Quantitative fitness effects of infection in a gene-for-gene system

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Received: *29 April 2009*
Accepted: *19 May 2009*

New Phytologist (2009) **184**: 485–494
doi: 10.1111/j.1469-8137.2009.02959.x

Key words: *Arabidopsis thaliana*, cost-benefit balance, isogenic lines, plant–pathogen interactions, *Pseudomonas syringae*, *RPS5 R* gene.

Summary

• It is often assumed that pathogen infection decreases plant fitness, thereby driving the evolution of plant resistance (*R*) genes. However, the impact of bacterial pathogens on fitness has been shown to be relatively subtle, ranging from positive to negative.

• In this study, we focus on the *Rps5*-mediated resistance in *Arabidopsis thaliana* and examine the fitness effects of resistance by experimentally infecting resistant (*R*) and susceptible (*S*) plants with a natural avirulent *Pseudomonas syringae* strain at each of three initial infection dosage levels. Our methodology ensured control of the plant genetic backgrounds; within each of two natural accessions we created isolines varying in the presence or absence of *Rps5*.

• In terms of lifetime fitness, *R* plants outperformed their *S* controls by 9.6–32% when infected by a pathogen carrying an associated *Avr* gene, depending on the initial dosage levels and genetic backgrounds.

• We also found that the naturally *R* line, Col-0, is more tolerant than the naturally *S* accession, Ga-0. The negative impact of infection on fitness was 20% less in Col-0 than Ga-0. We found no effect of *Rps5* itself on the tolerance of either accession. We therefore failed to find evidence for a trade-off between tolerance and resistance.

Introduction

Plants have developed elaborate defense strategies through the coevolutionary process (Jones & Dangl, 2006). In the classic gene-for-gene model of host–pathogen interactions, plant resistance (*R*) gene products recognize pathogen elicitors, encoded by avirulence (*Avr*) genes, and initiate signal transduction pathways (Flor, 1971; Keen, 1990). Induced resistance responses include the hypersensitive response (HR), a form of programmed plant cell death, cell-wall strengthening, and the expression of various defense-related genes (Dangl & Jones, 2001). Resistance gene-mediated resistance is a host-specific defense and can only be activated when both *R* gene and corresponding *Avr* gene are present (Staskawicz *et al.*, 1995); the absence of either component results in disease, which is typically associated with damage and a reduction in yield of the host plant (Jarosz & Davelos, 1995).

Molecular genetic studies of plant *R* genes have revealed extensive variation in *R* gene loci (Caicedo *et al.*, 1999; Mauricio *et al.*, 2003; Rose *et al.*, 2004; Bakker *et al.*, 2006). In several cases, the resistance and susceptibility alleles have been maintained in natural populations for millions of years (Stahl *et al.*, 1999; Tian *et al.*, 2002). Mathematical models generally suggest that a trade-off between costs of resistance in pathogen-free environments and benefits of resistance under infection is required to explain the coexistence of resistant and susceptible individuals (Stahl *et al.*, 1999; Bergelson *et al.*, 2001). Theories without a cost–benefit balance instead require a spatial structure or a trade-off between alternative defensive strategies (Thrall & Burdon, 2002). It is generally agreed that the magnitudes of both costs and benefits of resistance vary among different resistance traits, genetic backgrounds and environmental conditions (Bergelson, 1994; Bergelson & Purrington, 1996; Kover & Schaal, 2002).

Costs of resistance have been successfully detected in various plant–pathogen systems (Baldwin, 1990; Bergelson, 1994; Mauricio, 1998; Tian *et al.*, 2003), generally by utilizing genetically controlled lines to compare the performance of resistant and susceptible plants. Surprisingly, a less consistent picture emerges regarding the benefits of resistance. Whereas

substantial empirical support for benefits of resistance exists for plant–fungus systems (Jarosz & Burdon, 1992; Melendez & Ackerman, 1993; Korves & Bergelson, 2004; Kniskern & Rausher, 2006), plant–bacterial systems reveal fitness effects of infection ranging from negative (reducing fitness) to positive (increasing fitness) as a consequence of variation in resistance and tolerance among genetic backgrounds (Goss & Bergelson, 2007). These studies focus on overall quantitative resistance traits of plants subject to natural infection, and little has been shown about the fitness effects resulting from *R* gene mediated resistance (but see Korves & Bergelson, 2004).

There are still several gaps in our understanding of the evolutionary ecology of host defense, especially to bacterial pathogens. First, as pointed out in Goss & Bergelson (2007), fitness effects of bacterial pathogens on plant hosts have generally been measured using isolates collected from other host species (Kover & Schaal, 2002; Korves & Bergelson, 2004; Kover *et al.*, 2005; but see Goss & Bergelson, 2007). Experiments conducted with wild plants and their naturally occurring pathogens are preferential in having direct evolutionary relevance. Second, surveys of pathogens in naturally occurring plants reveal a wide range of bacterial titers in plant tissues (Dunning, 2008), and this variation appears to be important in shaping plant–bacteria interactions. For example, a study by Korves & Bergelson (2003) revealed different developmental responses of *Arabidopsis thaliana* hosts when infected by three initial densities of the bacterial pathogen, *Pseudomonas syringae*. It is thus instructive to test for fitness effects of infection at a range of titers. Finally, there remains the challenge of distinguishing between resistance and tolerance – two mechanisms that prevent plant fitness loss under infection (Mauricio *et al.*, 1997). By definition, resistance traits reduce pathogen growth and spread whereas tolerance traits diminish the impact of infection on fitness (Simms & Triplett, 1994). It has been hypothesized that there should be a negative correlation between resistance and tolerance abilities such that susceptible plants are more tolerant (Fineblum & Rausher, 1995; Mauricio *et al.*, 1997). In order to address whether resistance and tolerance are two exclusive defense strategies, at least regarding infection by one particular pathogen isolate, one can measure tolerance within a resistant accession and within a susceptible accession, and compare these estimates.

