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THE EFFECTS OF PATHOGEN INFECTION ON NITROGEN REMOBILIZATION IN *ARABIDOPSIS THALIANA*

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ABSTRACT

The natural enemies of plants are ubiquitous and can reduce plant fitness. Plants have evolved two defense strategies to ameliorate the fitness cost associated with natural enemy attack. The first strategy, resistance, reduces the frequency and/or severity of natural enemy damage. The second strategy, tolerance, attenuates the fitness cost of natural enemy damage. Very little is known about the traits through which tolerance is manifested, particularly with respect to plant-pathogen systems (pathosystems). Diseased and naturally senescing leaves are often similar in their visible symptoms and molecular activities, suggesting that they may involve similar processes. One process that may be shared by the two phenomena is the efficient remobilization of nitrogen, a limiting nutrient that is heavily remobilized during natural leaf senescence. Nitrogen metabolism during foliar infections is largely unexplored, although plants are known to remobilize nitrogen from diseased leaves. Efficient remobilization of nitrogen from diseased leaves may ameliorate the fitness cost of infection, thereby manifesting tolerance to infection. Using the model pathosystem *Arabidopsis thaliana – Pseudomonas syringae* we asked the following questions: 1) Does infection by *P. syringae* pathovar *tomato* strain DC3000 (*Pst* DC3000) affect the amount of nitrogen remobilized from leaves? 2) Is there a relationship between the amount of nitrogen remobilized from infected leaves and plant tolerance to infection? To our knowledge, our study is the first to explore the effect of infection on leaf nitrogen remobilization in the context of tolerance.

Results show that infected *A. thaliana* leaves remobilized nitrogen, however infection substantially reduced the amount of nitrogen remobilized. Plant fitness was inversely correlated with the amount of nitrogen retained by infected, senesced leaves, suggesting that the infection-caused impairment of nitrogen remobilization imposed a fitness cost. We detected little genetic variation in the effect of infection on the amount of nitrogen remobilized from infected leaves among 10 *A. thaliana* accessions. Similarly, we detected little genetic variation in *A. thaliana* symptom severity and tolerance to infection by *Pst* DC3000. The latter results contradict recent studies of this pathosystem,

indicating that estimates of the broad-sense heritability of resistance and tolerance in this system are highly conditional. The challenge involved with understanding tolerance in an evolutionary context is discussed. We explored the effects of infection on additional *A. thaliana* traits and found that infected *A. thaliana* plants produce shorter main stems. The inverse correlation between the nitrogen content of senesced, infected leaves and fitness supports efficient nitrogen remobilization as a promising candidate tolerance trait.

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1. INTRODUCTION

Plant natural enemies (herbivores and pathogens) are ubiquitous. Interactions between plants and their natural enemies can have community- and ecosystem-level consequences. For example, plant-natural enemy interactions can affect the interactions of plants with other community members (Strauss and Irwin 2004), and nutrient cycling within ecosystems can be affected by natural enemy-induced changes in plant quality (Chapman et al. 2003). Natural enemies can affect trait evolution in plants, and vice versa, through reciprocal selection/coevolution (Janzen 1980; Thompson 1999; Rausher 2001).

Plants have evolved resistance and tolerance in response to natural enemies. Resistance is manifested through traits that reduce plant availability, palatability, and/or nutritive quality with respect to their natural enemies. Resistance traits include structural barriers (e.g. trichomes, latex and reinforced cell walls), secondary chemical compounds (e.g. cardiac glycosides and glucosinolates), and premature leaf senescence. Resistance can also be achieved through phenological shifts. Resistance traits can be present before exposure to natural enemies (constitutive), or they can be induced following natural enemy attack (induced). Inducing resistance traits allows plants to circumvent the fitness costs of the traits when they are unnecessary. Induced resistance traits may also contribute to a moving target strategy whereby plants impede the evolution of counter-resistance in natural enemies by imposing shifting selection pressures on them (Adler and Karban 1994). When induced resistance traits confer a net fitness benefit to plants when in the presence of natural enemies, they represent adaptive plasticity. Whether induced or constitutive, when a resistance trait confers a net fitness advantage to the plants that possess it, the resistance trait qualifies as a defense trait (Agrawal 1999).

Tolerance is an alternative defense strategy to resistance that plants can employ. Tolerance compliments resistance by attenuating the fitness cost of natural enemy damage when plants are incompletely resistant. Tolerance traits may allow for a general

response to a diverse suite of natural enemies (Jokela et al. 2000). The evolution of broadly effective counter-resistance traits is difficult due to the diffuse nature of plant-natural enemy interactions, particularly in reference to specialists (Hougen-Eitzman and Rausher 1994). Relative to resistance traits little is known of the traits that manifest tolerance, particularly tolerance to pathogen infection (Stowe et al. 2000). Knowledge of tolerance traits is critical to our understanding of the evolutionary ecology of plant-natural enemy systems. In addition, natural enemy damage results in substantial crop yield losses in agriculture (Oerke and Dehne 1997); knowledge of the traits that contribute to tolerance can be applied in crop selection (Clarke 1986). Selecting for enhanced tolerance traits in crops may offer a more evolutionarily stable means of defending crop plants than selecting for increased resistance because tolerance does not impose reciprocal selection on natural enemies, whereas resistance does (Stowe et al. 2000).

Mechanisms of tolerance can be associated with two general characteristics of plants: resource allocation patterns and architecture (Stowe et al. 2000). With respect to the timing of natural enemy damage, tolerance traits can be pre-existing or induced (Stowe et al. 2000). Pre-existing mechanisms of tolerance to herbivory include high levels of carbon storage in roots (Strauss and Agrawal 1999), high numbers of dormant lateral meristems, and a highly integrated vascular system (Stowe et al. 2000). Induced mechanisms of tolerance to herbivory include the ability to transport stored carbon from roots to shoots, increased branching following herbivore-imposed release of apical dominance, increased rates of photosynthesis, changes in flowering phenology (Strauss and Agrawal 1999), and potentially, enhanced integration of the vascular system (Stowe et al. 2000). Little is known about the mechanisms of tolerance to pathogen infection. Clarke (1986) suggests candidate mechanisms of tolerance to pathogen infection, including pre-existing large root systems and post-infection increases in leaf production, root growth, and photosynthetic rates of uninfected leaves.

Tolerance is a relative measure that is based on two variables: the amount of natural enemy damage sustained, and plant fitness. The amount of damage sustained is a function of the resistance level of the plant. Plant resistance can be measured in either of the two following ways: the inverse of the amount of plant damage accrued per unit time, or the inverse of natural enemy performance (e.g. biomass attained or fecundity). Basing resistance measures on the performance of the natural enemy is particularly useful when plant damage is difficult to quantify. Plant damage due to pathogen infection (disease) is challenging to measure because some aspects of disease are invisible (Clarke 1986), obscuring the relationship between visible disease symptoms and fitness (Gaunt 1995).

A common approach to evaluating tolerance is the norm of reaction approach (Simms 2000), which measures tolerance as the slope of the line obtained by regressing fitness over a damage gradient. The norm of reaction approach allows one to compare the tolerance levels of multiple genotypes that vary in overall vigor. Intolerant genotypes exhibit a negative relationship between damage and fitness; completely tolerant genotypes exhibit no relationship between damage and fitness (a slope of zero), while higher damage levels correspond to higher fitness values in overcompensating genotypes. Genetic variation in tolerance is demonstrated by differences in the regression slopes of fitness over the damage gradient. In pathosystem studies, measures of tolerance are often based on visible disease symptoms, while invisible components of disease are not taken into account (Gaunt 1995). Clarke (1986) describes a measure of "overall" tolerance, which relates the relationship between pathogen density and plant fitness and thus includes plant tolerance to the pathogen and to the primary and secondary components of disease. When measuring overall tolerance, variation in plant resistance to the pathogen will not affect comparisons of overall tolerance between genotypes because (assuming an approximately linear relationship between damage and fitness) the slope of the regression of fitness over pathogen density is independent of the distance between control and infected pathogen densities.

Measures of tolerance to foliar infections are often based on visible disease symptoms (Gaunt 1995). Two visible symptoms that are commonly evaluated are chlorosis (yellowing as a result of chloroplast degradation) and necrotic lesions (regions of cell death). Tissues exhibiting these disease symptoms are increasingly thought to have undergone a type of premature/pathogen-induced senescence program; similarities exist between diseased and naturally senescing leaves with respect to visible symptoms and molecular activities (e.g. Greenberg and Ausubel 1993; Pérez-García et al. 1995; Morel and Dangl 1997; Butt et al. 1998; Pérez-García et al. 1998a; Pérez-García et al. 1998b; Weaver et al. 1998; Quirino et al. 1999; Obregón et al. 2001; Robatzek and Somssich 2001; Cots et al. 2002; Navabpour et al. 2003; Olea et al. 2004; Pageau et al. 2006). Premature senescence also occurs in plant-herbivore systems. In response to green peach aphid attack, *Arabidopsis thaliana* leaves prematurely senesce, possibly reducing green peach aphid performance and thus contributing to resistance (Pegadaraju 2005). During natural leaf senescence, nutrients are remobilized and redistributed by the plant (e.g. for storage or reproduction; Lim et al. 2003). The relative efficacies of pathogen-induced and natural leaf senescence in remobilizing nutrients have not been compared, although several studies have detected reduced nitrogen remobilization from diseased leaves (Dimmock and Gooding 2002; Barbottin et al. 2002). In addition, Barbottin et al. (2005) detected genetic variation in wheat in the rate and efficiency of nitrogen remobilization from diseased leaves. Nitrogen is an important limiting nutrient, and genetic variation in the effect of infection on nitrogen remobilization may explain genetic variation in tolerance to infection. To our knowledge, there has been no study that explored genetic variation in nutrient remobilization following pathogen infection in the context of tolerance.

Here we explore the link between the amount of nitrogen remobilized from infected leaves and tolerance to infection using the *Arabidopsis thaliana – Pseudomonas syringae* pathosystem. This pathosystem is a model system for examining mechanisms of plant resistance to pathogens (Katagiri et al. 2002). Kover and Schaal (2002) demonstrated that tolerance is present in this pathosystem. Moreover, genetic variation in tolerance has

been detected in this pathosystem (Kover and Schaal 2002; M.B. Unpublished) and symptom severity has been shown to serve as a reasonable estimate of pathogen density when using the norm of reaction approach to measure overall tolerance (Kover and Schaal 2002; Korves and Bergelson 2003; Korves and Bergelson 2004). In addition, increased branch and inflorescence production and changes in flowering phenology are potential candidate tolerance traits in this pathosystem (Korves and Bergelson 2003) warranting further study. Our study used 10 genetically disparate *A. thaliana* accessions (Table 1) to address the following questions:

1. Do *A. thaliana* genotypes vary in their susceptibility to the bacterial pathogen *Pseudomonas syringae*?
2. Is there genetic variation for the effect of infection on the amount of nitrogen remobilized from infected rosette leaves?
3. Is there genetic variation in the effect of infection on plant fitness?
4. Does genetic variation in the effect of infection on the amount of nitrogen remobilized from rosette leaves explain genetic variation in plant fitness?

