Costs and benefits of priming for defense in *Arabidopsis*

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Induced resistance protects plants against a wide spectrum of diseases; however, it can also entail costs due to the allocation of resources or toxicity of defensive products. The cellular defense responses involved in induced resistance are either activated directly or primed for augmented expression upon pathogen attack. Priming for defense may combine the advantages of enhanced disease protection and low costs. In this study, we have compared the costs and benefits of priming to those of induced direct defense in *Arabidopsis*. In the absence of pathogen infection, chemical priming by low doses of β-aminobutyric acid caused minor reductions in relative growth rate and had no effect on seed production, whereas induction of direct defense by high doses of β-aminobutyric acid or benzothiadiazole strongly affected both fitness parameters. These costs were defense-related, because the salicylic acid-insensitive defense mutant *npr1-1* remained unaffected by these treatments. Furthermore, the constitutive priming mutant *edr1-1* displayed only slightly lower levels of fitness than wild-type plants and performed considerably better than the constitutively activated defense mutant *cpr1-1*. Hence, priming involves less fitness costs than induced direct defense. Upon infection by *Pseudomonas syringae* or *Hyaloperonospora parasitica*, priming conferred levels of disease protection that almost equaled the protection in benzothiadiazole-treated wild-type plants and *cpr1* plants. Under these conditions, primed plants displayed significantly higher levels of fitness than noninduced plants and plants expressing chemically or *cpr1*-induced direct defense. Collectively, our results indicate that the benefits of priming-mediated resistance outweigh the costs in environments in which disease occurs.

induced resistance | innate immunity | plant defense

Plants resist the attacks of harmful microorganisms and insects through constitutive and inducible defenses. It is generally believed that inducible defenses have evolved to save energy under enemy-free conditions, but costs still arise upon activation of these defenses under hostile conditions. These costs can result from allocation of limited resources to defensive compounds or toxicity of the defense to the plant's own metabolism (1). In addition, costs can arise from external factors, when the defensive trait affects a beneficial interaction with another organism in the environment. It is therefore reasonable to assume that plants express their inducible defenses only if the benefits (i.e., protection against the attackers) outweigh the costs of the resistance.

Various studies have demonstrated costs related to jasmonic acid (JA)-inducible defense against herbivory. These costs can affect plant growth and reproductive traits (2–4). There are also studies that demonstrated ecological benefits of JA-inducible defense. Agrawal (5) showed that induction of defense in wild radish against insects correlated with enhanced seed production. Additionally, JA-induced defense in wild populations of *Nicotiana attenuata* conferred enhanced seed production in populations exposed to herbivory (3). Hence, costs of JA-inducible defenses are outweighed by the benefits of protection when plants are attacked by herbivores. A cost–benefit balance of salicylic acid (SA)-inducible defenses against pathogens has also been supposed. In wheat, Heil *et al.* (6) demonstrated costs of SA-inducible defenses on growth and seed set. In *Arabidopsis*, Cipollini (7) showed that exogenously applied SA reduced seed production. Recently, Heidel *et al.* (8) performed a field experiment with two sets of *Arabidopsis* genotypes: one group that is blocked in SA-inducible defenses and another group that constitutively expresses SA-inducible defenses. Both classes of genotypes were negatively affected in growth and seed set, suggesting that plant fitness reaches an optimum at a certain intermediate level of resistance that balances fitness and defense.

Upon appropriate stimulation, plants can increase their level of resistance against future pathogen attack. This phenomenon is known as induced resistance. Based on differences in signaling pathways and spectra of effectiveness, different types of induced resistance have been defined. The classic form of induced resistance is referred to as systemic acquired resistance (SAR) and occurs in distal plant parts upon localized infection by a necrosis-inducing pathogen (9). SAR is controlled by a signaling pathway that depends on endogenous accumulation of SA and the defense regulatory protein NPR1 (10) and is predominantly effective against biotrophic pathogens (11). Selected strains of nonpathogenic rhizobacteria can also induce systemic resistance, which is referred to as induced systemic resistance (ISR) (12). In *Arabidopsis*, ISR triggered by *Pseudomonas fluorescens* WCS417r functions independently of SA but requires NPR1 and responsiveness to JA and ethylene (13). *P. fluorescens* WCS417r-mediated ISR has a different spectrum of effectiveness than SAR, and is predominantly effective against pathogens that are sensitive to JA- and ethylene-dependent basal resistance (11). A third type of induced resistance is activated upon application of the chemical β-aminobutyric acid (BABA). The signaling pathway controlling BABA-induced resistance (BABA-IR) (14) differs from that of SAR and ISR. Although BABA-IR against *Pseudomonas syringae* depends solely on SA and NPR1 (15), against pathogenic fungi and oomycetes it is controlled by a pathway that involves abscisic acid- and phosphoinositide-dependent signaling (16, 17). BABA-IR is effective against biotrophic and necrotrophic pathogens, as well as certain types of abiotic stress (14–20).