In this study, we sought to fill these gaps in our understanding through a detailed investigation of the interaction between *A. thaliana* and *P. syringae*, both of which are well-known model species. In particular, we used resistant (*R*) and susceptible (*S*) isolines, constructed in one naturally resistant line and one naturally susceptible line, to measure the benefit of resistance and to assess the relationship between tolerance and *R* gene resistance in *A. thaliana*. We examined the *R* gene, *Rps5*, which segregates for an insertion/deletion polymorphism in which susceptible plants have the entire locus deleted (Simonich & Innes, 1995; Tian *et al.*, 2002). The *Rps5* gene confers resistance to the *P. syringae* pv. tomato carrying *avrPphB* (Jenner *et al.*, 1991) and to a *P. syringae* isolate recently found in naturally occurring *A. thaliana* leaves in Midwest USA (J. M. Kniskern, pers. comm.). Two sets of plant lines with controlled genetic backgrounds differing only in the presence or absence of a functional *Rps5* gene were cross-infected with this wild *P. syringae* strain at three initial infection dosages. In conducting these inoculations, we were interested in whether there is a fitness advantage of *R* plants relative to *S* plants when infected with pathogens carrying an *Avr* gene, and how this fitness advantage varies over three initial infection dosages. Second, we compared tolerance abilities and asked whether there is a negative relationship between *Rps5* resistance and tolerance.

Materials and Methods

Plant lines

Arabidopsis thaliana (L.) Heynh is a favored model species for studying plant–pathogen interactions (Mauch-Mani & Slusarenko, 1993). Its rapid generation time makes it convenient to obtain fitness estimates in 2–3 months and, as a primarily selfing species (selfing rate 99%; Abbot & Gomes, 1989), the total seed production of *A. thaliana* can be used as a reasonable estimate of combined male and female fitness. Four pairs of isogenic lines were used in our experiment. In each pair, *R* and *S* lines share the same genetic background except for the presence or absence of a functional *Rps5* gene (Table 1). This allowed us to attribute any differences in disease symptoms and yield-related measurements between *R* and *S* lines directly to the *Rps5* gene.

For this infection experiment, we used two pairs of lines created in the accession Ga-0, which is a naturally susceptible accession lacking the entire *Rps5* locus, and two pairs lines created in Col-0, which is a naturally resistant accession containing the entire *Rps5* locus. The procedures for constructing the pairs of plant lines are described below.

Construction of *Cre-lox* lines in Ga-0

Ga-0 is a fast-growing wild-type accession that lacks the *Rps5* locus (Tian *et al.*, 2002). The rationale for creating paired isolines in this naturally susceptible accession involved using

Table 1 *Arabidopsis thaliana* plant lines used in infection experiment

Name	Genetic manipulation	Accession	Resistance
C1+, C2+	T-DNA transformed	Ga-0	*Rps5*$^+$
C1–, C2–	*Cre-lox* excision of transgene	Ga-0	*Rps5*$^-$
E1+, E3+	Backcrossed sibs	Col-0	*Rps5*$^+$
E1–, E3–	EMS mutagenesis, backcrossed	Col-0	*Rps5*$^-$

Paired isogenic lines are designated with the same line name but differentiated by *Rps5*$^+$ or *Rps5*$^-$ (i.e. C1+/C1–).

a Cre-lox system to introduce *Rps5* plus a selectable marker, kanamycin resistance, into the genome and then excise *Rps5* out. In particular, to excise the *Rps5* locus, recombination was triggered by crossing a transgenic Ga-0 *Rps5* line to an isogenic Ga-0 line containing *Cre* recombinase (Bayley *et al.*, 1992). In this way, pairs of *R* and *S* lines with an insertion in the same chromosomal location were created: the *R* lines contain *Rps5*, the selectable marker and vector sequence, whereas the *S* lines contain only the selectable marker and vector sequence. We created two such pairs of lines; these lines differ in the chromosomal location of the inserts.

***Rps5*-containing T-DNA vector** The first step in line creation is to make a construct with which to introduce *Rps5*, flanked by lox sites, into Ga-0 through plant transformation. A 4 kb DNA fragment consisting of the entire *Rps5* coding sequence and its natural promoter (1.26 kb 5′ sequence) and terminator (58 bp 3′ sequence) from a natural resistant ecotype, Col-0, was shown to complement *Rps5* mutants (unpublished). A bluescript vector containing this region, provided by R. Innes (Indiana University), was digested to produce a 4.27 kb fragment containing the 4 kb region and *c*. 100 bases on both sides. The binary vector pCAMBIA2300 was modified to contain two 34-base *lox* recombination signals flanking the cloning site by inserting a *lox*-containing sequence from a plasmid pBS246 into the EcoRI (position 8359) and SmaI (position 8377) sites of the multicloning region. The modified binary vector pCAMBIA2300 contains a unique SmaI site between the two *lox* sites where the 4.27 kb *Rps5* fragment obtained from the bluescript vector was subcloned. The binary vector pCAMBIA2300 also confers kanamycin resistance which was used to screen for transformants.

