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Yeast Increases Resistance in *Arabidopsis* Against *Pseudomonas syringae* and *Botrytis cinerea* by Salicylic Acid–Dependent as Well as –Independent Mechanisms

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Cell-wall and glucopeptide components of yeast have been reported to exhibit elicitor activity. The mode of action of defense activation by yeast is not known so far. In this study, we used the model plant *Arabidopsis* to investigate the activation of defense responses by yeast, the effect on resistance against different pathogens, and the mode of action. Treatment of *Arabidopsis* plants with an autoclaved yeast suspension induced the expression of systemic acquired resistance–related genes and accumulation of the phytoalexin camalexin. Symptom development and bacterial growth after infection with a virulent strain of the pathogen *Pseudomonas syringae* was reduced in yeast-pretreated plants. No protection was detectable in mutants affected in the salicylate pathway, while mutants in the jasmonate or camalexin pathway were protected by yeast, indicating that the salicylate pathway is necessary for the yeast-induced resistance against *P. syringae*. Yeast also reduced symptom development after challenge with *Botrytis cinerea*. This protection was detectable in all mutants tested, indicating that it is independent of the salicylate, jasmonate, and camalexin pathway.

Plants are continuously attacked by a broad range of microorganism. Only a small proportion of these challenges end up in successful pathogen-spreading, because plants are resistant against the majority of microorganisms. This phenomenon is termed nonhost resistance and consists of preformed and induced mechanisms (Heath 2000a). The preformed defense includes structural barriers and constitutively present antimicrobial substances. The induced defense responses comprise the accumulation of stress signaling molecules such as salicylic acid (SA) and jasmonic acid (JA), expression of proteins with antimicrobial properties, or activities involved in the synthesis of phytoalexins and cell-wall reinforcement (Dangl and Jones 2001). Not only the contact with a living pathogen activates these induced defense responses but also recognition of several pathogen-derived substances. These so-called general elicitors or pathogen-associated molecular patterns (PAMP) comprise (poly)peptides, glycoproteins, oligosaccharides, or lipids (Nurnberger et al. 2004).

If a pathogen can overcome this nonhost resistance, it can spread in its host plant. Defense mechanisms are also activated in this interaction. These defense responses will slow down pathogen invasion but not prevent disease. This phenomenon is termed basal resistance because a defect in basal resistance will result in increased susceptibility. Besides nonhost resistance, plants have also developed specific resistance that is based on gene-for-gene interaction (Staskawicz et al. 1995). In this interaction, the plant recognizes the pathogen very early in the infection process. This specific recognition leads to very rapid activation of the defense program and local cell death called the hypersensitive response (HR) (Heath 2000b). Interestingly, defense responses besides this HR are very similar in specific, basal, and nonhost resistance with the major difference between these interactions in the timecourse of responses (Navarro et al. 2004; Tao et al. 2003). Therefore, the activation of the inducible defense reactions by nonhost microorganisms or PAMP may result in enhanced resistance against virulent pathogens.

Enhancing the resistance of plants is potentially attractive for agricultural application. For this application, the elicitor should be nontoxic, biodegradable, and cost effective. Yeast meets all these requirements and has been used in different studies as an elicitor of defense responses in cell cultures and whole plants. Phytoalexin biosynthesis, expression and activity of the enzyme phenylalanine ammonia lyase, and accumulation of the oxylipins JA and 12-oxo-phytodienoic acid (OPDA) were induced by yeast in different plant cell cultures (Basse and Boller 1992; Blechert et al. 1995; Parchmann et al. 1997; Suzuki et al. 2005). Less is known about the effect of yeast on whole plants. Treatment of soybean increased phytoalexin accumulation (Hahn and Albersheim 1978). The application of yeast cell-wall extracts on barley enhanced the resistance to powdery mildew (Reglinski et al. 1994). However, the mechanisms responsible for this increased resistance are unknown.

We are addressing this question using the model plant *Arabidopsis thaliana*. This plant provides the advantage that mutants in different pathways are available as a powerful tool to elucidate the mechanisms contributing to resistance. In addition, different pathosystems for this plant are established. Here, we show that treatment of *Arabidopsis* plants with sterile yeast suspension increases resistance to bacterial and fungal pathogens and we provide results on the mechanisms involved.

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RESULTS

Yeast activates defense responses and enhances resistance to bacterial and fungal pathogens.

In order to investigate if treatment with yeast induces defense responses in *Arabidopsis* plants, the accumulation of the phytoalexin camalexin was analyzed as an indicator for the elicitation of defense reactions. After spraying plants with a sterile yeast suspension, camalexin levels increased in comparison with plants sprayed with water (Fig. 1). Maximum levels were detectable five days after spraying and reached 54 nmol per gram of fresh weight (fw) (which equals 11 µg per gram of fw). This level is within the range of levels after pathogen attack, which have been reported to be between 8 (Tsuji et al. 1992) and 280 µg/g (Tierens et al. 2002). Different treatments of plants with sterile yeast suspension were tested. Spraying plants with a concentration of 0.3 g/ml was more effective than lower concentrations (data not shown). However, higher concentrations led to reduced growth of the plants. Spraying was more effective than drenching the soil, probably because the yeast did not easily distribute in the soil (data not shown). In order to find out if induction of camalexin is a more general feature of yeast and independent of the supplier, camalexin levels after treatment with yeast from three different suppliers were analyzed. Treatment with yeast from any of the sources resulted in camalexin accumulation, with levels more than 20-fold higher after five days as compared with control plants (data not shown).

Since camalexin accumulation was elicited by yeast, we investigated if resistance to two different pathogens was altered. *Pseudomonas syringae* is a hemibiotrophic bacterial pathogen, whereas *Botrytis cinerea* is a necrotrophic fungus. To assess the susceptibility to *B. cinerea*, plants were infected with a spore suspension five days after yeast treatment. Challenge with B. cinerea resulted in lesions consisting of a necrotic center with dead tissue and a surrounding chlorotic area. For quantitation, the size of both necrotic and chlorotic areas was determined separately. The area of necrotic spots was about sixfold smaller in the yeast-pretreated leaves 72 h after infection (Fig. 2A). Similarly, the size of chlorotic areas was fourfold smaller in the yeast-pretreated plants after 72 h (Fig. 2B).

Symptom development after infection with *P. syringae* was clearly reduced by pretreatment with yeast (Fig. 3A). A protection was detectable between five and 11 days after yeast treatment (data not shown). In order to analyze if symptoms are correlated with the spreading of the pathogen, the amount of *P. syringae* after different incubation times was quantified. In agreement with symptom development, bacterial growth was significantly reduced but not completely inhibited in the plants pretreated with yeast. The amount of bacteria was about four- and sixfold lower than in the control after 24 and 48 h respectively (Fig. 3B). This experiment was repeated six times, and the reduction in bacterial growth ranged between a factor of 2.5 and 8.

The SA pathway is upregulated upon yeast treatment.