For a long time, it was assumed that protection by induced resistance is based on direct activation of defenses by the resistance-inducing agent. Accumulation of pathogenesis-related proteins is an example that occurs directly upon induction of SAR. However, the suggested contribution of pathogenesis-related proteins to resistance is uncertain and appears insufficient to explain the broad spectrum of protection by SAR (21). Moreover, both rhizobacteria-mediated ISR and BABA-IR are not associated with direct activation of defense-related genes (12, 14). Interestingly, plants expressing SAR, ISR, or BABA-IR exhibit a faster and stronger
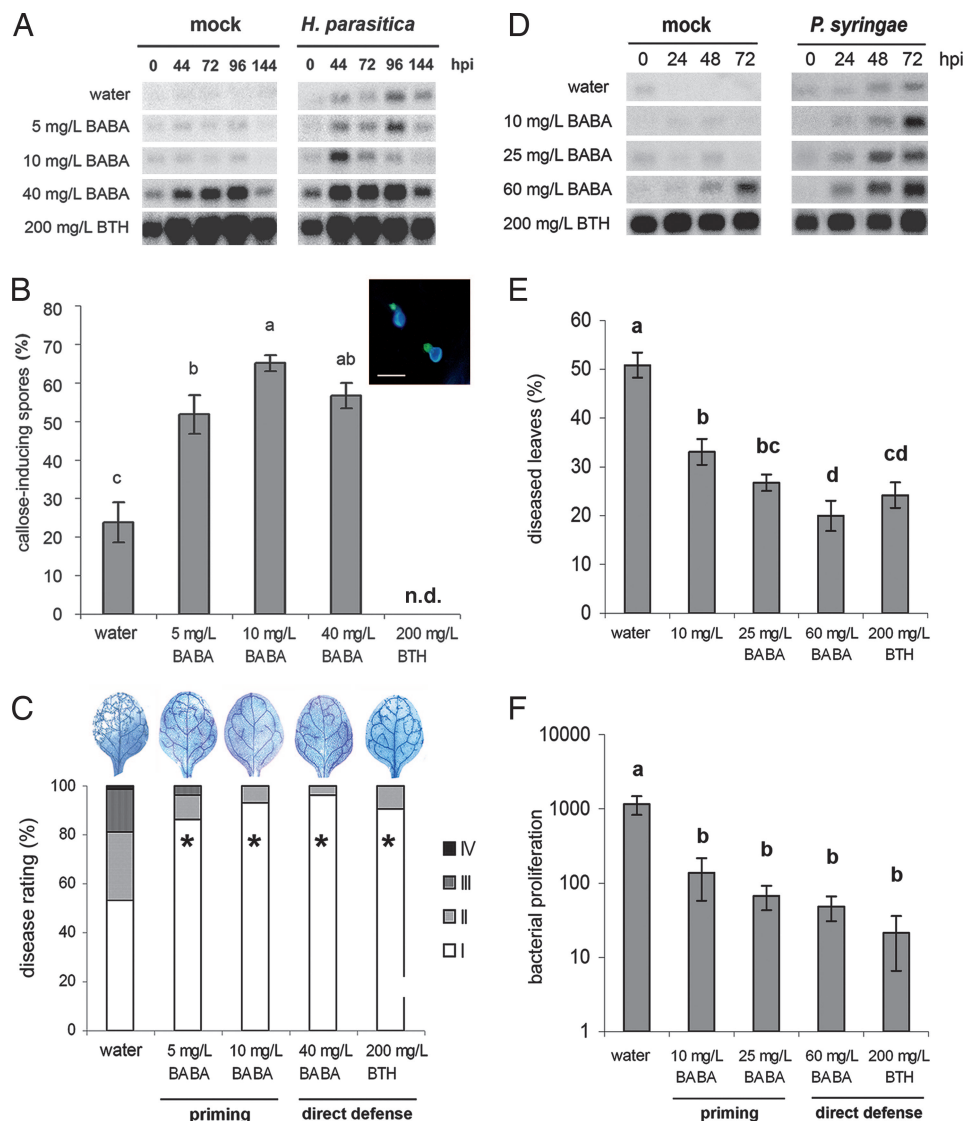
Fig. 1. Chemical induction of priming and direct defense against *H. parasitica* WACO9 (*A–C*) and *P. syringae* pv. *tomato* DC3000 (*D–F*). Col-0 plants were soil-drenched with increasing concentrations of BABA or sprayed with BTH and pathogen-inoculated 2 days later. (*A*) *PR-1* gene expression in 3-week-old control plants or BABA- or BTH-treated plants at different time points after inoculation. hpi, hours postinoculation. (*B*) Callose deposition 2 days after *H. parasitica* inoculation. (*Inset*) A representative example of *H. parasitica* spores triggering callose deposition in epidermal cells. (Scale bar, 20 μm.) n.d., not determined. (*C*) Induced resistance against *H. parasitica* at 8 days after inoculation. Asterisks indicate statistically different distributions of disease severity classes compared with the water control (χ^2 test; $\alpha = 0.05$). Colonization by the pathogen was visualized by lactophenol/trypan blue staining and light microscopy. (*D*) *PR-1* gene expression in 6-week-old control plants or BABA- or BTH-treated plants at different time points after inoculation. (*E*) Induced resistance against *P. syringae*. Shown are means ± SEM ($n = 15–20$) of the percentage of leaves with symptoms at 3 days after inoculation. Different letters indicate statistically significant differences (least significant difference test; $\alpha = 0.05$). (*F*) Growth of *P. syringae* over a 3-day time interval. Shown are means ± SD ($n = 5–10$). Different letters indicate statistically significant differences (least significant difference test; $\alpha = 0.05$). All experiments shown were repeated with comparable results.

activation of specific defense responses after they have been infected by a pathogen. This capacity for augmented defense expression is called priming (22).