***Cre*-containing T-DNA vector** The second step in line creation is to make a construct used to introduce *Cre* into the Ga-0 genetic background. The *Cre*-containing T-DNA plasmid was constructed by inserting a 3.43 kb sequence containing the *Cre* gene driven by the 35S *Cucumber mosaic virus* (CMV) promoter and the Nos 3′ terminator (Osborne *et al.*, 1995), into the binary vector pCAMBIA3301. This vector contains a resistance gene conferring resistance to the herbicide Basta (glufosinate; Bayer Crop Science, Monheim am Rhein, Germany) and the *GUS* gene.

***Cre-lox* transgenic plants construction** The final steps in line creation involve transforming Ga-0 with each of the above-mentioned constructs and then crossing the transformants to obtain *R* and *S* isolines. Transformation for both *lox-Rps5-lox* and *Cre* transgenic plants in Ga-0 was carried out by vacuum infiltration (Bechtold *et al.*, 1993). Plants were grown under long days until flowering, and were infiltrated with *Agrobacterium tumefaciens* carrying either *Rps5* or *Cre* on the binary vector pCAMBIA2300 or pCAMBIA3301, respectively. To excise *Rps5* using the *Cre-lox* system, two homozygous lines carrying single independent insertions of the *Rps5* transgene in noncoding regions were crossed with each of four *Cre* transgenic lines. In the F_1 generation, those seedlings without a *Cre*-bearing chromosome were excluded by spraying a 1 : 500 dilution of Basta and those without a *Rps5*-bearing chromosome were then excluded with a quick PCR screen performed directly on leaf tissue (Thompson & Henry, 1995). Our unpublished data from previous experiments has suggested that the F_1 plants with somatic loss of *Rps5* were more likely to show recombinational excision of the transgene, thus it was these F_1 plants with which we proceeded. In the F_2 generation, seedlings were sprayed with a 1 : 1100 dilution of Basta to distinguish *Cre*-bearing and non*Cre*-bearing plants without substantial seedling mortality of either genotype, and those non*Cre*-bearing plants were then screened for recombinational excision of the *Rps5* transgene using a PCR test based on vector sequence. Heterozygous ($Rps5^+/Rps5^-$) F_2 plants were self-pollinated and homozygous $Rps5^+$ and $Rps5^-$ siblings were selected (F_3). These homozygous siblings were further self-pollinated for two more generations (F_4–F_5) to obtain the desired isolines. In each generation, the presence or absence of *Rps5* was tracked by PCR and direct sequencing, and the absence of *Cre* was confirmed with additional PCR screening for GUS. To locate the genomic position of the transgenic *Rps5* gene, we performed TAIL (thermal asymmetric interlaced) PCR to amplify the fragments between a set of nested specific T-DNA border primers and the AD (Arbitrary Degenerate) primers (Liu & Whittier 1995). Two of the resulting pairs of *Cre-lox* isogenic lines (C1+/– and C2+/–) were used in the experiment; both of these lines contained inserts in noncoding regions to minimize transgenic impacts.

Ethyl methanesulfonate (EMS) mutant lines in Col-0

To complement the experiment using *Cre-lox* isolines in a naturally susceptible ecotype, we examined the fitness effect of *Rps5* in a naturally resistant genetic background, Col-0, which is a fast-growing accession that naturally contains a functional copy of *Rps5*. By screening 3000 EMS mutants, the *Arabidopsis* TILLING (Targeting Induced Local Lesions IN Genomes) Project (Till *et al.*, 2003) has identified five missense mutations and one truncation mutation in the first 1 kb region of *Rps5*. The particular mutant line that we used has a stop codon (caused by TGG → TAG point mutation) in place of the 319th amino acid (out of 889) of the *RPS5* protein.

To create isolines for our experiment, we first backcrossed the mutant line with its parental Col-0 lines for three generations to reduce the number of background mutations present. Two independent backcross lines, E1 and E3, were created; each presumably possesses different background mutations that persisted after backcrossing. F_1 plants of the last backcross of each of these lines were self-pollinated to obtain homozygous F_2 plants differing in the presence or absence of $Rps5^-$ (with the early stop codon) but segregating for the same pool of

remaining background mutations. Hence, the EMS mutants both contain the same early stop codon in the *Rps5* gene but differ in any background mutations that may be segregating in the *R* and *S* pairs.

Pathogen strain

Pseudomonas syringae is commonly found in wild *A. thaliana* plants in Midwest USA and was confirmed to be a natural pathogen (Jakob *et al.*, 2002). It is capable of infecting the aerial parts of its host plants, and most isolates interact with its host in a gene-for-gene fashion (J. M. Kniskern and J. Bergelson, unpublished). We used a wild *P. syringae* strain (strainPNA29.1a) isolated from a natural *A. thaliana* plant in the Midwest USA. Strain PNA29.1a carries an *avirulence* allele that is recognized by the *Rps5* gene product (J. M. Kniskern & J. Bergelson, unpublished), and thus it interacts in a gene-for-gene fashion with *A. thaliana.*

Glasshouse experimental methods

We used three initial concentrations, 10^3, 10^5 and 10^7, corresponding to the range of titers observed in natural *Arabidopsis* populations (Dunning, 2008), to compare plant performances under different levels of selective pressure. This resulted in four treatment types (three concentrations + one mock) for each of our eight plant lines. Each treatment and plant line combination was replicated eight times, thus our experiment included a total of 256 plants that were scored for symptoms and fitness. These plants were distributed among eight flats, with adjacent plants in each flat separated by a distance of 2.5 cm. This distance was designed to introduce root competition, and is close to the densities observed in natural conditions (Donohue *et al.*, 2005). Experimental plants were placed in a randomized order, with wild-type Col-0 plants placed on the edges of each flat to control for edge effects. Seeds were sown in a 1:1 mixture of Promix BX (Premier Horticulture, Quakerstown, PA, USA) and Metro Mix 200 (Sun Grow J=Horticulture, Bellevue, Washington, USA), cold-stratified for 3 d, and then transferred into a growth room with short day (12 h light) at 20°C. Flats were rotated across the growth room every other day to homogenize lighting and other conditions. Plants were irrigated once every other day and received no fertilization.

