

Fitness Benefits of Systemic Acquired Resistance During *Hyaloperonospora parasitica* Infection in *Arabidopsis thaliana*

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ABSTRACT

We investigated the fitness benefits of systemic acquired resistance (SAR) in *Arabidopsis thaliana* using a mutational and transformational genetic approach. Genetic lines were designed to differ in the genes determining resistance signaling in a common genetic background. Two mutant lines (*cpr1* and *cpr5*) constitutively activate SAR at different points in SAR signaling, and one mutant line (*npr1*) has impaired SAR. The transgenic line (*NPRI-H*) has enhanced resistance when SAR is activated, but SAR is still inducible similarly to wild type. The fitness benefits were also investigated under two nutrient levels to test theories that preventing pathogen damage and realized resistance benefits may be affected by nutrient availability. Under low-nutrient conditions and treatment with the pathogenic oomycete, *Hyaloperonospora parasitica*, wild type had a higher fitness than the mutant that could not activate SAR, demonstrating that normal inducible SAR is beneficial in these conditions; this result, however, was not found under high-nutrient conditions. The mutants with constitutive SAR all failed to show a fitness benefit in comparison to wild type under a *H. parasitica* pathogen treatment, suggesting that SAR is induced to prevent an excessive fitness cost.

A resistance to a pathogen or herbivore is likely to persist only if the resistance benefits the organism by reducing the fitness loss caused by a pathogen or herbivore. Benefits are assumed in many cases to be balanced by the fitness cost incurred by expressing resistance in the absence of the pathogen/herbivore. While fitness costs of resistance have rarely been unambiguously found in natural populations, less effort has been devoted to finding benefits of resistance.

Benefits have been recorded in a few cases of herbivory resistance for both induced (AGRAWAL 1999; HEIL 2004) and constitutive resistances (MAURICIO and RAUSHER 1997), for herbicide resistance (BAUCOM and MAURICIO 2004), and for pathogen resistance (VAN HULTEN *et al.* 2006). In other cases, no fitness benefit has been found even though the resistance does reduce damage by herbivores (SIMMS and RAUSHER 1987; ELLE *et al.* 1999). In many previous studies of fitness costs, benefits have been presumed (KAKES 1989; BRYANT and JULKUNEN-TIITTO 1995), and it may be a reasonable assumption in most cases that a benefit exists under some environmental conditions, but it is still useful to know under what conditions there is a benefit. Benefits have been shown to depend on resource availability (ELLE *et al.* 1999), a logical result given that costs also

depend on resources (PURRINGTON and BERGELSON 1997; HEIL *et al.* 2000).

Induced resistance mechanisms are hypothesized to reduce costs and maximize benefits, assuming that such resistance mechanisms have no cost until induction (RHOADES 1979; KARBAN and BALDWIN 1997). Some plants have a mix of inducible and constitutive defenses in different parts of the plant with the constitutive defenses protecting the most valuable reproductive organs while the inducible defenses protect the more expendable roots and leaves (ZANGERL and RUTLEDGE 1996). Under an optimal scenario, the level of activation of induced defenses should match the predicted threat. However, induced resistances could also be unbeneficial if a low level of attack on a host stimulates a resistance pathway that is costly, providing excessive protection against a minor threat. The mechanisms of induction and signaling compounds have been extensively studied in multiple plant systems (FARMER *et al.* 1992; ALBRECHT *et al.* 1993; MOURA and RYAN 2001; LI *et al.* 2002); however, this information does not allow easy determination of the costs and benefits as there are many interacting signals leading to a complex response involving many genes (MALECK *et al.* 2000; HEIDEL and BALDWIN 2004).

Systemic acquired resistance (SAR) is an induced resistance that has been found (with some variation) in all angiosperms tested (HEIL 1999). SAR has been shown to have significant fitness costs in a variety of environmental conditions (HEIDEL *et al.* 2004). Even though the

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benefits of SAR against a known pathogen have not been measured beyond the first qualitative observation by Ross (1961), they have been found for the related primed resistance (VAN HULTEN *et al.* 2006). Pathogen-produced necrotic lesions induce SAR and lead to resistance against a wide spectrum of pathogens, including viruses, bacteria, fungi, and oomycetes (RYALS *et al.* 1996). The exact mechanism by which SAR is manifested and the range of pathogens that this resistance protects against are not fully understood. However, SAR is believed to be a result of the expression of a variety of defensive proteins and small defensive compounds. Individual components of some of the signaling pathways that lead to resistance to particular pathogens have been successfully identified. A group of proteins upregulated during SAR are known as the pathogenesis-related (PR) proteins and many are known to possess antibacterial or antifungal/oomycete properties (DATTA and MUTHUKRISHNAN 1999).

