The effect of environmental heterogeneity on *RPW8*-mediated resistance to powdery mildews in *Arabidopsis thaliana*

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**Background and Aims** The biotic and abiotic environment of interacting hosts and parasites may vary considerably over small spatial and temporal scales. It is essential to understand how different environments affect host disease resistance because this determines frequency of disease and, importantly, heterogeneous environments can retard direct selection and potentially maintain genetic variation for resistance in natural populations.

**Methods** The effect of different temperatures and soil nutrient conditions on the outcome of infection by a pathogen was quantified in *Arabidopsis thaliana*. Expression levels of a gene conferring resistance to powdery mildews, *RPW8*, were compared with levels of disease to test a possible mechanism behind variation in resistance.

**Key Results** Most host genotypes changed from susceptible to resistant across environments with the ranking of genotypes differing between treatments.Transcription levels of *RPW8* increased after infection and varied between environments, but there was no tight association between transcription and resistance levels.

**Conclusions** There is a strong potential for a heterogeneous environment to change the resistance capacity of *A. thaliana* genotypes and hence the direction and magnitude of selection in the presence of the pathogen. Possible causative links between resistance gene expression and disease resistance are discussed in light of the present results on *RPW8*.

**Key words:** Genotype × environment interaction, *RPW8*, *Arabidopsis thaliana*, Golovinomyces orontii, powdery mildew, qPCR, temperature, plant × pathogen interaction, disease resistance.

**INTRODUCTION**

Natural plant and animal populations often harbour genetic variation for disease resistance and susceptible and resistant individuals co-occur despite a fitness disadvantage of susceptible genotypes in the presence of the parasite (e.g. Jarosz and Burdon, 1992; Jeffery and Bangham, 2000). So why has natural selection not eliminated the susceptible genotypes? The answer is important because the processes acting to maintain variation in disease resistance in uncontrolled environments will determine disease dynamics and can potentially undermine any of our attempts to control disease (Gandon et al., 2001).

Evolutionary theory provides a number of testable hypotheses as to what maintains variation in disease resistance in natural populations, in the form of adaptive (Dawkins and Krebs, 1979; Simms and Rausher, 1987; Jeffery and Bangham, 2000) and non-adaptive processes (Sasaki, 2000; Thrall and Burdon, 2002; Tellier, 2009). However, relatively little attention has been given to the role of environmental heterogeneity in maintaining such variation (Thompson, 2005; Laine and Tellier, 2008; Lazzaro and Little, 2009; Wolinska and King, 2009). A spatially or temporally varying environment may affect the function of genes differentially and hence cause variation in the expression of host and parasite traits (Lynch and Walsh, 1998). Any variation in traits related to infection and disease can lead to variation in the favoured host or parasite genotype and result in heterogeneous selection for host tolerance and resistance and parasite infectivity and virulence (Levene, 1953; Gillespie and Turelli, 1989). The potential importance of heterogeneous selection in host–parasite co-evolution and in maintaining variation in disease resistance is apparent when the performance of host or parasite genotypes varies under different abiotic conditions (e.g. Price et al., 2004; Laine, 2007; Vale et al., 2008), or when distinct host genotypes perform differently with different parasite genotypes (e.g. Burdon and Jarosz, 1991; Salvaudon et al., 2005; Dubuffet et al., 2007).

In plants, the interactive effect of host genotype, parasite genotype and environmental factors on disease outcome has long been recognized (Burdon, 1987). Empirical evidence is plentiful but largely limited to studies at the whole-plant and parasite level and therefore provides little understanding of the underlying genetic mechanisms behind differential disease responses to the environment. Plants rely on their innate immune system to recognize pathogen-associated molecules and trigger an immune response (Jones and Dangl, 2006). Central to this process of recognition and defence response are the disease resistance (*R*) genes. The activation of *R*-genes and their encoded proteins by pathogen effectors are potentially strong defence responses which often lead to the death of infected cells and hence the arrest of the infection (Jones and Dangl, 2006). A few *R*-genes and other pathogenesis-related genes are reported to vary in expression across different temperatures, light or humidity levels (e.g. Wang et al., 2001; Yang and Hua, 2004; Noutoshi et al., 2005).
of the species (Jorgensen and Emerson, 2008). The same combination of RPW8-1 and RPW8-2 alleles can be associated with resistance in some individuals and susceptibility in others, suggesting that genetic factors other than allelic variation at RPW8 are involved in the resistance reaction (Jorgensen and Emerson, 2008). Members of the protein family 14-3-3 have, for example, been suggested as possible regulators of RPW8-2 (Yang et al., 2009). Long-established natural populations of A. thaliana have been shown to have considerable allelic variation at the RPW8 locus (Jorgensen and Emerson, 2008).

