alternaria* sp. Utah 10 ($n = 20$), *F. solani* Utah 4 ($n = 25$) and *F. brachygibbosum* Utah 4 ($n = 20$) infection, respectively.

infected plants compared with non-infected plants at 2 dai (ANOVA, $F_{4;10} = 44.956$, $P \leq 0.001$) and 4 dai (ANOVA, $F_{4;10} = 44.956$, $P = 0.004$). However, JA levels were significantly higher in infected plant only at 4 dai (ANOVA, $F_{4;10} = 4.389$, $P = 0.006$).

We also analysed changes in ABA and other key oxylipins, for example JA-Ile and (9S, 13S)-12-oxo-phytodienoic acid (OPDA), divinylethers (DVE) and C_{12} derivatives (C12) including 12-oxo-(9Z)-dodecenoic acid (traumatin), 9-hydroxy-12-oxo-(10E)-dodecenoic acid (OH-traumatin) and (2E)-dodecenedioic acid (traumatic acid). However, only *Alternaria* sp. Utah 10 but not *F. brachygibbosum* Utah 4-infected plants had significant increased levels of C_{12} derivatives including traumatin, OH-traumatin and traumatic acid compared with control plants at 2 or 8 dai (Supporting Information Fig. S2). Levels of ABA, JA-Ile, OPDA and DVE did not change significantly for any of the tested fungal pathogens.

JA signalling plays a central role in *Fusarium* resistance but not in *Alternaria* resistance

To further explore the role of the induced phytohormones (SA and JA) in *N. attenuata*'s defence against the native Utah fungal pathogens, *N. attenuata* plants which were silenced in JA biosynthesis (ir-*gla1*, as-*lox3*) (Halitschke & Baldwin 2003; Bonaventure *et al.* 2011), JA-Ile production (ir-*jar4/6*) (Wang *et al.* 2008), JA-Ile perception (ir-*coi1*) and pathogen-induced SA accumulation (ov-*nahG*) (Gilardoni *et al.* 2011) were experimentally infected with fungal isolates (Fig. 4a). Infection with *Alternaria* sp. Utah 10 and *F. brachygibbosum* Utah 4 not only resulted in differences in chlorosis, necrosis, plant growth and development, but also differently influenced JA and SA accumulation patterns over time. Hence, we chose these two fungal isolates to examine the impact of JA and SA on disease progression.

Ir-*gla1* and as-*lox3* were the most susceptible genotypes to *F. brachygibbosum* Utah 4 infection. Infected ir-*gla1* and as-*lox3* plants were the first ones to show wilting symptoms. At 34 dai, 60% of the leaf area of infected ir-*gla1* and as-*lox3* plants wilted which was a significantly higher percentage than that of infected WT and other transgenic plants (ANOVA, $F_{5;144} = 14.920$, $P < 0.001$) (Fig. 4b). After they started wilting, infected ir-*gla1* plants dried out rapidly while infected as-*lox3* plants turned chlorotic and dried out more slowly. Infected as-*lox3* plants also had a greater percentage of chlorosis (53% of leaf area) at 34 dai in comparison with those of infected WT and other transgenic plants (ANOVA, $F_{5;144} = 7.890$, $P < 0.001$) (Fig. 4b). Infected ir-*gla1* and as-*lox3* plants had the largest number of dead plants (ir-*gla1*: 56%; as-*lox3*: 36%) at 44 dai compared with other genotypes (G-test, $G > 8.972$, 1d.f., $P < 0.002$) (Fig. 4c). In addition,