SAR is dependent on the production of salicylic acid and the level and activity of NPR1, a protein that interacts with transcription factors that regulate the expression of defense-related genes (PENNINCKX *et al.* 1996; ZHANG *et al.* 1999; FAN and DONG 2002; WANG *et al.* 2005). In *Arabidopsis thaliana*, SAR can be monitored with the *PR-1* gene as a molecular marker. Another inducible resistance mechanism is dependent on production of jasmonic acid and ethylene, involves the marker gene *PDF1.2*, and is effective against fungal/oomycete pathogens (PENNINCKX *et al.* 1996; CLARKE *et al.* 2000). Evidence has accumulated that there are interactions between these pathways whereby induction of one pathway may inactivate the other (DOARES *et al.* 1995a,b; FIDANTSEF *et al.* 1999; THALER *et al.* 1999).

In *A. thaliana*, a number of mutants have been isolated in the SAR pathway. The mutant *npr1* suppresses SAR against the bacteria *Pseudomonas syringae* and against the oomycete *Hyaloperonospora parasitica* (CAO *et al.* 1994; BOWLING *et al.* 1997), and overexpression of *NPR1* in the *NPR1-H* genotype leads to enhanced resistance to pathogen challenges (CAO *et al.* 1998). The mutations in *cpr1*, *cpr5*, and *cpr6* cause SAR to switch from inducible to constitutive resistance (BOWLING *et al.* 1994; BOWLING *et al.* 1997; CLARKE *et al.* 1998, respectively). Analysis of double mutants has determined how these mutations affect the pathways and how the effects are dependent on the signaling compounds salicylic acid and jasmonic acid (CLARKE *et al.* 1998, 2000).

To answer the question of resistance costs and benefits, transgenic and mutant organisms may be useful to discover specific effects of traits that would not be possible otherwise (TATAR 2000). Furthermore, it has been stated that benefits of resistance cannot be determined until genetic manipulation allows inhibiting defense so that plants can be attacked with and without resistance (STAMP 2003). Using the SAR mutants and one transgenic line (Table 1), we were able to

TABLE 1
SAR phenotypes

Genotype	Phenotype
Wild type	Normal wild-type SAR
<i>npr1</i>	Cannot induce SAR
<i>cpr1</i>	Constitutive SAR
<i>cpr5</i>	Constitutive SAR
<i>NPR1-H</i>	SAR is inducible, but stronger than wild type when induced

perform this experiment. Under a pathogen challenge, we compared the fitness of wild-type plants that have SAR against the fitness of *npr1* plants that have suppressed SAR. In addition, we tested the efficacy of induced defense *vs.* constitutive defense by comparing the fitness of wild-type plants to *cpr* plants while under pathogen challenge. These benefits are further tested under two nutrient levels to determine the impact of nutrient limitation on benefits.

MATERIALS AND METHODS

A. thaliana (Brassicaceae) is a short-lived selfing annual plant species found in agricultural fields and waste places throughout the world. The Col-0 ecotype (Landsberg, Germany) was used in this study, since the mutants used were originally isolated in this strain.

Mutant and transgenic genotypes: The *npr1*, *cpr1*, and *cpr5* mutants and the *NPR1-H* transgenic line have been previously described (HEIDEL *et al.* 2004), but their SAR phenotypes are summarized in Table 1.

Pathogen: The oomycete pathogen *H. parasitica* (formerly *Peronospora parasitica*) was the pathogen in this study. *H. parasitica* is a natural pathogen of *A. thaliana* in Great Britain (HOLUB *et al.* 1994) and in the United States where the infection rates have been measured to reach 54% of the plants in a population (HEIDEL 2002). The isolate Noco2 (Norwich, Norfolk, UK) was used since Col-0 carries no functional resistance gene against this isolate.