Since the discovery of priming in plant cell suspension cultures by Kauss *et al.* (23), priming has been demonstrated in different plant species against pathogens, insects, and abiotic stress (22). Hence, priming appears to be a common feature of a plant's immune system that offers protection against a wide spectrum of environmental stresses. In *Arabidopsis*, Kohler *et al.* (24) demonstrated that SAR-induced *Arabidopsis* expressed augmented levels of the defense-related *PAL* gene upon infection by *P. syringae*. Van Wees *et al.* (25) and Verhagen *et al.* (26) demonstrated that treatment of *Arabidopsis* with ISR-inducing *P. fluorescens* WCS417r bacteria did not directly activate defense-related genes; however, *Arabidopsis* was primed for enhanced expression of JA- and ethylene-inducible genes upon infection by *P. syringae*. Treatment with BABA primes *Arabidopsis* for SA-dependent defenses (15, 20) and enhanced formation of callose-rich papillae that functions independently from SA and NPR1 (16). Recently, we showed that these forms of priming require specific cellular signaling components (17), suggesting a regulation mechanism that is exclusively dedicated to priming. Priming accelerates and increases the plant's ability to activate the defense that is best adapted to cope with a certain stress

situation. In this perspective, priming represents an important ecological adaptation to resist environmental stress.

As most studies on costs and benefits of induced resistance have focused on situations in which the defense is activated directly by the inducing agent, the possibility of studying costs and benefits of priming has so far been overlooked. This lack of data prompted us to determine the costs and benefits of priming in *Arabidopsis* and compare those to the costs and benefits of induced direct defense. By using BABA as a chemical inducer of priming and a constitutive priming mutant of *Arabidopsis*, we compared fitness parameters in the absence and presence of pathogen attack. We demonstrate that priming involves considerably fewer costs than induction of direct defense. In addition, we demonstrate that the benefits of priming outweigh the costs when infection by pathogens occurs.

Results

Effectiveness of Chemically Induced Priming and Direct Defense. To compare the effectiveness of priming and direct defense, 3- and 6-week-old *Arabidopsis* plants (Col-0) were treated with increasing concentrations of BABA or benzothiadiazole (BTH). Two days later, the 3-week-old plants were mock or challenge inoculated with *Hyaloperonospora parasitica*, whereas the 6-week-old plants were mock-inoculated or challenge-inoculated with *P. syringae*. To determine the level of priming and/or direct defense, leaves were
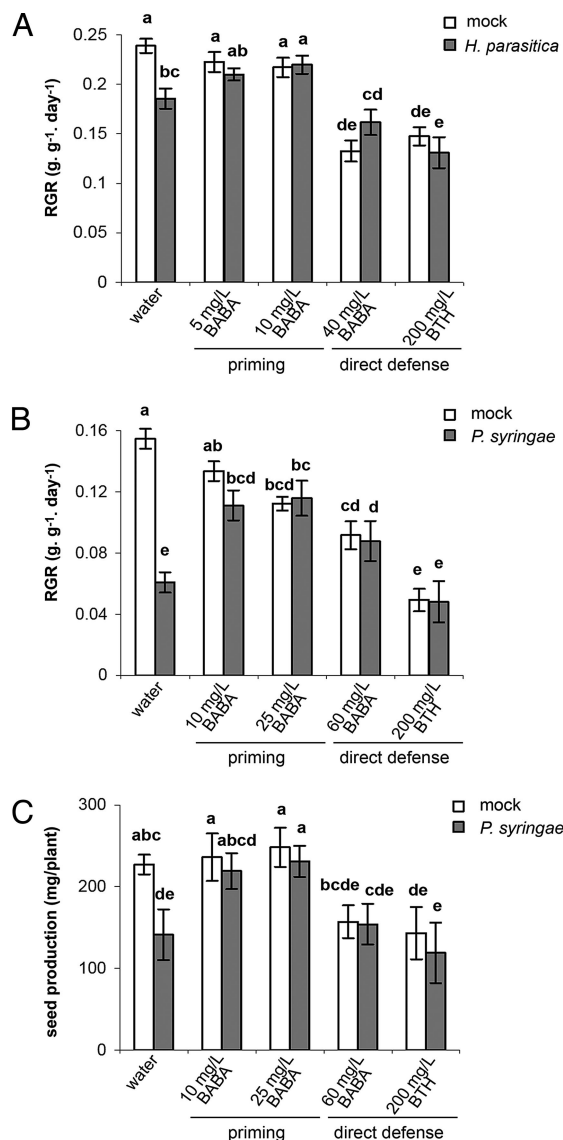
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Fig. 2. Costs and benefits of chemically induced priming and direct defense in the absence and presence of *H. parasitica* (*A*) or *P. syringae* (*B* and *C*). Plants were treated as described in the legend of Fig. 1. (*A*) RGR of mock- and *H. parasitica*-inoculated plants (3–4 weeks old) over the 12-day period from chemical treatment. Shown are mean values ± SEM (*n* = 8–12). (*B*) RGR of mock- and *P. syringae*-inoculated plants (6–7 weeks old) over the 12-day period from chemical treatment. (*C*) Seed production of mock- and *P. syringae*-inoculated plants. Shown are mean values ± SEM (*n* = 8–12) of the seed weight per plant. Different letters indicate statistically significant differences (least significant difference test; *α* = 0.05). All experiments shown were repeated with comparable results.