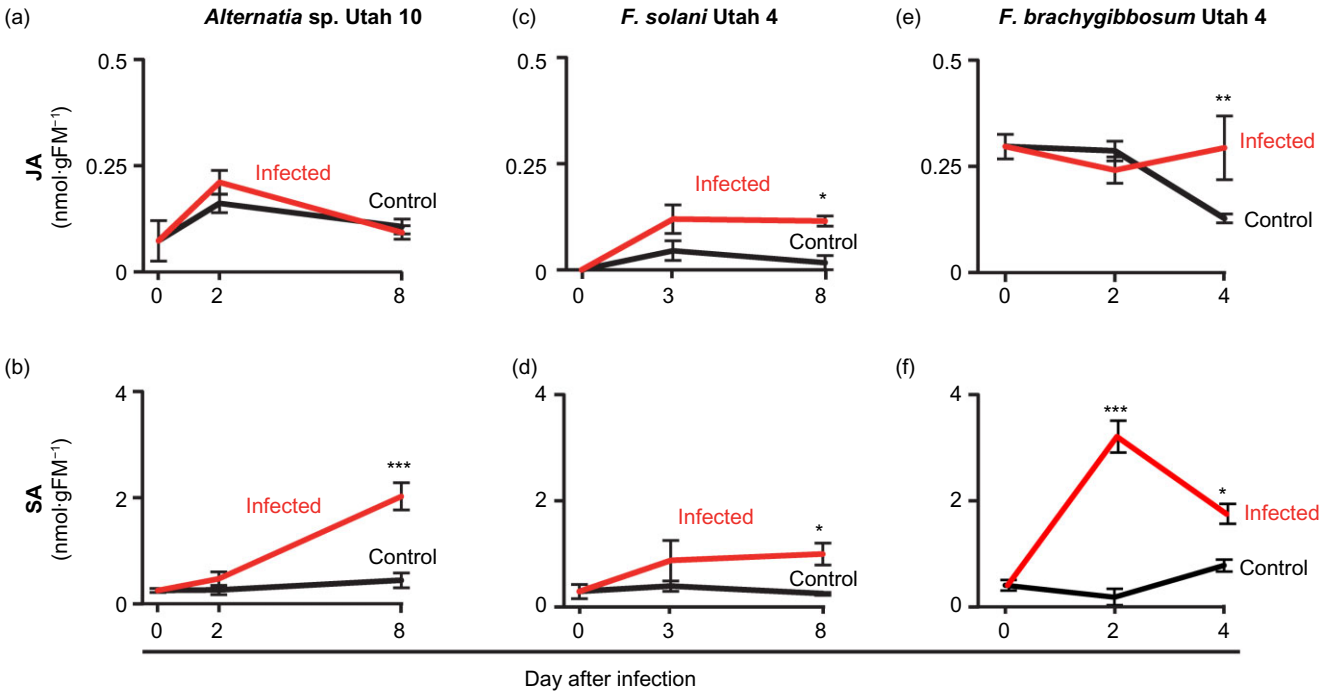

Figure 3. *Alternaria* and *Fusarium* infection differentially induce JA and SA level in *N. attenuata*. JA and SA levels in *N. attenuata* plants infected with *Alternaria* sp. Utah 10 (a, b), *F. solani* Utah 4 (c, d) and *F. brachygibbosum* Utah 4 (e, f). nmol·gFM^{-1}: nmol per gram of fresh mass. The error bars represent standard errors from five biological replicates. Black and red lines represent phytohormone levels of control and infected plants, respectively. Asterisks indicate significant differences between infected and non-infected plants at a given time point (one-way ANOVA with LSD *post hoc* test, ***$P \leq 0.001$, **$P \leq 0.01$, *$P \leq 0.05$).

F. brachygibbosum Utah 4-infected ir-*gla1* and as-*lox3* plants showed a much stronger reduction in rosette diameter (ir-*gla1*: 37.8%; as-*lox3*: 50.1%) and stalk height (ir-*gla1*: 42.5%; as-*lox3*: 49.3%) compared with infected WT plants (rosette diameter: 9.5%; stalk height: 10.2%) (Fig. 4c and Supporting Information Fig. S3). The rate of plant development as indicated by bolting time was more delayed in infected ir-*gla1* (about 3 d) and as-*lox3* plants (about 2 d) compared with those of infected WT plants which were not significantly delayed compared with uninfected WT plants (Student's *t*-test, $P < 0.01$) (Fig. 4b). Flowering time was also more delayed in infected ir-*gla1* (5 d) compared with infected WT plants (Student's *t*-test, $P < 0.001$). These results indicate that products derived from the LOX3 pathway (including JA) increase the resistance of *N. attenuata* to *F. brachygibbosum* Utah 4.

Although *F. brachygibbosum* Utah 4-infected ir-*jar4/6* plants showed no significant difference in visible disease symptoms (number of dead plants, percentage of wilting and chlorosis symptoms) compared with infected WT (Fig. 4b,c), they had smaller rosette diameters (29.1% reduction) and stalk heights (18.6% reductions) in comparison with infected WT plants (rosette diameter: 9.5%; stalk height: 10.2%) (Fig. 4c and Supporting Information Fig. S3). Thus JA-Ile may increase the resistance of *N. attenuata* plant to *F. brachygibbosum* Utah 4, but may not be as important as other products derived from the LOX3 pathway.