RPW8 acts as a signalling component in the basal resistance against powdery mildew pathogens by inducing the expression of common pathogenesis-related genes and the production of salicylic acid (Xiao et al., 2005; Wang et al., 2007). Resistance conferred by RPW8 results in a hypersensitive reaction (HR) at the site of infection with rapid, localized cell death that limits the spread of the invading pathogen (Xiao et al., 2001). One locus, RPW8-2, may have a dual role in pathogen defence by also acting directly at the plant–pathogen interface to limit the development of the pathogen haustorium (WM Wang et al., 2009). As with many other R-genes, transcripts of RPW8-1 and RPW8-2 are present at low levels in unchallenged plants but increase continuously for more than 7 d after pathogen infection in resistant genotypes (Xiao et al., 2005). Successful infection by powdery mildew pathogens will decrease leaf biomass and hence cause negative effects on plant fitness (Orgil et al., 2007).

**MATERIALS AND METHODS**

**The study system**

The gene RPW8 confers broad-spectrum resistance to powdery mildew pathogens in Arabidopsis thaliana and contains a total of six paralogues that differ in their presence and copy number among individuals (Xiao et al., 2001; Fig. 1). Paralogues RPW8-1 and RPW8-2 have been shown to be directly responsible for resistance to powdery mildews in the A. thaliana genotype Ms-0 (Xiao et al., 2001) and a prerequisite for, but not exclusively associated with, resistance in other genotypes of six paralogues directly involved in disease resistance, RPW8-1 and RPW8-2, in the three RPW8 types used in the study (not to scale). Allele names refer to those of Jorgensen and Emerson (2008). (B) The organization of the six paralogues at the RPW8 locus on chromosome 3 in Arabidopsis thaliana resistant ecotype Ms-0 (GenBank accession no. AF273059) and susceptible Col-0 (TAIR database, http://www.arabidopsis.org/index.jsp) (after Xiao et al., 2001).

Plant material

Seeds of A. thaliana were collected from locations in East Anglia, UK, and had been used previously in a study of natural variation at RPW8 in this species (Jorgensen and Emerson, 2008). All wild collections were grown and self-seeded in the greenhouse for one generation. Eleven accessions were chosen so that a total of three RPW8 genotypes were...
represented by three or four unrelated accessions each (Fig. 1). Accessions with the same RPW8 genotype had identical alleles at RPW8.1 and RPW8.2 but differed at a minimum of four of 14 unlinked microsatellite loci distributed across the rest of the genome (Jorgensen and Emerson, 2008).

Experimental conditions

Multiple seeds from one maternal plant per accession were sown in Levinton F2 compost containing insecticide (Intercept, Scotts, http://www.scottsprofessional.com/) and 12 % grit, and germinated in a growth chamber under short day conditions (8 h light, approx. 100 μmol m⁻² s⁻¹, 22 °C and high humidity, >90 %). Seedlings were transplanted to individual 90-mL pots when 2–3 leaves had emerged. Pots contained either Levinton F2 compost with Intercept (N/P/K = 150 : 200 : 200 mg L⁻¹) for a high nutrient treatment or a mixture of F2 compost (1/3) and sterile horticultural sand (2/3) with intercept for a low nutrient treatment. Seven days after transplanting, plants were transferred to two identical growth cabinets (Fitotron, Weiss-Gallenkamp) with short day conditions (8 h light, approx. 150 μmol m⁻² s⁻¹, approx. 85 % humidity) and regular watering. Two temperature regimes were applied. One cabinet had a constant temperature of 20 °C, whereas the other was kept at 20 °C for 16 h followed by an 8-h cold period at 10 °C inserted 4 h into the dark period. Ramping between temperatures was performed over 30 min within the 8-h cold period. Eight plants represented each accession, two in each of the four treatments: (A) nutrient-rich soil/constant temperature, (B) nutrient-rich soil/cold nights, (C) nutrient-poor soil/constant temperature and (D) nutrient-poor soil/cold nights. Treatment A reflects standard growth conditions for experimental studies of the A. thaliana – powdery mildew interaction (Xiao et al., 2003; Jorgensen and Emerson, 2008), while the use of sandy soils and the introduction of a cold night period represent more realistic aspects of natural growth conditions for A. thaliana in East Anglia. Soil nutrient status was manipulated by the addition of sand because the focus was on the general nutrient condition of the plant and not any specific nutrient component. Plants were randomized within each cabinet every 3–4 d.

