PLANT-ANIMAL INTERACTIONS - ORIGINAL PAPER

Ontogenetic changes in tolerance to herbivory in *Arabidopsis*

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Abstract Tolerance to herbivory—the ability of plants to maintain fitness despite herbivore damage—is expected to change during the life cycle of plants because the physiological mechanisms underlying tolerance to herbivory are linked to growth, and resource allocation to growth changes throughout ontogeny. We used the model plant *Arabidopsis thaliana* to test two hypotheses: that tolerance increases as plants grow, and that tolerance decreases at the onset of reproduction. We chose three accessions previously reported to vary for resistance to herbivory in order to explore whether tolerance and resistance are inversely related. Cabbage looper (*Trichoplusia ni*) larvae were allowed to feed on plants at either the four-leaf, six-leaf, or 1st-flower developmental stage until 50% of the leaf area was removed. Overall, we found a trend for increased tolerance with ontogenetic stage, but there were important differences among accessions in their response to herbivory at different stages. Tolerance did not decrease with the onset of flowering, nor did we find any correlation between resistance and tolerance levels. Three main plant traits correlated strongly with tolerance: stem mass, an earlier onset of reproduction and a longer fruiting period. This study suggests there may be considerable variation in ontogenetic patterns of tolerance in natural populations of *A. thaliana*, and warrants further investigations with more accessions or natural populations, and detailed measurements of traits purported to contribute to tolerance in our quest to understand the mechanisms of tolerance to herbivory.

Keywords Ontogeny · Tolerance · Growth analysis · Herbivory · *Arabidopsis thaliana*

Introduction

According to optimal defense theory, natural selection favors the allocation of resources to defense against herbivores when the fitness benefits of defenses outweigh their cost (McKey et al. 1979; Rhoades et al. 1979). However, the benefit:cost ratio of resource allocation to defense is expected to change throughout development because of proximate and ultimate causes: ontogenetic changes in the morphology and physiology of plants alter both the capacity of plants to acquire resources, and also the fitness value of defense traits. Therefore, the extent to which particular tissues and organs should be defended is expected to vary throughout the development and growth of individuals (Boege and Marquis 2005; McKey 1974).

Considering that defense in plants may be achieved through resistance (decreasing the amount of tissue lost to herbivores) and tolerance (maintaining fitness despite the loss of tissue to herbivores; Karban and Baldwin 1997; Strauss and Agrawal 1999), any attempt to predict how defense changes throughout development must examine the ontogenetic changes in the costs and benefits of

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allocation to both resistance and tolerance traits. Direct costs of resistance are derived from the commitment of resources to resistance traits such as the production and maintenance of secondary metabolites, physical barriers, and special structures that deter herbivore attack (simple and glandular trichomes, extrafloral nectaries; Simms and Fritz 1992; Simms and Rausher 1989). As seedlings grow into juvenile plants, the number of source organs available for the acquisition of soil nutrients and C or light increases. Generally, growth results in a greater capacity to acquire resources, which, in turn, may translate to a relative decrease in the cost of resistance. Therefore, as plants get larger they should invest more resources in resistance (Boege and Marquis 2005; del Val and Dirzo 2003).

However, changes in the fitness value of resistance traits throughout development are not easy to predict. The fitness value of making tissues resistant to herbivores depends on the fitness value of the tissue in question and the risk of losing it to herbivores. At pre-reproductive stages, the production of more vegetative tissue should have high priority (Harper 1977), but protecting the existing tissue through resistance is also important. Therefore, plants should invest in resistance only if the shunting of resources to resistance does not slow down vegetative growth to the extent that fitness decreases more than it would from the loss of vegetative tissue to herbivores (Huhta et al. 2003, 2009; Avila-Sakar and Laarakker, in preparation). In order to quantify this fitness cost of herbivory, the rate of loss of fitness as a function of the amount of tissue removed by herbivores, i.e., tolerance, must be determined.

Similarly, the costs and benefits of tolerance are bound to vary with ontogeny: The ability to tolerate damage has been proposed to depend on the availability of meristems and stored resources, and the physiological capacity to increase photosynthesis and to translocate resources from storage tissues to tissues actively involved in growth and reproduction (Strauss and Agrawal 1999; Tiffin 2000). Additionally, leaf morphology, canopy architecture, and the onset of senescence have been identified as other endogenous factors contributing to tolerance (Trumble et al. 1993). Since the availability of meristems, and the capacity to garner and store resources generally increase with size, tolerance to herbivory should also increase throughout ontogeny (Bardner and Fletcher 1974; Boege and Marquis 2005). However, there may be developmental stages during which tolerance traits do not increase with plant size because of the diversion of resources to other functions (e.g., during the reproductive phase in semelparous plants; Boege and Marquis 2005).