Experimental design: The five genotypes (wild type, *cpr1*, *cpr5*, *npr1*, and *NPR1-H*) were grown in all combinations of two nutrient treatments and with and without pathogen treatment. Plants ($n = 855$) were grown in growth chambers at the Duke University Phytotron in 18 flats, each consisting of an array of "cells," each 4.5×5.9 cm and 6.4 cm deep. Every flat was divided as equally as possible among the five genotypes with random placement of genotypes within each flat. Half of the flats received the nutrient supplement described below and half did not. Three or four seeds of one genotype were planted in each cell after 10 days at 4° in water. One week after planting, plants were randomly thinned to one per cell.

An autoclaved 1:1:1 mix of sand, gravel, and surface was used as the growth medium for all plants. In the high-nutrient treatment, each cell received 5 ml of half-strength Hoagland's solution weekly for the first 2 months of the experiment. In the low-nutrient treatment, each cell received 5 ml of distilled water at the same time. All plants were watered as needed at least twice a day during the experiment except during the pathogen treatment when watering was not needed.

Five flats in each nutrient treatment were given the pathogen treatment. For this treatment, 2 μ l of a spore

suspension of *H. parasitica* Noco2 was placed on each cotyledon of a seedling. This was done once a day for 3 consecutive days, starting 1 week after planting with spore concentrations of 5.2×10^4 /ml, 4.0×10^4 /ml, and 12.5×10^4 /ml, respectively, on those 3 days. These were the highest concentrations achievable following dilution of the original spore sample gathered that day from stock material. The other four flats in each nutrient treatment had 2 μ l of distilled water placed on each cotyledon at the same time at which the other flats were infected. One week after the last inoculation, plants were assayed for disease when the disease phenotype was fully developed. The disease rating was determined by the coverage of conidiospores on the two cotyledons, which were the only leaves present on most plants. The disease rating scale was the following: 0 for no spores, 1 for spores covering <50% of one leaf, 2 for spores covering >50% of one leaf, 3 for spores covering <50% of both leaves, 4 for spores covering >50% of one leaf and >50% of the other leaf, and 5 for spores covering >50% of both leaves. This scale was used for all statistical analyses, but categories 2, 3, and 4 contained few individuals so categories 2, 3, and 4 are joined with category 1 in the figures.

H. parasitica requires high humidity, usually >95% relative humidity, to sporulate and therefore at the start of the experiment the flats had transparent plastic domes over them to make the conditions permissible for the pathogen. Additionally, the domes prevented the spread of conidiospores from infected flats to uninfected flats. The domes raised the humidity level to ~100%. The temperature inside the domes was kept at ~16° during the day and 14° during the night by maintaining the temperature in the growth chamber at 9° during the day and 14° during the night. At 2.5 weeks after planting, after the disease rating was measured, the plastic domes were removed and the temperature in the chamber was changed to 22° in the day and 14° in the night with 60% relative humidity to prevent further sporulation and spread of the pathogen. Additionally, all plants were sprayed thoroughly with distilled water when the domes were removed, washing spores off infected plants. No further infections or sporulation was seen once spores were washed off and the humidity lowered. Throughout, the light/dark cycle was 14 hr/10 hr with a light level of 450 mE/sec/m². Flat locations were randomized within the chamber every week and, starting at 1 month, cells were randomized between flats within nutrient treatments.

At 97 days after planting, all plants had senesced, and seed yield was determined for each plant. The total number of siliques was counted on the main stem and side shoots, respectively. The number of seeds per silique was determined separately for the main and side stems by counting the number of seeds in every fifth intact silique for the high-nutrient treatment and by counting the number of seeds in every intact silique for the low-nutrient treatment. The number of seeds for the main stem and side shoots was determined by multiplying the average seeds per silique by the number of siliques. The seeds in the main stem and side shoots were then added together to give the total number of seeds per plant. Nine plants were not included in seed yield results because they had lost too many seeds from their siliques to make accurate estimations of their seed yield.

RNA analysis: One leaf was removed from each plant in the experiment at day 45. All leaves of the same genotype within a flat (8–9 plants/genotype/flat if all plants survived) were bulked together for further analysis. RNA was extracted and probed as described by CLARKE *et al.* (1998). Briefly, tissue was frozen in liquid N₂ and ground to a powder, and then RNA was extracted with a LiCl buffer. RNA was precipitated with sodium acetate and ethanol and run on a 1.2% agarose gel with formaldehyde. RNA was transferred overnight to a cellulose

membrane, crosslinked by UV, and then probed with the appropriate probe containing radiolabeled phosphorus. Probes were used for *PR-1*, *PDF1.2*, and *UBQ5*. *UBQ5* (an ubiquitin gene) expression was used as a control since *UBQ5* concentration is constant between various treatments and normalization by *UBQ5* adjusts for different total amounts of RNA loaded on the gel. Expression of mRNA was quantified using ImageQuant software and sequentially normalized by *UBQ5* expression and then by the mean wild-type expression over all treatments. Normalization by wild-type expression reduces the blot effect and results in more accurate estimation of relative expression between genotypes.