Bacterial infection was conducted on 2-wk-old seedlings. One day before infection, a single colony of the pathogen strain was suspended in King's B liquid media, incubated at 28°C overnight, then diluted 1 : 10 on the following morning and allowed to grow to an optical density of approx. 1–1.2 at 600 nm. The bacterial cultures were centrifuged and resuspended in 10 mM $MgSO_4$. Serial dilutions were made to obtain three concentrations for the pathogen strain: 10^3, 10^5 and 10^7 colony forming units (CFU) per ml. For each experimental plant, the first two true leaves were infiltrated with the bacterial suspension or the 10 mM $MgSO_4$ buffer (mock), using a blunt-ended syringe. Plants were randomly assigned to infection treatment. Lids were left on the flats for one night to maintain high humidity and thus promote infection.

Disease symptoms scoring and fitness-traits measurement

Severity of disease symptoms (Symptom 4-D) was recorded 4 d after inoculation, when symptoms reached their peak for the two accessions. Each infected leaf received a score from 0 to 3, with 0 corresponding to no symptoms and 1, 2 or 3 corresponding to mild, medium, or severe symptoms, based on both the proportion of leaf area showing disease symptoms and the severity of visible chlorosis/dryness of the diseased leaf. We summed the scores for the two infected leaves per plant. Thus, for each experimental plant, this resulted in a score between 0 (both inoculated leaves healthy) and 6 (both showing severe lesions). It has been shown that post-disease symptoms are highly correlated with the in-leaf bacterial titers (Kover & Schaal, 2002), so we used this score as a measure of bacterial fitness.

Plant yield was estimated by total silique length per shoot, which has been shown to correlate well with seed production for *A. thaliana* accessions (Roux *et al.*, 2004). We calculated this quantity by multiplying the total number of siliques with the average silique length. To get a consistent measurement, average silique length was obtained by taking the average of the lengths of the 3rd, 5th and 7th siliques on the main stem.

Data analysis

For testing quantitative effects on fitness and symptom, we performed a general linear model (GLM) analysis containing the following main effects: (1) *Flat* accounts for the difference in micro-environments for the eight experimental blocks; (2) *Accession* (Col-0 vs Ga-0) stands for plant genetic background effect; (3) *Line* nested in accession is equivalent to the position effect of the transgenic insertion event in the Cre-lox lines and represents the effects of remaining mutations in the EMS lines; (4) *Resistance* designates the presence or absence of the *Rps5* gene (*R* vs *S*); and (5) *Dose* represents the four infection levels: mock, 10^3, 10^5 and 10^7. In summary, factor 1 is environmental, factors 2–4 are plant-related, and, factor 5 is treatment related. All factors, except for flat were fully crossed with each other and were treated as fixed effects. We chose not to treat either accession or line as a random effect since levels of neither factor were random samples of a population to which we intended to extrapolate.

In order to test quantitative effects on tolerance, the 'Dose' factor was replaced by a 'Symptom 4-D' factor in the GLM described above. We made this substitution because we were particularly interested in comparing the ability of plant lines to maintain fitness in the face of disease symptoms. Again, all factors except *Flat* were treated as fixed factors.

All the statistical tests were performed on the raw untransformed data as well as on data standardized by the performance of mock plants of the same lines. Both raw and standardized total silique lengths were then log-transformed to satisfy the normality and equal variance assumption of linear regression. Model fitting was conducted using Proc GLM in SAS 9.1 (SAS Institute Inc., Cary, NC, USA). Since the statistical analyses led to the same biological conclusions, only the results using the raw data (log transformed) are presented.

We conducted several pairwise comparisons within the regression model to examine the significance of particular effects of interest after adjusting for other factors in the model. This was done using the 'Estimate' or 'Contrast' statement in Proc GLM in SAS 9.1.

Results

Mock plants

Plants that received a mock treatment never showed any disease symptoms. Their fitness, measured by total silique lengths, was affected only by genetic background (Table 2); accession Ga-0 has more than twice the total seed production of Col-0 (Fig. 1). We did not observe any difference between lines within each accession ($P = 0.82$) suggesting the lack of a position effect for the *Cre-lox* lines and relative homogeneity in genetic background of the EMS mutant lines.

Note that we did not detect any reduction in the fitness of *Rps5*$^+$ lines relative to *Rps5*$^-$ lines ($P = 0.96$). There are two possible explanations for our failure to detect a cost of resistance. First, the sample size for each line in this experiment is very small ($n = 8$). Second, it is well known that environmental factors affect the magnitude (and presence) of costs of resistance, and that relatively benign glasshouse conditions, such as those used in our experiment, can be less likely to reveal costs of resistance relative to more stressful (e.g. field) conditions (Bergelson & Purrington, 1996).