To assess the mode of action of yeast-induced protection, the regulation of gene expression was investigated, using a cDNA array comprising 1,400 genes. Expression of genes encoding PR1, PR2, and PR5, all SA-responsive genes,

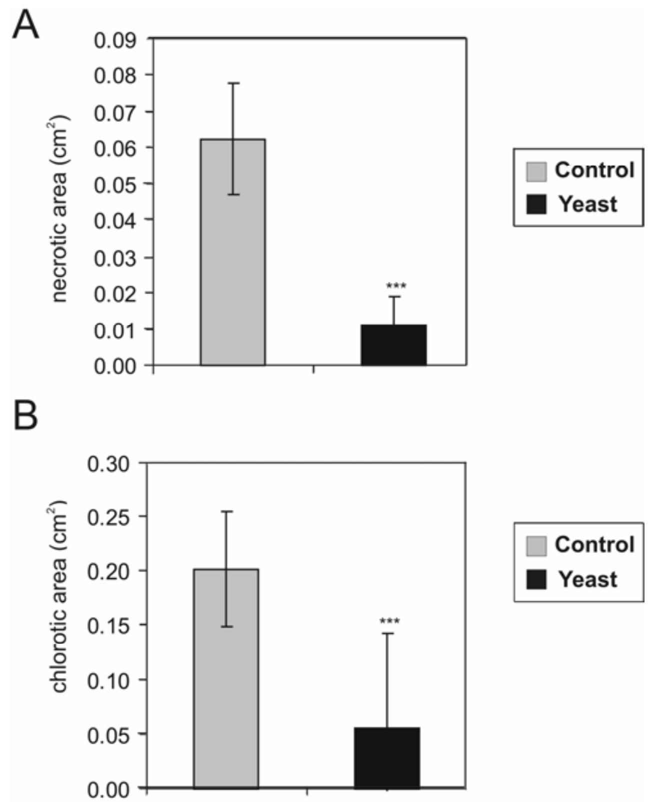

Fig. 2. Effect of yeast pretreatment on *Botrytis cinerea* disease development in leaves of *Arabidopsis* plants. Plants were sprayed with water (control) or yeast suspension, and after 5 days, leaves were infected with *B. cinerea.* **A,** Necrotic and **B,** chlorotic areas were determined 72 h after inoculation. Shown are the mean values of 24 samples ± standard deviation. The experiment was repeated six times with similar results. Stars indicate significant differences of the yeast-treated sample as compared with the water-treated control according to *t*-test (***$P < 0.001$).

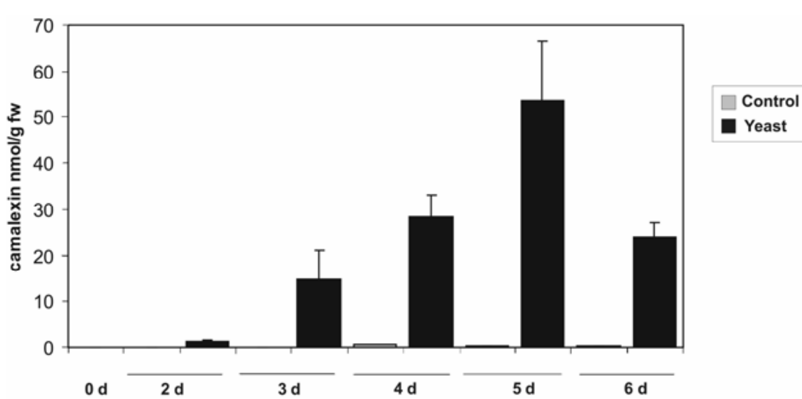

Fig. 1. Accumulation of camalexin in *Arabidopsis* leaves after treatment with yeast suspension. Plants were sprayed with water or yeast suspension, and leaves were harvested after the time indicated. Shown are the mean values of three independent samples ± standard deviation. The experiment was repeated four times with similar results.

showed a clear induction two days after yeast treatment (Table 1). Additionally, genes belonging to the detoxification system were upregulated. Induction was evident for two glutathion-*S*-transferases (*Gst2*, *Gst11*) and a UDP-glucosyl transferase. In contrast, expression of genes positively regulated by JA and ethylene was not increased. The only gene significantly down-regulated in our microarray experiments was *Asa1*, encoding a JA-inducible antranilate synthase involved in secondary metabolism (Table 1).

Northern blot analysis was used to verify this result and, additionally, to analyze expression at different timepoints. The SA-responsive genes *Pr1* and *Pr2* showed clear induction at

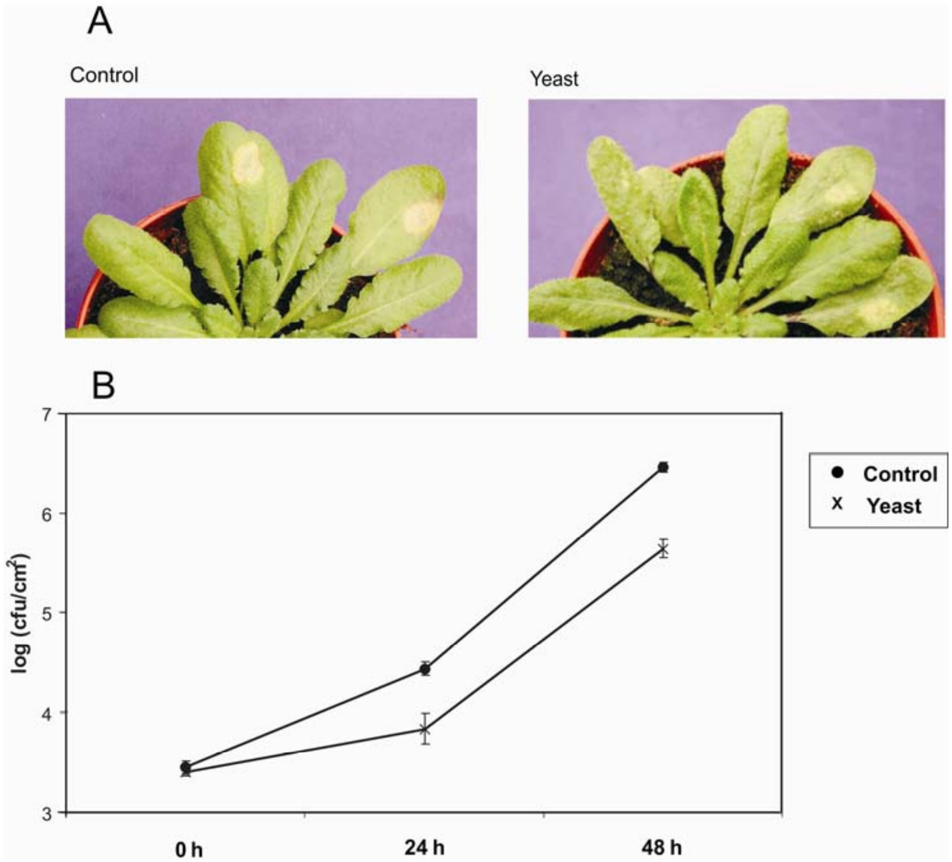

Fig. 3. Effect of yeast pretreatment on *Pseudomonas syringae* infection. **A,** *Arabidopsis* plants were sprayed with either water (control) or a yeast suspension, and after 5 days, leaves were infiltrated with *P. syringae*. Shown is a representative picture taken 72 h after inoculation. **B,** Plants were sprayed with water or yeast suspension, and after 7 days leaves were infiltrated with *P. syringae* and were harvested at the indicated times after inoculation. Shown are the mean values of five independent samples ± standard deviation. For each sample, four leaf disks originating from two plants were homogenized. At 24 and 48 h, the difference between control and yeast was significant according to *t*-test with $P < 0.001$.