Statistical analysis: The uninfected plants in this study have been previously analyzed separately (HEIDEL *et al.* 2004) and are used here only in comparison to the infected plants. Disease rating results were analyzed by logistic regression with genotype and nutrient treatment as fixed effects, and “flat” as a random effect nested within the nutrient treatments and disease rating as an ordinal variable.

The seed yield results were analyzed by analysis of variance (ANOVA), with genotypes, nutrient treatments, and infection treatments as fixed effects (flats nested within nutrient treatments and infection treatments) and flat as a random effect. There were significant differences in mortality between genotypes, so in the measure of fitness, all plants that died before reproduction were given a seed yield of 0. Seed yield data were transformed by various methods (see table legends) to conform to the assumptions of ANOVA. Although the flats are not classical “blocks” since the cells were later randomized between them, the original flat for each cell was found to have a significant effect. Therefore, the original flat for each plant was included in ANOVAs.

PR gene expression results were analyzed by ANOVA with genotype, nutrient treatments, and infection treatments as fixed effects and the blot as a random effect. The blot (particular cellulose membrane used in the RNA hybridization) was treated as an effect because the probe may hybridize and be washed off at different levels between blots. Moreover, different amounts of probe on blots will cause different levels of RNA to be detected between blots even if identical amounts of RNA are on the blots. Expression data were transformed (see table legend) to conform to the assumptions of ANOVA.

In all ANOVAs, all possible interaction terms were tested and if they were not significant, they were removed from the analysis. After ANOVAs, contrast tests between wild type and all other genotypes were performed as orthogonal planned comparisons. All other contrasts were performed as Tukey–Kramer tests. The data used in all figures and the sample sizes for each category are given in supplemental Table 1 at <http://www.genetics.org/supplemental/>. All statistical analyses were performed using JMP 4 (SAS Institute).

RESULTS

Disease rating of wild-type and SAR mutants: A comparison of the disease ratings demonstrated that both the genotype and the nutrient treatment had significant effects (Table 2 and Figure 1). There was a significantly higher disease rating for plants in the low-nutrient treatment ($n = 236$) compared to the high-nutrient treatment ($n = 228$). The absence of a significant genotype by nutrient interaction indicated that a lack of nutrients generally weakened resistance instead of affecting one genotype more than others. The genotypes expressing SAR constitutively (*cpr1* and

TABLE 2

Effect of genotype and nutrients on disease rating: effect tests

Source	d.f.	χ^2
Genotype	4	18.44**
Flat (nutrient)	8	14.62
Nutrient	1	3.95*

*Significant differences at $P < 0.05$; **significance at $P < 0.01$.

cpr5) had (1) significantly lower disease ratings, (2) a higher percentage of plants with a disease rating of 0, and (3) a lower percentage of plants with a disease rating of 5 than other genotypes. All three points suggest that *cpr1* and *cpr5* acted as expected and had higher resistance than wild type. However, there was no difference between the disease rating of wild type and the *npr1* or *NPRI-H* genotypes.

The *cpr* mutants were initially identified by their expression of resistance in experiments conducted under lower light levels and using a different soil mix than used in this study. Therefore, it was possible that the expression of resistance would be seen only under the original environmental conditions. The lowered disease rating found here for *cpr* mutants (Table 2; Figure 1) demonstrates that their resistance is expressed in more than one environment.

Seed yield of wild-type and SAR mutants: Genotype, flat, nutrient treatment, infection treatment, and genotype \times nutrient all had significant effects on seed yield, but the genotype \times infection interaction was not significant (Table 3). The infection treatment lowered fitness as expected (Figure 2).