To date, greater tolerance at later developmental stages has been found in the annuals *Cakile edentula*, *Raphanus sativus* (Brassicaceae), *Coryspermum hyssopifolium* (Chenopodiaceae), grasses, one woody species,

Casearia nitida (Salicaceae; Boege 2005; Boege et al. 2007; del Val and Crawley 2005; Gedge and Maun 1994); and several species of agronomic interest (Bardner and Fletcher 1974).

Here, we report on an experiment that assessed tolerance to herbivore damage at three ontogenetic stages of plants. We used a simple plant–herbivore system (*A. thaliana–Trichoplusia ni*) under controlled conditions to test the hypothesis that tolerance increases with developmental stage, from the seedling to the pre-reproductive stages as suggested for semelparous plants (Bardner and Fletcher 1974; Boege and Marquis 2005). We also hypothesized that tolerance would decrease at the onset of reproduction as resources were shunted to reproductive structures and plants had less time to recover from damage and fewer meristems available for re-growth (Boege and Marquis 2005; Tuomi et al. 1994). In an exploratory approach, we used three accessions that span a wide range in resistance levels with the intention of sampling from a wide range of tolerance levels. We expected such variation in tolerance because of the negative correlation between resistance and tolerance proposed to occur as a consequence of a tradeoff in resource allocation between two sets of costly traits with a high degree of redundancy in their function (Mauricio et al. 1997). However, detecting this tradeoff was not a main goal of this study.

Materials and methods

Study organisms

Arabidopsis thaliana (L.) Heynh. (Brassicaceae) is a self-pollinating annual with a short life cycle (Baskin and Baskin 1983; Pigliucci 2002). Natural populations of *A. thaliana* adapted to particular conditions (ecotypes; Jander et al. 2001; Kilian et al. 1985) show genetic variability for traits related to resistance (van Poecke 2007), including glucosinolate content (Bidart-Bouzat and Kliebenstein 2008; Magrath et al. 1994), trichome density (Handley et al. 2005; Larkin et al. 1996), and epicuticular wax composition (Rashotte et al. 1997). Levels of florivory and frugivory also vary among populations (Arany et al. 2009). *A. thaliana* is a prolific model system in plant genetics, evolution, ecology and developmental biology (Mitchell-Olds 2001; Pigliucci 2002; van Poecke and Dicke 2004). Together with other model crucifers (e.g., rapid cycling *Brassica*), research in *Arabidopsis* is relevant to the production of annual crops, particularly those in the same family (canola, mustard, broccoli, etc.; Gavloski and Lamb 2000; Oerke 2006).

Trichoplusia ni Hübner (Lepidoptera: Noctuidae), is a generalist herbivore that commonly feeds on a wide variety of crop plants, including those of the family Brassicaceae

(Jander et al. 2001; Pilson 2000). It has been widely used in studies of plant–herbivore interactions (Grant-Petersson and Renwick 1996; Jander et al. 2001; Johnson and Dowd 2004).

Feeding experiment and estimation of tolerance

In order to sample plants with a range of resistance levels, we used three ecotypes (hereafter referred to as "accessions"; Alonso-Blanco and Koornneef 2000) of *A. thaliana* with low (L), mid-high (MH), and high (H) resistance to *T. ni* (respectively, CS20, CS1092 and CS6180) as determined by a previous study in which plants were exposed to feeding by larvae of *T. ni* (Jander et al. 2001). We planted 200 seeds directly into 4-inch pots containing Promix Bx (Premier Horticulture, Rivière-du-Loup) to which 5 ml of 0.00076 g/ml 20-20-20 All-purpose Fertilizer (Plant Products, Brampton) had been added, as recommended by the manufacturer. We planted two seeds per pot and thinned them to one, haphazardly selected seedling per pot 14 days after planting. Plants were grown in two growth chambers (Conviron ATC26; Controlled Environments, Winnipeg), under a 16:8 h light:dark regime, at 22/18°C and 60% constant humidity. Plants were rotated on a daily basis to minimize positional effects and fertilized weekly as indicated above. Concurrently, eggs of *T. ni* obtained from the Insect Rearing lab at the Southern Crop Protection & Food Research Centre of Agriculture & Agri-Food Canada (London, ON) were raised on artificial diet (Chippendale and Beck 1965) for later use in the application of damage treatments.