Table 1. Expression of genes involved in systemic acquired resistance, detoxification, and the jasmonate/ethylene pathway in response to yeast treatment

Locus	Pathway[a]	Description	Log ratios[b]	SD[c]	CV[d]
At2g14610	SAR	PR1	3.30	1.00	30.36
At3g57160	SAR	PR2; 1,3-β-glucanase	2.60	0.78	34.29
At1g75040	SAR	PR5	1.96	0.52	26.38
At4g02520	Detox	GST2, glutathione-*S*-transferase	1.90	0.62	45.70
At2g36800	Detox	UGT73C5, UDP-glucosyl-transferase	1.37	0.64	46.59
At1g02920	Detox	GST11, glutathione-S-transferase	1.31*	0.29	22.11
At3g15210	JA/ET	ERF4, ethylene-responsive element-binding factor 4	0.42	0.46	110.75
At1g05010	JA/ET	ACC oxidase	0.35	0.26	59.28
At5g44420	JA/ET	PDF 1.2, defensin	0.22	1.22	547.53
At5g20700	JA/ET	Senescence-associated protein	0.20*	0.62	318.24
At3g45140	JA/ET	LOX2, lipoxygenase	–0.22	0.26	117.08
At4g11280	JA/ET	ACS6, aminocyclopropane carboxylate synthase	–0.42*	0.46	109.14
At5g42650	JA/ET	AOS, allene oxide synthase	–0.46	0.46	101.44
At1g66340	JA/ET	ETR1, ethylene receptor, putative	–0.54	0.45	83.24
At3g23150	JA/ET	ETR2, ethylene receptor, putative	–0.82*	0.95	116.24
At5g24780	JA/ET	VSP1, vegetative storage protein	–2.15*	1.49	69.40
At5g05730	JA/ET	ASA1, antranilate synthase	–1.78	0.18	10.01

[a] SAR = systemic acquired resistance, Detox = detoxification, and JA/ET = the jasmonate/ethylene pathway.
[b] Shown is the ratio of the mean expression in yeast-treated and water-treated plants, two days after treatment measured in microarray experiments. Asterisks indicate low signal intensity.
[c] SD = standard deviation.
[d] CV = coefficient of variation.

24 and 48 h after yeast treatment, which is in agreement with the array analysis data (Fig. 4). Also increased expression of glutathion-*S*-transferases between 3 and 48 h was detected. In agreement with the array results, expression of *Gst2* was strongly induced at 24 and 48 h. Expression of *Gst1* was strongest at 3 h after yeast treatment and only slightly higher than the control at later timepoints. No clear differential expression in control and yeast-treated plants was observed for the JA-responsive genes *PDF1.2*, *Aos*, and *Lox2*.

The SA pathway is necessary for the protective effect of yeast against *P. syringae*.

To further elucidate the mechanism responsible for the protection elicited by yeast, mutants in the SA and JA pathway and camalexin biosynthesis were analyzed. Two mutants affected in each pathway were tested. The *NahG*-expressing plants do not accumulate SA and the *npr1* mutant is insensitive to SA (Cao et al. 1994; Delaney et al. 1994). For the JA pathway, mutants defective in JA biosynthesis (*opr3*, *dde2)* and JA signaling (*jin1)* were analyzed (Berger et al. 1996; von Malek et al. 2002; Stintzi and Browse 2000). *dde2* is not able to synthesize OPDA and JA, while *opr3* accumulates OPDA but not JA. *jin1* is defective in the gene *Atmyc2*, encoding a transcription factor (Lorenzo et al. 2004). In the mutants *pad3* and *cyp79B2/3*, the biosynthesis of camalexin is strongly reduced (Glawischnig et al. 2004; Glazebrook and Ausubel 1994). In agreement with reported data, bacterial growth was higher in *NahG* and *npr1* plants and lower in *dde2* and *opr3* plants than in the corresponding wild type (Delaney et al. 1994; Raacke et al., in press).

Pretreatment with yeast did not result in lower bacterial growth or reduced symptoms in either of the mutants affected in the SA pathway (Fig. 5A). In contrast, mutants in camalexin biosynthesis and JA biosynthesis or signaling exhibited a similar protective effect of yeast pretreatment as the wild types. Bacterial growth in *cyp79B2/3*, *pad3*, *dde2*, *opr3*, and *jin1* was two- to fivefold lower in the yeast-pretreated than in the water-pretreated mutant plants (Fig. 5A to C). This indicates that the SA pathway is necessary for the protection effect inferred by yeast against *P. syringae*, while JA and camalexin are not important contributors.

The protection of yeast against *B. cinerea* is not dependent on SA, JA, or camalexin.

In order to find out if the same mechanism is responsible for the protection effect of yeast against *B. cinerea*, symptom development after challenge with *B. cinerea* was tested in the same set of mutants used for analysis of the susceptibility to *P. syringae* (discussed above). Comparison of lesion development of the water-pretreated wild-type and mutant plants revealed that chlorotic areas were larger in all mutants except *jin1*, which exhibited smaller chlorotic areas than the corresponding wild type (Fig. 6A, B). These results are consistent with reported data (Ferrari et al. 2003; Nickstadt et al. 2004; Raacke et al., in press). Surprisingly, yeast treatment conferred a protection against *B. cinerea* in all mutants tested, based on lesion size. The size of necrotic areas was smaller in the yeast pretreated plants, with factors ranging between 3 and 7. This indicates that none of the pathways tested is indispensable for the protection by yeast against this pathogen.

An alternative mechanism would be a direct protection effect of yeast independent of the plant response. To test whether the protection is based on the presence of yeast rather than on defense responses activated in the plant, the yeast was removed from the leaves by rinsing 4 days after spraying and plants were inoculated the next day. Lesion development was compared with control plants that were rinsed 4 days after spraying with water and were infected the following day. Removal of the yeast prevented a significant decrease in the size of the chlorotic area (data not shown). In addition, we tested if the protection effect is already present before the five-day incubation period that is necessary for full development of the protection against *P. syringae*. Already 1 day after spraying, yeast pretreatment resulted in four times smaller lesion development upon *B. cinerea* infection than did water pretreatment. Shorter incubation times could not be tested because, several hours after yeast spraying, droplets of spores were spreading on the leaf surface, resulting in different symptoms than in standard experiments. These results are in favor of a direct effect of yeast. A possible mechanism for the protective effect against *B. cinerea* would be an inhibition of *B. cinerea* growth by yeast. In order to test this possibility, *B. cinerea* was grown on plates with yeast. No inhibitory effect of yeast could be detected, and additionally, *B. cinerea* was able to grow on plates consisting of only yeast and agar (data not shown), suggesting that a different mechanism is responsible for the protection.

DISCUSSION

Activation of inducible defense mechanisms in plants is potentially suitable to enhance the resistance of plants against virulent pathogens. The advantage of disease control by activators such as the yeast suspension used in this study versus fungicides is the avoidance of toxic compounds, which is important in organic agriculture. On the other hand, activating the plant immune system to increase resistance shows variable performance in the field, due to the complexity of plant-pathogen interactions and the influence of external factors (Elmer

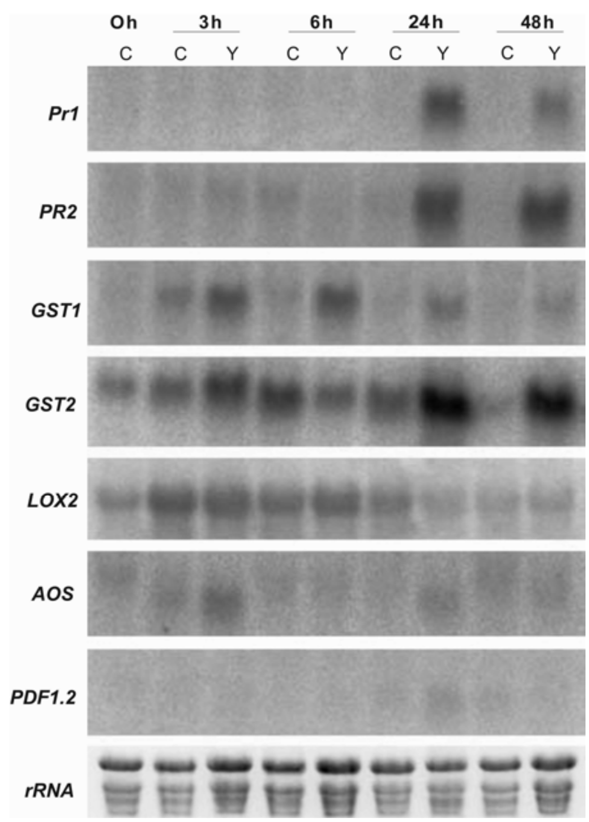

Fig. 4. Regulation of gene expression in leaves of *Arabidopsis* plants in response to yeast treatment. Plants were sprayed with water (C) or yeast suspension (Y), and leaves were harvested at the times indicated. The experiment was repeated four times. A representative Northern blot is shown. A total of 8 µg of RNA was loaded per lane. Gel loading was monitored by EtBr-staining of the gel.

and Reglinski 2006). Therefore, efficacy is poorly calculable and depends on environmental conditions and on plant genotypes. In addition, decrease in yield despite a protection against pathogens has been observed, due to negative effects on growth or on resistance against insects (Heil and Bostock 2002). Therefore, careful investigations of the influence of an activator treatment on a broad range of parameters are necessary.