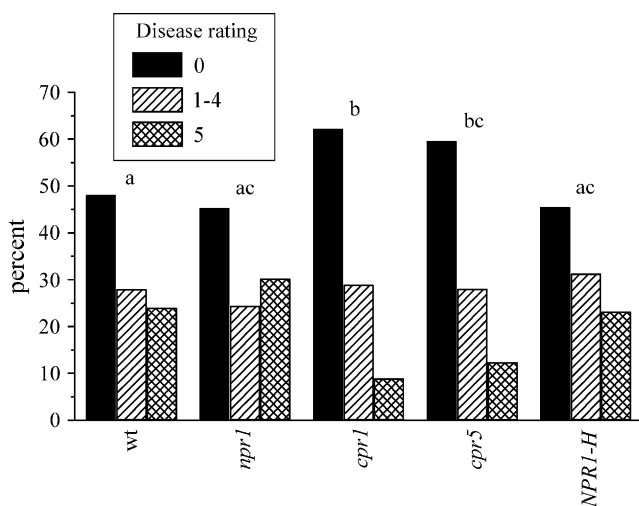


FIGURE 1.—Disease rating across both nutrient treatments. Disease ratings 1–4 were grouped together (bars with diagonals). Letters indicate significant differences in disease rating among genotypes (i.e., “a” is significantly different from “b,” but is not significantly different from “ac”). wt, wild type.

TABLE 3

Effect of genotype, nutrient, and infection on fitness: effect tests

Source	d.f.	SS	F-value
Genotype	4	2963	35.1***
Nutrient	1	648	30.3***
Infection	1	104	4.9*
Flat (nutrient, infection)	15	2979	9.4***
Genotype \times nutrient	4	885	10.5***

ANOVA of effects on fitness with genotype, nutrient, and infection as fixed effects and “flat” as a random effect. Seed-yield data were cube root transformed to conform to assumptions of ANOVA. SS, sum of squares. *Significant F-values at $P < 0.05$; ***significance at $P < 0.001$.

Within infected plants in the high-nutrient treatment, both *cpr1* and *cpr5* had lower fitness than wild type (Figure 2A; Table 4). Within infected plants in the low-nutrient treatment, *cpr1* had lower fitness than both wild type and *NPRI-H*, and *npr1* had lower fitness than wild type (Figure 2B; Table 4). Disease severity as measured by disease rating had a significant effect on

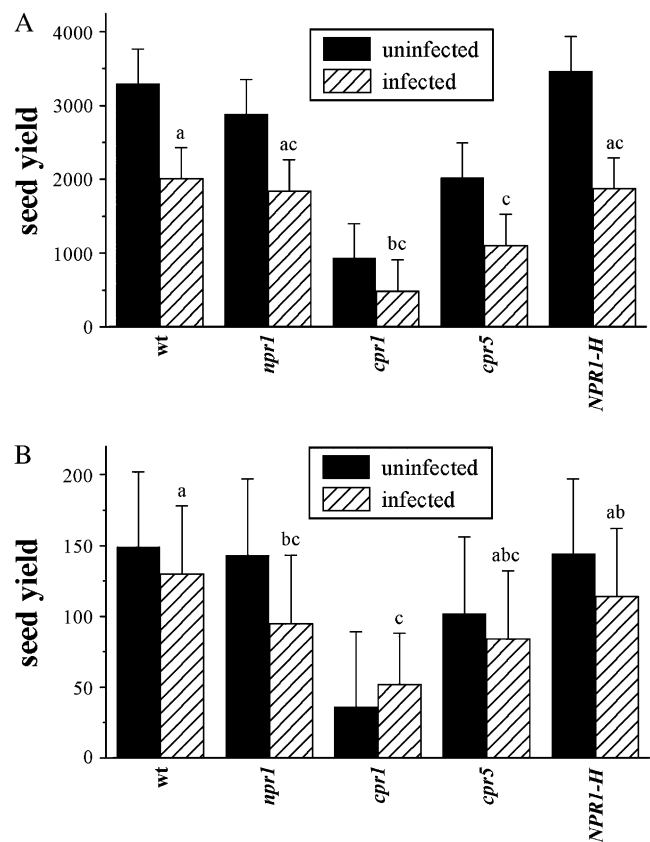


FIGURE 2.—Fitness of genotypes. Mean seed yield values are shown with \pm SE. Letters indicate significantly different values within the infected treatment. (A) Seed yield under high-nutrient treatment. (B) Seed yield under low-nutrient treatment.