Study System

Arabidopsis thaliana (mouse ear cress) is an annual belonging to the family Brassicaceae (mustards). The geographic range of *A. thaliana* extends across Asia, Europe and North America; North American populations are believed to have been introduced within the last 200 years. The ecology of *A. thaliana* is relatively poorly characterized with respect to its natural enemies, despite its prevalence in biological research.

Pseudomonas syringae is an economically important bacterial pathogen that infects hundreds of plant species worldwide. Infections by *P. syringae* are non-systemic, and typical symptoms in susceptible hosts include foliar spots and blights (Alfano and Collmer 1996). *Pseudomonas syringae* is categorized into over forty pathovars (pv.) according to degree of pathogenicity (ability to cause disease). Host range further divides

Table 1. The names of the accessions used in our experiments and their stock number at the Arabidopsis Information Management System.

Code	Stock	Accession
1	CS6673	Col-0
2	CS6674	Ct-1
3	CS6736	Hi-0
4	CS1380	Mt-0
5	CS6805	No-0
6	CS6839	Po-0
7	CS6850	Rsch-4
8	CS6874	Tsu-0
9	CS6889	Wil-2
10	CS6897	Wu-0

Kover and Schaal (2002).

pathovars into strains, for example *P. syringae* pv. *tomato* strain DC3000. *Pseudomonas syringae* has been described as a biotrophic pathogen, a necrotrophic pathogen, and a hemibiotrophic pathogen (Thaler et al. 2004). These descriptors represent a continuum relating the vitality status of the host cell to nutrient acquisition by the pathogen. Biotrophic pathogens obtain resources from living cells, necrotrophs from dead cells, and hemibiotrophs exhibit an initial biotrophic phase followed by a necrotrophic phase.

Natural colonies of *P. syringae* can be found on leaf surfaces and within leaf apoplasts, although the majority of *P. syringae* populations are epiphytic, presumably consuming leaf leachates. The *P. syringae* cells can emigrate from surrounding vegetation to leaf surfaces via aerial transport, rain-splash, or insect transport (Hirano and Upper 2000). In addition, *P. syringae* cells contained within the seeds can colonize the surfaces of seedlings. Both the density of earlier colonists and host resistance to infection probably determine colonization success. It appears that once epiphytic *P. syringae* populations reach a certain size, the bacteria enter the intercellular space of the leaf (the apoplast) through openings in the cuticle (stomata and lesions). The apoplast is believed to be relatively dry and nutrient poor, unlike host cell cytoplasm. Within the apoplast, *P. syringae* constructs a pilus to act as a needle to inject over thirty "effector" proteins through the host cell walls to the cytoplasm. If the host cell is resistant, an effector can act as an avirulence factor, triggering a host defense response called the hypersensitive response (HR). The HR involves rapid cell death around the infection site and is thought to limit the growth of certain pathogens, including strains of *P. syringae* (Katagiri et al. 2002). When a host is susceptible, effector proteins quantitatively act as "virulence" factors, presumed functions of which include facilitating water and nutrient leakage from the host cell and suppressing defense responses.

2. MATERIALS AND METHODS

Arabidopsis thaliana growth conditions

Seeds were planted in 5.72 cm x 5.72 cm pots containing equal parts Premier High Porosity soil, Palmetto Vermiculite Co. Inc. vermiculite, and Krum Horticultural perlite. To induce seed germination we stored the seeds in the dark at 4 °C for three days. The seeds were then transferred to a Percival Scientific growth chamber set at 21 °C, 70% humidity, with a photoperiod of 14 hours of light, 10 hours of dark. The trays containing the pots (50 pots per tray) were haphazardly rearranged every few days to reduce possible positional effects. The plants were watered as needed. During the course of each experiment, 500 mL of diluted (as instructed) Peters Concentrated Liquid Plant Food were added twice to each tray. In experiments 1 and 2, fertilizer was added 22 and 36 days after planting. In experiment 3, fertilizer was added 22 and 42 days after planting.

Pst DC3000 culturing and inoculum preparation

For each experiment the treatment solutions were prepared using the following protocol. The *Pst* DC3000 (obtained from American Type Culture Collection; USDA permit # 69487) were cultured for 30 hours on NYG agar plates containing 50 µg/mL of the antibiotic Rifampicin. *Pst* DC3000 is genetically modified to be resistant to Rifampicin. The presence of Rifampicin prevents contamination of the agar by other microorganisms. The bacteria were removed from the plates using a sterile cell spreader. They were then gradually added to 1 L of 10 mM $MgCl_2$ until the cell suspension reached an optical density of 0.4 when measured at a wavelength 600 nm using a GeneQuant pro spectrophotometer. This optical density corresponds to a concentration of 2×10^8 colony forming units/mL (Katagiri et al. 2002). Lastly, we added 200 µL of Silwet L-77, a surfactant. The presence of the surfactant facilitates infection by causing the treatment solutions to adhere to and spread over the leaf surfaces to which they are applied.

The control solution consisted of 1 L of 10 mM $MgCl_2$ with 200 µL of Silwet L-77. We heavily doused the plants with the treatment solutions using spray bottles and covered the trays with propagation domes for one day. *Pst* DC3000 enters the leaf apoplast through natural leaf openings, primarily the stomata. Maintaining high humidity aids the infection process by increasing the frequency and aperture of stomata opening.

Assaying *A. thaliana* symptom severity

In experiments 1 and 3 (described below) we estimated *A. thaliana* symptom severity in response to infection by *Pst* DC3000 by quantifying the proportion of total rosette area that exhibited chlorosis, necrosis, and purpling (disease symptoms; Figure 1). Symptomatic areas were quantified from digital photographs taken with a Nikon Coolpix 5900 mounted on a tripod positioned over the plants. The symptomatic areas of the rosette were separated from the green areas using Adobe Photoshop 7.0.1 (Adobe Systems Inc. 2002). All pixels were then filled with black. In Image J (Rasband, W.W., Image J, U.S. National Institutes of Health, Bethesda, Maryland, USA, http://rsb.info.nih.gov/ij/ 1997-2006), the numbers of pixels corresponding to the symptomatic rosette areas and green rosette areas were counted using the programs "measure" option. The symptomatic proportion of the rosette (our measure of susceptibility) was then calculated by dividing symptomatic rosette area (in pixels) by total rosette area (in pixels).

Statistical analyses

All of our experimental designs were based on an analysis of variance (ANOVA) framework. We used nonparametric (NP) tests to analyze our data because the data did not meet ANOVA assumptions of multivariate normality and homogeneity of variance-covariance matrices. The NP tests described below are sensitive to differences in multivariate dispersion. Dispersion is measured as the sum of squared distances between

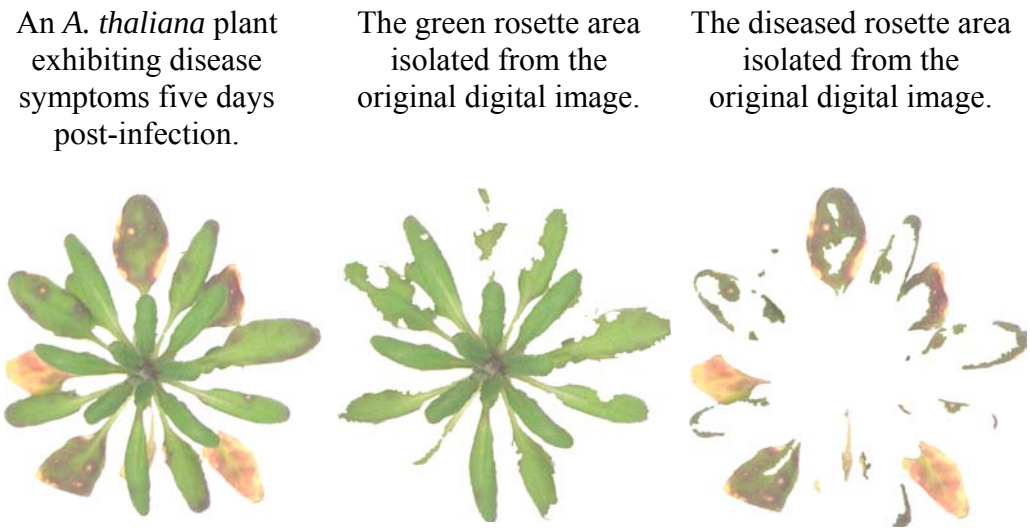


Figure 1: Quantifying *A. thaliana* disease symptom severity five days after infection with *Pst* DC3000.

observations and their treatment group centroid in multivariate space. All NPANOVA and NP multivariate analyses of variance (NPMANOVA) were accompanied by a test for homogeneity of treatment group dispersions using the computer program PERMDISP (Anderson 2004; Anderson 2006). Heterogeneity in treatment group dispersions can result in rejection of the null hypothesis in NPANOVA and NPMANOVA. Unless otherwise noted in the results, treatment group dispersions were not different. All analyses were based on Euclidean distances and involved 999 permutations of standardized (to z-scores) response variables. Pairwise comparisons were performed under significant model terms. The sequential Bonferroni procedure was used to adjust the significance level for multiple comparisons (Holm 1979). We used a significance level of $\alpha = 0.05$ for all analyses.

We conducted all NPANOVA and NPMANOVA using permutational analysis of variance (PERMANOVA; Anderson 2001a; Anderson 2001b; McArdle and Anderson 2001). PERMANOVA is a computer program that randomly permutes the sampling units within a response variable data matrix. The program calculates a pseudo *F*-statistic for the original response variable data matrix and for each permutation of the data matrix by dividing the within-group sum of squared distances by the total sum of squared distances between observations. The null expectation is that the random permutations of the sampling units will not affect the value of the pseudo *F*-statistic (this would indicate no treatment effects on sampling unit values). A *P*-value is derived by comparing the pseudo *F*-statistic associated with the original response variable data matrix to the null distribution of pseudo *F*-statistics associated with the random permutations.