In addition to inducing resistance, elicitors have been reported to increase the synthesis of secondary metabolites, which might be useful for the production of plant-derived compounds that are used as therapeutics or flavors. Treatment of *Arabidopsis* plants with yeast increased the accumulation of the secondary metabolite camalexin. This is in agreement with other studies reporting an increase in the levels of phytoalexins in soybean cotyledons and cell cultures upon addition of yeast preparations (Blechert et al. 1995; Hahn and Albersheim 1978). Interestingly, in *Arabidopsis* plants, the accumulation of camalexin is typically correlated with the development of lesions. This study shows that a strong increase in camalexin (300-fold) is possible without lesion development. The dramatic increase of camalexin levels after yeast treatment indicates future potential applications of this elicitor in enhancing secondary metabolism.

Based on studies with cell cultures, it was hypothesized that the induction of secondary metabolite production by yeast elicitors is mediated via an activation of the JA pathway (Mueller et al. 1993). In contrast, we found that spraying *Arabidopsis* plants with yeast suspension led to an activation of the SA but not the JA pathway, based on regulation of gene expression. Our results suggest that the effects of yeast on *Arabidopsis* plants are not mediated by the JA pathway. Recently, differences in gene regulation by JA and yeast have been reported for *Medicago* suspension-cell cultures (Suzuki et al. 2005), supporting our results that the JA pathway is not a relevant mediator of yeast-induced responses.

Whether the SA or the JA pathway or camalexin accumulation is activated, either singly or in any combination thereof, depends on the choice of the inducer, e.g., the pathogen, elicitor, or chemical. Figure 7 compares the profile of defense activation in *Arabidopsis* by yeast with the profile of microorganism, PAMP, and chemical inducers. Living pathogens, e.g., virulent and avirulent strains of *P. syringae* and fungi such as *Alternaria brassicicola* and *B. cinerea*, induce the most complex set of responses, including accumulation of SA, JA, and camalexin, even though with differences in kinetics and magnitude (Govrin and Levine 2002; Heck et al. 2003; Thomma et al. 2001b). These differences are crucial for the outcome of the interaction. The defense responses are triggered by recognition of the pathogens as well as the destruction of plant tissue at later stages. In contrast, living nonpathogenic and nonhost microorganisms activate mainly the JA pathway (Ryu et al. 2004; Verhagen et al. 2004; Zimmerli et al. 2004). The bacterial pep-

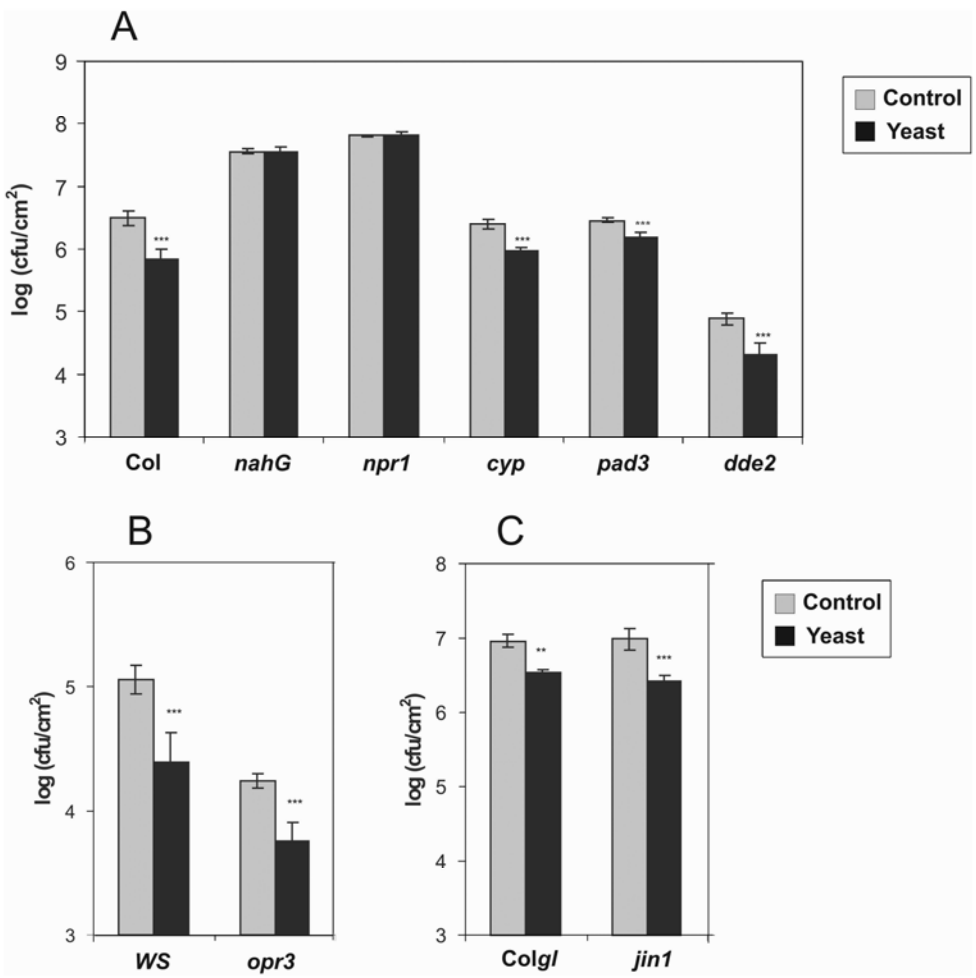

Fig. 5. Effect of yeast pretreatment on growth of *Pseudomonas syringae* in *NahG* plants and the *Arabidopsis* mutants *npr1*, *cyp79B2/3 (cyp)*, *pad3*, *dde2*, *opr3*, *jin1*, and corresponding wild-types **A,** Col, **B,** WS, and **C,** Col*gl*. Plants were sprayed with a water (control) or yeast suspension, and after 5 days, leaves were infiltrated with *P. syringae* and were harvested 48 h after inoculation. Shown are the mean values of at least five independent samples ± standard deviation. For each sample, four leaf disks originating from two plants were homogenized. The experiment was repeated at least three times with similar results. Stars indicate significant differences between the yeast-treated sample and the water-treated control according to *t*-test (****P* < 0.001, ***P* < 0.01).

tide flagellin, one of the most intensively studied PAMP, induces SA and JA pathways in *Arabidopsis* (Gomez-Gomez et al. 1999); the induction of phytoalexin accumulation has not been determined by this PAMP. Chemicals, such as β-aminobutyric acid (BABA) and benzo(1,2,3)thiadiazole-7-carbothioic acid *S*-methylester (BTH), prime or activate the SA pathway (von Rad et al. 2005; Zimmerli et al. 2000). It has been hypothesized that transient activation of JA biosynthesis genes combined with a more sustained induction of genes involved in SA-associated defense and detoxification is a general feature of plant activators (von Rad et al. 2005). The profile of yeast-induced responses showed the highest concordance with the profile of chemical plant activators. This is supported by the fact that all yeast-induced genes are also induced by the BTH-containing plant activator BION, which was previously shown using the same array (von Rad et al. 2005). However, regulation of camalexin accumulation has not been reported for most plant activators except BABA which, in contrast to yeast, exerts a negative effect on camalexin accumulation (Zimmerli et al. 2000). Interestingly, there was only low similarity between the activation profiles of yeast and living nonpathogenic microorganisms, even though yeast could be classified as one. Breakage of the fungal cells during sterilization might be responsible for this difference in defense activation and results in making the sterilized yeast suspension more similar to PAMP or chemical inducers.