TABLE 4
Effect of genotype within infected plants: effects tests

Source	Seed yield in high-nutrient treatment			Seed yield in low-nutrient treatment		
	d.f.	SS	F-value	d.f.	SS	F-value
Genotype	4	8.17×10^7	10.8***	4	608	4.3**
Flat	5	1.53×10^8	20.3***	4	4268	30.5***

Seed-yield data were not transformed in the high-nutrient treatment and were square root transformed in the low-nutrient treatment. SS, sum of squares. **Significant F-values at $P < 0.01$; ***significance at $P < 0.001$.

fitness (Table 5; Figure 3), indicating the logical negative correlation between increased disease symptoms and lower fitness. In a comparison of fitness of different disease rating categories, plants with a disease rating of 0 had significantly higher seed yield than plants with a disease rating of 3, 4, or 5 by Tukey–Kramer, and there were no other significant differences.

PR-1 and PDF1.2 expression: Genotype, nutrient treatment, blot, and genotype \times nutrient treatment had significant effects on *PR-1* expression with higher expression in the low-nutrient treatment (Table 6; Figure 4). Infection treatment had no significant effect on *PR-1* expression. The effect of genotype was largely caused by *cpr1* and *cpr5*. Other than a blot effect (results not shown), there were no significant effects on *PDF1.2* expression.

DISCUSSION

Is SAR beneficial?: We investigated the benefits of resistance by comparing the response of a mutant that cannot induce SAR, *npr1*, to wild-type plants. For resistance to be beneficial, resistant plants should have higher fitness than nonresistant plants under a pathogen treatment. The significantly lower fitness of infected *npr1* vs. infected wild type under low-nutrient conditions (Figure 2) indicates that normal inducible SAR as it exists in wild-type plants is beneficial under these conditions. There was no benefit found under high-nutrient conditions, but SAR was not found to have

a negative effect on fitness. SAR in the wild-type plants could have lowered fitness if the cost of the induced resistance were greater than the benefit of the resistance. These results, in addition to the fact that *npr1* plants had lower fitness than wild type in a field trial (HEIDEL *et al.* 2004), suggest that normal inducible SAR does have an adaptive purpose.

The *NPRI-H* genotype allowed us to test whether it is beneficial to have a stronger-than-wild-type induction of SAR. The similar fitness of wild type and *NPRI-H* under both nutrient treatments demonstrates that stronger SAR is not beneficial, but does not have an extra cost. Perhaps *NPRI-H* can be beneficial only with a longer and more damaging pathogen attack (Cao *et al.* 1998).

Why induced resistance?: We tested whether induced or constitutive defense is more effective against a single pathogen attack by comparing the response of wild-type plants to *cpr* plants. The constitutive resistance in the *cpr* plants did not lead to a fitness benefit for the plants in

TABLE 5

Effect of disease rating on fitness within infected treatment

Source	d.f.	SS	F-value
Genotype	4	1446	19.5***
Nutrient	1	162	8.8*
“Flat” (nutrient)	8	1985	13.4***
Disease rating	5	820	8.9***
Genotype \times nutrient	4	521	7.05***

Seed-yield data were cube root transformed. Genotype, nutrient, and disease rating (ordinal variable) are fixed effects and “flat” is a random effect. SS, sum of squares. *Significant F-values at $P < 0.05$; ***significance at $P < 0.001$.

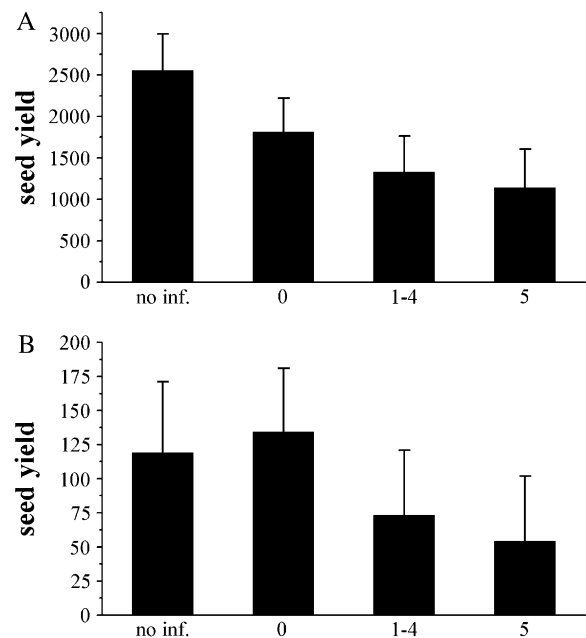


FIGURE 3.—Effect of disease rating on seed yield. Mean seed yield for plants within each disease-rating category (“no inf.” refers to plants in the uninfected treatment) are shown with \pm SE. (A) Within high-nutrient treatment category. (B) Within low-nutrient treatment category.