Plants from each accession were divided into four groups: the control group (undamaged), and the four-leaf, six-leaf and 1st-flower groups, designated according to the ontogenetic stage (hereafter, "ontogeny") at which plants were subjected to herbivory by third-instar *T. ni* larvae. The date at which individual plants were damaged varied up to 12 days within groups due to variation in developmental rates. The herbivory treatment consisted in allowing one or two larvae to feed on each plant until approximately 50% of the leaf area was removed, a damage intensity previously seen to produce significant declines in fruit and seed production (Avila-Sakar and Tucker, unpublished data). The actual percentage of leaf area lost was determined later from digital pictures of each plant taken before and after damage (Winfolia Pro 2006a; Regent Instruments, Quebec). Larvae took 0.5, 1.0, and 2 h to consume the prescribed leaf area on plants at the four-leaf, six-leaf and 1st-flower stages, respectively. Within ontogeny groups, L plants were more readily consumed (C. Tucker, personal observation). Larvae damaged only foliar tissue.

We started 100 plants from each accession, but mortality (independent of accession or ontogeny) reduced our sample size to 75 individuals per accession with a minimum of ten individuals per accession-by-ontogeny combination. Following damage, plants were left to grow and produce fruit. As fruits ripened, they were collected daily until the last fruit was removed. As plants died, we harvested and separated them into roots, stems, and leaves. After removing soil from the roots, all parts were dried to constant weight, and weighed separately to the last 0.0001 g.

We estimated individual seed production as the product of the number of fruits produced by a plant and the average number of seeds per fruit obtained from a sample of ten to 15 plants for each accession-by-ontogeny combination. Because both damage and ontogeny might influence seed viability, we tested for these effects on seed germination. We intended to use the product of the number of seeds and the germination rate as an estimate individual fitness, but preliminary analyses showed that this variable yielded no more information than the number of seeds alone.

We estimated tolerance as the slope of the relation between fitness (seed production) and level of damage (each accession is a family of full-sibs generated through self-pollination; Pilson 2000). We also estimated individual compensatory ability as:

$$\omega(y)_{ijk} = \frac{y_{ijk} - \tilde{y}_{ic}}{\tilde{y}_{ic}} = \frac{y_{ijk}}{\tilde{y}_{ic}} - 1$$

where y is the number of seeds, i is the accession, j is the ontogenetic stage, k is the individual in a particular accession-by-ontogeny combination, and c is the control group for a particular accession. We used medians ($\tilde{y}$) instead of means in order to reduce the influence of outliers. A value of zero for ω indicates equal compensation, while significant negative or positive deviations from zero indicate under- or overcompensation, respectively. Therefore, this estimate reflects the level of tolerance of an individual in a particular accession-by-ontogeny combination (Boege 2005; Strauss and Agrawal 1999; Avila-Sakar and Laarakker, in preparation).

We also measured the effects of the timing of damage on the time from germination to bolting, first flower, first fruit, and cessation of fruit production, as well as the period between the production of the first fruit and last fruit because we were interested in understanding some of the mechanisms potentially involved in changes in fitness after damage, particularly those related to the timing of development of the reproductive structures.

Growth analysis

Since growth rate may influence tolerance (Hilbert et al. 1981; Maschinski and Whitham 1989), we calculated relative growth rates (RGR) as the increment in total biomass (*m*) between successive stages (*i*) divided by the total mass

at the previous stage and the number of days between stages (d) $\mathrm{RGR}_i = (m_i - m_{i-1})m_{i-1}^{-1}d^{-1}$. Because of the destructive sampling required to measure biomass, concurrent to the feeding trial experiment, another 18 plants from each accession were grown and randomly divided into four-leaf, six-leaf, and 1st-flower groups as above. When plants reached the desired developmental stage they were harvested, separated into roots, stems and leaves, dried and weighed as the rest of the plants. Final biomass values from the control plants of the feeding experiment, which were harvested at senescence, were used to calculate RGR from 1st-flower to senescence.