Which compound or PAMP of yeast suspension comprises the actual elicitor for camalexin accumulation and PR gene expression? The suspension used in this study contains all con-

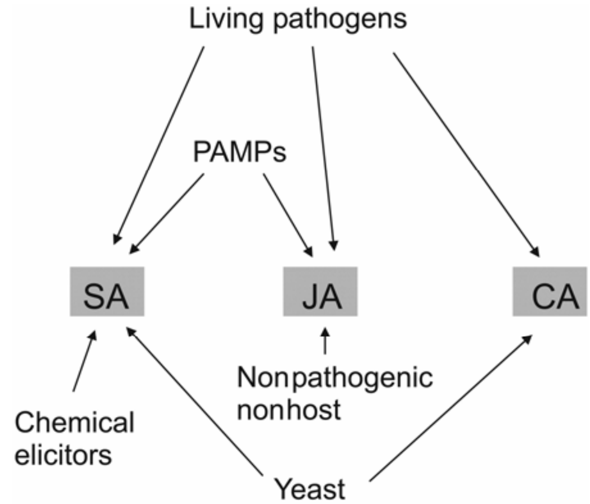

Fig. 7. Comparison of the activation of inducible defense responses in *Arabidopsis*. Data were derived from living pathogens *Pseudomonas syringae*, *Alternaria brassicicolae*, and *Botrytis cinerea* (Govrin and Levine 2002; Heck et al. 2003; Thomma et al. 2001b), nonpathogenic rhizobacteria *P. fluorescence* (Verhagen et al. 2004), and *Serratia marcescens* 90-166 (Ryu et al. 2004), nonhost *Blumeria graminis hordei* (Zimmerli et al. 2004), chemical elicitors benzo(1,2,3)thiadiazole-7-carbothioic acid *S*-methylester (BTH) (Rad et al. 2005), and β-aminobutyric acid (BABA) (Zimmerli et al. 2000), pathogen-associated molecular pattern (PAMP) flagellin (Gomez-Gomez et al. 1999). The accumulation of camalexin has not been reported for flagellin, *B. graminis*, or rhizobacteria.

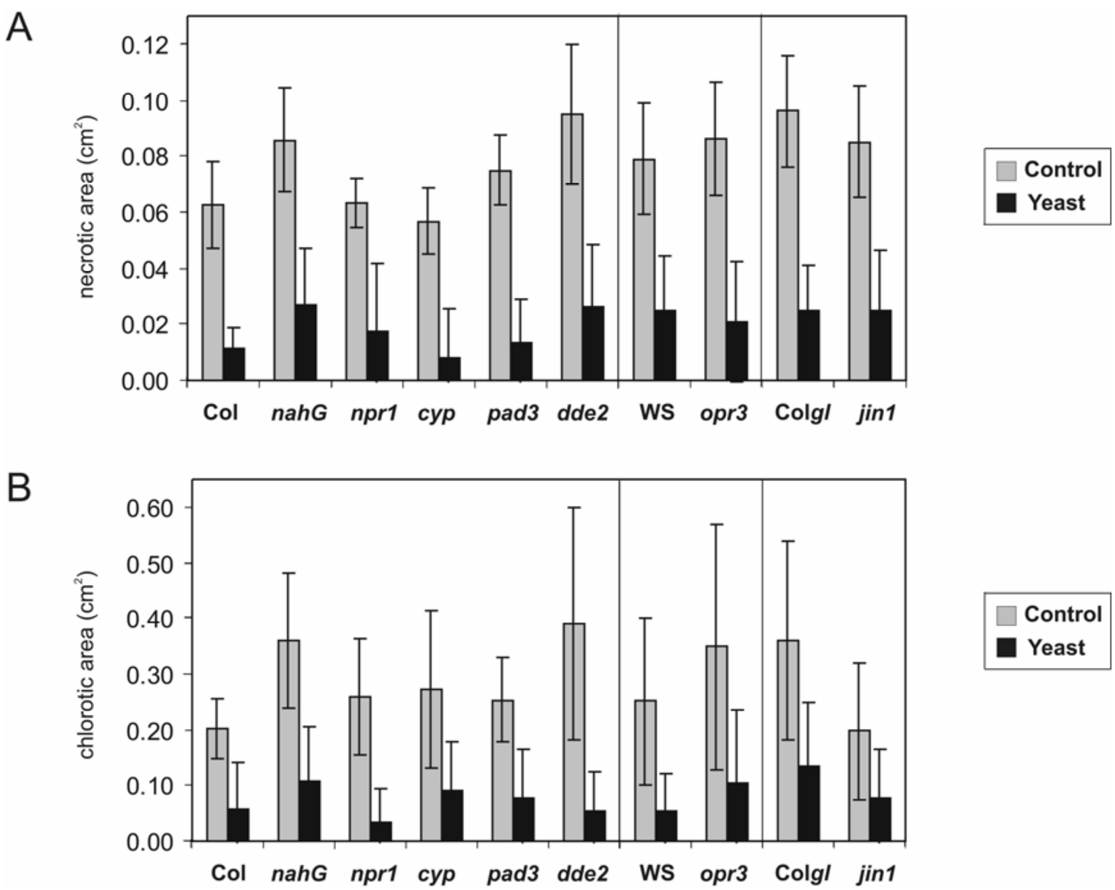

Fig. 6. Effect of yeast pretreatment on *Botrytis cinerea* disease development in *NahG* plants and the *Arabidopsis* mutants *npr1*, *cyp79B2/3 (cyp)*, *pad3*, *dde2*, *opr3*, *jin1*, and corresponding wild-types Col, WS, and Col*gl*. Plants were sprayed with water (control) or a yeast suspension, and after 5 days, leaves were infected with *B. cinerea.* **A,** Necrotic and **B,** chlorotic areas were determined 72 h after inoculation. Shown are the mean values of 24 samples ± standard deviation. The experiment was repeated at least three times with similar results. In all wild types and mutants, the difference between control and the yeast-treated sample was significant according to *t*-test with $P < 0.001$.

stituents of the fungal cell, including proteins as well as fungal cell walls. For most of the experiments reported, cell wall preparations or a glucan derived from the cell wall have been used (Blechert et al. 1995; Hahn and Albersheim 1978; Suzuki et al. 2005), but glucopeptides and glucoproteins also showed elicitor activity (Basse and Boller 1992). This indicates that yeast suspension contains several PAMP with elicitor activity. Similarly, for preparations of bacteria, it was proposed that they contain at least one additional PAMP besides flagellin (Zipfel et al. 2004). Using a suspension of yeast provides the advantage that a combination of PAMP might be more effective than a single elicitor and that no expensive isolation of one elicitor is necessary. Another advantage is a greater chance that a mixture of PAMP rather than a single PAMP may be active in a broad range of plant species. Nevertheless, as discussed above, which pathways are activated varies with the plant species, which renders the application of this resistance induction in crop plants less predictable.