Table 2 Effects of accession and *RPS5* resistance on *Arabidopsis thaliana* plant fitness under mock treatment

		Total silique length		
Source	df	MS	*F*	*P*
Flat	7	0.04	2.87	*
Line[a]	2	0.01	0.77	
Accession	1	14.13	1089.22	***
Resistance	1	0.01	0.60	
Line × *Resistance*	2	0.02	1.24	
Accession × *Resistance*	1	0.02	1.62	
Error	49	0.01		
R^2		0.958		

[a]*Line* is nested in *Accession*.
*, $0.05 > P > 0.01$; **, $0.01 > P > 0.001$; ***, $P < 0.001$.

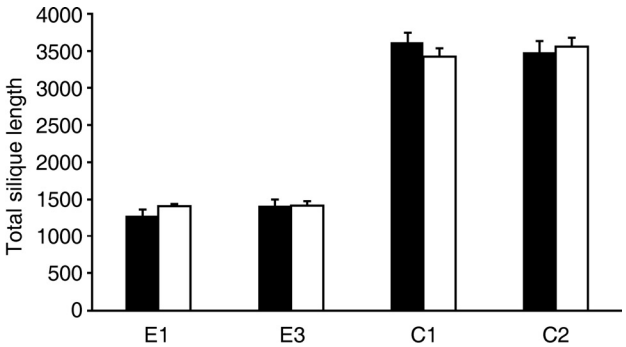

Fig. 1 *Arabidopsis thaliana* plants under mock treatment. Total silique lengths (mean ± SEM) for resistant (*R*) and susceptible (*S*) lines of each isogenic pair were plotted. E1 and E3 are EMS mutant lines in Col-0 accession, while C1 and C2 are *Cre-lox* lines in Ga-0 accession. Sample size is eight for each line.

Quantitative effects on plant fitness and disease symptoms

Results from the nested analyses of variance for both plant fitness and disease symptom traits are reported in Table 3; both models fit well to our data, as indicated by the relatively high R^2 values (> 0.87). There is general consistency in the factors that affect disease symptoms and plant fitness, as one would expect. *Rps5* resistance strongly ameliorated symptoms and enhanced plant fitness in the presence of infection. Four days after infection, *Rps5*$^-$ lines had significantly more disease symptoms than *Rps5*$^+$ lines. In our experiment, the accession Col-0 presented significantly more symptoms than Ga-0 at the same *Dose*, and there was additionally a significant interaction between *Dose* and *Accession*. These results suggest that the *Rps5* allele is not the sole determinant of resistance to the natural *P. syringae* strain PNA29.1a. In addition, there is a strong and consistent effect of dosage on fitness and symptoms; both *Rps5*$^-$ and *Rps5*$^+$ plants showed significantly more severe disease symptoms (Fig. 2), and produced fewer seeds, as initial infection dosage increased (Fig. 3). That said, there was a significant interaction between *Dose* and *Resistance* for both disease symptoms and total silique length, suggesting that the benefit of resistance changes with dosage.

The lack of an interaction between *Accession* and *Resistance* indicates that the effects of the *Rps5* allele on disease symptoms and fitness were not dependent on the genetic background. Furthermore, the lack of an effect of *Line* nested in *Accession* indicates consistency in the two isolines in the Ga-0 background and in the two isolines in the Col-0 background.

Disentangling plant genotype effects on fitness

It has been shown that the impact of pathogen infection varies among genetic backgrounds of host plants. Plant genotype effects consist of two main components: first, the 'resistance genotype' (*Rps5*$^+$ vs *Rps5*$^-$), in our case, the presence or absence

Table 3 Quantitative effects of infection on disease symptoms and plant fitness in *Arabidopsis thaliana*

		Symptom 4d			TSL		
Source	df	MS	*F*	*P*	MS	*F*	*P*
Flat	7	1.78	1.88		0.07	2.10	*
Line[a]	2	0.10	0.10		0.10	2.82	
Accession	1	4.25	4.50	*	43.89	1250.87	***
Resistance	1	13.60	14.38	**	1.02	28.99	***
Dose	3	439.00	464.34	***	6.31	179.78	***
Line × *Resistance*	2	0.25	0.27		0.09	2.63	
Line × *Dose*	6	0.34	0.36		0.07	2.04	
Accession × *Resistance*	1	3.28	3.47		0.02	0.47	
Accession × *Dose*	3	3.30	3.49	*	0.84	23.90	***
Resistance × *Dose*	3	8.08	8.54	***	0.18	5.07	**
Line × *Resistance* × *Dose*	6	1.62	1.71		0.06	1.61	
Accession × Resistance × Dose	3	1.62	1.71		0.04	1.24	
Error	217	0.95			0.04		
R^2		0.872			0.900		

[a]*Line* is nested in *Accession*.

Results are from general linear model analysis on disease symptoms 4 d after infection (Symptom 4d) and estimated total silique length (TSL). *, 0.05 > *P* > 0.01; **, 0.01 > *P* > 0.001; ***, *P* < 0.001.