Inoculations and harvesting

Inoculations with Golovinomyces orontii strain MGH (Plotnikova et al., 1998) were performed after 23 d of growth when all plants had formed a minimum of eight proper leaves. This obligate parasite is one of four powdery mildew isolates (G. cichoracearum, G. cruciferarum and Oidium lycopersici) that have been shown to cause near-identical disease phenotypes in a number of different A. thaliana accessions (Xiao et al., 2004). Reproduction and dispersal in powdery mildew species are primarily by asexual spores (conidia) formed in conidiophores from upright hyphae (Plotnikova et al., 1998). Prior to the inoculations, one leaf per plant (20–30 mg each) was harvested, snap-frozen in liquid nitrogen and stored at −80 °C for subsequent RNA extraction. All plants were transferred to long day conditions and 22 °C (16 h light, approx. 100 μmol m⁻² s⁻¹) for immediate inoculation. Only the host plants therefore experienced different temperatures while the pathogen experienced constant temperatures (22 °C) across treatment groups. Conidia from heavily infected plants of the susceptible genotype Col-gl1 (Col-0 harbouring the glabrous mutation 1) were brushed evenly on each individual leaf rosette. Col-0 was used as positive control in the inoculations. Plants were kept at high humidity (>90 %) for 48 h to allow favourable conditions for spore germination after which relative humidity was decreased to 65–75 %. One leaf per plant was harvested 4 d (95–96 h) after inoculation and stored in 70 % ethanol for later staining and measurement of fungal growth. At this stage, fungal colonies had formed asexual spores but were still at a size where single colonies could be easily distinguished. Leaves were harvested for RNA extraction 2 d later (143–145 h after inoculation), snap-frozen in liquid nitrogen and stored at −80 °C for later RNA extraction. The later harvesting for RNA extraction was chosen to increase the chance of detecting a difference in RPW8 expression after infection because RPW8.1 and RPW8.2 transcription is known to increase continuously at least 7 d after infection in resistant genotypes (Xiao et al., 2005). In summary, two leaves from two independent plants per accession per treatment were available for analyses of fungal growth and another two leaves per accession per treatment for analyses of RPW8 expression.

Staining and measurements

Leaves stored in ethanol were stained with Trypan Blue (Koch and Slusarenko, 1990), mounted in 50 % glycerol and investigated under a microscope. The first ten germinated spores (hereafter termed colonies) that were encountered on a leaf were used for measurements of fungal growth. The longest hyphae in a colony was measured (curved length) using the Auto-Montage microscopy image analysis software (Syncroscopy). The number of conidiophores was counted for each colony.

RNA extraction and qPCR

Total RNA was extracted from two combined leaves (one from each replicate plant) using the RNasy Plant mini kit (Qiagen) and including a DNase digestion step according to the manufacturer’s protocol. A spectrophotometer (ND-1000; NANODrop, Wilmington, DE, USA) was used to assess RNA purity and concentration. Reverse transcription was performed by incubating 1 μg of RNA with 1.5 μg random primers (Invitrogen) in a total volume of 11 μL at 70 °C for 10 min followed by the addition of 200 U Superscript II Reverse Transcriptase with buffer and DTT (Invitrogen), 1 μL 10 mM dNTP (Roche) and 40 U RNase inhibitor (Promega) in a final volume of 20 μL and incubation at 42 °C for 1 h. The cDNA was stored at −20 °C. Primers for quantitative real-time PCR were designed by PrimerDesign Ltd to amplify a 105-bp product of RPW8-1 (GenBank accession number AF273059) (forward: 5′GTTGGAAAGTCCACATTAAGG, reverse: 5′TA CTTTGTCTGGTCTGAGTT), a 124-bp product of RPW8-2 (GenBank accession number AF273059) (forward: 5′CCGT CAAAAGGCCCAAAAGATAG, reverse: 5′TTCTTCATTT TCACCCTGAACCTCT) and five reference genes (At2g28390, At4g26410, At5g55840, Gapdh and At5g46630) included in
the GeNorm Arabidopsis Housekeeping Gene Kit (PrimerDesign Ltd). PCR reactions were performed with an ABI Prism 7700 Sequence Detection System (Applied Biosystems) using SYBR Green to monitor double-stranded DNA synthesis. Reactions contained 10 μL 2 × SYBR Green Master Mix (Applied Biosystems), 4.5 ng cDNA (assuming 100% efficiency in the reverse transcription reaction) and 1 μL of primer-mix (PrimerDesign Ltd) in a final volume of 20-μL reactions. Conditions for the PCR reaction were 2 min at 50°C, 10 min at 95°C, and then 50 cycles, each consisting of 15 s at 95°C and 1 min at 60°C. Amplicon dissociation curves were recorded after cycle 50 according to the manufacturer’s instructions (Applied Biosystems) in order to detect primer dimers and non-specific products in the reaction.