TABLE 6
Effects on *PR-1* expression: effect tests

Source	<i>PR-1</i> expression		
	d.f.	SS	F-value
Genotype	4	59.5	4.70**
Nutrient	1	29.8	9.41**
Infection	1	3.5	1.10
Blot	3	42.0	4.42**

Expression data was boxcox transformed. SS, sum of squares. **Significance at $P < 0.01$.

the pathogen treatment (Figure 2), demonstrating that the known fitness cost of constitutive resistance (HEIDEL *et al.* 2004) was too high to give a net benefit under the conditions of this experiment. This effect is seen in both nutrient treatments, indicating that this finding is not dependent on nutrient availability. Constitutive resistance might have been beneficial if the resistance had the ability to prevent pathogen damage before the induced resistance was sufficiently induced; however, this was not the case. Similarly to *NPRI-H*, however, constitutive expression could potentially be beneficial when under a longer pathogen attack, especially when secondary within-plant infections are likely to arise.

These results suggest that SAR is not constitutive because the reduction in pathogen damage from constitutive resistance fails to adequately compensate for the costs despite reducing pathogen damage. These results are partially consistent with the results in a native tobacco species where herbivore resistance induced by methyl jasmonate reduced herbivory and increased fitness in some but not all situations (BALDWIN 1998). When resistance is chemically induced with methyl jasmonate, the resistance occurs over a shorter time frame than when resistance is constitutively activated by genetic changes (as in the *cpr* mutants); therefore, the chemical induction might be less costly, assuming equal intensities of resistances.

Tolerance effects: Any infection can have deleterious effects due to direct damage by the pathogen and/or as a result of the cost of mounting inducible host defenses. Overall, infection significantly reduced the fitness of all plants (Table 3; Figure 2). Consistent with this, plants with greater disease symptoms had decreased fitness (Table 5; Figure 3). Other than *cpr1*, the effect of infection on fitness was similar across genotypes as demonstrated by the lack of a significant genotype \times infection interaction (Table 4).

However, there is not necessarily a direct linkage between disease severity, as measured here, and pathogen-caused reductions in fitness; two equally strong infections, as determined visually, may have different effects on fitness, depending on undetected levels of damage and tolerance. Tolerance differences have been found in response to herbivory (TIFFIN and RAUSHER 1999) and

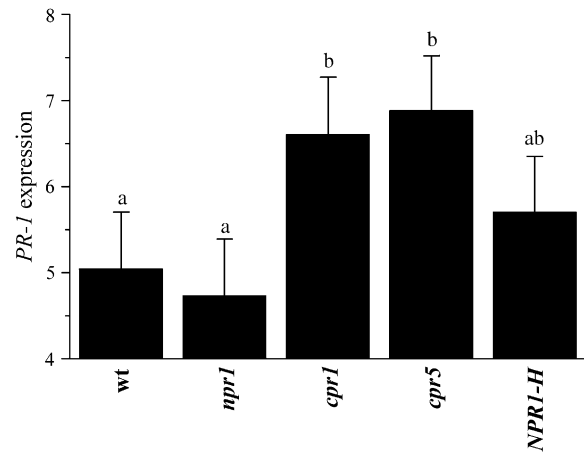


FIGURE 4.—Relative expression of *PR-1* across all infection and nutrient treatments. Mean values (\pm SE) of transformed data are shown. Letters indicate significantly different values.

plant pathogens (BENT *et al.* 1992; KOVER and SCHAAAL 2002; SCHURCH and ROY 2004), and these tolerance differences can have implications for the study of benefits.

The lack of a linkage between disease severity and reductions in fitness was not an important complication for the *cpr* mutants since they had lower disease severity than wild type, and disease severity is correlated to fitness in this experiment. Tolerance could not have been the explanation for the fitness differences between the *cpr* mutants and wild type because similar fitness differences between these genotypes were found without the pathogen (Figure 2). In contrast, tolerance could be important for explaining the data from *npr1*. Even though the *npr1* mutants had a similar disease rating to wild type, they have lower fitness under low-nutrient conditions. This fitness difference could be caused by a less severe infection in wild type due to induced resistance in a manner not captured in our disease rating or by a difference in tolerance (*i.e.*, wild type has a higher tolerance). While it would be useful from a mechanistic view to know whether resistance or tolerance is responsible, it can be argued that, in an evolutionary sense, it is irrelevant because the fitness of *npr1* is lower regardless of whether resistance or tolerance is responsible.