Interestingly, the susceptibilities of ir-*coi1* and WT plants to *F. brachygibbosum* Utah 4 were very similar. Infected ir-*coi1* plants did not differ significantly in death rate, wilting or chlorosis (Fig. 4b,c). Also, no significant difference in reduction of rosette diameter and stalk height was found in infected ir-*coi1* compared with infected WT (Fig. 4c and Supporting Information Fig. S3). Most infected plants from the ir-*coi1 N. attenuata* genotype were asymptomatic, suggesting that JA-Ile perception by COI1 does not play an important role in the resistance of *N. attenuata* to *F. brachygibbosum* Utah 4.

Alternaria sp. Utah 10-infected as-*lox3*, ir-*coi1* and ir-*jar4/6* plants showed a reduction in chlorosis at 8 dai compared with infected WT plants (Fig. 4c). While *Alternaria* infection reduced rosette diameters of ir-*jar4/6* plants, as-*lox3* plants were unaffected and ir-*coi1* plants benefited from being infected by a significantly increased rosette diameter (Student's *t*-test at 24 dai, $P < 0.01$) (Fig. 4c and Supporting Information Fig. S3). Moreover, infection of ir-*jar4/6* plants reduced stalk heights compared with non-infected ir-*jar4/6* plants (Student's *t*-test at 48 dai, $P = 0.012$) and this reduction was greater than that observed in infected WT plants (Fig. 4c and Supporting Information Fig. S3). These results demonstrate that canonical jasmonate signalling involving three components, JA, JA-Ile and COI1, in a linear cascade cannot fully account for the defence of *N. attenuata* against *Alternaria* sp. Utah 10 infections.