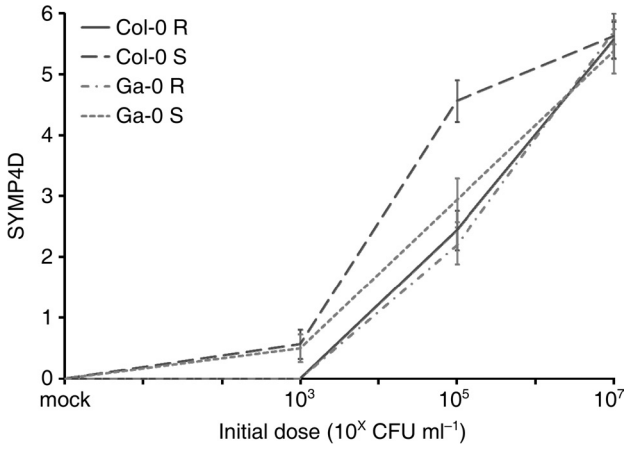

Fig. 2 Effect of *Arabidopsis thaliana* plant genotype (accession, *Rps5*-resistance) on disease symptoms under three infection conditions. Means and SEM for disease symptom 4 d after infection were plotted by initial infection dosages.

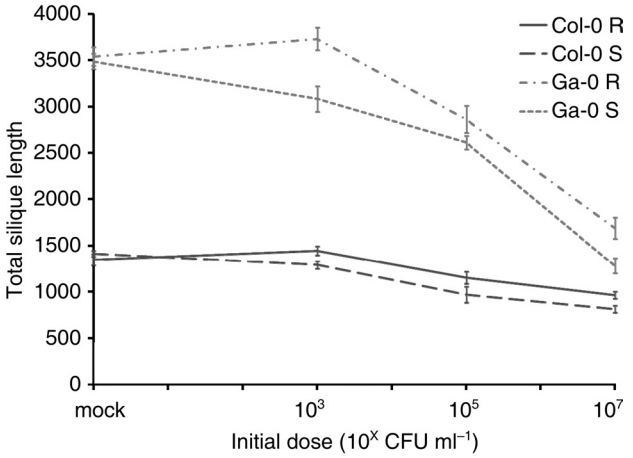

Fig. 3 Effect of *Arabidopsis thaliana* plant genotype (accession, *Rps5*-resistance) on plant fitness under three infection conditions. Means and SEM of total silique length were plotted by initial infection dosages and are based on raw data. Planned comparisons were made for resistant (*R*) vs susceptible (*S*) within each accession for each dose level.

of the *Rps5* gene, and second, the 'accession effect', which includes all other genetic differences between the two accessions (e.g. Col-0 vs Ga-0). The properly controlled isolines in each accession allow us to distinguish the effects of resistance from other differences in the genetic background.

The benefit of *Rps5* resistance as a function of inoculation dosage *Rps5*$^+$ genotypes were consistently fitter than *Rps5*$^-$ plants in the Col-0 genetic background, although this effect is marginal for the lowest infection density (10^3 CFU ml^{-1}; Table 4, Fig. 3). Similarly, *Rps5*$^+$ genotypes were generally fitter than *Rps5*$^-$ plants in the Ga-0 genetic background, except for the intermediate infection density (10^5 CFU ml^{-1}; Table 4, Fig. 3). If we define the benefits of *Rps5* resistance as the difference in fitness between *Rps5*$^+$ and *Rps5*$^-$ plants at a particular level of infection, we find that the magnitude of the benefits resulting from *Rps5* range from 9.6% to 32% across initial dose levels and accessions (Table 4). The highest selection differentials were found at the high and intermediate initial dosages for Col-0 and at the low and high initial dosages for Ga-0. This suggests that natural selection may favor the resistant plants when the pathogen is prevalent.

Table 4 Effect of *Arabidopsis thaliana* plant genotype (accessions, *Rps5*-resistance) on plant fitness under three infection dosages

Dose	Comparison	*F*	*P*	Significant effect	Magnitude of the benefit (%)[a]
10^3	Ga-0 *R* vs Ga-0 *S*	9.13	**	Ga-0 Resistance benefit	21.0
	Col-0 *R* vs Col-0 *S*	2.93	$P = 0.09$		12.0
10^5	Ga-0 *R* vs Ga-0 *S*	1.50			9.6
	Col-0 *R* vs Col-0 *S*	9.56	**	Col-0 Resistance benefit	18.8
10^7	Ga-0 *R* vs Ga-0 *S*	17.00	***	Ga-0 Resistance benefit	32.0
	Col-0 *R* vs Col-0 *S*	7.43	**	Col-0 Resistance benefit	19.0

[a]The magnitudes of benefits are based on least-square means generated by the general linear model described in Table 3.
*, $0.05 > P > 0.01$; **, $0.01 > P > 0.001$; ***, $P < 0.001$.

Table 5 Effects of disease symptoms on *Arabidopsis thaliana* plant fitness

		TSL		
Source	df	MS	*F*	*P*
Flat	7	0.06	1.34	
Symp4d	1	17.14	355.26	***
Line[a]	2	0.06	1.19	
Accession	1	30.95	641.26	***
Resistance	1	0.06	1.27	
Symp4d × *Line*	2	0.08	1.58	
Symp4d × *Accession*	1	1.47	30.42	***
Symp4d × *Resistance*	1	0.15	3.06	
Line × *Resistance*	2	0.13	2.73	
Accession × *Resistance*	1	0.12	2.54	
Symp4d × *Line* × *Resistance*	2	0.22	4.52	*
Symp4d × *Accession* × *Resistance*	1	0.07	1.48	
Error	233	0.05		
R^2		0.852		

[a]*Line* is nested in *Accession*.
Results are from a general linear model analysis on estimated total silique length (TSL); Symp4d, disease symptoms 4 d after infection.
*, $0.05 > P > 0.01$; **, $0.01 > P > 0.001$; ***, $P < 0.001$.