collected at different time points after inoculation for quantification of *PR-1* gene activation and callose deposition. In 3-week-old plants, treatment with 5 and 10 mg/liter BABA did not activate the *PR-1* gene, whereas treatment with 40 mg/liter BABA and 200 mg/liter BTH triggered *PR-1* expression directly (Fig. 1*A*). After challenge with *H. parasitica*, *PR-1* was induced transiently in water-treated control plants, reaching a maximum at 96 h after inoculation. Treatment with 5 mg/liter BABA did not accelerate this pathogen-induced activation of *PR-1*. However, treatment with 10 mg/liter BABA conferred enhanced levels of *PR-1* expression at 44 and 72 h after inoculation, indicating that this treatment primed *Arabidopsis* for SA-inducible defenses. All treatments with BABA primed *Arabidopsis* for callose deposition, as evidenced by enhanced num-
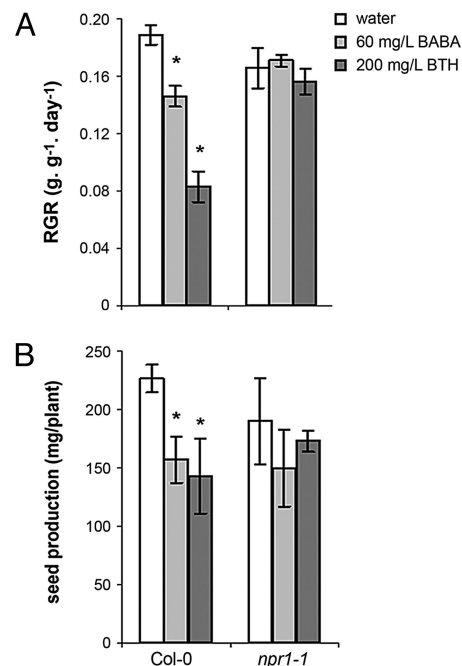

Fig. 3. Effects of direct defense-inducing amounts of BABA and BTH on RGR (*A*) and seed production (*B*) in Col-0 and *npr1-1*. Six-week-old plants were soil-drenched with water or 60 mg/liter BABA or were sprayed with 200 mg/liter BTH. See the legend of Fig. 2 for further details. Asterisks indicate statistically significant differences compared with the water control (*α* = 0.05, Student's *t* test).

bers of callose-rich papillae at the sites of spore germination (Fig. 1*B*). Resistance against *H. parasitica* was quantified by determining disease symptom severity and pathogen colonization at 8 days after inoculation. Compared with water-treated control plants, all treatments with BABA and BTH strongly reduced colonization by *H. parasitica* and disease severity (Fig. 1*C*). Apparently, BABA-induced priming is equally effective against *H. parasitica* as induction of direct defense by BABA or BTH. In 6-week-old *Arabidopsis*, treatment with 10 or 25 mg/liter BABA did not induce *PR-1* expression directly, but it did prime plants for earlier and stronger *PR-1* expression upon *P. syringae* infection (Fig. 1*D*). Treatment with BTH induced *PR-1* directly in noninfected plants of this age. In addition, 60 mg/liter BABA induced *PR-1* expression in non-infected plants, although it did so much later and to a lower extent than BTH (Fig. 1*D*). Induced resistance against *P. syringae* was quantified by determining the percentage of diseased leaves and the extent of bacterial proliferation in the leaves at 3 days after inoculation. Compared with control plants, priming-inducing concentrations of BABA significantly reduced the level of disease and bacterial growth (Fig. 1 *E* and *F*). Activation of direct defense with 60 mg/liter BABA or 200 mg/liter BTH induced only slightly higher levels of resistance, indicating that priming is almost as effective against *P. syringae* as induction of direct defense.