Two other studies have measured the fitness effect of *H. parasitica* infection. KORVES and BERGELSON (2004) demonstrated that the same strain, Noco2, lowered the seed yield of *A. thaliana* but paradoxically only in lines carrying the cognate-specific resistance gene and under conditions of intraspecific competition. Lines lacking the R-gene did not have a significant reduction in fitness when infected by the pathogen. In our experiment, all the genotypes lack the R-gene recognizing Noco2, yet there was an overall fitness reduction from infection, as might be typically expected. The difference between the two studies might result from either growth conditions

or time of infection. In our study, infection was performed on cotyledons 1 week after germination, while in the KORVES and BERGELSON (2004) study, infection was carried out on true leaves 2 weeks after germination. However, VAN HULTEN *et al.* (2006) found that infection by *H. parasitica* lowers fitness, consistent with our results.

Nutrient effects: Different theories of how nutrient stress affects resistance have been suggested (KARBAN and BALDWIN 1997). If it were possible to measure the amount of energy and resources devoted to SAR, the effects of nutrient stress might be more easily elucidated. However, determination of the resources and energy used by SAR is difficult, since SAR is a complex resistance mechanism and does not result from the production of a single antimicrobial protein or compound (MALECK *et al.* 2000). Even if the energy required for biosynthesis of one primary protein can be calculated, the relation of this energy expense to fitness is complex due to further potential interactions with regulatory networks. Without knowing the components of the complete resistance mechanism, it is difficult to test the effects of limited nutrients on the production of SAR resistance mechanisms.

A large number of proteins are produced during SAR (WARD *et al.* 1991; MALECK *et al.* 2000), and high concentrations of PR proteins occur in some species following infection (in tobacco, two PR proteins increased to 3% of soluble protein; VOGEL-LANGE *et al.* 1988). It is therefore likely that SAR probably requires a significant amount of nitrogen. Under nitrogen limitation (such as found in the low-nutrient treatment), there are two possible outcomes for PR protein biosynthesis: (1) PR proteins may continue to be synthesized, but the fitness cost of producing such proteins is greater. Or (2) the synthesis of PR proteins can be lowered, decreasing the fitness cost of resistance but also increasing the risk of damage from pathogens. The first option has been found by PURRINGTON and BERGELSON (1997) where a plant expressing herbicide resistance had a greater cost under nutrient limitation. In that case, herbicide resistance resulted from changes in the target enzyme that make it less effective in its normal role. In our study, the observation that all genotypes had a higher disease rating under low-nutrient conditions (Figure 2) and that there was no significant genotype \times nutrient interaction suggests that resistance was downregulated under low-nutrient conditions independently of the SAR-affecting mutations. An inhibitory mechanism is more likely to exist for SAR than for the herbicide resistance found by PURRINGTON and BERGELSON (1997) because herbicide resistance has occurred only very recently, giving little time for regulation to develop. Furthermore, this specific type of herbicide resistance cannot be easily downregulated since the herbicide resistance and the cost directly results from a mutation to an essential enzyme.

Relation of mRNA expression to fitness: As already noted, connecting the activation of specific molecular pathways to fitness is difficult. Despite this problem, expression of certain mRNAs can be used as markers of costly processes regardless of whether the synthesis of the specific marker mRNA and its protein are costly. In this view, *PR-1* is a good marker for costly processes because its expression is higher in *cpr1* and *cpr5* (which have the lowest fitness relative to wild type), *NPR1-H*, and *npr1* (Figure 4), while *PDF1.2* is not a good marker since it has no correlation with resistance costs in this study. This is consistent with previous results in a natural environment, demonstrating that *PR-1* is associated with costly resistance while *PDF1.2* is not (HEIDEL *et al.* 2004). The infection treatment did not have a significant effect on the expression of either *PR-1* (Table 6) or *PDF1.2*. This is probably due to the fact that the leaf samples used for gene expression studies were collected 1 month after disease expression, and by this time differences between infected and uninfected plants may have largely subsided. As demonstrated in this study, induced resistances can be costly if they are left activated for a long time, so it is logical that SAR and its marker genes would be deactivated quickly to conserve resources.

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