Yeast treatment decreased the susceptibility to virulent *P. syringae.* In mutants defective in the SA pathway, this protection against *P. syringae* could not be induced by yeast, showing that the SA pathway is responsible for yeast-induced resistance against *P. syringae*. This mechanism is similar to the mechanism effective in basal resistance, which has been documented by the higher susceptibility of mutants in the SA pathway against *P. syringae* (Delaney et al. 1994). In contrast, mutants in camalexin biosynthesis or in the JA pathway showed a reduction in bacterial growth by yeast pretreatment similar to that of wild type, indicating that these pathways do not contribute to the protection conferred by yeast. This situation is, again, similar to basal resistance. While JA and camalexin accumulate after *P. syringae* challenge, they are not effective or necessary in defending this pathogen (Block et al. 2005; Glazebrook and Ausubel 1994; Thomma et al. 2001a). Taken together, these results show great similarity between the defense mechanism in basal and in nonhost resistance and provide evidence for the hypothesis that the defense responses activated by PAMP greatly overlap with mechanisms important for basal resistance (Zimmerli et al. 2004).

It has been reported that camalexin contributes to the resistance against necrotrophic fungi such as *B. cinerea* (Ferrari et al. 2003). Indeed, we found reduced sensitivity of plants with increased camalexin levels after pretreatment with yeast. Surprisingly, this yeast-induced reduction in sensitivity was also obtained in mutants defective in camalexin biosynthesis, leading to the conclusion that camalexin is not important for the yeast-induced protection against *B. cinerea*. According to a general model, the JA pathway plays an important role in the defense against necrotrophic pathogens (Thomma et al. 2001a). Intriguingly, mutants defective in JA biosynthesis or signaling also could be protected by yeast. This indicates that either a different plant pathway is involved in this protection or that resistance against *B. cinerea* is independent of the defense mechanism of the plant. Possible candidates for a signaling mechanism involved are reactive oxygen species (ROS). ROS lead to accumulation of camalexin and induction of expression of glutathione-*S*-transferases, which is in agreement with our results on the effect of yeast treatment. However, six genes putatively encoding NADPH oxidases involved in ROS production are present in the *Arabidopsis* genome, rendering a conclusive loss of function approach difficult.

Testing the direct effect of yeast on *B. cinerea* revealed no inhibitory effects. Another direct effect would be a decrease in hydrophobicity of the surface, which could delay germination of *B. cinerea* spores. Alternatively, the yeast present on the leaf could be used by *B. cinerea* as a source of nutrients. Since attacking the plant leaf and killing the host cells requires more energy and effort from the pathogen than just taking up the nutrients supplied, this would be a much more cost-effective lifestyle for the fungus. The reality might be a combination of several of the mechanisms discussed, contributing in concert to the protection effect so that eliminating just one signaling pathway does not result in loss of protection.

MATERIALS AND METHODS

Plant and pathogen cultivation.

Wild-type *Arabidopsis thaliana* Col-0 was used as standard wild type. Mutants used in this work in the Col-0 background were *nahG*, *npr1*, *dde2*, *pad3*, and *cyp79B2/3*. The corresponding wild types for *jin1* and *opr3* were Col*gl1* and WS, respectively. Plants were grown in soil with a 9-h light period (light intensity, 180 μmol quanta m^{-2} s^{-1}) for 5 weeks.

The bacterial strain *Pseudomonas syringae* pv. *tomato* DC3000 (provided by B. Staskawicz, Berkley, CA, U.S.A.) was used. The bacteria were cultured in Kings B medium containing 50 mg of rifampicin per liter. For inoculation, bacteria were resuspended in 10 mM $MgCl_2$, adjusted to an optical density at 600 nm of 0.2, which is equivalent to 10^8 CFU/ml, and were diluted to 10^6 CFU/ml. Growth and spore harvesting of the fungus *Botrytis cinerea* (strain MUCL30158, Mycothèque Université Catholique de Louvain, Louvain-la-Neuve, Belgium) was done as described previously (Thomma et al. 1999). For testing the direct effect of yeast on *B. cinerea*, fungal growth was monitored on potato dextrose agar plates containing a layer of autoclaved suspension of commercially available bakers yeast (0.3 g/ml) and on plates consisting of yeast (0.3 g/ml) and 1.5% agar.

Treatment of plants.

Plants were sprayed with an autoclaved suspension (0.3 g/ml) of commercially available bakers yeast (Deutsche Hefewerk GmbH, Nuernberg, Germany). For the comparison of different yeast sources, yeast was additionally obtained from Uniform GmbH (Werne, Germany) and Dr. Oetker (Bielefeld, Germany). The cell density of the yeast suspensions used in different experiments was between 4×10^9 and 9×10^9 cells per milliliter. The protein content of the suspensions was between 1.4 and 1.9 mg per milliliter; protein determination was done according to Bradford (1976). Plants were sprayed with approximately 1 ml per plant until leaves were fully wet. For determination of susceptibility to *P. syringae*, leaves were infiltrated with a bacterial suspension in 10 mM $MgCl_2$ at a density of 10^6 CFU/ml, using a needleless syringe. Infiltration was applied on one half of the leaf with approximately 20 μl of bacterial suspension. Bacterial growth in the leaves was analyzed as by Whalen and associates (1991); leaf disks with a diameter of 1.4 cm were used.

For the *B. cinerea* disease susceptibility assays, two needle-prick wounds were applied to each leaf of 5-week-old *Arabidopsis* plants, and the fresh wounds were covered with 5-μl drops of a suspension of 9×10^5 conidial spores per milliliter in 12 g/liter potato dextrose broth (Difco, Detroit). Plants were incubated after the infection in transparent boxes, to maintain high humidity. Necrotic and chlorotic areas were quantified with the program SURFACE, as described by Rostas and associates (in press).

For isolation of RNA and camalexin, leaves were harvested at the timepoints indicated, were immediately frozen in liquid nitrogen, and were stored at -80^{o}C.

RNA isolation and Northern blot analysis.

Arabidopsis leaf material (100 mg) was ground and RNA was extracted with Tritidy (Applichem, Darmstadt, Germany) according to the manufactures protocol. Additionally, the RNA pellet was washed once with 3 M $LiCl_2$. RNA was separated on 1.2% agarose gels and was blotted on nitrocellulose membrane. Hybridization was performed according to Ehness and associates (1997) with radioactively labeled DNA. Filters were exposed to a screen for 24 to 48 h, and the screen was scanned with a PhosphorImager (BAS, Fuji, Tokyo).

DNA from the following genes was used as probes: *Pr1* At2g14610, *Pr2* At3g57260, *Gst1* At1g02930, *Gst2* At4g02520, *Lox2* At3g45140, *Aos* At5g42650, and *Pdf1.2* At5g44420.

cDNA array analysis.

The *A. thaliana* DNA microarray used consisted of longer fragments of synthetic or complementary DNA. Sequences were derived from databases, as polymerase chain reaction (PCR)-amplified partial open reading frames or specific 3′ untranslated region (UTR) sequences, or were provided by others (Huang et al. 2002). The array contained about 1,400 spots corresponding to 1,164 genes associated with plant defense and various cDNAs associated with either primary metabolism, housekeeping, or both. For members of the family of ABC-transporters, cytochrome P450 monooxygenases, glycosyltransferases, glutathione-*S*-transferases, and aquaporins specific 3′ UTR sequences of 125 to 300 bp were used (Glombitza et al. 2004). Members of other gene families were represented by partial or complete coding sequences of at least 450 bp. These expressed sequence tag clones were available from the Arabidopsis Biological Resource Center (Columbus, OH, U.S.A.) or were designed at the National Research Center for Environment and Health (Oberschleissheim, Germany). Microarray analyses were performed with some modifications, as described previously (Loeffler et al. 2005; von Rad et al. 2005). Briefly, amino-modified PCR products were arrayed onto silylated microscope slides (CSS-100 silylated slides; CEL Associates, Houston, TX, U.S.A.) using a DNA array robot (model GMS 417; Genetic Microsystems Robotics, Cambridge).