Costs and Benefits of Chemically Induced Priming and Direct Defense.
Costs and benefits of BABA- and BTH-induced resistance on plant growth were analyzed by quantifying the relative growth rate (RGR). In 3- to 4-week-old plants, induction of priming by 5 or 10 mg/liter BABA did not lead to statistically significant reductions in RGR in the noninfected plants (Fig. 2 *A* and *B*). In contrast, the direct defense-inducing treatments, 40 mg/liter BABA and 200 mg/liter BTH, reduced RGR by 44% and 39%, respectively (Fig. 2*A*). In 6- to 7-week old plants, induction of priming by 10 mg/liter BABA had no significant effect on RGR, whereas 25 mg/liter BABA resulted in a 27% reduction of RGR (Fig. 2*B*). The direct
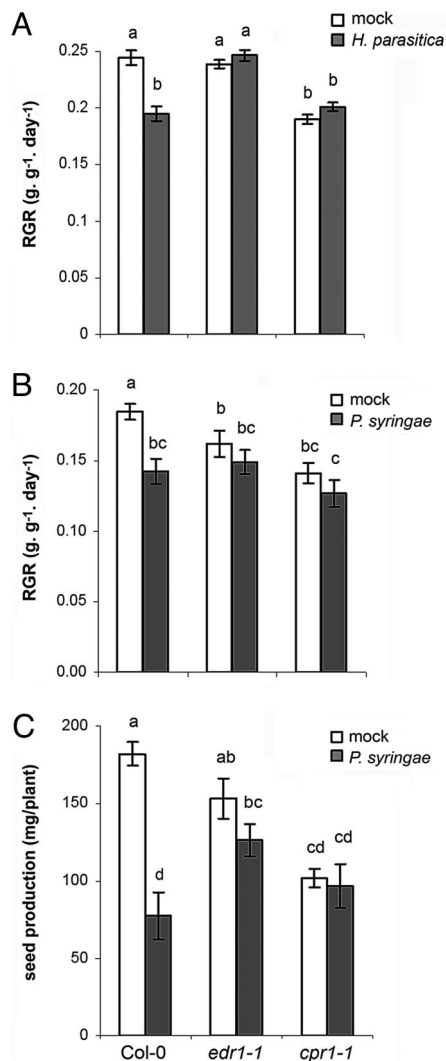
Fig. 4. Priming in the *edr1-1* mutant and constitutive direct defense in the *cpr1-1* mutant against *H. parasitica* (*A–C*) and *P. syringae* (*D–F*). (*A*) *PR-1* gene expression in 3-week-old plants at different time points after inoculation. hpi, hours postinoculation. (*B*) Callose deposition at 2 days after challenge with *H. parasitica*. (*C*) Induced resistance against *H. parasitica* 8 days after inoculation. (*D*) *PR-1* gene expression in 6-week-old plants at different time points after inoculation. (*E*) Induced resistance against *P. syringae*. (*F*) Growth of *P. syringae* over a 3-day time interval. See the legend of Fig. 1 for details.

defense-inducing treatments 60 mg/liter BABA and 200 mg/liter BTH caused significantly stronger reductions in RGR (41% and 71%, respectively). Hence, induction of direct defense involves higher costs on plant growth than induction of priming. Upon inoculation with *H. parasitica*, control plants were significantly reduced in RGR in comparison with noninfected control plants (24%) (Fig. 2*A*). Infection with *P. syringae* caused an even stronger reduction in RGR (62%) (Fig. 2*B*). These disease-related costs were absent in all BABA- and BTH-treated plants (Fig. 2*A* and *B*). Interestingly, of all pathogen-inoculated plants, the primed plants displayed the highest growth rates (Fig. 2*A* and *B*), which indicates that priming yields optimal levels of plant growth under conditions of disease pressure.

To further examine the impact of priming and direct defense on plant fitness, seed production was determined in mock- and *P. syringae*-inoculated plants. In mock-inoculated plants, the priming-inducing treatments did not affect seed production, whereas the direct defense-inducing treatments, 60 mg/liter BABA and 200 mg/liter BTH, reduced seed production by 31% and 37%, respectively (Fig. 2*C*). Inoculation of control plants with *P. syringae* reduced seed production by 38% (Fig. 2*C*). In contrast, inoculation with *P. syringae* had no significant effect on seed production in plants treated with priming- or direct defense-inducing concentrations of BABA and BTH. Most importantly, the primed plants produced the highest amount of seeds of all *P. syringae*-inoculated plants. Hence, priming provides enhanced fitness under conditions of disease pressure.

Fitness Reduction by BABA and BTH Is Caused by NPR1-Dependent Defenses. To exclude the possibility that the fitness-reducing effects of high concentrations of BABA and BTH are caused by direct

phytotoxicity of the chemicals, RGR and seed production were quantified in *npr1-1* plants, which are unable to express SA-dependent defenses (27). In contrast with wild-type plants, *npr1-1* was not affected in growth or seed production after treatment with 60 mg/liter BABA or 200 mg/liter BTH (Fig. 3), indicating that the BABA- and BTH-induced reductions in plant fitness are not caused by direct phytotoxicity but are due to costs of NPR1-dependent resistance mechanisms.

Effectiveness of *edr1*-Induced Priming and *cpr1*-Induced Defense. The *edr1-1* mutant has been described as having an enhanced response to infection by biotrophic pathogens (28) and could be regarded as a constitutive priming mutant. In contrast, the *cpr1-1* mutant displays constitutive expression of SA-inducible defenses (29). To confirm these phenotypes in our bioassay systems, both mutants were tested for *PR-1* expression, papillae formation, and resistance against *H. parasitica* and *P. syringae*. Noninfected *edr1* plants did not express elevated levels of *PR-1* gene expression (Fig. 4*A*). However, after inoculation with either *H. parasitica* or *P. syringae*, *edr1* activated this marker gene faster and/or stronger than wild-type plants (Fig. 4 *A* and *D*), confirming that *edr1* is constitutively primed for SA-inducible defenses. In addition, *edr1* deposited more callose-rich papillae at 2 days after inoculation with *H. parasitica* than did wild-type and *cpr1* plants (Fig. 4*B*). These findings indicate that *edr1* is also constitutively primed for enhanced callose deposition. The *cpr1* mutant showed enhanced *PR-1* expression in the absence of a pathogen (Fig. 4 *A* and *D*), confirming its constitutive defense phenotype. Upon infection with *P. syringae* or *H. parasitica*, *cpr1* and *edr1* displayed enhanced levels of resistance compared with wild-type plants (Fig. 4 *C*, *E*, and *F*). However,