To test for effects of the independent variables on our response variables, while taking into account the correlation structure of the response variables, and to understand the contribution of original response variables to distinguishing treatment groups from one another in multivariate space, we conducted canonical analyses of principal coordinates (CAP) using the CAP computer program (Anderson and Robinson 2003; Anderson and Willis 2003; Anderson 2004). CAP uses principle coordinate analysis (PCO) to reduce

the dimensionality of the response data cloud. It then conducts either a canonical discriminant analysis (CDA) or a canonical correlation analysis on all or a subset of the resulting PCO axes. CDA constructs linear combinations of the principal coordinates that minimize within-group variation and maximize between-group variation in canonical space. CAP tests the goodness of fit of the CDA using the "leave-one-out" approach (Lachenbruch and Mickey 1968). Finally, CAP produces the canonical correlations of the original response variables with the canonical axes. Response variables that have strong absolute correlations with the canonical axes represent variables that are important in distinguishing the treatment groups in canonical space.

Experiment 1: assaying *A. thaliana* symptom severity

In order to obtain measures of overall tolerance to infection, we required an estimate of the density of *Pst* DC3000 in the *A. thaliana* leaves. Kover and Schaal (2002) and Korves and Bergelson (2003) demonstrated that the density of *Pst* DC3000 strongly correlates with *A. thaliana* symptom severity four days post-infection. We assayed symptom severity (described above) using 15 replicates of each *A. thaliana* accession. The plants were infected 50 days after they were planted and were photographed five days post-infection. We used NPANOVA to test for genetic variation in *A. thaliana* susceptibility under the imposed experimental conditions.

Experiment 2: effects of infection on nitrogen remobilization, fitness, and other plant traits

Effects of infection on the remobilization of nitrogen from infected leaves

A potential consequence of infection with respect to immature leaves is reduced subsequent growth. Effects of infection on subsequent leaf growth may affect the original amount of leaf nitrogen available for remobilization during senescence (induced or natural). As a result, tests for an effect of infection on the amount of nitrogen

remobilized from leaves may be confounded. Ideally, infection and control treatments would be applied when *A. thaliana* rosettes were mature, but had not yet begun natural senescence. We treated our plants when the rosettes were large, and when individuals within a few of the accessions had begun to bolt. The rosettes did not exhibit natural senescence symptoms at this time. To test for an effect of treatment on subsequent leaf growth, we used NPANOVA with independent variables accession, treatment status, and accession*treatment status. As a proxy for subsequent leaf growth (our dependent variable), we used the mass of the dried, sampled, senesced rosette leaves (described below). A significant effect of treatment status on dry leaf sample mass would indicate that our tests for the effect of infection on leaf nitrogen remobilization may be confounded by differences in leaf growth following infection.

To test for an effect of infection on the amount of nitrogen remobilized from infected leaves, we infected *A. thaliana* with *Pst* DC3000 and measured the nitrogen content of senesced, treated leaves. For each accession we planted fifteen replicates per treatment. The plants were infected 49 days after they were planted. The first 12 leaves to senesce on each plant were collected, wrapped in Kraft ® paper, and stored at room temperature. For each plant, all of the collected leaves were combined and ground to a powder in a Wig-L-Bug ® ball mill. The samples were dried, weighed, and assessed for nitrogen content at the Department of Soil Sciences Laboratory at the University of North Carolina, Raleigh. Nitrogen content was measured using a PerkinElemer 2400 CHN analyzer. To evaluate the effect of infection on the amount of nitrogen remobilized from infected *A. thaliana* leaves, and to explore genetic variation in the effect of infection on the amount of nitrogen remobilized from leaves, we used NPANOVA with subsequent pairwise comparisons under significant model terms. We used the proportion of nitrogen with respect to the total sample mass as the response variable.

The effect of infection on plant fitness and genetic variation in tolerance to infection

To examine the effect of infection on *A. thaliana* fitness, we estimated seed number and average seed size for each plant. We further explored the effects of infection on *A. thaliana* fitness by testing for trans-generational effects of infection on progeny size and susceptibility to *Pst* DC3000. Fruits were collected for 4.5 months, at which time fruit production was still occurring, but at very low levels. We assumed that continuing fruit collection would have little bearing on our plant fitness estimates.

Mature fruits were removed and stored in coin envelopes prior to dehiscence. Each coin envelope represented a collection period of seven to ten days. Occasionally, fruits dehisced before they were collected; we represented the number of seeds lost from these fruits with the average number of seeds per fruit collected within the respective seven to ten day collection period. Approximately 4% of the seeds in this experiment were lost due to fruit dehiscence prior to collection.

We collected 43,051 fruits; we used the 35,693 fruits obtained during the first five collection periods for each plant to estimate plant fitness. The number of seeds from the remaining collection periods was estimated by multiplying their number of fruits by the average number of seeds per fruit associated with envelopes 4 and 5. We used envelopes 4 and 5 because later fruits appeared to be smaller than earlier fruits, and they were associated with smaller average numbers of seeds per fruit.

To prepare the seeds for the photographs we removed them from their husks using 7.62 cm stainless steel sieves with size 40 stainless steel mesh (W.S. Tyler, Mentor, Ohio, U.S.A.). We placed the seeds on a white background and spread them out with a dissecting needle (Figure 2). The seeds were then photographed from above using a Nikon Coolpix 5900 mounted on a tripod. To allow comparability of seed size between samples, the same level of zoom was used for each photograph. We programmed a macro in Image J to process the images. The macro converted the images to 8-bit



Figure 2: An example of the digital images processed in order to obtain an estimate of the number of seeds produced by each plant, and average seed size.

grayscale images, used the watershed function to enhance the separation of touching seeds, and the analyze particles function yielded seed number and area. To prevent the inclusion of small debris in our data, we adjusted the bin width under analyze particles so that particles smaller than 10 pixels were omitted (average seed area was approximately 23 pixels). We used NPMANOVA to examine the effects of accession, treatment, and accession*treatment on seed number and size.

To test for trans-generational effects of infection on progeny size and susceptibility to *Pst* DC3000 (measures of performance), we infected a single representative of the progeny of 12 plants per parent treatment combination with *Pst* DC3000 46 days after they were planted. To control for the potential effect of seed production date with respect to parent phenology, only seeds produced during the first two collection periods were used. We assayed progeny susceptibility as described above. To test for effects of accession, parent treatment status, and the accession*parent treatment status interaction on progeny size and susceptibility, we used NPMANOVA.

Effects of infection on plant traits and links to tolerance

To explore the effects of infection on plant traits and to identify traits that may be associated with tolerance to infection, we documented the number of days to bolting, flowering, and fruiting; measured the height of the main stem, and counted the number of inflorescences, primary branches, and secondary branches. The number of days to bolting, flowering, and fruiting were very highly correlated. We used the number of days to flowering to represent phenology in CAP analyses for each accession because, of the three, it is the most accurately measured. CAP analyses allowed us to explore treatment effects on plant traits while accounting for their correlation structure. In order to identify traits linked to tolerance, we looked for inconsistencies between tolerant and intolerant accessions (as identified above) in the traits that were highly correlated with the canonical axes that separated the treatment groups in canonical space.

3. RESULTS

Due to seedling mortality, experiment 2 treatment groups contained unequal numbers of replicates. To balance the design for statistical analyses, we selected the appropriate number of individuals from each treatment group at random to drop from the final data set. Accession 8 was omitted from experiment 2 analyses due to a considerable level of early mortality. The final replication numbers for experiment 2 were 14 per treatment group in the parental generation, and 11 per treatment group in the progeny generation.

Experiment 1: assaying *A. thaliana* symptom severity

All *A. thaliana* accessions exhibited disease symptoms five days post-infection. Median symptomatic rosette proportion was 0.75, with a range of 0.13 to 0.94. We observed genetic variation in symptom severity (Table 2); accession explained approximately 43% of the variation in symptom severity. Subsequent pairwise comparisons between accessions indicated that one accession was responsible for the observed genetic variation in symptom severity (Table 3). Accession data dispersion differences did not explain significant pairwise comparisons (Tables 4 and 5).

Experiment 2: effects of infection on nitrogen remobilization, fitness, and plant traits

Effects of infection on the remobilization of nitrogen from infected leaves

Arabidopsis thaliana leaves were fully expanded or near full expansion when treated; leaf mass was not affected by treatment status (Table 6). The amount of nitrogen remobilized from infected leaves was affected by infection (Table 7); infection approximately doubled the median amount of nitrogen retained in senesced leaves. The median amount of nitrogen retained in control leaves was 0.0100, with a range of 0.0069-0.0182. The median amount of nitrogen remaining in infected leaves was 0.0183, with a range of

Table 2. NPANOVA results testing for genetic variation in the symptomatic proportion of the rosette five days post-infection.

Source	df	SS	SS%	MS	*F*	P-Value
Accession	9	63.56	42.66	7.06	11.57	0.001
Residual	140	85.44	57.34	0.61		
Total	149	149				

Table 3. Pairwise comparisons between accessions for the symptomatic proportion of the rosette five days post-infection.

Groups	t	P-value	Holm-Adjusted P-Value
*1,10	3.4301	0.001	0.001
*3,10	4.0788	0.001	0.001
*6,10	2.8853	0.001	0.001
7,10	3.5326	0.002	0.001
4,10	3.3585	0.003	0.001
2,10	3.0934	0.004	0.001
8,10	3.0785	0.006	0.001
5,10	2.7955	0.012	0.001
3,9	2.3662	0.026	0.001
6,9	1.7832	0.044	0.001
9,10	1.8344	0.073	0.001
2,3	1.6864	0.102	0.001
1,9	1.6774	0.103	0.002
3,4	1.6735	0.109	0.002
7,9	1.7201	0.115	0.002
4,9	1.4153	0.169	0.002
2,6	1.2882	0.185	0.002
3,8	1.2454	0.212	0.002
8,9	1.257	0.212	0.002
4,6	1.2262	0.232	0.002
3,5	1.2059	0.246	0.002
2,9	1.174	0.248	0.002
6,8	1.1693	0.262	0.002
5,6	1.1975	0.271	0.002
5,9	1.0461	0.32	0.002
3,7	0.8625	0.4	0.003
6,7	0.9895	0.417	0.003
2,7	0.7829	0.436	0.003
1,2	0.7816	0.44	0.003
1,6	0.9341	0.472	0.003
4,7	0.6426	0.478	0.003
1,3	0.6755	0.524	0.004
1,4	0.6494	0.538	0.004

Significant comparisons at $\alpha = 0.05$ are indicated with an asterisk.

Table 3. Continued.

Groups	t	P-value	Holm-Adjusted P-Value
1,5	0.5671	0.579	0.004
5,7	0.5349	0.6	0.005
1,8	0.5045	0.62	0.005
7,8	0.468	0.645	0.006
3,6	0.6682	0.745	0.006
2,4	0.2526	0.804	0.007
2,8	0.2212	0.835	0.008
5,8	0.1202	0.895	0.01
4,5	0.1137	0.905	0.013
1,7	0.0865	0.928	0.017
2,5	0.0604	0.949	0.025
4,8	0.0315	0.98	0.05

Significant comparisons at $\alpha = 0.05$ are indicated with an asterisk.