Cycle quantification \( C_q \) (Bustin et al., 2009) was the cycle number at which amplification entered the exponential phase. Each PCR reaction was performed in triplicate on separate plates. Twofold serial dilutions of one sample covering the range of 18–0.5625 ng RNA were performed and used in separate PCR reactions to calculate efficiencies for each primer pair (Ramakers et al., 2003).

Data analyses

GeNorm v. 3.5 (Vandesompele et al., 2002) was used to analyse the expression stability of the five reference genes in 15 samples and select the most stable reference gene for use in all samples. Quantification of \( RPW8.1 \) and \( RPW8.2 \) expression relative to the chosen reference gene was performed from \( C_q \) values using the standard curve method (Pfaffl, 2001).

The effect of temperature and soil nutrient conditions on fungal growth and \( RPW8 \) expression was analysed with factorial analyses of variance in R (http://cran.r-project.org/). \( RPW8 \) genotype (hereafter \( RPW8 \) type 1, 2 and 3, Fig. 1) and accessions nested within \( RPW8 \) type were entered as explanatory variables to separate effects of the \( RPW8 \) locus (‘\( RPW8 \) type’) from that of other genetic factors (‘accessions within \( RPW8 \) type’). Soil nutrient status and temperature were entered as factors to test the effect of different environments on fungal growth and \( RPW8 \) expression. Infection status (before or after inoculation) was included in the analyses of \( RPW8 \) expression to test the effect of pathogen infection on transcription levels. Log-transformed measures of hyphal length and \( RPW8 \) expression were entered as response variables. Effects of inoculation within each treatment were tested with one-way ANOVAs. Residual plots confirmed that data were approximately normally distributed.

RESULTS

There was a strong positive correlation between average hyphal length of \( G. orontii \) colonies and the proportion of colonies that had formed conidiophores \( (r = 0.804, P \leq 0.001, n = 52, \text{data not shown}) \). Hyphal length was therefore a good indicator of the amount of asexual reproduction by \( G. orontii \) in this experiment. No conidiophores were observed on colonies with hyphal length less than 342 μm long \( (n = 520) \) which was therefore taken as the size below which the host plant is resistant.

Growth of \( G. orontii \) differed significantly between the three \( RPW8 \) types (Table 1). There were no significant interactions between \( RPW8 \) type and soil nutrient conditions or temperature. The overall effect of the \( RPW8 \) locus on fungal growth was therefore independent of environmental conditions. However, interaction terms between accessions nested within \( RPW8 \) type, soil nutrient conditions and temperature indicate that hyphal length differed significantly across environmental conditions among accessions of each \( RPW8 \) type (Fig. 2, Table 1). Accessions with identical \( RPW8.1 \) and \( RPW8.2 \) alleles did not therefore have similar disease phenotypes across treatments.

Efficiencies of PCRs ranged between 2.04 and 2.29 \( (R^2 \text{ for standard curve} > 0.99) \). All five reference genes had high expression stabilities in GeNorm \( (M < 0.53) \). The most stable, At5g46630 \( (M = 0.45) \), was selected for further use.