Fig. 5. Costs and benefits of *edr1*-induced priming and *cpr1*-induced defense in the absence and presence of *H. parasitica* (*A* and *B*) and *P. syringae* (*C*). (*A*) RGR in 3- to 4-week-old plants over the 10-day period after mock or challenge inoculation with *H. parasitica*. Shown are mean RGR values ± SEM (*n* = 8–12). (*B*) RGR in 6- to 7-week-old plants over the 12-day period after mock or challenge inoculation with *P. syringae*. (*C*) Seed production by mock- and *P. syringae*-inoculated plants. Shown are mean values ± SEM (*n* = 8–12) of the seed weight per plant. See the legend of Fig. 2 for further details.

the level of resistance against both pathogens was consistently higher in *cpr1* than in *edr1*.

Costs and Benefits of *edr1*-Induced Priming and *cpr1*-Induced Direct Defense. Costs of *edr1*-induced priming and *cpr1*-induced direct defense were quantified by RGR and seed production. Noninfected, 3- to 4-week-old *edr1* plants showed similar growth rates as wild-type plants (Fig. 5*A*), whereas 6- to 7-week-old *edr1* plants grew significantly slower than the wild-type (Fig. 5*B*), indicating that *edr1*-inducing priming involves costs on plant growth only at the relatively old age of a plant. The *edr1* mutant did not show a significant reduction in seed production (Fig. 5*C*). Compared with the wild type, *cpr1* displayed strongly reduced RGR, irrespective of its age, and produced significantly less seeds than wild-type and *edr1* plants (Fig. 5). After inoculation with *H. parasitica* or *P. syringae*, wild-type plants were significantly reduced in RGR compared with noninfected plants (Fig. 5 *A* and *B*). Inoculation with *P. syringae* also decreased seed production in wild-type plants (Fig. 5*C*). These

disease-related effects were considerably less pronounced in the more resistant *edr1* and *cpr1* plants (Fig. 5). Upon infection by *H. parasitica*, *edr1* showed a higher RGR than wild-type and *cpr1* plants (Fig. 5*A*). This effect was not evident among the *P. syringae*-infected plants (Fig. 5*B*). However, seed production upon infection with *P. syringae* was significantly higher in *edr1* than in wild-type plants, whereas that of *cpr1* did not significantly differ from the wild-type (Fig. 5*C*). Hence, *edr1*-induced priming yields a higher level of fitness than *cpr1*-induced defense upon infection by *H. parasitica* or *P. syringae*.

Discussion

In this study, we have shown that priming by BABA or the *edr1* mutation is associated with only marginal reductions in growth, whereas induction of direct defense by high concentrations of BABA, BTH, or the *cpr1* mutation, causes much stronger reductions in plant growth and even reduced seed production (Figs. 2 and 5). Thus, induction of priming involves fewer costs than direct defense. Despite the fact that chemically and *cpr1*-induced direct defense yielded high levels of resistance (Figs. 1 and 4), the costs on plant growth and seed production were in the same order of magnitude as the disease-related costs in noninduced plants (Figs. 2 and 5). Therefore, it can be concluded that the costs of induced direct defense are not outweighed by the benefits of the enhanced protection. In contrast, primed plants were almost equally resistant to *P. syringae* and *H. parasitica* but suffered from considerably fewer fitness costs. Moreover, after pathogen infection, primed plants displayed higher levels of fitness than noninduced plants and plants expressing direct defense, which strongly indicates that the benefits of induced resistance through priming outweigh the costs under conditions of disease pressure.

An important challenge for the future will be to assess the role of priming under field conditions. However, at this stage, there are no clear genetic, molecular, or physiological markers available to quantify the state of priming. Therefore, the only way to experimentally determine priming is by measuring the speed and intensity by which defense is activated after disease exposure. Such experiments require two conditions: (*i*) no disease pressure before the measurements of defense and (*ii*) sufficient disease pressure during the measurements of defense. Unfortunately, both conditions are very difficult to control under field conditions.

Interestingly, the effects of the *edr1* mutation on plant growth were age-dependent: 3- to 4-week-old *edr1* plants displayed similar growth rates as wild-type plants (Fig. 5*A*), whereas 6- to 7-week-old *edr1* plants were significantly reduced in growth compared with wild-type plants (Fig. 5*B*). Hence, costs of *edr1*-induced priming are only apparent in relatively old plants. This age-dependency might be explained by the enhanced senescence phenotype of the *edr1* mutant (30).