Table 4. PERMDISP results testing for homogeneity of accession dispersion for the symptomatic proportion of the rosette five days post-infection.

Source	df	SS	SS%	MS	*F*	P-Value
Accession	9	7.17	10.68	0.8	1.86	0.039
Residual	140	59.93	89.32	0.43		
Total	149	67.1				

Table 5. PERMDISP pairwise comparisons between accessions for dispersion in the symptomatic proportion of the rosette five days post-infection.

Groups	t	P-Value	Holm-Adjusted P-Value
*4,10	3.4773	0.001	0.001
4,9	3.0467	0.005	0.001
4,8	2.9101	0.008	0.001
7,10	2.7183	0.01	0.001
2,10	2.7275	0.012	0.001
3,10	2.7504	0.012	0.001
4,6	1.5888	0.023	0.001
4,5	2.1409	0.025	0.001
7,9	2.0847	0.043	0.001
1,10	2.2122	0.044	0.001
3,9	2.117	0.046	0.001
2,9	2.0882	0.064	0.001
8,10	1.8899	0.069	0.002
3,6	1.3329	0.13	0.002
1,9	1.5122	0.133	0.002
6,7	1.3593	0.133	0.002
3,8	1.4741	0.142	0.002
2,6	1.322	0.146	0.002
7,8	1.4383	0.155	0.002
2,8	1.4329	0.156	0.002
2,4	1.3681	0.173	0.002
5,7	1.3365	0.173	0.002
3,4	1.2988	0.188	0.002
5,10	1.3887	0.192	0.002
1,6	1.2084	0.216	0.002
2,5	1.2851	0.224	0.003
3,5	1.3146	0.226	0.003
1,4	1.1265	0.264	0.003
8,9	1.0521	0.285	0.003
4,7	0.9149	0.351	0.003
9,10	0.9096	0.402	0.003
1,5	0.8752	0.416	0.004
1,8	0.7703	0.423	0.004

Significant comparisons at $\alpha = 0.05$ are indicated with an asterisk.

Table 5. Continued.

Groups	t	P-Value	Holm-Adjusted P-Value
6,8	0.9828	0.454	0.004
5,9	0.5778	0.549	0.005
5,6	0.8427	0.554	0.005
1,7	0.3746	0.704	0.006
6,9	0.6058	0.776	0.006
1,3	0.2869	0.79	0.007
5,8	0.3149	0.799	0.008
1,2	0.2527	0.826	0.01
2,7	0.1897	0.841	0.013
3,7	0.1452	0.888	0.017
6,10	0.1704	0.947	0.025
2,3	0.0506	0.956	0.05

Significant comparisons at $\alpha = 0.05$ are indicated with an asterisk.

Table 6. NPANOVA results testing for effects of accession, treatment status, and their interaction on the mass of the collected, senesced rosette leaves.

Source	df	SS	SS%	MS	*F*	P-Value
Accession [A]	8	47.01	18.73	5.88	6.84	0.001
Treatment Status [T]	1	0.17	0.07	0.17	0.2	0.654
A X T	8	2.94	1.17	0.37	0.43	0.903
Residual	234	200.89	80.03	0.86		
Total	251	251				

Note: Bracketed letters represent shorthand notation for the independent variables.

Table 7. NPANOVA results testing for effects of accession, treatment status, and their interaction on the nitrogen content of the collected, senesced rosette leaves.

Source	df	SS	SS%	MS	*F*	P-Value
Accession [A]	8	29.1	11.59	3.64	8.91	0.001
Treatment Status [T]	1	112.93	44.99	112.93	276.75	0.001
A X T	8	13.48	5.37	1.69	4.13	0.001
Residual	234	95.49	38.04	0.41		
Total	251	251				

Note: Bracketed letters represent shorthand notation for the independent variables.

0.0099-0.0436. There was genetic variation in the effect of infection on the amount of nitrogen remobilized from infected leaves; however one accession explained the majority of the genetic variation (Tables 8-10). Within accession and treatment status, data dispersions were different (Tables 11 and 12) however these differences do not affect our interpretation of the rejection of the null in the NPANOVA. Interestingly, data dispersion was higher for the infected plants (Figure 3).

The effect of infection on plant fitness and genetic variation in tolerance to infection

Of all of the fitness correlates measured in our study (seed number, average seed size, progeny size, and progeny resistance to *Pst* DC3000), only seed number was affected by infection (Tables 13-17). Infection reduced median seed number by approximately 11% (4696 control: 4202 infected; Figure 4). We did not detect genetic variation in overall tolerance to infection, as there was no interaction effect between accession and treatment status on seed number, and experiment 1 showed all 10 *A. thaliana* accessions to harbor similar pathogen densities (as inferred by symptom severity). We found that there was a significant, negative correlation between the amount of nitrogen remaining in infected, senesced leaves and seed number (Spearman's $\rho = -.3463$, $p < .0001$; Figure 5), suggesting that plants that inefficiently remobilized nitrogen (potentially as a result of environmental heterogeneity) had relatively poor fitness.

Effects of infection on plant traits and links to tolerance

Infection by *Pst* DC3000 affected *A. thaliana* traits. There were significant differences in multivariate treatment group locations in canonical space for eight of the nine accessions included in the CAP analyses (Table 18). Main stem height was consistently reduced in infected plants (Table 19; Figure 6). Other plant traits also correlated strongly with the canonical axis separating treatment groups, but these traits were inconsistent among the accessions. We further explored the effect of infection on main stem height using NPANOVA. Accession, treatment status, and accession*treatment status explained a

Table 8. Pairwise comparisons between accessions within the control treatment of the nitrogen content of the collected, senesced rosette leaves.

Groups	t	P-Value	Holm-Adjusted P-Value
*3,6	3.8407	0.001	0.001
*3,7	5.0296	0.001	0.001
*3,9	4.0569	0.001	0.001
*5,7	4.2205	0.001	0.002
*1,3	3.398	0.002	0.002
3,4	3.039	0.003	0.002
5,9	3.09	0.004	0.002
2,3	3.2072	0.006	0.002
3,10	3.1693	0.006	0.002
5,6	2.8569	0.009	0.002
1,7	2.5278	0.018	0.002
7,10	2.3569	0.02	0.002
2,5	2.2268	0.028	0.002
1,5	2.3766	0.029	0.002
6,7	2.186	0.04	0.002
5,10	2.1592	0.044	0.002
4,7	2.0869	0.048	0.003
7,9	2.1271	0.051	0.003
4,5	2.0569	0.057	0.003
2,7	2.0153	0.066	0.003
3,5	1.1092	0.266	0.003
9,10	0.8035	0.429	0.003
1,9	0.7964	0.457	0.004
6,10	0.5896	0.548	0.004
4,9	0.6719	0.568	0.004
2,9	0.5344	0.586	0.005
1,6	0.5476	0.633	0.005
4,6	0.4901	0.652	0.006
2,6	0.3494	0.737	0.006
6,9	0.2358	0.832	0.007
2,10	0.1744	0.879	0.008
1,10	0.1007	0.902	0.01
2,4	0.131	0.907	0.013

Significant comparisons at $\alpha = 0.05$ are indicated with an asterisk.

Table 8. Continued.

Groups	t	P-Value	Holm-Adjusted P-Value
1,2	0.0945	0.931	0.017
1,4	0.0568	0.966	0.025
4,10	0.0316	0.984	0.05

Significant comparisons at $\alpha = 0.05$ are indicated with an asterisk.

Table 9. Pairwise comparisons between accessions within the infected treatment of the nitrogen content of the collected, senesced rosette leaves.

Groups	t	P-Value	Holm-Adjusted P-Value
*1,3	5.3206	0.001	0.001
*3,4	5.1854	0.001	0.001
*3,6	3.988	0.001	0.001
*3,7	4.4855	0.001	0.002
*3,10	3.8812	0.002	0.002
*3,5	3.4764	0.004	0.002
3,9	2.9985	0.005	0.002
2,3	3.0969	0.008	0.002
1,9	2.1204	0.032	0.002
1,6	2.1526	0.04	0.002
4,9	2.0838	0.04	0.002
1,10	2.1104	0.044	0.002
4,10	2.0065	0.049	0.002
1,2	1.8421	0.056	0.002
4,6	2.0281	0.061	0.002
2,4	1.8246	0.087	0.002
4,5	1.4759	0.152	0.003
1,5	1.4781	0.174	0.003
7,9	1.2649	0.203	0.003
1,7	1.094	0.284	0.003
4,7	1.09	0.286	0.003
2,7	1.0319	0.325	0.003
7,10	0.9191	0.346	0.004
6,7	0.891	0.368	0.004
5,7	0.6514	0.523	0.004
6,9	0.6278	0.576	0.005
5,9	0.5225	0.605	0.005
9,10	0.5636	0.609	0.006
2,6	0.4097	0.725	0.006
2,10	0.3527	0.752	0.007
2,5	0.3453	0.754	0.008
2,9	0.1651	0.874	0.01
1,4	0.1246	0.912	0.013

Significant comparisons at $\alpha = 0.05$ are indicated with an asterisk.

Table 9. Continued.

Groups	t	P-Value	Holm-Adjusted P-Value
6,10	0.0674	0.947	0.017
5,10	0.0538	0.963	0.025
5,6	0.0039	0.996	0.05

Significant comparisons at $\alpha = 0.05$ are indicated with an asterisk.

Table 10. NPANOVA results testing for effects of accession, treatment status, and their interaction on the nitrogen content of the collected, senesced rosette leaves, excluding accession 3.

Source	df	SS	SS%	MS	*F*	P-Value
Accession [A]	7	5.64	2.53	0.88	1.68	0.113
Treatment Status [T]	1	112.52	50.46	112.52	233.98	0.001
A X T	7	4.82	2.16	0.69	1.43	0.210
Residual	208	100.02	44.85	0.48		
Total	223	223				

Note: Bracketed letters represent shorthand notation for the independent variables.

Table 11. PERMDISP results testing for homogeneity of treatment group nitrogen data dispersion.

Source	df	SS	SS%	MS	*F*	P-Value
Accession [A]	8	3.74	6.94	0.47	3.16	0.001
Treatment Status [T]	1	13.36	24.81	13.36	90.36	0.001
A X T	8	2.16	4.02	0.27	1.83	0.087
Residual	234	35.55	66.02	0.15		
Total	251	53.85				

Note: Bracketed letters represent shorthand notation for the independent variables.