Transcript levels of \( RPW8.1 \) and \( RPW8.2 \) increased after infection in all \( RPW8 \) types and independently of environmental conditions (Tables 2 and 3, Fig. 3). However, accessions with the same \( RPW8 \) type differed significantly in their response to infection across soil nutrient conditions and temperature. Transcript levels of \( RPW8.1 \) were mainly affected by soil nutrient conditions (Table 2) while transcript levels of \( RPW8.2 \) were affected by both soil nutrient conditions and temperature (Table 3). Standard growth conditions for controlled experiments with \( A. thaliana \) (treatment A) in most cases resulted in increased \( RPW8.1 \) and \( RPW8.2 \) expression after infection, which is in line with previous findings for genotype Ms-0 (Xiao et al., 2005).

Transcript levels of \( RPW8.1 \) and \( RPW8.2 \) after infection were not directly associated with the amount of fungal growth across the 11 \( A. thaliana \) accessions. There was no significant correlation between average \( RPW8.1 \) expression after infection and average hyphal length (Pearson’s correlation \( r = -0.207, P = 0.178, n = 44 \)) although high levels of expression were never associated with long hyphae (Fig. 4). Likewise, no significant correlation was found between average \( RPW8.2 \) expression after infection and average hyphal length \( (r = 0.084, P = 0.589, n = 44, \text{Fig. 4}) \) or between average \( RPW8.1 \) expression and average \( RPW8.2 \)

### Table 1. Analyses of variance in hyphal length of Golovinomyces orontii grown on 11 Arabidopsis thaliana accessions representing three different \( RPW8 \) types

<table>
<thead>
<tr>
<th></th>
<th>d.f.</th>
<th>Mean square</th>
<th>( F_{(\Delta)} )</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( RPW8 ) type (R)</td>
<td>2</td>
<td>4.887</td>
<td>8.88(2,8)</td>
<td>0.009</td>
</tr>
<tr>
<td>Soil (S)</td>
<td>1</td>
<td>0.018</td>
<td>0.03(1,8)</td>
<td>0.860</td>
</tr>
<tr>
<td>Temperature (T)</td>
<td>1</td>
<td>0.624</td>
<td>0.48(1,8)</td>
<td>0.509</td>
</tr>
<tr>
<td>( R \times S )</td>
<td>2</td>
<td>1.097</td>
<td>1.99(2,8)</td>
<td>0.199</td>
</tr>
<tr>
<td>( R \times T )</td>
<td>2</td>
<td>1.015</td>
<td>0.27(2,8)</td>
<td>0.767</td>
</tr>
<tr>
<td>( S \times T )</td>
<td>1</td>
<td>2.818</td>
<td>5.12(1,8)</td>
<td>0.054</td>
</tr>
<tr>
<td>( R \times S \times T )</td>
<td>2</td>
<td>1.542</td>
<td>2.80(2,8)</td>
<td>0.120</td>
</tr>
<tr>
<td>Accession within ( RPW8 ) type (R[A])</td>
<td>8</td>
<td>4.404</td>
<td>5.08(8,396)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>R[A] × S</td>
<td>8</td>
<td>3.341</td>
<td>3.86(8,396)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>R[A] × T</td>
<td>8</td>
<td>1.806</td>
<td>2.09(8,396)</td>
<td>0.036</td>
</tr>
<tr>
<td>R[A] × S × T</td>
<td>8</td>
<td>6.373</td>
<td>7.36(8,396)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Error</td>
<td>396</td>
<td>42.876</td>
<td>5.08(8,396)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
DISCUSSION