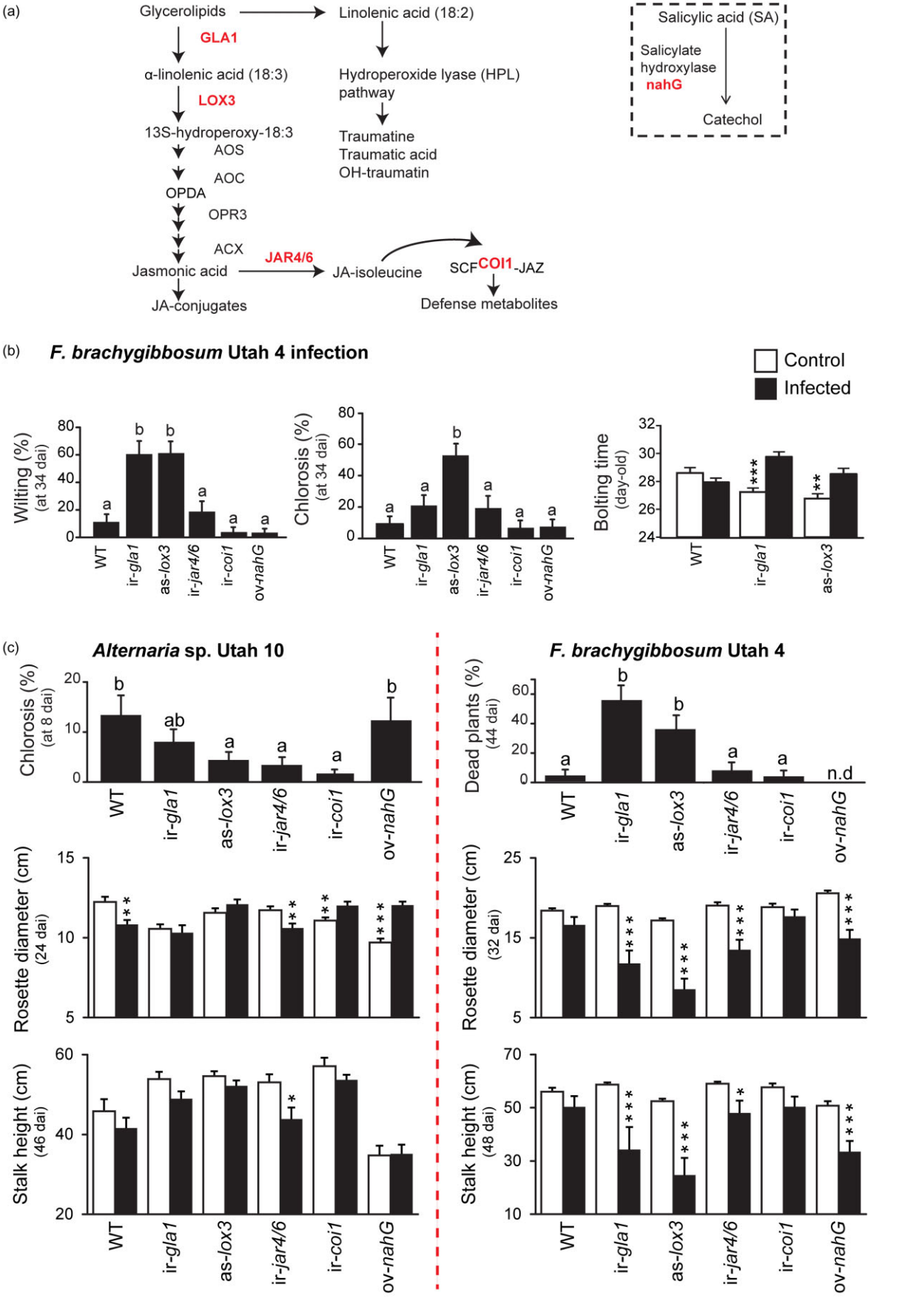

Figure 4. Transgenic *N. attenuata* plants impaired in JA biosynthesis but not JA perception and SA accumulation are differently affected by *Alternaria* and *Fusarium* infection. (a) Schematic representation of JA biosynthesis pathway in *N. attenuata*. α-linolenic acid (18:3) is released by glycerolipase 1 (GLA1) from chloroplast membrane glycerolipids, oxidized by lipoxygenase 3 (LOX3) at C_{13} to form 13S-hydroperoxy-18:3 (13S-OOH-18:3). 13S-OOH-18:3 is the substrate for allene oxide synthase that forms 12,13-epoxy-18:3 which is subsequently cyclized to 12-oxo-phytodienoic acid (OPDA) by allene oxide cyclase. OPDA which is then reduced by OPDA reductase 3 undergoes three cycles of β-oxidation, involving acetyl-CoA transferase 1 and finally forming (+)-7-iso-jasmonic acid (JA). JA can be further conjugated to isoleucine (Ile) by JASMONATE RESISTANT 4 and 6 (JAR4 and JAR6) to form JA-Ile. JA-Ile activates the SCF^{COI1}-JAZ complex which transcriptionally activates genes involved in the biosynthesis of defence molecules. The proteins given in red font (GLA1, LOX3, JAR4/6 and COI1) refer to proteins that have been silenced in transgenic lines (ir-*gla1*, as-*lox3*, ir-*jar4/6* and ir-*coi1*) using RNA interference technique. Ov-*nahG* plants ectopically express the bacterial salicylate hydroxylase gene to inactivate SA signalling in *N. attenuata*. (b) Disease symptoms (wilting, chlorosis and delay in bolting time) of *F. brachygibbosum* Utah 4-infected transgenic (ir-*gla1*, as-*lox3*, ir-*jar4/6*, ir-*coi1*, ov-*nahG*) and wild type (WT) plants. (c) Disease symptoms (percentage of chlorosis and number of dead plants) and plant growth (average of rosette diameter and stalk height) of *Alternaria* sp. Utah 10 and *F. brachygibbosum* Utah 4-infected ir-*gla1*, as-*lox3*, ir-*jar4/6*, ir-*coi1*, ov-*nahG* and WT plants and the respective control plants. Solid bars represent infected plants while open bars represent control plants. Error bars represent standard errors from 25 biological replicates. Different letters (a,b) indicate significantly different data groups determined by one-way ANOVA with LSD *post hoc* test, $P \leq 0.05$. Asterisks indicate significant differences between infected and non-infected plants within one genotype (Student's *t*-test, $***P \leq 0.001$, $**P \leq 0.01$, $*P \leq 0.05$).

SA may increase the resistance of *N. attenuata* to *Fusarium* infection but not to *Alternaria*

The infection by *F. brachygibbosum* Utah 4 caused no significant differences in the percentage of chlorosis and wilting symptoms in infected ov-*nahG* plants compared with infected WT (Fig. 4b). In addition, infected ov-*nahG* plants did not suffer mortalities until 44 dai (Fig. 4c). However, infected ov-*nahG* plants showed stronger reductions in both rosette diameter (27.4 versus 9.5%) and stalk height (34 versus 10.2%) compared with infected WT plants (Fig. 4c and Supporting Information Fig. S3). These results point to SA production enhancing *N. attenuata*'s resistance to *F. brachygibbosum* Utah 4.

Similarly, *Alternaria* sp. Utah 10-infected ov-*nahG* plants showed no significant differences in chlorosis compared with infected WT (Fig. 4c). However, while *Alternaria* infection slightly reduced stalk heights of infected WT plants, stalk heights of infected ov-*nahG* plants remained unaffected (Fig. 4c and Supporting Information Fig. S3). Moreover, while infected WT plants showed a reduction in rosette diameters (11.4%) compared with non-infected controls (Student's *t*-test at 24 dai, $P = 0.002$), infected ov-*nahG* plants benefited from being infected by having a significantly increased rosette diameters (24%) compared with uninfected ov-*nahG* (Student's *t*-test at 24 dai, $P < 0.001$) (Fig. 4c and Supporting Information Fig. S3). These results indicate that SA production may enhance disease severity for *Alternaria* sp. Utah 10-infected *N. attenuata*.