Table 12. PERMDISP pairwise comparisons between accessions for nitrogen data dispersion.

Groups	t	P-Value	Holm-Adjusted P-Value
*1,3	3.3519	0.001	0.001
3,6	2.7215	0.009	0.001
3,7	2.5554	0.013	0.001
3,4	2.4304	0.014	0.002
3,10	2.5077	0.023	0.002
1,5	2.1368	0.037	0.002
1,2	1.8832	0.053	0.002
1,4	1.5178	0.13	0.002
1,9	1.5225	0.131	0.002
3,9	1.559	0.133	0.002
5,6	1.4381	0.176	0.002
5,10	1.2386	0.205	0.002
3,5	1.2191	0.21	0.002
2,6	1.2412	0.224	0.002
1,7	1.2235	0.229	0.002
2,3	1.2458	0.235	0.002
5,7	1.2655	0.237	0.003
1,10	1.1364	0.256	0.003
1,6	1.034	0.285	0.003
4,5	1.113	0.299	0.003
2,10	1.0657	0.316	0.003
2,7	1.0845	0.32	0.003
2,4	0.9399	0.4	0.004
6,9	0.8729	0.436	0.004
7,9	0.7179	0.497	0.004
9,10	0.7072	0.515	0.005
4,9	0.5667	0.616	0.005
4,6	0.4731	0.644	0.006
5,9	0.4043	0.701	0.006
2,9	0.3088	0.757	0.007
4,7	0.2393	0.805	0.008
4,10	0.2366	0.819	0.01
6,7	0.2198	0.834	0.013

Significant comparisons at $\alpha = 0.05$ are indicated with an asterisk.

Table 12. Continued.

Groups	t	P-Value	Holm-Adjusted P-Value
6,10	0.1968	0.834	0.017
2,5	0.0803	0.941	0.025
7,10	0.0103	0.99	0.05

Significant comparisons at $\alpha = 0.05$ are indicated with an asterisk.

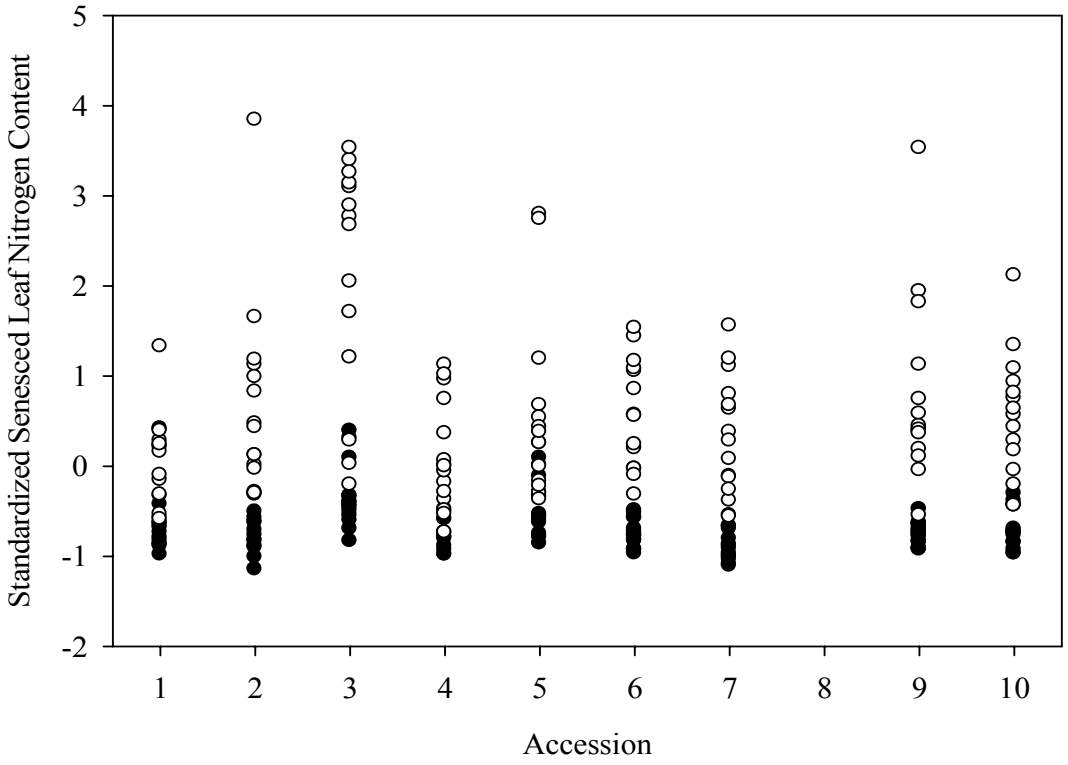


Figure 3: Nitrogen data dispersion by accession. Closed circles represent control plant standardized nitrogen values; open circles represent infected plant standardized nitrogen values.

Table 13. NPMANOVA results testing for effects of accession, treatment status, and their interaction on seed number and average seed size.

Source	df	SS	SS%	MS	*F*	P-Value
Accession [A]	8	168.22	33.51	21.03	15.37	0.001
Treatment Status [T]	1	5.23	1.04	5.23	3.83	0.028
A X T	8	8.39	1.67	1.05	0.77	0.748
Residual	234	320.15	63.78	1.37		
Total	251	502				

Note: Bracketed letters represent shorthand notation for the independent variables.

Table 14. PERMDISP results testing for homogeneity of treatment group seed number and average seed size dispersion.

Source	df	SS	SS%	MS	*F*	P-Value
Accession [A]	8	11.66	8.76	1.46	2.92	0.002
Treatment Status [T]	1	0.24	0.18	0.24	0.49	0.529
A X T	8	4.22	3.17	0.53	1.06	0.409
Residual	234	116.9	87.88	0.5		
Total	251	133.02				

Note: Bracketed letters represent shorthand notation for the independent variables.

Table 15. NPANOVA results testing for effects of accession, treatment status, and their interaction on seed number.

Source	df	SS	SS%	MS	*F*	P-Value
Accession [A]	8	154.18	30.71	19.27	13.48	0.001
Treatment [T]	1	5.93	1.18	5.93	4.15	0.015
A X T	8	7.33	1.46	0.92	0.64	0.852
Residual	234	334.55	66.64	1.43		
Total	251	502				

Note: Bracketed letters represent shorthand notation for the independent variables.

Table 16. NPANOVA results testing for effects of accession, treatment status, and their interaction on average seed size.

Source	df	SS	SS%	MS	*F*	P-Value
Accession [A]	8	121.38	48.36	15.17	28.13	0.001
Treatment Status [T]	1	0.23	0.09	0.23	0.42	0.516
A X T	8	3.2	1.27	0.4	0.74	0.674
Residual	234	126.2	50.28	0.54		
Total	251	251				

Note: Bracketed letters represent shorthand notation for the independent variables.

Table 17. NPMANOVA results testing for effects of accession, parent treatment status, and their interaction on progeny size and the symptomatic portion of the rosette five days post-infection.

Source	df	SS	SS%	MS	*F*	P-Value
Accession [A]	9	8.64	10.47	0.96	2.72	0.005
Parent Treatment Status [P]	1	0.26	0.32	0.26	0.75	0.39
A X P	9	3.15	3.82	0.35	0.99	0.43
Residual	200	70.48	85.41	0.35		
Total	219	82.52				

Note: Bracketed letters represent shorthand notation for the independent variables.

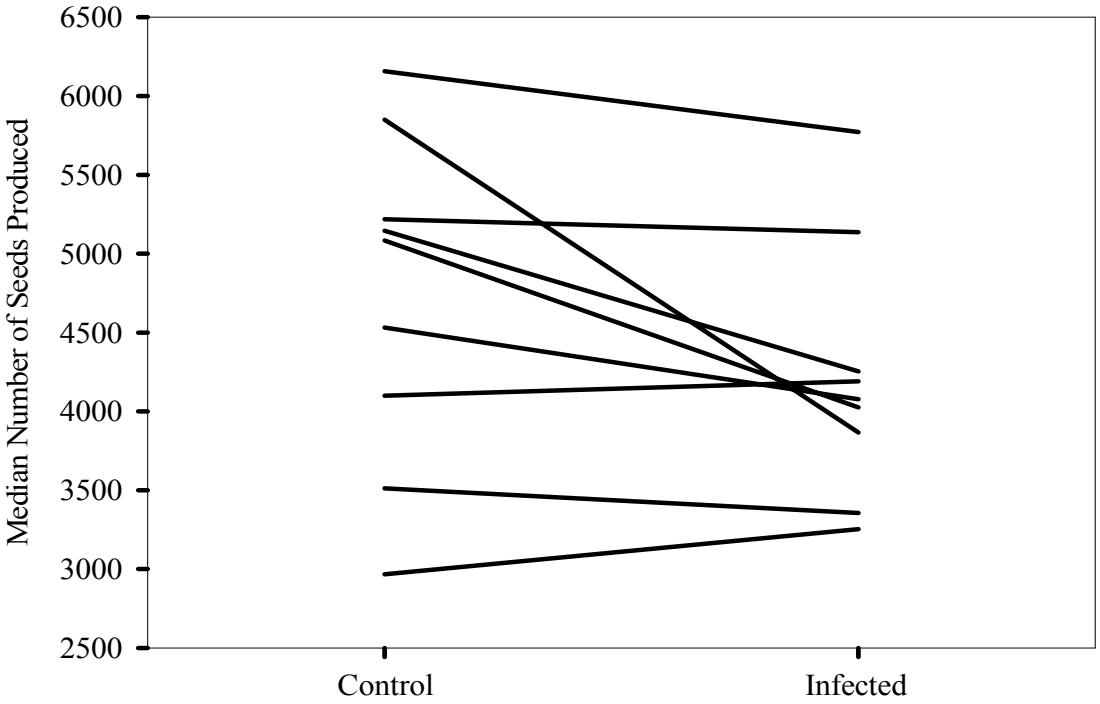


Figure 4: The median number of seeds produced by each accession by control and infected treatment groups.