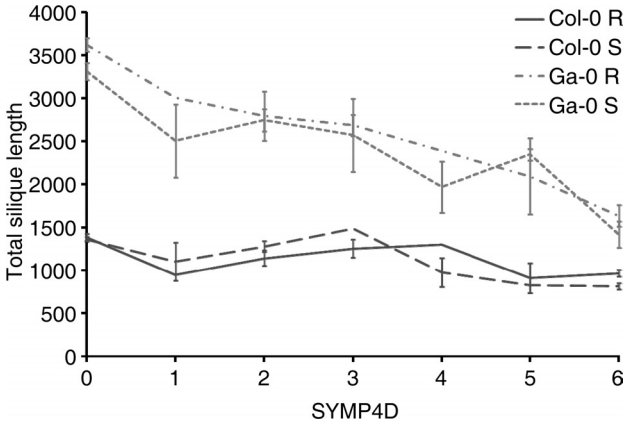

Fig. 4 Effects of disease symptoms on *Arabidopsis thaliana* plant fitness. Means and SEM of total silique length were plotted by symptom levels and are based on raw data.

The effect of genetic background as a function of inoculation dosage We observed a strongly significant difference between *Accessions* in the effect of *Dose* on fitness ($F = 23.90$, $P < 0.001$). At a pathogen dosage of 10^7 CFU ml^{-1}, $Rps5^+$ individuals in the Col-0 background suffered a 29% reduction in fitness relative to mock controls whereas $Rps5^+$ individuals in the Ga-0 background suffered a 52% reduction in fitness (Fig. 3). The same is true for $Rps5^-$ individuals, where infected plants in the Col-0 background suffer a 43% reduction in fitness relative to mock controls whereas those in the Ga-0 background suffer a 63% reduction (Fig. 3). Thus, the negative impact of infection is approx. 20% greater in Ga-0 than Col-0 for a given inoculation dosage. This difference exists despite the fact that Ga-0 experienced fewer symptoms than Col-0 when infected at the same dose (see subsection on Quantitative effects on plant fitness and disease symptoms).

The relationship between resistance and tolerance

A common approach for assessing tolerance is to examine the ability of particular host genotypes to sustain seed output given equivalent damage levels (Simms & Triplett, 1994). Here, we examine the relationship between fitness and symptoms as a measure of tolerance. Overall, total seed production decreased with symptom scores measured 4 d after infection (Table 5); the correlation between these two traits is −0.71 ($P < 0.001$). The interaction between *Accession* and *Symptoms* is highly significant, indicating differences in the rate at which the fitness of Col-0 and Ga-0 declines with damage (Table 5). Within the conditions of our experiment, Ga-0 always has higher fitness than Col-0, but the gap between accessions (i.e. tolerance effect) decreases with an increase in symptom scores (Fig. 4). In other words, Ga-0 suffers a stronger decline in fitness for a given increase in symptoms and is thus less tolerant than Col-0.

Within each accession, no significant effect of *Rps5* on the relationship between symptoms and fitness was detected (Table 5, Fig. 4). While, admittedly, it is unlikely that we would see a trade-off between *Rps5* resistance and tolerance in the Ga-0 background, since the *R* gene was introduced

transgenically with no opportunity to coadapt to the rest of the genome, the absence of a trade-off in the Col-0 background is more meaningful. Furthermore, a comparison across accessions failed to reveal any evidence of a trade-off: the naturally *Rps5* resistant genetic background, Col-0, is more tolerant than the naturally *Rps5* susceptible background. We thus conclude that the *Rps5* gene plays a crucial role in symptom development and plant yield (Table 3, Figs 2, 3) but does not affect tolerance directly.

Discussion

Pseudomonas syringae as a potential selective agent for *R* gene evolution

It is widely accepted that pathogens can impose selection on their native hosts and shape the evolution of plant resistance (Jarosz & Davelos, 1995). Nevertheless, surprisingly few studies measure the fitness impact of bacterial pathogens on plant hosts in natural (nonagricultural) systems. One particularly relevant study demonstrated that *A. thaliana* plants grown in a natural population but protected from bacteria with the antibiotic, Agrimycin, harbor lower bacterial titers and produce 56% more seeds than controls subjected to natural bacterial colonization (Traw *et al.*, 2007). Pre-induction of systemic acquired resistance (SAR) similarly protects *A. thaliana* hosts by reducing bacterial titers in leaves and elevating plant fitness by 54% relative to controls (Traw *et al.*, 2007). The bacterial species affected by SAR and antibiotic include several well-known pathogens, including *P. syringae, Pseudomonas viridiflava* and *Xanthamonas campestris* (Kniskern *et al.*, 2007; Traw *et al.*, 2007). While the observed fitness impacts cannot be attributed to any single bacterial species, it is clear that the bacterial community as a group has the potential to impose selection on *A. thaliana*.

Pseudomonas syringae is nonetheless likely to be an important pathogen of *A. thaliana*; it has been found on wild *A. thaliana* plants in Midwest USA and confirmed to be a pathogen (Jakob *et al.*, 2002; J. M. Kniskern and J. Bergelson, unpublished). Furthermore, there are many effectors of *P. syringae* that are known to be recognized by *A. thaliana*; some examples include *avrRpt2* (Kunkel *et al.*, 1993), *avrRpm1* (Dangl *et al.*, 1992), *avrB* (Bisgrove *et al.*, 1994), and *avrPphB* (Simonich & Innes, 1995). Population-level studies reveal that some of these cognizant *R* genes segregate for resistant and susceptible alleles (reviewed in Bergelson *et al.*, 2001), and negative frequency-dependent selection between hosts and pathogens may be largely responsible for this variation (Stahl *et al.*, 1999). All of these patterns suggest that the interaction between *A. thaliana* and *P. syringae* is both ecologically and evolutionarily important.