The abiotic environment can have significant effects on the interaction between *A. thaliana* and the powdery mildew pathogen *G. orontii*. The majority of host genotypes in the experiment presented here change from susceptible to resistant across temperatures and soil nutrient conditions with the ranking of genotypes changing between treatments. There is therefore strong potential for a heterogeneous environment to change the sign and magnitude of selection on host genotypes in the presence of the pathogen and hence to influence the maintenance of variation in *Arabidopsis* powdery mildew resistance in spatially structured populations.
Fig. 3. The effect of temperature, nutrients and infection on transcript levels of RPW8-1 and RPW8-2 in A. thaliana. Names of accessions in each bar chart refer to those of Jorgensen and Emerson (2008). Bars represent averages of three replicates + s.e. for each treatment: A, nutrient-rich soil/constant temperature; B, nutrient-rich soil/cold nights; C, nutrient-poor soil/constant temperature; D, nutrient-poor soil/cold nights. Black bars: before infection; white bars: 6 d after infection with G. orontii. Simple effects of infection within a treatment are presented for each accession (*P < 0.05, **P < 0.01, ***P < 0.001, all other others: no significant effect; no corrections for multiple tests). #No statistics reported due to missing data (fewer than three data points).
The impact of temperature on the phenotypes of interacting plants and pathogens has been widely reported (e.g. Chongo and Bernier, 2000; de Jong et al., 2002; Yan and Chen, 2008). A general expectation is that higher temperatures will accelerate the breakdown of resistance, either through higher pathogen pressure or through a negative effect on genes mediating resistance (Webb et al., 2010). However, the empirical support for this prediction is ambiguous. Reports can be found for decreasing (Gijzen et al., 1996; Chongo and Bernier, 2000; Y. Wang et al., 2009) as well as increasing host resistance with increasing temperature (Yan and Chen, 2008; Fu et al., 2009; Webb et al., 2010). Likewise, the effect of host nutritional status on the plant–pathogen interaction appears to vary between host and pathogen species and, importantly, with the form in which the nutrients are available to the host (Agrios, 1997). For example, powdery mildews are known to have increased fitness on host plants with high levels of nitrogen but other nutrients may have the opposite effect (Jensen and Munk, 1997; Sander and Heitfuss, 1998). In the A. thaliana – G. orontii interaction investigated here it is impossible to generalize about the effect that changing temperature and nutrient conditions have on host resistance because of the significant differences among host genotypes. RPW8 type determines the average level of host resistance independent of environmental conditions but accessions with the same RPW8 type have different disease phenotypes across different environments. This is in line with our previous work showing that other genetic factors must interact with RPW8 to mediate resistance (Jorgensen and Emerson, 2008) and suggests that these genetic factors are affected by environmental conditions. The results not only demonstrate the effect that multiple genotypes can have on the level of infection in a population as a whole in fluctuating environments, they also highlight the importance of including multiple host genotypes in the assessments of disease phenotypes across environmental gradients.

At present we have limited knowledge about the genetic factors involved in RPW8-mediated resistance and therefore few candidate genes to explain the variation across environments (Xiao et al., 2005; Wang et al., 2007; Yang et al., 2009). Studies on other R-genes reveal many factors that affect R-gene signalling upstream and downstream of activation (Eitas and Dangl, 2010; Elmore et al., 2011) and some of these are known to respond to variable environments. For example, mitogen-activated protein kinase cascades are functionally linked protein kinases involved in signal transduction downstream of R-genes (Pedley and Martin, 2005) and may also respond to abiotic stresses (e.g. Shi et al., 2011). Temperature-sensitive heat shock proteins (HSPs) act to fold and stabilize proteins and one family, HSP90, appears to be essential for the upstream regulation of several R-genes (reviewed in Shirasu, 2009). As a first step to understand the importance of RPW8 regulation, I investigated whether the significant phenotypic differences in host response to G. orontii infection across treatments are associated with variation in the expression of RPW8. In the present study, transcription levels differed between infected and non-infected individuals independent of environmental conditions when averaged across accessions with the same RPW8 type. However, in no case did accessions with the same RPW8-I and RPW8-2 alleles show the same change in gene expression across treatments. There are a few reports of other R-genes varying in expression between infected and non-infected plants (Yoshimura et al., 1998; Wang et al., 1999; Mohr et al., 2010), across abiotic environments (Yoshimura et al., 1998; Wang et al., 2001; Yang and Hua, 2004; Noutoshi et al., 2005) or between different tissue types or developmental stages (Collins et al., 1999). Yet other R-genes are found to be expressed at the same low level across infection regimes and other environmental gradients (Century et al., 1999; Tan et al., 2007; Millett et al., 2009). None of these studies reports quantitative measures of host resistance and we therefore know little about the effect that variation in R-gene expression has on the host phenotype. However, a number of scenarios can be envisaged. It is possible that increased R-gene expression will increase the general ability (sensitivity)

![Fig. 4. Associations between G. orontii growth and transcript levels of RPW8-I and RPW8-2 6 d after infection. Each symbol represent different A. thaliana accessions in four different treatments (see text). Black symbols are RPW8 type 1, grey symbols are type 2 and open symbols are type 3 (see Fig. 1).](http://aob.oxfordjournals.org/)