Because the defense arsenal in primed plants remains dormant until pathogen infection, priming does not confer major fitness costs under pathogen-free conditions (Figs. 2 and 5). But even upon infection, the augmented defense expression in primed plants remains mostly localized at the sites of pathogen attack. For instance, BABA-induced priming leads to augmented callose deposition only in cells that were in contact with the invading pathogen (15, 16). Hence, priming provides the plant with a timely and spatially efficient strategy to deploy its defensive mechanisms. Yet, priming caused low but statistically significant reductions in plant fitness (Figs. 2 and 5). These priming-related costs could be explained by the observations that *Arabidopsis* shows enhanced expression of signaling-related genes upon induction of priming (ref. 31 and J.T. and S. van der Ent, unpublished results). Because the defense is not activated before pathogen attack, it is assumed that the corresponding signal transduction pathways remain inactive until the plant is

exposed to pathogen attack. Nevertheless, enhanced expression of inactive signaling proteins could already involve small allocation costs. Hence, only if the environment imposes relatively high levels of disease pressure will the plant benefit from priming.

In this study, we have shown that induced resistance through induction of active defense is not an optimal strategy for disease protection, because the associated costs outweigh the benefits of protection. Conversely, induced resistance by means of priming offers an efficient form of plant protection with significant benefits under conditions of disease occurrence. Hence, knowledge about the molecular mechanisms underlying priming can be instrumental in the development of new concepts for disease control, because it provides broad-spectrum resistance without major trade-offs on growth and fruit and seed set. Our data show that priming has significant effects on plant growth and seed production that depend on the extent of disease pressure. Given the fact that growth and seed set are crucially important for the ecological performance of plants, it is plausible that priming plays an important role in nature. Hence, investigating the evolutionary and ecological function of priming will be an exciting challenge for future research.

Materials and Methods

Plants and Pathogens. *Arabidopsis* Col-0 mutants *npr1-1* (27), *cpr1-1* (29), and *edr1-1* (28) were kindly provided by X. Dong (Duke University, Durham, NC) and R. Innes (Indiana University, Bloomington, IN), respectively. Seedlings were grown in sand for 2 weeks and subsequently transferred to 60-ml pots containing a potting soil–sand (12:5) mixture as described in ref. 13. Plants were cultivated in a growth chamber with a 9-h day (24°C) and 15-h (20°C) night cycle at 60–70% relative humidity. *P. syringae* pv. *tomato* strain DC3000 (32) and *H. parasitica* strain WACO9 were cultured as described in ref. 11.

RNA Extraction and Blotting. For RNA extraction, leaves from 4–10 plants were collected. RNA extraction, RNA blotting, and labeling of the *PR-1* probe was performed as described in ref. 33. Equal loading was verified by ethidium bromide staining of the gel.

P. syringae Bioassays. For the chemically induced resistance assays, 6-week-old plants were soil-drenched with water (control) or a BABA solution. BTH was administered by spraying the leaves with a BTH solution containing wettable powder. Two days later, plants were inoculated by dipping the leaves in a suspension of *P. syringae*, as described in ref. 17. Mock inoculation was performed by dipping the leaves into a similar solution without bacteria. Three days after

inoculation, the percentage of leaves with symptoms was determined per plant ($n = 15$–20). Leaves showing water-soaked lesions surrounded by chlorosis were scored as diseased. Bacterial proliferation over a 3-day time interval was determined as described in ref. 17. Plant material for RGR analysis was collected at the day of chemical treatment and 12 days later. During the experiments with *edr1* and *cpr1*, material was collected 1 day before pathogen inoculation and 12 days later. Plants started to flower in the period of RGR analysis after inoculation, enabling disease progression into the flower stalk.

H. parasitica Bioassays. For the chemically induced resistance assays, BABA and BTH were applied to 3-week-old plants as described above. Two days later, *H. parasitica* inoculation was performed by spraying the leaves with 10 mM $MgSO_4$ containing 5×10^4 sporangia per milliliter. To ensure infection, plants were put at 17°C and kept at 100% relative humidity for 24 h after inoculation. At 6 days after inoculation, plants were returned to 100% relative humidity to trigger sporulation. Disease symptoms were scored in 40 plants per treatment ($\approx$200 leaves) at 8 days after inoculation. Disease rating was expressed as severity of disease on each leaf: I, no sporulation; II, trailing necrosis; III, <50% of the leaf area covered by sporangia; IV, >50% of the leaf area covered with sporangia, with additional chlorosis. Leaves from five plants were stained with lactophenol trypan blue and examined microscopically (34). Callose staining was performed as described in ref. 17. Callose deposition was quantified as the proportion of the attempted penetration sites per leaf with callose depositions. For RGR analysis, plant material was collected at the day of chemical treatment and 10 days later. During the experiments with *edr1* and *cpr1*, plant material was collected at 1 day before pathogen inoculation and 10 days later.

Fitness Parameters. RGR ($g \cdot g^{-1} \cdot day^{-1}$) was calculated according to RGR $= (\ln W_2 - \ln W_1)/t_2 - t_1$, where W_1 and W_2 is plant dry weight at time points t_1 and t_2, respectively. At each time point, rosette dry weights from 8–12 randomly collected plants were determined (dried for 48 h at 70°C). After time point t_2, plants were transferred to a 16-h day/8-h night cycle. Seeds from 8–12 plants per treatment were collected in Aracon tubes (Beta Developments, Gent, Belgium). Plants were watered regularly until they had fully senesced. Seed production was expressed as the average seed weight per plant.