An indirect aminoallyl labeling method (described on The Institute for Genomic Research website) was used for preparing probes. Reverse transcription of RNA samples (one control and one treated sample for each slide) was done in the presence of Cy3-dUTP or Cy5-dUTP (Amersham Pharmacia Biotech, Munich, Germany). Purification of the Cy3- and Cy5-labeled probes was performed according to standard protocols to remove unincorporated nucleotides.

The Cy3- and Cy5-labeled probes were hybridized to microarray glass slides (Loeffler et al. 2005; von Rad et al. 2005). Arrays were scanned using an AXON GenePix 4000A scanner (Molecular Devices, Menlo Park, CA, U.S.A.). The GenePix Pro 6.0 and Accuity 4.0 (AXON; Molecular Devices) software packages were used to identify differentially expressed genes. Background fluorescence was calculated as the median fluorescence signal of nontarget pixels around each gene spot. Spots showing less than 50% difference between background and signal were excluded. Normalization was over all features including: i) all features printed on the array that met the quality criteria that at least 55% of the pixels in both signals (635 and 532 nm) of a given spot were stronger than the background plus standard deviation; ii) background uniformity [Rgn R^2 (635/532)] was higher than 0.5; ii) only spots with less than 3% saturated pixels were considered; and iv) undetected spots or weak signals (sum of medians >500) were excluded. Gene expression was considered as induced or repressed if the transcript level showed a minimum of 2.0-fold change (corresponds to a log ratio of 1.0/–1.0). Four technical replicates of array hybridizations and a dye-swap for each biological replicate were performed. Three independent biological replicates were analyzed. We applied the following selection procedure to our expression data: i) signal intensities of less than twofold above local background level were excluded, and ii) only expression log ratios higher than 1.0 (lower than –1.0) values with coefficient of variation values below 50 were regarded as significant. These very rigorous criteria ensure that our procedure ignores genes with relatively low basal expression ratios.

Determination of camalexin.

An internal standard of 6-fluoroindole-3-carboxaldehyde (50 µg) (Sigma, Taufkirchen, Germany) was added to leaf material (200 mg) prior to extraction with 500 µl of methanol/water (80%, vol/vol) in an ultrasonic water bath for 10 min. Extraction was repeated, and the combined extracts were partitioned against 3 × 1 ml of petrol ether. The upper-petrol ether phases were discarded, and the remaining methanol/water phase was subjected to high-pressure liquid chromatography analysis on a Purospher STAR RP-18 ec column (250 × 4.6 mm; 5 µm; Merck, Darmstadt, Germany). Water and acetonitrile were solvents A and B, respectively. Solvent B was linearly increased from 0 (0 min) to 10 (1 min), 20 (6 min), 20 (16 min), 55 (33.5 min), 55 (34 min), and 100% (45 min) at a flow rate of 1 ml min^{-1}. A fluorescent detector (λ_{ex} = 305 nm, λ_{em} = 364 nm) was used to monitor camalexin.

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LITERATURE CITED

Basse, C. W., and Boller, T. 1992. Glycopeptide elicitors of stress responses in tomato cells. Plant Physiol. 98:1239-1247.

Berger, S., Bell, E., and Mullet, J. E. 1996. Two methyl jasmonate-insensitive mutants show altered expression of atvsp in response to methyl jasmonate and wounding. Plant Physiol. 111:525-531.

Blechert, S., Brodschelm, W., Hoelder, S., Kammerer, L., Kutchan, T., Mueller, M. J., Xia, Z.-Q., and Zenk, M. H. 1995. The octadecanoid pathway: Signal molecules for the regulation of secondary pathways. Proc. Natl. Acad. Sci. U.S.A. 92:4099-4105.

Block, A., Schmelz, E., Jones, J., and Klee, H. J. 2005. Coronatine and salicylic acid: The battle between *Arabidopsis* and *Pseudomonas* for phytohormone control. Mol. Plant Pathol. 6:79-83.

Bradford, M. M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of proteins utilizing the principle of protein-dye binding. Anal. Biochem. 72:248-254.

Cao, H., Bowling, S. A., Gordon, A. S., and Dong, X. N. 1994. Characterization of an Arabidopsis mutant that is nonresponsive to inducers of systemic acquired resistance. Plant Cell 6:1583-1592.

Dangl, J. L., and Jones, J. D. 2001. Plant pathogens and integrated defence responses to infection. Nature 411:826-833.

Delaney, T., Uknes, S., Vernooij, B., Friedrich, L., Weymann, K., Negrotto, D., Gaffney, T., Gut-Rella, M., Kessmann, H., Ward, E., and Ryals, J. 1994. A central role of salicylic acid in plant disease resistance. Science 266:1247-1250.

Ehness, R., Ecker, M., Godt, D. E., and Roitsch, T. 1997. Glucose and stress independently regulate source and sink metabolism and defense mechanisms via signal transduction pathways involving protein phosphorylation. Plant Cell 9:1825-1841.

Elmer, P. and Reglinski, T. 2006. Biosuppression of *Botrytis cinerea* in grapes. Plant Pathol. 55:155-177.

Ferrari, S., Plotnikova, J. M., Lorenzo, G. D., and Ausubel, F. M. 2003. *Arabidopsis* local resistance to *Botrytis cinerea* involves salicylic acid and camalexin and requires EDS4 and PAD2, but not SID2, EDS5 or PAD4. Plant J. 35:193-205.

Glawischnig, E., Hansen, B. G., Olsen, C. E., and Halkier, B. A. 2004. Camalexin is synthesized from indole-3-acetaldoxime, a key branching point between primary and secondary metabolism in *Arabidopsis*. Proc. Natl. Acad. Sci. U.S.A. 101:8245-8250.

Glazebrook, J., and Ausubel, F. M. 1994. Isolation of phytoalexin-deficient mutants of *Arabidopsis thaliana* and characterization of their interactions with bacterial pathogens. Proc. Natl. Acad. Sci. U.S.A. 91:8955-8959.

Glombitza S., Dubuis, P.-H., Thulke, O., Welzl, G., Bovet, L., Götz, M., Affenzeller, M., Geist, B., Hehn, A., Asnaghi, C., Ernst, D., Seidlitz, H. K., Martinoia, E., Werck-Reichhart, D., Mauch, F., and Schäffner, A. R. 2004. Crosstalk and differential response to abiotic and biotic stressors reflected at the transcriptional level of effector genes from secondary metabolism. Plant Mol. Biol. 54:817-835.

Gomez-Gomez, L., Felix, G., and Boller, T. 1999. A single locus determines sensitivity to bacterial flagellin in *Arabidopsis thaliana*. Plant J. 18:277-284.

Govrin, E. M., and Levine, A. 2002. Infection of *Arabidopsis* with a necrotrophic pathogen, *Botrytis cinerea*, elicits various defense responses but does not induce systemic acquired resistance (SAR). Plant Mol. Biol. 48:267-276.

Hahn, M. G., and Albersheim, P. 1978. Host-pathogen interactions. Plant Physiol. 62:107-111.

Heath, M. C. 2000a. Nonhost resistance and nonspecific plant defenses. Curr. Opin. Plant Biol. 3:315-319.

Heath, M. C. 2000b. Hypersensitive response-related death. Plant Mol. Biol. 44:321-334.

Heck, S., Grau, T., Buchala, A., Métraux, J.-P., and Nawrath, C. 2003. Genetic evidence that expression of NahG modifies defence pathways independent of salicylic acid biosynthesis in the *Arabidopsis-Pseudomonas syringae* pv. *tomato* interaction. Plant J. 36:342-352.

Heil, M., and Bostock, R. M. 2002. Induced systemic resistance (ISR) against pathogens in the context of induced plant defences. Ann. Bot. 89:503-512.