DISCUSSION

Unraveling the dynamics of the fungal disease outbreak in native *N. attenuata* population

Effective management of plant diseases requires knowledge of their dynamics, that is, the spatial and temporal change in disease incidence and severity (Ericson *et al*. 1999). In this study, we addressed the dynamics of a fungal disease outbreak observed in native *N. attenuata* populations (Schuck *et al*. 2014b). In these populations, within only 16 d, more than half of all diseased plants recovered completely while at the same time many other previously asymptomatic plants developed disease symptoms. In addition, no clear spatial pattern could be observed on the population or subpopulation scale. The subpopulations with high recovery rate and with a high abundance of newly diseased plants were separated by less or completely unaffected subpopulations. Within a subpopulation, diseased plants were usually surrounded by healthy plants. The microspatial heterogeneity of fungal pathogen density within the soil is hypothesized to be an explanation for the distribution of diseased plants. In addition, Schuck *et al*. (2014b) also suggested that the great genetic diversity of native *N. attenuata* populations could augment the phenotypic plasticity that occurs within individuals and could explain the coexistence of individuals with different defence strategies, leading to the dynamic in disease incidence and severity of individual plant in short period of time.

The heterogeneous distribution of fungal species as well as their variability in virulence (Schuck *et al*. 2014b) may contribute to the observed variation in disease susceptibility within natural populations. In our study, we could demonstrate that infection by three different fungal species (*Alternaria* sp. and *Fusarium* spp.) resulted in pathogen-specific plant responses with regard to observable disease symptoms. *Alternaria* sp. Utah 10-infected plants mainly showed symptoms of chlorosis (Fig. 1) which may be caused by the tentoxin production of this fungal species (Holland *et al*. 1997; Slavov *et al*. 2004; Lawrence *et al*. 2008). Interestingly, *Alternaria* sp. Utah 10-infected *N. attenuata* plants were able to recover from chlorosis symptoms, similar as many diseased plants in the native population. *F. solani* Utah 4 not only caused chlorosis, but also curly leaves and abnormal flowers (shortened, bent flowers with protruding pistils) (Supporting Information Fig. S1) which could result from the production of a fungal toxin named dihydrofusarubin (Albrecht *et al*. 1998). This phenotype generated under laboratory conditions was similar to that observed on plants within the wild population during the disease outbreak (Supporting Information Fig. S1). However, *F. solani* Utah 4 could

not be isolated directly in the abnormal flowers collected from a natural population (data not shown), and it remains unclear if root infection by this fungus is responsible for these flower symptoms through systemically transmitted plant responses. In contrast, the symptoms of *F. brachygibbosum*-infected plants were distinct from *Alternaria* sp. and *F. solani*-infected plants. Plants infected by *F. brachygibbosum* Utah 4 exhibited asymptomatic growth for an extended time, followed by a late occurrence of chlorosis and necrosis (Fig. 1). The necrosis in the root crown of *F. brachygibbosum* Utah 4-infected *N. attenuata* plants (Supporting Information Fig. S1) could be an explanation for the reduced water uptake (Agrios 2005) most likely responsible for the observed wilting symptoms. For *F. graminearum*, it is known that the trichotecene toxin deoxynivalenol is associated with tissue necrosis occurring during wheat crown rot disease (Stephens *et al*. 2008). Therefore, we speculate that toxins produced by *F. brachygibbosum* Utah 4 might also be responsible for chlorosis and necrosis on *N. attenuata* infected by this pathogen. In short, these pathogen-specific plant responses provide a plausible explanation for the diverse change of disease symptoms observed in native *N. attenuata* populations.

Interestingly, *Alternaria*-infected *N. attenuata* plants were able to recover from chlorosis symptoms, similar as the diseased plants in the native population. In contrast, *Fusarium*-infected plants could not recover from the infection at least under laboratory conditions. Interestingly, we observed an induction of C_{12} derivatives such as traumatin, traumatic acid and OH-traumatin after *Alternaria* sp. Utah 10 infection (Supporting Information Fig. S2). These C_{12} derivatives of the Hydroperoxide lyase pathway (HPL) pathway have been shown to be involved in growth-stimulating and wound-healing activities in plants (Zimmerman & Coudron 1979). The application of these compounds on *N. attenuata* leaves induced numerous disease resistance genes, for example genes encoding for PATHOGENESIS-RELATED PROTEIN 5 and DISEASE RESISTANCE RESPONSE PROTEIN 206. In addition, genes involved in mitogenesis were also up-regulated (Kallenbach *et al*. 2011). Based on these data, we hypothesize that the C_{12} derivatives might contribute to the recovery of *Alternaria*-infected plants from disease symptoms.