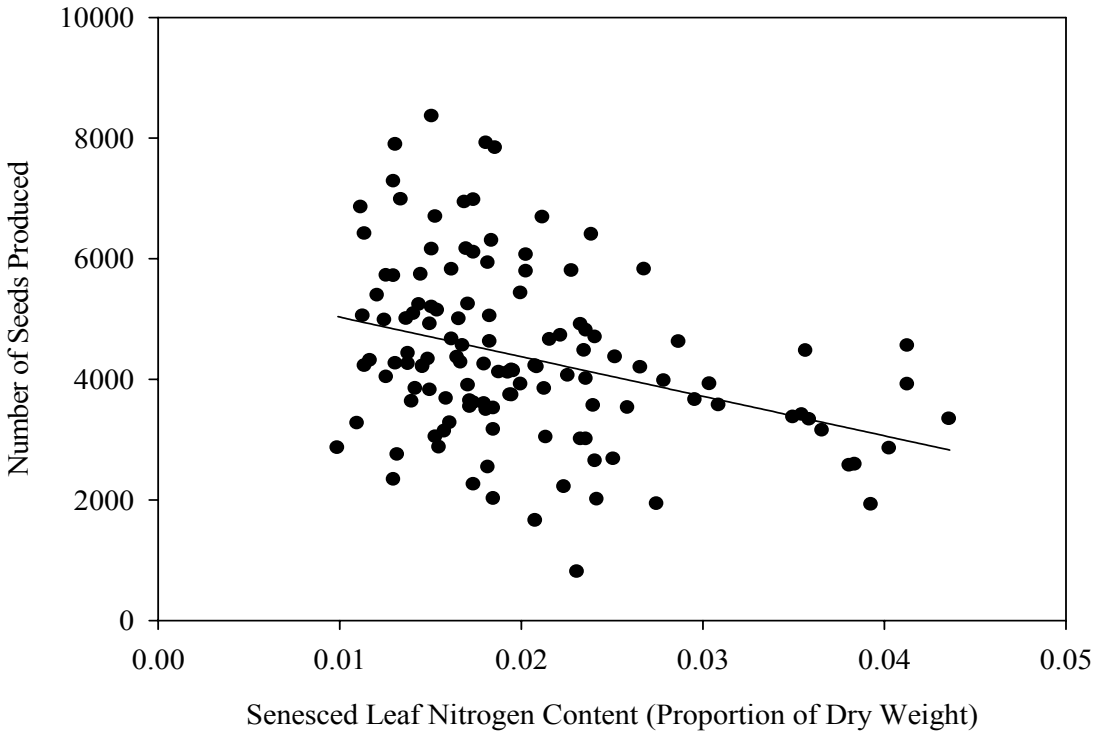


Figure 5: The association between the amount of nitrogen remaining in senesced, infected leaves, and seed number.

Table 18. The results of treatment group separation (by accession) using the CAP procedure on z-transformed performance response variables.

Accession	Number of PCO Axes	Variation Explained by the PCO axes (%)	δ^2	Permutation Test P-value
1	5	96.225	0.408	0.027
2	5	93.126	0.758	0.001
3	5	94.316	0.392	0.036
4	4	89.435	0.330	0.032
5	3	72.558	0.583	0.001
6	2	61.522	0.022	0.785
7	5	100.000	0.309	0.090
9	6	100.000	0.516	0.005
10	3	79.502	0.266	0.054

δ2 = the squared canonical correlation of the canonical axis.

Table 19. Correlations of the z-transformed, original variables with the canonical axis

Days to Flower	Number of Inflorescences	Main Stem Height	1° Branches	2° Branches	Average Number of Seeds per Fruit
0.3679	-0.3851	-0.6033	0.8069	0.1189	-0.2127
0.2392	-0.0682	0.7653	0.3435	0.4949	-0.4793
-0.2896	-0.4878	-0.9095	0.0033	0.1024	-0.2469
0.0077	-0.3714	-0.7098	0.4871	0.3765	-0.1917
0.3892	0.4213	-0.9209	-0.3125	-0.0244	0.0473
-0.196	N/A	0.6479	-0.7727	-0.7378	0.2282
-0.1573	N/A	0.5708	-0.1895	0.2738	-0.3094
0.0412	-0.089	-0.7366	0.2083	-0.0504	-0.0824
-0.4717	0.4044	0.7319	-0.0059	0.3399	0.8955

N/A indicates not applicable (there was no variation in the number of inflorescences for accessions 6 and 7).

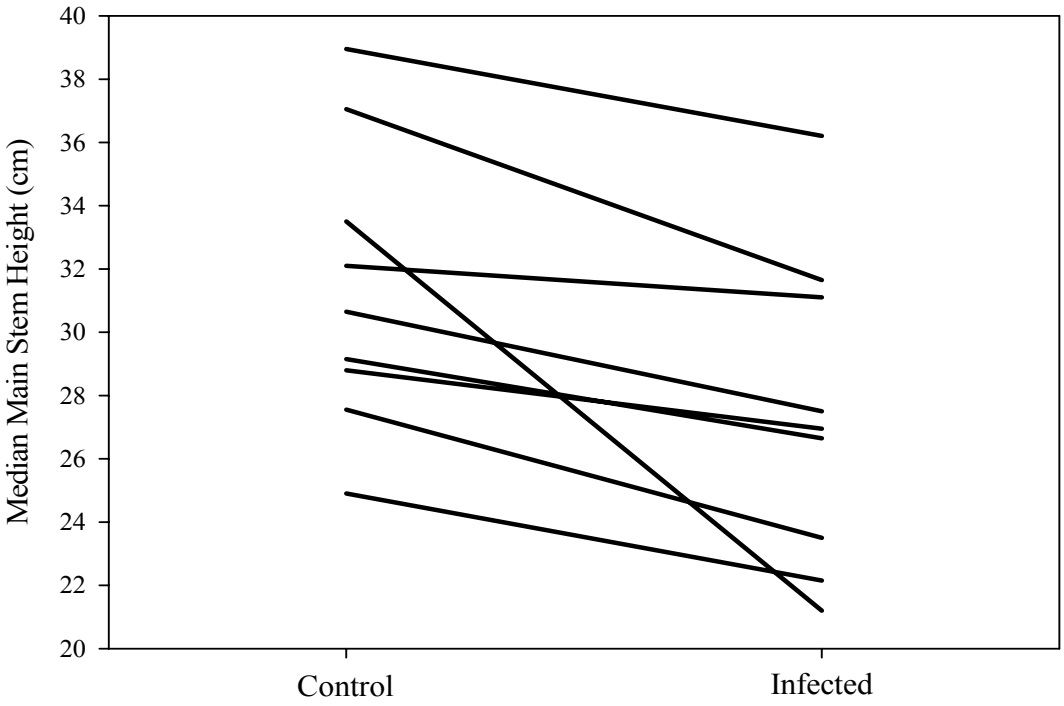


Figure 6: The effect of infection on main stem height; median main stem heights by accession, by control and infected treatment groups.

significant portion of the variation in main stem height (Table 20). Had we detected genetic variation in tolerance, we would have tested for a genetic correlation between main stem height and overall tolerance.

Table 20. NPANOVA results testing for effects of accession, treatment status, and their interaction on main stem height.

Source	df	SS	SS%	MS	*F*	P-Value
Accession [A]	8	98.74	39.34	12.34	26.19	0.001
Treatment Status [T]	1	27.71	11.04	27.71	58.81	0.001
A X T	8	14.28	5.69	1.79	3.79	0.001
Residual	234	110.27	43.93	0.47		
Total	251	251.00				

Note: Bracketed letters represent shorthand notation for the independent variables.

4. DISCUSSION

We assayed *A. thaliana* susceptibility to *Pst* DC3000 in two experiments. Plant susceptibility to pathogen infection is inversely related to resistance to pathogen infection, which is often measured in terms of pathogen density in pathosystem studies. Kover and Schaal (2002) and Korves and Bergelson (2003) found that the extent of chlorosis and necrosis in infected *A. thaliana* correlates strongly with *Pst* DC3000 density, indicating that symptom severity can be used as a proxy for susceptibility in this pathosystem. The plants in the experiments described above were grown under equivalent conditions; however for the progeny plants in the second experiment, there was a six-day delay in the second and final application of fertilizer. Consistent with previous studies (Kover and Schaal 2002; Kover et al. 2005; M.B. Unpublished), we detected genetic variation in *A. thaliana* susceptibility to *Pst* DC3000 in both experiments. The results of our two experiments were surprisingly different, however. In the first experiment, we detected very little genetic variation in susceptibility, and the median susceptibility level was relatively high (0.72). In the second experiment, we observed substantial genetic variation in susceptibility (Table A-1), and the median susceptibility level was relatively low (0.50). The inconsistencies in susceptibility observed between the two experiments indicate that measures of susceptibility in this pathosystem are highly sensitive to experimental conditions.

The timing of infection, relative to the ontogeny of the plants, may have contributed to the discrepancy in the results of our two experiments. The plants of the second experiment were noticeably smaller, and exhibited a lower frequency of bolting, relative to the plants of the first experiment. Although no senescence symptoms were visible in our plants when infected, because bolting is tightly correlated with leaf senescence in *A. thaliana* (Levey and Wingler 2005), their leaves may have begun the senescence process. Kus et al. (2002) documented reduced *P. syringae* growth in older *A. thaliana* plants (age-related resistance), possibly as a result of prior initiation of natural leaf senescence in the older plants. Senescence and foliar disease show substantial overlap in their visible

symptoms and molecular activities. The more developed plants of the first experiment may have initiated leaf senescence prior to infection, and thereby accelerated the development of senescence symptoms (explaining the high median symptom severity), and in turn obscured the detection of genetic variation in susceptibility. Plant resistance is often a function of ontogenetic stage; Boege and Marquis (2005) recommend that resistance studies include plant ontogenetic stage as a determining factor of resistance. In addition, constitutive and induced resistance markers in *A. thaliana* have been shown to be dependent on nitrogen supply (Dietrich and Heil 2004). The difference in the fertilization regime between the two experiments could have contributed to the difference in the resistance levels observed between the two experiments. Because genetic variation in the expression of resistance varies substantially between plant ontogeny and the abiotic conditions such as fertilization regime, it is challenging to understand the evolution of resistance in an evolutionary context.

Relative to resistance mechanisms little is known about the traits through which plants manifest tolerance to pathogens, despite their importance in the evolutionary ecology of pathosystems. We investigated the link between a foliar infection and the remobilization of nitrogen (a limiting nutrient) in the context of tolerance. Several recent studies involving the interaction between tomato plants and *P. syringae* pv. *tomato* have provided strong evidence that tomato plants remobilize nitrogen from infected tissues (Pérez-García et al. 1995; Pérez-García et al. 1998a; Pérez-García et al. 1998b). We found that *A. thaliana* leaves infected with *P. syringae* pv. tomato contained approximately twice the nitrogen content of healthy leaves when senesced. Using the recently reported estimate of 5% for *A. thaliana* leaf nitrogen content (Devienne-Barret et al. 2006), we estimate that infection inhibited the remobilization of 20% of leaf nitrogen content in the infected plants. Studies of agricultural pathosystems have similarly found that diseased leaves are compromised in their ability to remobilize nitrogen (Barbottin et al. 2005; Dimmock and Gooding 2002; Garry et al. 1996). To our knowledge, our study is the first to have investigated nitrogen remobilization from diseased leaves in the context of overall tolerance to infection. The nitrogen content of senesced leaves in our

study was negatively correlated with the number of seeds produced, suggesting that nitrogen remobilization may (in part) ameliorate the fitness cost of infection. Genetic variation in nitrogen remobilization efficiency from diseased leaves may in turn explain genetic variation in overall tolerance.