Statistical analyses

The effects of accession, ontogeny, and their interaction on seed germination were analyzed by means of a logistic regression analysis using the logit link function (PROC LOGISTIC; SAS Institute 1999). Tolerance estimates were obtained from a fixed effects general linear model with accession, ontogeny and their interaction as fixed factors, the proportion of leaf area damaged as a covariate, and seed production as response variable. Initially, a full model with all possible interactions was tested, but since the only significant interaction involving the covariate was that with ontogeny, we present results for a more reduced model. We used fixed effects general linear models to assess the effects of accession, ontogeny, and their interaction on compensatory ability. We used the Tukey–Kramer method for multiple comparisons (PROC GLM; SAS Institute 1999).

In order to examine possible mechanisms of tolerance, we tested several models that included covariates related to growth and reproductive phenology in addition to accession, ontogeny, and their interaction. Leaf, stem and root biomass were considered indicators of resource allocation to vegetative growth. We used total plant leaf area before damage as an estimate of plant size at the time of damage in lieu of biomass measurements because the latter would have required destructive sampling. Time to bolting was used as an indicator of the switch from vegetative growth to reproduction, and the time from bolting to the collection of the last mature fruit (reproductive period) was used as a measure of the period during which plants allocated most of their resources to reproduction. We hypothesized that compensatory ability would be greater in plants with the ability to store resources in leaves, stems or roots, and with longer reproductive periods, but it would be lower in plants with an earlier onset of reproduction because they would have produced less tissue with which to garner resources. We selected the model with the highest R^2_{adj} that contained only significant predictors ($\alpha = 0.05$). All models shown met the assumptions of normality and homoscedasticity.

By definition, the control group does not appear in any analyses of compensatory ability.

Results

To assess for biases in the application of the damage treatments, we tested for the effects of accession and ontogeny on the proportion of leaf area removed. Overall, the proportion of leaf area damaged by *T. ni* was of $51.1 \pm 1.3\%$ (mean $\pm$ SD, $n = 164$). However, there was a significant accession-by-ontogeny interaction effect: L plants damaged during the 1st-flower stage received less damage than the two accession-by-ontogeny combinations with the highest mean damage ($F = 3.29$, $df = 4$, $P = 0.01$). In order to correct for this unintended bias in the application of damage treatments, we excluded from the analyses four plants which received either too little (<20%) or too much (>75%) damage.

Seed germination

Most plants had a 100% germination rate (mean $\pm$ SE, $94.1 \pm 1.5\%$; median $= 100\%$). However, a logistic regression analysis revealed highly significant effects of accession, ontogeny, and their interaction on the proportion of seeds that germinated (Table 1). We found lower germination rates among the MH plants and also among those damaged at the six-leaf stage, with the lowest mean germination rate precisely in MH plants damaged at the six-leaf stage (five plants with germination rates below 50%). These effects were not strong enough to override the effects of accession and ontogeny on seed production (see below). We did not observe a relationship between seed production and germination rate (data not shown). Effects of accession and ontogeny, but not of their interaction, were found for other reproductive variables and for biomass at harvest (Tucker 2008). In general, damage had a

Table 1 Logistic regression analysis for the effects of accession and the ontogenetic stage at which plants were damaged (ontogeny) on seed germination

Source	df	Wald's χ^2
Accession (A)	2	250.06****
Ontogeny (O)	3	103.02****
A × O	6	177.42****
Overall model evaluation	df	χ^2
Likelihood ratio test	11	915.72****
Score test	11	978.42****
Wald test	11	542.03****

**** $P < 0.0001$

Table 2 Means (SE) of reproductive variables and final plant biomass for plants damaged at different ontogenetic stages and for undamaged controls

Ontogenetic stage	Seeds	Fruits	Seeds per fruit	Seed germination (%)	Biomass at harvest (g)			n
					Whole plant	Fruit	Vegetative[a]	
Control	13,830 (432)	323 (12)	43.6 (0.8)	95.8 (2.4)	0.9987 (0.0323)	0.3559 (0.0121)	0.6427 (0.0259)	57
Four-leaf	7,855 (468)	188 (10)	41.2 (0.8)	96.9 (1.6)	0.6678[b] (0.0333)	0.1950[b] (0.0110)	0.4693 (0.0246)	51
Six-leaf	9,554 (314)	228 (9)	42.8 (0.8)	88.8 (3.7)	0.8135 (0.0307)	0.2702 (0.0095)	0.5433 (0.0265)	54
1st-flower	11,039 (563)	258 (13)	43.0 (0.7)	96.6 (1.6)	0.8540 (0.0344)	0.2847 (0.0122)	0.5693 (0.0253)	59