1. Heil, M. (2002) *Curr. Opin. Plant. Biol.* **5,** 345–350.
2. Agrawal, A. A., Strauss, S. Y. & Stout, M. J. (1999) *Evolution* **53,** 1093–1104.
3. Baldwin, I. T. (1998) *Proc. Natl. Acad. Sci. USA* **95,** 8113–8118.
4. Van Dam, N. M. & Baldwin, I. T. (2001) *Funct. Ecol.* **15,** 406–415.
5. Agrawal, A. A. (1998) *Science* **279,** 1201–1202.
6. Heil, M., Hilper, A., Kaiser, W. & Linsenmair, K. E. (2000) *J. Ecol.* **88,** 645–654.
7. Cipollini, D. F. (2002) *Oecologia* **131,** 514–520.
8. Heidel, A. J., Clarke, J. D., Antonovics, J. & Dong, X. (2004) *Genetics* **168,** 2197–2206.
9. Ryals, J. A., Neuenschwander, U. H., Willits, M. G., Molina, A., Steiner, H.-Y. & Hunt, M. D. (1996) *Plant Cell* **8,** 1808–1819.
10. Dong, X. N. (2004) *Curr. Opin. Plant Biol.* **7,** 547–552.
11. Ton, J., Van Pelt, J. A., Van Loon, L. C. & Pieterse, C. M. J. (2002) *Mol. Plant–Microbe Interact.* **15,** 27–34.
12. Van Loon, L. C., Bakker, P. A. & Pieterse, C. M. J. (1998) *Annu. Rev. Phytopathol.* **36,** 453–483.
13. Pieterse, C. M. J., Van Wees, S. C. M., Van Pelt, J. A., Knoester, M., Laan, R., Gerrits, H., Weisbeek, P. J. & Van Loon, L. C. (1998) *Plant Cell* **10,** 1571–1580.
14. Jakab, G., Cottier, V., Toquin, V., Rigoli, G., Zimmerli, L., Metraux, J.-P. & Mauch-Mani, B. (2001) *Eur. J. Plant Pathol.* **107,** 29–37.
15. Zimmerli, L., Jakab, G., Métraux, J.-P. & Mauch-Mani, B. (2000) *Proc. Natl. Acad. Sci. USA* **97,** 12920–12925.
16. Ton, J. & Mauch-Mani, B. (2004) *Plant J.* **38,** 119–130.
17. Ton, J., Jakab, G., Toquin, V., Flors, V., Iavicoli, A., Maeder, M. N., Metraux, J.-P. & Mauch-Mani, B. (2005) *Plant Cell* **17,** 987–999.
18. Cohen, Y. R. (2002) *Plant Dis.* **86,** 448–457.
19. Jakab, G., Ton, J., Flors, V., Zimmerli, L., Metraux, J.-P. & Mauch-Mani, B. (2005) *Plant Physiol.* **139,** 267–274.
20. Zimmerli, L., Métraux, J.-P. & Mauch-Mani, B. (2001) *Plant Physiol.* **126,** 517–523.
21. Van Loon, L. C. (1997) *Eur. J. Plant Pathol.* **103,** 753–765.
22. Conrath, U., Pieterse, C. M. J. & Mauch-Mani, B. (2002) *Trends Plant Sci.* **7,** 210–216.
23. Kauss, H., Theisinger-Hinkel, E., Mindermann, R. & Conrath, U. (1992) *Plant J.* **2,** 655–660.
24. Kohler, A., Schwindling, S. & Conrath, U. (2002) *Plant Physiol.* **128,** 1046–1056.
25. Van Wees, S. C. M., Luijendijk, M., Smoorenburg, I., Van Loon, L. C. & Pieterse, C. M. J. (1999) *Plant Mol. Biol.* **41,** 537–549.
26. Verhagen, B. W. M., Glazebrook, J., Zhu, T., Chang, H.-S., Van Loon, L. C. & Pieterse, C. M. J. (2004) *Mol. Plant–Microbe Interact.* **17,** 895–908.
27. Cao, H., Bowling, S. A., Gordon, A. S. & Dong, X. (1994) *Plant Cell* **6,** 1583–1592.
28. Frye, C. A. & Innes, R. W. (1998) *Plant Cell* **10,** 947–956.
29. Bowling, S. A., Guo, A., Cao, H., Gordon, A. S., Klessig, D. F. & Dong, X. (1994) *Plant Cell* **6,** 1845–1857.
30. Tang, D., Christiansen, K. M. & Innes, R. W. (2005) *Plant Physiol.* **138,** 1018–1026.
31. Maleck, K., Levine, A., Eulgem, T., Morgan, A., Schmid, J., Lawton, K. A., Dangl, J. L. & Dietrich, R. A. (2000) *Nat. Genet.* **26,** 403–410.
32. Whalen, M. C., Innes, R. W., Bent, A. F. & Staskawicz, B. J. (1991) *Plant Cell* **3,** 49–59.
33. Ton, J., De Vos, M., Robben, C., Buchala, A., Métraux, J.-P., Van Loon, L. C. & Pieterse, C. M. J. (2002) *Plant J.* **29,** 11–21.
34. Koch, E. & Slusarenko, A. (1990) *Plant Cell* **2,** 437–445.

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