We detected genetic variation in the amount of nitrogen remobilized from diseased *A. thaliana* leaves. Genetic variation in nitrogen remobilization from diseased leaves has also been reported for wheat (Barbottin 2005). Contrary to previous studies (Kover and Schaal 2002; M.B. Unpublished) we did not detect genetic variation in *A. thaliana* tolerance to infection. The incongruence between the current and previous studies cannot be explained by differences in the methods used to estimate tolerance. Symptom severity, which is correlated with pathogen density, was measured using similar methods, as was seed number. Similar to *A. thaliana* susceptibility, the condition-specific manifestation of genetic variation in *A. thaliana* tolerance makes understanding the evolution of tolerance a challenge. The condition-specific expression of tolerance has been documented in other pathosystems. For example, Kniskern and Rausher (2006) detected dramatic effects of environmental heterogeneity on the expression of tolerance in the *Ipomoea purpurea – coleosporium ipomoeae* pathosystem, ranging from intolerance to overcompensation. Korves and Bergelson (2004) showed that the fitness response of *A. thaliana* to infection by *Pst* DC3000 is dependent on the presence or absence of competition; plants growing alone exhibited overcompensation, while those experiencing competition showed reduced fitness when infected.

In addition to having effects on *A. thaliana* fitness and nitrogen remobilization efficiency, infection by *Pst* DC3000 consistently reduced main stem height. Other traits were affected as well (Table 18b), but the trait responses were idiosyncratic. We also detected an effect of infection on the carbon content of senesced leaves; however this effect was, also, idiosyncratic (Table A-2; Figure A-1). Previous studies have documented that the responses of *A. thaliana* to infection by *Pst* DC3000 have included earlier flowering and an increase in the number of basal branches (Korves and Bergelson 2003 and 2004).

These traits appear to have been affected in a few of the accessions in our study as well. Pathogens are known to affect host plant morphology and phenology. Some of these changes may reduce the fitness cost of infection, and thus be tolerance traits. Nitrogen remobilization in response to infection remains a promising and enigmatic means by which plants may tolerate natural enemy damage.

Tolerance is an important element of the evolutionary ecology of plants and their natural enemies because it ameliorates the effects of natural enemy damage to plant fitness without imposing selection for the evolution of counter-defense in natural enemies. Our results suggest that in the *A. thaliana – P. syringae* pathosystem, minor differences in experimental conditions can have a dramatic influence on the severity and broad-sense heritability of measures of defense. Nitrogen remobilization in the context of plant tolerance is a promising trait that warrants further investigation.

LIST OF REFERENCES

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1. Adler F.R. and Karban R. (1994) Defended fortresses or moving targets? Another model of inducible defenses inspired by military metaphors. In: *The American Naturalist*, pp. 813-832
2. Agrawal A.A. (1999) Induced plant defense: evolution of induction and adaptive phenotypic plasticity. In: *Inducible Plant Defenses against Pathogens and Herbivores: Biochemistry, Ecology, and Agriculture*, pp. 251–268. American Phytopathological Society Press, St. Paul
3. Alfano J.R. and Collmer A. (1996) Bacterial pathogens in plants: life up against the wall. In: *The Plant Cell*, pp. 1683-1698
4. Anderson M.J. (2001) A new method for non-parametric multivariate analysis of variance. In: *Austral Ecology*, pp. 32-46
5. Anderson M.J. (2002) CAP: a FORTRAN computer program for canonical analysis of principal coordinates. Department of Statistics, University of Auckland, New Zealand
6. Anderson M.J. (2004) PERMDISP: a FORTRAN computer program for permutational analysis of multivariate dispersions (for any two-factor ANOVA design) using permutation tests. Department of Statistics, University of Auckland, New Zealand
7. Anderson M.J. (2005) PERMANOVA: a FORTRAN computer program for permutational multivariate analysis of variance. Department of Statistics, University of Auckland, New Zealand
8. Anderson M.J. (2006) Distance-based tests for homogeneity of multivariate dispersions. In: *Biometrics*, pp. 245-53
9. Anderson M.J. and Robinson J. (2003) Generalized discriminant analysis based on distances. In: *Australian and New Zealand Journal of Statistics*, pp. 301-318
10. Anderson M.J. and Willis T.J. (2003) Canonical analysis of principal coordinates: a useful method of constrained ordination for ecology. In: *Ecology*, pp. 511-525
11. Barbottin A., Lecomte C., Bouchard C. and Jeuffroy M.H. (2005) Nitrogen remobilization during grain filling in wheat. In: *Crop Science*, pp. 1141-1150
12. Boege K. and Marquis R.J. (2005) Facing herbivory as you grow up: the ontogeny of resistance in plants. In: *Trends in Ecology & Evolution*, pp. 441-448
13. Butt A., Mousley C., Morris K., Beynon J., Can C., Holub E., Greenberg J.T. and Buchanan-Wollaston V. (1998) Differential expression of a senescence-enhanced metallothionein gene in Arabidopsis in response to isolates of *Peronospora parasitica* and *Pseudomonas syringae*. In: *Plant Journal*, pp. 209-221
14. Chapman S.K., Hart S.C., Cobb N.S., Whitham T.G. and Koch G.W. (2003) Insect herbivory increases litter quality and decomposition: an extension of the acceleration hypothesis. In: *Ecology*, pp. 2867-2876
15. Clarke D.D. (1986) Tolerance of parasites and disease in plants and its significance in host-parasite interactions. In: *Advances in Plant Pathology*, pp. 161–197
16. Cots J., Fargeix C., Gindro K. and Widmer F. (2002) Pathogenic attack and carbon reallocation in soybean leaves (*Glycine max.* L.): reinitiation of the glyoxylate cycle as a defence reaction. In: *Journal of Plant Physiology*, pp. 91-96

17. Devienne-Barret F., Richard-Molard C., Chelle M., Maury O. and Ney B. (2006) Ara-Rhizotron: an effective culture system to study simultaneously root and shoot development of *Arabidopsis*. In: *Plant and Soil*, pp. 253-266
18. Dietrich R., Ploss K. and Heil M. (2004) Constitutive and induced resistance to pathogens in *Arabidopsis thaliana* depends on nitrogen supply. In: *Plant, Cell and Environment*, pp. 896-906
19. Dimmock J. and Gooding M.J. (2002) The influence of foliar diseases, and their control by fungicides, on the protein concentration in wheat grain: a review. In: *Journal of Agricultural Science*, pp. 349-366
20. Garry G., Tivoli B., Jeuffroy M.H. and Citharel J. (1996) Effects of Ascochyta blight caused by *Mycosphaerella pinodes* on the translocation of carbohydrates and nitrogenous compounds from the leaf and hull to the seed of dried-pea. In: *Plant Pathology*, pp. 769-777
21. Gaunt R.E. (1995) The relationship between plant disease severity and yield. In: *Annual Review of Phytopathology*, pp. 119-144
22. Greenberg J.T. and Ausubel F.M. (1993) *Arabidopsis* mutants compromised for the control of cellular damage during pathogenesis and aging. In: *The Plant Journal*, pp. 327-41
23. Hirano S.S. and Upper C.D. (2000) Bacteria in the leaf ecosystem with emphasis on *Pseudomonas syringae* - a pathogen, ice nucleus, and epiphyte. In: *Microbiology and Molecular Biology Reviews*, pp. 624-653
24. Holm S. (1979) A simple sequentially rejective multiple test procedure. In: *Scandinavian Journal of Statistics*, pp. 65-70
25. Hougen-Eitzman D. and Rausher M.D. (1994) Interactions between herbivorous insects and plant-insect coevolution. In: *The American Naturalist*, pp. 677-697
26. Janzen D.H. (1980) When is it coevolution? In: *Evolution*, pp. 611-612
27. Jokela J., Schmid-Hempel P. and Rigby M.C. (2000) Dr. Pangloss restrained by the Red Queen–steps towards a unified defence theory. In: *Oikos*, p. 267
28. Katagiri F., Thilmony R. and He S.Y. (2002) The *Arabidopsis thaliana-Pseudomonas syringae* interaction. In: *The Arabidopsis Book* (eds. Meyerowitz EM and Somerville CR), pp. DOI 10.1199-tab.0039. American Society of Plant Biologists, Rockville
29. Kniskern J.M. and Rausher M.D. (2005) Environmental variation mediates the deleterious effects of *Coleosporium ipomoeae* on *Ipomoea purpurea*. In: *Ecology*, pp. 675-685
30. Korves T. and Bergelson J. (2004) A novel cost of R gene resistance in the presence of disease. In: *The American Naturalist*, pp. 489-504
31. Korves T.M. and Bergelson J. (2003) A developmental response to pathogen infection in Arabidopsis. In: *Plant Physiology*, pp. 339-347
32. Kover P.X. and Schaal B.A. (2002) Genetic variation for disease resistance and tolerance among *Arabidopsis thaliana* accessions. In: *Proceedings of the National Academy of Sciences of the United States of America*, pp. 11270-11274
33. Kover P.X., Wolf J.B., Kunkel B.N. and Cheverud J.M. (2005) Genetic architecture of *Arabidopsis thaliana* response to infection by *Pseudomonas syringae*. In: *Heredity*, pp. 507-517