In contrast to domesticated crop plants, wild *N. attenuata* populations are genetically diverse (Baldwin 2001; Sime & Baldwin 2003; Bahulikar *et al*. 2004) and this genetic diversity could translate into different defensive responses towards fungal pathogens, eventually leading to different levels of plant resistance. In addition, *N. attenuata* is known to germinate into monoculture-like populations with a high density of potential host plants (Baldwin 2001). The increase in host population density has recently been shown to be positively correlated with an increase of host resistance against fungal pathogens (Jousimo *et al*. 2014). The potentially higher gene flow in dense and genetically diverse populations such as formed by *N. attenuata* may result in rapid selection towards pathogen resistance and the evolution of the according plant resistance traits. By using different transgenic *N. attenuata* genotypes silenced in JA and SA accumulation or JA perception, we show that these different plant genotypes responded differently to pathogen infection (Fig. 4 and Supporting Information Fig. S3) consistent with the hypothesis that plant genetic diversity in phytohormone inducibility within natural *N. attenuata* populations might reflect the observed diversity in disease susceptibility. With regard to herbivores, individual *N. attenuata* plants within natural populations have been shown to be highly variable in their ability to elicit JA bursts after elicitation, a feature useful in identifying *N. attenuata* mutants naturally deficient in JA production (Kallenbach *et al*. 2012). A similar phytohormone depended variability in resistance against fungal pathogens is a reasonable hypothesis for *N. attenuata* to account for the observed variability in disease dynamics within populations (Schuck *et al*. 2014b).

JA is essential for *N. attenuata*'s resistance to *Fusarium*

While JA is known to increase resistance of plants to necrotrophic pathogens, both SA and JA are required for resistance against hemibiotrophic pathogens (McDowell & Dangl 2000; Glazebrook 2005). In our study, *F. brachygibbosum* Utah 4 was able to induce both SA and JA in *N. attenuata* plants, suggesting a hemibiotrophic lifestyle of this fungal species. However, ir-*gla1* and as-*lox3* plants which are reduced in JA accumulation (Halitschke & Baldwin 2003; Bonaventure *et al*. 2011) were highly susceptible to *F. brachygibbosum* Utah 4 infection compared with WT plants (Fig. 4 and Supporting Information Fig. S3), pointing to the important role of JA in *N. attenuata*'s resistance towards *F. brachygibbosum* Utah 4. Interestingly, ir-*gla1 N. attenuata* plants seemed to be even more susceptible to *F. brachygibbosum* Utah 4 than as-*lox3* plants and showed a greater number of dead plants (Fig. 4). Silencing GLA1 expression reduces the levels of oxylipins different from JA in response to pathogen attack (Schuck *et al*. 2014a), and these GLA1-dependent compounds might account for the differences in symptoms between *F. brachygibbosum*-infected ir-*gla1* and as-*lox3 N. attenuata* genotypes.

Prior attack from insect herbivores elicits systemic induced resistance to subsequent fungal challenge (Eyles *et al*. 2007). Resistance against chewing lepidopteran herbivores is known to be frequently mediated by JA-signalled responses, which are also important for defence against necrotrophic pathogens, whereas, resistance to attack from phloem-sucking hemipterans is often associated with SA responses (Stout *et al*. 2006; Thaler *et al*. 2010). For example, prior herbivory by the beetle *Gastrophysa viridula* decreased infection of the rust fungus *Uromyces rumicis* on their host plant *Rumex obtusifolius* (Hatcher & Paul 2000). Similarly, previous herbivory of jack pine budworm (*Choristoneura pinus pinus* Freeman; JPBW) increase resistance to *Grosmannia clavigera* Robinson-Jeffrey & Davidson fungi in jack pine (Colgan & Erbilgin 2011). In our plant model system, JA production has been previously shown to be highly induced by both biotic stress (*Manduca sexta*) and abiotic factors (wounding and UVB) (McCloud & Baldwin 1997; Dinh *et al*. 2013). The prior exposure to these biotic or

abiotic JA-inducers may explain why a large proportion of *N. attenuata* plants were resistant to *Fusarium* infection and remained healthy during the fungal disease outbreak in the native population.