Given this suggestive evidence, we sought to assess whether *P. syringae* may act as a selective agent for *R* gene evolution by incurring fitness benefits and costs of resistance. Previous studies that directly measure the effect of *Pseudomonas* species on plant fitness have revealed a variety of impacts that depend on host genotypes and the environment (Kover & Schaal, 2002; Goss & Bergelson, 2006, 2007). While these studies have laid a solid base for exploring general patterns among host accessions in how they respond to bacterial pathogen infection, they do not allow one to extrapolate how pathogens select on particular resistance traits because clean comparisons between resistant and susceptible plants were not utilized. One exception is the study of Korves & Bergelson (2004), in which the fitness benefits of resistance against *P. syringae* carrying *avrRpt2* (conferred by *Rps2* gene) were found in the presence of intraspecific competition.

In this study, we created isolines in each of two genetic backgrounds to test the impact of pathogen infection on an *R* gene mediated resistance, *Rps5*. By comparing the total silique length, a measurement for lifetime seed production, in infected $Rps5^+$ and $Rps5^-$ plants, we found that $Rps5^+$ plants outperformed their $Rps5^-$ counterparts by 9.6–32%, depending on the initial dosage and accessions. Although a complete assessment of the selective role of *P. syringae* attack on *Rps5* will require measuring both costs and benefits under natural conditions, here we have demonstrated some evidence for a potential benefit for the *Rps5*-mediated resistance.

Our data suggest that initial infection dose plays an important role in host fitness. Damage to $Rps5^+$ and $Rps5^-$ plants is minor or even nondetectable when the infection dosage is low (10^3 CFU ml^{-1}). When infected with a higher titer of bacteria, even $Rps5^+$ plants suffer a significant reduction in fitness, although $Rps5^-$ plants suffer more. The effectiveness of host resistance or tolerance is maximized at medium to high infection dose, where the benefit of the *Rps5* allele is *c*. 21%. Although the benefit of *Rps5* gene is higher than its associated cost (8%; L. Gao and J. Bergelson, unpublished), one needs to keep in mind that the larger benefit is only present at relatively high initial dosages (10^5 or above). Field surveys of natural *Arabidopsis* populations have found that only a small percentage of plants are infected and, when infected, *A. thaliana* typically harbors a bacterial titer of roughly 10^3 (Dunning, 2008).

Resistance vs tolerance of host plants

Many studies have estimated the cost–benefit trade-off for resistance and/or tolerance to herbivores (Fineblum & Rausher, 1995; Mauricio & Rausher, 1997; Lennartsson *et al.*, 1998; Mauricio, 1998; Tiffin & Rausher, 1999; Fornoni & Núñez-Farfán, 2000; Honkanen & Jormalainen, 2005). Other host–enemy systems, including plant–holoparasitic plant (Koskela *et al.*, 2002; Puustinen *et al.*, 2004) and plant–pathogen (Simms & Triplett, 1994; Peters, 1999; Korves & Bergelson, 2003; Kniskern & Rausher, 2006; Goss & Bergelson, 2007), have received relatively less attention. In the case of bacterial pathogens, empirical studies investigating the joint pattern of pathogen-induced selection on resistance and tolerance are especially rare.

The use of isolines allows us to distinguish between tolerance and *Rps5* resistance clearly. We obtained estimates of *Rps5* resistance by comparing *R* vs *S* individuals within each accession. Our data suggest that *Rps5* resistance does not affect tolerance directly. Furthermore, we found that the naturally *Rps5* resistant accession, Col-0, is more tolerant than the naturally *Rps5* susceptible accession, Ga-0. Thus, we found no evidence in this study that tolerance and resistance, defined as either the *Rps5 R*-gene or genetic background associated with phenotypic resistance, are mutually exclusive.

Evidence of selection favoring the retention of both tolerance and resistance has been shown in various host–parasite systems (Leimu & Koricheva, 2006). For example, Mauricio *et al*. (1997) found the coexistence of tolerance and two resistance traits against herbivores (trichome density and total glucosinolate concentration) in natural populations of *A. thaliana*. Resistance and tolerance traits were measured in *Mimulus guttatus* when challenged with a generalist pathogen, Cucumber mosaic virus (CMV), and again, no evidence of a trade-off was found (Carr *et al*., 2006). A relatively recent theoretical study by Restif & Koella (2004) demonstrated various scenarios of simultaneous evolution of the two strategies of defense and concluded that resistance and tolerance need not be exclusive.

Although an infection experiment under field conditions would give more realistic and evolutionarily relevant results (Bergelson & Purrington, 1996), we chose to conduct a glasshouse experiment because it is much easier to control pathogens, especially when dealing with multiple infection dosages. Most of the previous infection studies were also conducted in glasshouse condition, partly for the same reason (Kover & Schaal, 2002; Korves & Bergelson, 2004). Our qualitative results can help one better identify and understand the key selective drivers of *R* gene evolution, such as benefits of resistance, co-existence of resistance and tolerance, and virulence effects of *avirulence* genes.

Acknowledgements

We are grateful to the staff of the University of Chicago glasshouse for their assistance during the experiment, two anonymous reviewers and Mark Rausher for constructive comments. Support was provided by NIH grant GM57994 and NSF grant MCB 603515 to J.B. and a V. Dropkin Foundation Fellowship to F.R.

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