34. Kus J.V., Zaton K., Sarkar R. and Cameron R.K. (2002) Age-related resistance in Arabidopsis Is a developmentally regulated defense response to *Pseudomonas syringae*. In: *The Plant Cell*, pp. 479-490
35. Lachenbruch P.A. and Mickey M.R. (1968) Estimation of error rates in discriminant analysis. In: *Technometrics*, pp. 1-11
36. Levey S. and Wingler A. (2005) Natural variation in the regulation of leaf senescence and relation to other traits in Arabidopsis. In: *Plant, Cell and Environment*, pp. 223-231
37. Lim P.O., Woo H.R. and Nam H.G. (2003) Molecular genetics of leaf senescence in *Arabidopsis*. In: *Trends in Plant Science*, pp. 272-278
38. McArdle B.H. and Anderson M.J. (2001) Fitting multivariate models to community data: A comment on distance-based redundancy analysis. In: *Ecology*, pp. 290-297
39. Morel J.B. and Dangl J.L. (1997) The hypersensitive response and the induction of cell death in plants. In: *Cell Death and Differentiation*, pp. 671-683
40. Navabpour S., Morris K., Allen R., Harrison E., Ah-Mackerness S. and Buchanan-Wollaston V. (2003) Expression of senescence-enhanced genes in response to oxidative stress. In: *Journal of Experimental Botany*, pp. 2285-2292
41. Obregón P., Martín R., Sanz A. and Castresana C. (2001) Activation of defence-related genes during senescence: a correlation between gene expression and cellular damage. In: *Plant Molecular Biology*, pp. 67-77
42. Oerke E.C. and Dehne H.W. (1997) Global crop production and the efficacy of crop protection-current situation and future trends. In: *European Journal of Plant Pathology*, pp. 203-215
43. Olea F., Pérez-García A., Cantón F.R., Rivera M.E., Cañas R., Ávila C., Cazorla F.M., Cánovas F.M. and de Vicente A. (2004) Up-regulation and localization of asparagine synthetase in tomato leaves infected by the bacterial pathogen *Pseudomonas syringae*. In: *Plant and Cell Physiology*, pp. 770-780
44. Pageau K., Reisdorf-Cren M., Morot-Gaudry J.-F. and Masclaux-Daubresse C. (2006) The two senescence-related markers, *GSI* (cytosolic glutamine synthetase) and *GDH* (glutamate dehydrogenase), involved in nitrogen mobilization, are differentially regulated during pathogen attack and by stress hormones and reactive oxygen species in *Nicotiana tabacum* L. leaves. *Journal of Experimental Botany*, 57, 547-557
45. Pegadaraju V., Knepper C., Reese J. and Shah J. (2005) Premature leaf senescence modulated by the Arabidopsis PHYTOALEXIN DEFICIENT4 gene is associated with defense against the phloem-feeding green peach aphid. In: *Plant Physiology*, pp. 1927-1934
46. Pérez-García A., Cánovas F.M., Gallardo F., Hirel B. and de Vincente A. (1995) Differential expression of glutamine synthetase isoforms in tomato detached leaflets infected with *Pseudomonas syringae* pv. *tomato*. In: *Molecular Plant-Microbe Interactions*, pp. 96-103
47. Pérez-García A., de Vicente A., Cantón F.R., Cazorla F.M., Codina J.C., Garcia-Gutiérrez A. and Cánovas F.M. (1998) Light-dependent changes of tomato

glutamine synthetase in response to *Pseudomonas syringae* infection or phosphinothricin treatment. In: *Physiologia Plantarum*, pp. 377-384
48. Pérez-García A., Pereira S., Pissarra J., García Gutiérrez A., Cazorla F.M., Salema R., de Vicente A. and Cánovas F.M. (1998) Cytosolic localization in tomato mesophyll cells of a novel glutamine synthetase induced in response to bacterial infection or phosphinothricin treatment. In: *Planta*, pp. 426-434
49. Quirino B.F., Normanly J. and Amasino R.M. (1999) Diverse range of gene activity during *Arabidopsis thaliana* leaf senescence includes pathogen-independent induction of defense-related genes. In: *Plant Molecular Biology*, pp. 267-278
50. Rausher M.D. (2001) Co-evolution and plant resistance to natural enemies. In: *Nature*, pp. 857-864
51. Robatzek S. and Somssich I.E. (2001) A new member of the *Arabidopsis* WRKY transcription factor family, AtWRKY 6, is associated with both senescence- and defence-related processes. In: *The Plant Journal*, pp. 123-133
52. Simms E.L. (2000) Defining tolerance as a norm of reaction. In: *Evolutionary Ecology*, pp. 563-570
53. Stowe K.A., Marquis R.J., Hochwender C.G. and Simms E.L. (2000) The evolutionary ecology of tolerance to consumer damage. In: *Annual Review of Ecology and Systematics*, pp. 565-595
54. Strauss S.Y. and Agrawal A.A. (1999) The ecology and evolution of plant tolerance to herbivory. In: *Trends in Ecology & Evolution*, pp. 179-185
55. Strauss S.Y. and Irwin R.E. (2004) Ecological and evolutionary consequences of multispecies plant-animal interactions. In: *Annual Review of Ecology, Evolution and Systematics*, pp. 435-66
56. Thaler J.S., Owen B. and Higgins V.J. (2004) The role of the jasmonate response in plant susceptibility to diverse pathogens with a range of lifestyles. In: *Plant Physiology*, pp. 530-538
57. Thompson J.N. (1999) The evolution of species interactions. In: *Science*, p. 2116
58. Weaver L.M., Gan S., Quirino B. and Amasino R.M. (1998) A comparison of the expression patterns of several senescence-associated genes in response to stress and hormone treatment. In: *Plant Molecular Biology*, pp. 455-469

APPENDIX

APPENDIX

Table A-1. Pairwise comparisons between accessions in the progeny generation for the symptomatic proportion of the rosette five days post-infection.

Groups	t	P-Value	Holm-Adjusted P-Value
*3,8	4.4727	0.001	0.001
*4,9	3.817	0.001	0.001
*5,9	5.6599	0.001	0.001
*8,9	5.623	0.001	0.001
*8,10	5.3825	0.001	0.001
*9,10	6.9711	0.001	0.001
*3,4	4.1232	0.001	0.001
*5,8	5.4746	0.001	0.001
*2,4	5.3589	0.001	0.001
*3,5	5.1189	0.001	0.001
*5,6	6.9565	0.001	0.001
*4,10	3.7488	0.001	0.001
*1,3	5.7586	0.001	0.002
*6,10	5.0408	0.001	0.002
*2,8	4.8911	0.001	0.002
*1,4	7.8804	0.001	0.002
*7,10	2.9852	0.001	0.002
*4,7	4.8155	0.001	0.002
*1,2	4.5434	0.001	0.002
*3,7	4.2685	0.001	0.002
*6,8	6.767	0.001	0.002
*1,9	4.555	0.001	0.002
*7,8	3.8572	0.001	0.002
*2,7	3.3713	0.002	0.002
7,9	2.6505	0.009	0.002
3,9	2.5515	0.011	0.003
2,10	2.4581	0.013	0.003
1,6	2.5066	0.019	0.003
1,5	2.3029	0.02	0.003
2,9	2.3804	0.024	0.003
4,5	2.0765	0.035	0.003
3,10	2.0558	0.036	0.004
2,3	2.2444	0.04	0.004
5,7	2.0356	0.041	0.004

Significant comparisons at a = 0.05 are indicated with an asterisk.

Table A-1. Continued.

Groups	t	P-Value	Holm-Adjusted P-Value
6,9	1.9826	0.054	0.005
1,8	1.8328	0.074	0.005
2,5	1.7248	0.097	0.006
1,7	1.6433	0.11	0.006
3,6	1.1207	0.3	0.007
2,6	0.7273	0.452	0.008
4,8	0.8086	0.456	0.010
4,6	0.5187	0.653	0.013
5,10	0.3291	0.75	0.017
6,7	0.2939	0.798	0.025
1,10	0.2573	0.822	0.050

Significant comparisons at a = 0.05 are indicated with an asterisk.

Table A-2. NPANOVA results testing for effects of accession, treatment status, and their interaction on the carbon content of the collected, senesced rosette leaves.

Source	df	SS	SS%	MS	*F*	P-Value
Accession [A]	8	136.37	54.33	17.05	39.90	0.001
Treatment Status [T]	1	0.84	0.34	0.84	1.97	0.170
A X T	8	13.81	5.50	1.73	4.04	0.001
Residual	234	99.98	39.83	0.43		
Total	251	251.00				

Note: Bracketed letters represent shorthand notation for the independent variables.

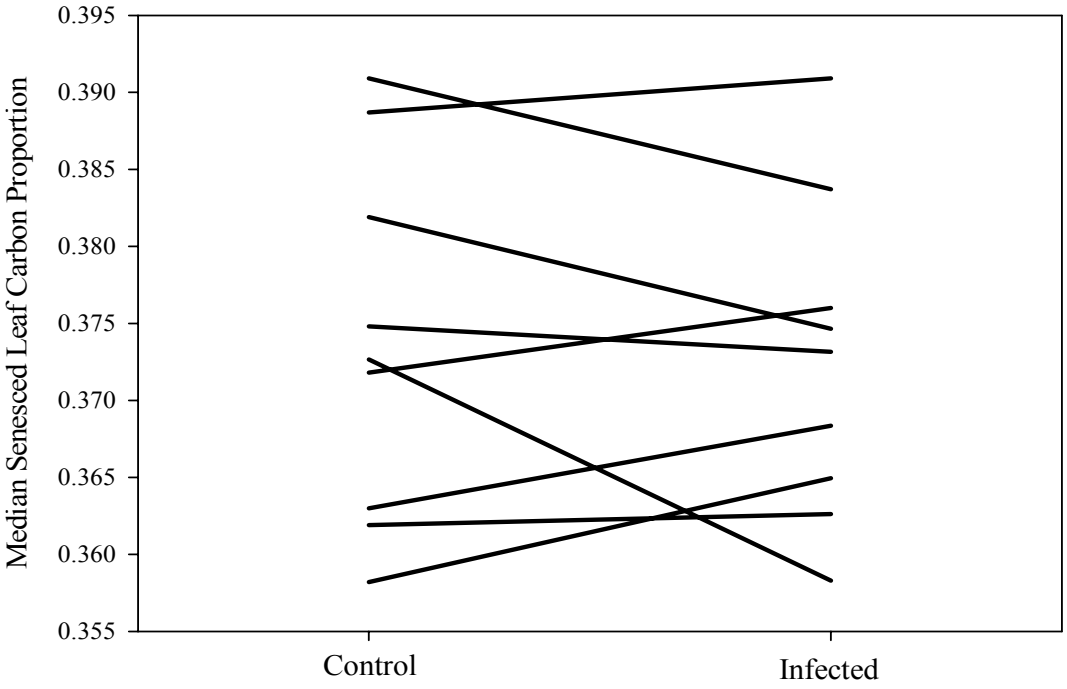


Figure A-1: The effect of infection on the carbon content of senesced rosette leaves, by control and infected treatment groups.

VITA

Michelle Boercker was born July 25th, 1979 in San Bernardino, California. She attended elementary, intermediate, and high school in Paradise, California, graduating from Paradise High School in 1997. In Bellingham, Washington, Michelle attended Western Washington University, acquiring a Bachelor of Science in Biology/Anthropology in the winter of 2001. In the fall of 2003, Michelle began the Masters Degree program in Ecology and Evolutionary Biology at the University of Tennessee, Knoxville, Tennessee. She graduated with a Master of Science degree in the fall of 2006.