...expression has on the host phenotype. However, a number of scenarios can be envisaged. It is possible that increased R-gene expression will increase the general ability (sensitivity)
of the plant to detect the pathogen and mount a defence response. High transcript levels would then be expected to be associated with resistance and low or no transcription to be associated with susceptibility. A study of transgenic A. thaliana lines containing RPW8.1 and RPW8.2 suggested such an association (Xiao et al., 2003) but it is clear from the present results that this pattern cannot be generalized to all A. thaliana genotypes. Instead, while high levels of RPW8.1 and, to some extent, RPW8.2 expression were only associated with resistance, low levels could be associated with both resistance and susceptibility. An alternative scenario is that only low concentrations of R-proteins are necessary for full pathogen recognition and/or defence response and that the degree of host resistance is independent of R-gene expression once it is above a certain threshold level. If R-genes are costly to express in evolutionary terms we would then expect a tight transcriptional control at constant low levels and not the highly variable expression levels we see at RPW8 (Brown, 2003).

Finally, it is possible that mRNA levels do not accurately reflect R-protein abundance. Post-transcriptional regulation has been reported for RPM1 in A. thaliana (Boyes et al., 1998) and may explain a general lack of correlation between expression and host resistance. The gene RPW8 is an essential component of resistance to powdery mildews in A. thaliana, but other (largely unknown) genetic factors are also involved in this process (Jorgensen and Emerson, 2008; Yang et al., 2009). Based on the present results, I hypothesize that high levels of RPW8.1 and/or RPW8.2 expression may result in resistance. However, in some genotypes and/or in some environments RPW8.1 and RPW8.2 expression levels are low, and here interacting genetic factors may instead function with RPW8.1 and/or RPW8.2 to mediate resistance. The function of these other genetic factors is also likely to be environment-dependent. This hypothesis remains to be tested. However, the suggested scenario provides a mechanism by which environmental heterogeneity can vary the resistance capacity of A. thaliana individuals and lead to the maintenance of variation in disease resistance in natural populations.

CONCLUSIONS

The abiotic conditions of hosts and parasites may have important effects on both interacting parties. The present experiment was designed to investigate the isolated effects of host–temperature and host–nutrient interactions on infection outcome in a plant–pathogen interaction. Host plants were grown under different temperature and soil-nutrient conditions before and after infection to avoid any direct effect of the different temperatures on the pathogen. This is a significant step forward compared with the vast majority of studies reporting host phenotypic responses to temperature gradients because previous experimental designs have rarely allowed the separation of effects on host and pathogen (but see Ramage and Sutherland, 1995). It is possible that in the present experiment the change in growth conditions immediately before infection (a move between growth cabinets) may have affected RPW8 expression independent of the pathogen infection. There is also a small risk that the effects of infection on RPW8 expression was confounded by a 6-d age difference in leaves harvested before and after inoculation or by the harvesting per se. However, expression patterns in the standard treatment A (nutrient-rich soil/constant temperature) before and after infection are similar to the patterns of RPW8 expression reported from studies where no change in growth conditions or harvesting were enforced (Xiao et al., 2005). Therefore, it seems unlikely that any change in RPW8 expression after infection in the present experiment is due to other factors than the pathogen.

Our understanding of the mechanisms of pathogen infection and host plant defence has been significantly advanced by the discovery of complex signalling pathways and highly variable recognition and effector genes across a wide range of interacting plant and pathogen species (Jones and Dangl, 2006; Oliva et al., 2010). It is clear, however, that the homogeneous laboratory conditions used in most mechanistic research fail to provide a full insight into the molecular and biochemical factors affecting phenotypic traits. The present study exemplifies how a host genotype is not consistently ‘resistant’ or ‘susceptible’ and how the expression (and hence potentially the function) of genes involved in resistance can vary considerably across environments. It is even possible that the loci and genetic pathways identified under laboratory conditions are not the same as those important in natural environments, as seen from studies of other traits (Weinig et al., 2002; Carroll et al., 2004). Ignoring the environmental conditions within which traits arose and persist is therefore likely to result in significantly biased interpretations of the mechanism, and the evolution, of infection and defence. It is clear that field studies will be the ultimate approach if we want to obtain a complete understanding of the mechanism and dynamics of disease in natural populations.

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LITERATURE CITED


Boyes DC, Nam J, Dangl JL. 1998. The Arabidopsis thaliana RPM1 disease resistance gene product is a peripheral plasma membrane protein that is degraded coincident with the hypersensitive response. Proceedings of the National Academy of Sciences of the USA 95: 15849–15854.


Levere H. 1953. Genetic equilibrium when more than one ecological niche is available. American Naturalist 87: 331–333.