Huang X., von Rad U., and Durner J. 2002. Nitric oxide induces the nitric oxide-tolerant alternative oxidase in *Arabidopsis* suspension cells. Planta 215:914-923.

Loeffler, C., Berger, S., Guy, A., Durand, T., Bringmann, G., Dreyer, M., von Rad, U., Durner, J., and Mueller, M. J. 2005. B1-phytoprostanes trigger plant defense and detoxification responses. Plant Physiol 137:328-340.

Lorenzo, O., Chico, J. M., Sanchez-Serrano, J. J., and Solano, R. 2004. JASMONATE-INSENSITIVE1 encodes a MYC transcription factor essential to discriminate between different jasmonate-regulated defense responses in *Arabidopsis*. Plant Cell 16:1938-1950.

Mueller, M. J., Brodschelm, W., Spannagl, E., and Zenk, M. H. 1993. Signaling in the elicitation process is mediated through the octadecanoid pathway leading to jasmonic acid. Proc. Natl. Acad. Sci. U.S.A. 90:7490-7494.

Navarro, L., Zipfel, C., Rowland, O., Keller, I., Robatzek, S., Boller, T., and Jones, J. D. G. 2004. The transcriptional innate immune response to flg22. Interplay and overplay with avr gene-dependent defense responses and bacterial pathogenesis. Plant Physiol. 135:1113-1128.

Nickstadt, A., Thomma, B. P. H. J., Feussner, I., Kangasjarvi, J., Zeier, J., Loeffler, C., Scheel, D., and Berger, S. 2004. The jasmonate-insensitive mutant *jin1* shows increased resistance to biotrophic as well as necrotrophic pathogens. Molec. Plant Pathol. 5:425-434.

Nurnberger, T., Brunner, F., Kemmerling, B., and Piater, L. 2004. Innate immunity in plants and animals: Striking similarities and obvious differences. Immunol. Rev. 198:249-266.

Parchmann, S., Gundlach, H., and Mueller, M. J. 1997. Induction of 12-oxo-phytodienoic acid in wounded plants and elicited plant cell cultures. Plant Physiol. 115:1057-1064.

Raacke, I. C, Mueller, M. J., and Berger, S. Defects in allene oxide synthase and OPDA reductase alter the resistance to *Pseudomonas syringae* and *Botrytis cinerea*. J. Phytopathol. In press

Reglinski T., Lyon, G. D., and Newton, A. C. 1994. Induction of resistance mechanisms in barley by yeast-derived elicitors. Ann. Appl. Biol. 124:509-517.

Rostas, M., Ton, J., Mauch-Mani, B., and Turlings, T. C. J. Fungal infection reduces herbivore-induced plant volatiles of maize but does not affect naive parasitoids. J. Chem. Ecol. In press

Ryu, C.-M., Murphy, J. F., Mysore, K. S., and Kloepper, J. W. 2004. Plant growth-promoting rhizobacteria systemically protect *Arabidopsis thaliana* against *Cucumber mosaic* virus by a salicylic acid and NPR1-independent and jasmonic acid-dependent signaling pathway. Plant J. 39:381-392.

Staskawicz, B. J., Ausubel, F. M., Baker, B. J., Ellis, J. G., and Jones, J. D. 1995. Molecular genetics of plant disease resistance. Science 268:661-666.

Stintzi, A., and Browse, J. 2000. The arabidopsis male-sterile mutant, *opr3*, lacks the 12-oxophytodienoic acid reductase required for jasmonate synthesis. Proc. Natl. Acad. Sci. U.S.A. 97:10625-10630.

Suzuki, H., Reddy, M. S. S., Naoumkina, M., Aziz, N., May, G. C., Huhman, D. V., Summer, L. W., Blount, J. W., Mendes, P., and Dixon, R. A. 2005. Methyl jasmonate and yeast elicitor induce differential transcriptional and metabolic re-programming in cell suspension cultures of the model legume *Medicago truncatula*. Planta 220:696-707.

Tao, Y., Xie, Z., Chen, W., Glazebrook, J., Chang, H. S., Han, B., Zhu, T., Zou, G., and Katagiri, F. 2003. Quantitative nature of *Arabidopsis* responses during compatible and incompatible interactions with the bacterial pathogen *Pseudomonas syringae*. Plant Cell 15:317-330.

Thomma, B. P., Eggermont, K., Tierens, K. F., and Broekaert, W. F. 1999. Requirement of functional ethylene-insensitive 2 gene for efficient resistance of *Arabidopsis* to infection by *Botrytis cinerea*. Plant Physiol. 121:1093-1102.

Thomma, B. P., Penninckx, I. A., Broekaert, W. F., and Cammue, B. P. 2001a. The complexity of disease signaling in Arabidopsis. Curr. Opin. Immunol. 13:63-68.

Thomma, B. P. H. J., Tierens, K. F. M., Penninckx, I. A. M. A., Mauch-Mani, B., Broekaert, W. F., and Cammue, B. P. A. 2001b. Different micro-organisms differentially induce *Arabidopsis* disease response pathways. Plant Physiol. Biochem. 39:673-680.

Tierens, K. F., Thomma, B. P., Bari, R. P., Garmier, M., Eggermont, K., Brouwer, M., Penninckx, I. A., Broekaert, W. F., and Cammue, B. P. 2002. *Esa1*, an *Arabidopsis* mutant with enhanced susceptibility to a range of necrotrophic fungal pathogens, shows a distorted induction of defense responses by reactive oxygen generating compounds. Plant J. 29:131-140.

Tsuji, J., Jackson, E., Gage, D., Hammerschmidt, R., and Somerville, C. 1992. Phytoalexin accumulation in *Arabidopsis thaliana* during the hypersensitive reaction to *Pseudomonas syringae* pv. *syringae*. Plant Physiol. 98:1304-1309.

Verhagen, B. W. M., Glazebrook, J., Zhu, T., Chang, H. S., van Loon, L. C., and Pieterse, C. M. J. 2004. The transcriptome of rhizobacteria-induced systemic resistance in *Arabidopsis*. Mol. Plant-Microbe Interact. 17:895-908.

von Malek, B., van der Graaff, E., Schneitz, K., and Keller, B. 2002. The *Arabidopsis* male-sterile mutant dde2-2 is defective in the allene oxide synthase gene encoding one of the key enzymes of the jasmonic acid biosynthesis pathway. Planta 216:187-192.

von Rad, U., Mueller, M. J., and Durner, J. 2005. Evaluation of natural and synthetic stimulants of plant immunity by microarray technology. New Phytol. 165:191-202.

Whalen, M., Innes, R. W., Bent, A. F., and Staskawicz, B. 1991. Identification of *Pseudomonas syringae* pathogens of *Arabidopsis thaliana* and bacterial gene determining avirulence on both *Arabidopsis* and soybean. Plant Cell 3:49-59.

Zimmerli, L., Jakab, G., Metraux, J. P., and Mauch-Mani, B. 2000. Potentiation of pathogen-specific defense mechanisms in arabidopsis by beta -aminobutyric acid. Proc. Natl. Acad. Sci. U.S.A. 97:12920-12925.

Zimmerli, L., Stein, M., Lipka, V., Schulze-Lefert, P., and Somerville, S. 2004. Host and non-host pathogens elicit different jasmonate/ethylene responses in *Arabidopsis*. Plant J. 40:633-646.

Zipfel, C., Robatzek, S., Navarro, L., Oakeley, E. J., Jones, J. D. G., Felix, G., and Boller, T. 2004. Bacterial disease resistance in *Arabidopsis* through flagellin perception. Nature 428:764-767.

AUTHOR-RECOMMENDED INTERNET RESOURCE

The Institute for Genomic Research (TIGR) aminoallyl labeling method: atarrays.tigr.org/PDF/Aminoallyl.pdf