Canonical JA signalling does not apply for *N. attenuata*'s interactions with *Fusarium* and *Alternaria*

In our study, canonical jasmonate signalling as characterized in Arabidopsis (*Arabidopsis thaliana*), involving JA, JA-Ile and COI1 in a linear cascade where these three components are known to interact with each other and control almost all well-characterized JA responses (Katsir *et al*. 2008), cannot fully account for the resistance of *N. attenuata* to its native *Fusarium* pathogens. Susceptibility to *F. brachygibbosum* Utah 4 decreased in the transgenic plants in the following order, as-*lox3* > ir-*jar4/6* > ir-*coi1* and *F. brachygibbosum* Utah 4-infected ir-*coi1* and WT plants did not differ in disease severity (Fig. 4 and Supporting Information Fig. S3), which suggests that products derived from JA biosynthesis pathway other than JA-Ile and its perception by COI1 are more important in resisting *Fusarium* infection. In other words, the resistance of *N. attenuata* plants to its native *Fusarium* pathogen could be also due to a COI1-independent defence response pathway. This interpretation could explain why JA but not JA-Ile induction was detected 4 d after *F. brachygibbosum* Utah 4 infection (Fig. 3). This is also in good agreement with the results of Thatcher *et al*. (2009) showing that there was no difference in the degree of *F. oxysporum* colonization between *A. thaliana coi1* mutants and WT plants until a late stage infection. A similar disconnect could be described in the herbivore resistance of *Solanum nigrum*, where ir-*lox3* and ir-*coi1* plants were highly susceptible to herbivory, while ir-*jar4* plants with reduced levels of JA-Ile accumulation did not differ from WT plants (VanDoorn *et al*. 2011).

The COI1-independent defence response of *N. attenuata* against *Fusarium spp*. could involve elements of the JA biosynthesis pathway or other phytohormones. Auxin has been also shown to be a part of the COI1-independent defence signalling in *A. thaliana* infected by *S. sclerotiorum* as AXR1, a central regulator of auxin responses, functions as a positive modulator or synergistic factor of JA-dependent responses (Stotz *et al*. 2011). In *N. attenuata*, cytokinin signalling could mediate plant defence responses employing JA-dependent, but COI1-independent signalling mechanisms.

The canonical jasmonate signalling also does not fully account for the resistance of *N. attenuata* to its native *Alternaria* pathogens. Ir-*coi*1 plants were more resistant to infection by *Alternaria* sp. Utah 10 compared with WT (Fig. 4 and Supporting Information Fig. S3), suggesting that the JA-Ile perception by COI1 has a negative effect on the resistance of *N. attenuata* against *Alternaria* pathogens. Silencing the COI1 receptor leads to a higher accumulation of JA-Ile (Paschold *et al*. 2008; Stotz *et al*. 2011; VanDoorn *et al*. 2011) and ir-*jar4/6* plants were more susceptible to *Alternaria* sp. Utah 10 infection compared with ir-*coi1* and as-*lox3* genotypes (Fig. 4 and Supporting Information Fig. S3). These results suggest that JA-Ile or its downstream functioning signalling molecules are involved in the defence response of *N. attenuata* against *Alternaria* sp. Utah 10, independently of their role in canonical jasmonate signalling. Again, these results were commensurate with the JA-Ile levels in *Alternaria* sp. Utah 10-infected plants, which showed a slight trend to increase at 2 dai, even though it is not significantly different from the uninfected controls (Supporting Information Fig. S2).

SA increases *N. attenuata*'s resistance to *Fusarium* but decreases the resistance to *Alternaria*

SA is a phytohormone typically produced by plants in response to biotrophic pathogen attack (Glazebrook 2005). In this study, SA production seemed to increase *N. attenuata*'s resistance to *Fusarium* but makes it more susceptible to *Alternaria*. We found that ov-*nahG* plants, which induce less SA by pathogen attack compared with WT plants (Gilardoni *et al*. 2011), were more susceptible to *F. brachygibbosum* Utah 4 (Fig. 4 and Supporting Information Fig. S3). Although assessing the role of SA based solely on phenotypic observations on ov-*nahG* plants could potentially be inaccurate (Glazebrook 2005), this result is consistent with the importance of SA in plant resistance against *Fusarium* pathogens (Shibata *et al*. 2010; Shcherbakova *et al*. 2011).

In contrast to the positive effect of SA on *N. attenuata*'s resistance to *F. brachygibbosum* Utah 10, SA seemed to increase *N. attenuata*'s susceptibility to *Alternaria* sp. Utah 10 as ov-*nahG* plants exhibited an enhanced resistance to this pathogen (Fig. 4, Supporting Information Figs S3 and S4). SA and JA are known to antagonize each other's effects (Glazebrook 2005). The reduction in SA production could lead to an enhancement of JA production which then acts in a COI1-independent manner to increase resistance of plants to *Alternaria* pathogens. Most of the *Alternaria* species are known to be necrotrophic (Thomma 2003) and *Alternaria* sp. Utah 10 most likely fits in this category, therefore SA-induced defence responses would be presumably less effective than those induced by JA, which provides a plausible explanation why induced SA accumulation does not result in increased *Alternaria* resistance and even increases plant susceptibility. SA signalling could be exploited by *Alternaria* to promote plant colonization and disease progression. To draw more solid conclusion on the role of SA in *N. attenuata*'s defence against its native fungal pathogens, experiments with various transgenic *N. attenuata* genotypes compromised not only in SA biosynthesis but also in SA signalling are needed.

CONCLUSIONS AND OUTLOOK

Taken together, our findings demonstrate that JA is essential for resistance of *N. attenuata* against its native *Fusarium* pathogens; however, the canonical jasmonate signalling involving JA, JA-Ile and COI1 in a linear cascade cannot

fully account for the resistance of this plant species to either *Fusarium* or *Alternaria* pathogens. In addition, our study suggests that the dynamic in disease incidence could be related to the individual plant's defensive status within a population. The trade-offs in plant defence against different species of pathogens as well as the genetically determined resistance level of each individual plant are important to describe spatiotemporal patterns of a disease outbreak in nature.

Because the effect of an entire pathogen community on plants might be substantially different from the effects of a single pathogen, experiments with several different pathogens which resemble a natural fungal community would be more appropriate and would allow us to better understand such complex interactions of plants and pathogens in nature, especially on how plants deal with multiple infections by pathogens differing in their infection strategies (hemibiotrophic versus necrotrophic). The insights gained from the study of these interactions will not only improve our knowledge of how native plants cope with pathogens, but will also be valuable for the development of crop protection programmes.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Figure S1. Disease symptoms of *N. attenuata* plants infected by different *Fusarium* species. (a, b) Native *N. attenuata* plant with curly leaves and bent flowers photographed in a natural population in the Great Basin Desert, Utah, during a disease outbreak in 2011. (c) Diseased plant with curly leaves caused by *F. solani* Utah 4 infection at 32 dai. (d) Bent flowers with protruding pistils (red arrow) of heavily diseased plants caused by *F. solani* Utah 4 infection at 42 dai. (e) Diseased plant with root crown canker (red arrow) caused by *F. brachygibbosum* Utah 4 infection in comparison with a non-infected plant at 54 dai.

Figure S2. Phytohormone levels in *N. attenuata* plants infected with *Alternaria*. Level of three major C_{12} derivatives (traumatin, traumatic acid and OH-traumatin), abscisic acid (ABA), JA conjugated to isoleucine (JA-Ile), (9S, 13S)-12-oxo-phytodienoic acid (OPDA) and colneleic acid (9-DVE-18:2) in *N. attenuata* plants infected with *Alternaria* sp. Utah 10. nmol·gFM^{-1}, nmol per gram of fresh mass. The error bars represent standard errors from five biological replicates. Open and solid bars represent phytohormone levels of control and infected plants, respectively. Asterisks indicate significant differences between infected and non-infected plants at a given time point (one-way ANOVA with LSD *post hoc* test, $^{***}P \leq 0.001$, $^{*}P \leq 0.05$). n.d., not detected.

Figure S3. Growth of *N. attenuata* WT and transgenic plants are differently affected by *Alternaria* and *Fusarium* infection. Relative difference to non-infected plants in rosette diameter, stalk height (cm) between ir-*gla1*, as-*lox3*, ir-*jar4/6*, ir-*coi1*, ov-*nahG*, WT plants inoculated with *Alternaria* sp. Utah 10 or *F. brachygibbosum* Utah 4. Twenty-five biological replicates were used for each treatment. Asterisks indicate significant differences between infected and non-infected plants at the final measured time point as indicated (Student's *t*-test, $^{***}P \leq 0.001$, $^{**}P \leq 0.01$,$^{*}P \leq 0.05$).

Table S1. Mean ± standard deviation of bolting (when plants started to elongate) and flowering (appearance of the first open flower) time of fungal infected and non-infected *N. attenuata* plants. *Alternaria* sp. Utah 10-infected plants showed a delay in flowering time (Student's *t*-test $P < 0.01$). $n = 25$ (for *Alternaria* sp. Utah 10 and *F. brachygibbosum* Utah 4 infection) and $n = 20$ (for *F. solani* Utah 4 infection).