[a] Vegetative biomass is the sum of leaf, stem and root biomass

[b] $n = 49$

Table 3 Estimation of tolerance by means of an analysis of covariance for the effect of ontogeny and accession on the number of seeds produced by plants using the proportion of leaf area damaged by *Trichoplusia ni* as covariate

Source	df	MS	F
Damage (D)	1	624,382,153	66.08****
Ontogeny (O)	2	2,122,394	0.22
D × O	2	46,197,669	4.89**
Accession	2	312,811,938	33.11****
Error	209	9,448,279	

Ontogenetic stage	Parameter estimates, mean ± SE (95% CI)	
	Intercept[a]	Slope (=tolerance)
Four-leaf	13,150 ± 937 (11,313, 14,986)	−9,958 ± 2,111 (−14,095, −5,820)
Six-leaf	13,548 ± 936 (11,714, 15,382)	−7,283 ± 2,401 (−11,284, −3,283)
1st-flower	12,927 ± 727 (11,502, 14,352)	−3,410 ± 1,465 (−6,281, −538)

** $P < 0.01$, **** $P < 0.0001$

[a] Intercepts were significantly lower by 2,068 seeds for accession L, and greater by 2,078 seeds for MH

negative effect on reproductive output and biomass accumulation, this being more pronounced at the four-leaf stage and less so at flowering (Table 2).

Tolerance

We found significant effects of damage, accession, ontogeny, and the accession-by-ontogeny and damage-by-ontogeny interactions on seed production (Table 3). Seed production decreased linearly with damage (no evidence of curvilinearity), but the slope of this relation decreased from the four-leaf stage to the 1st-flower stage. All slopes were significantly different from zero, and the slope for plants at the four-leaf stage was significantly different from that for plants at the 1st-flower stage ($t = 3.10$, $P = 0.0022$; Table 3). Slopes were parallel among accessions within ontogenetic stage, but intercepts were significantly lower for L and greater for MH. Thus, in the absence of herbivore damage L and MH had, respectively, the lowest and highest fitness of all three accessions.

Compensatory ability

An analysis of covariance using the proportion of leaf area damaged as covariate confirmed that the deletion of the four outliers for damage level eliminated any significant effects of the covariate or its interaction with accession, ontogeny or both on compensatory ability (data not shown). Therefore, we used the simpler model without the covariate.

Compensatory ability was significantly affected by accession ($F_{2,151} = 14.59$, $P < 0.0001$) and ontogeny ($F_{2,151} = 17.55$, $P < 0.0001$). Most importantly, a significant accession-by-ontogeny interaction revealed that the effect of ontogeny on compensatory ability varied among accessions ($F_{4,151} = 9.33$, $P < 0.0001$; Fig. 1). The lowest level of compensation was observed on L plants damaged at the four-leaf stage; L plants damaged at the six-leaf and 1st-flower stages did not differ significantly from one another in compensatory ability. The highest level of compensation was for MH plants damaged at the 1st-flower stage; MH plants damaged at the four-leaf and six-leaf
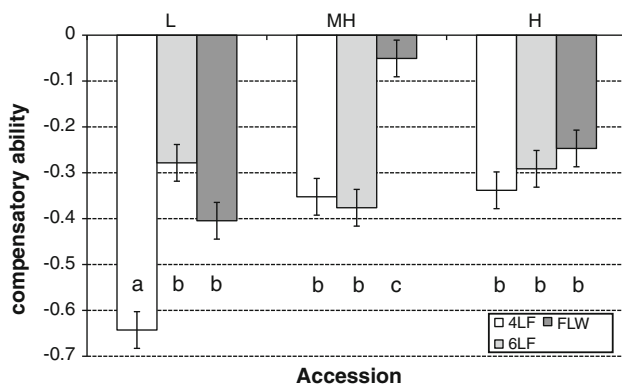
Fig. 1 Least squares means and SEs of compensatory ability estimated from seed production for three accessions of *Arabidopsis thaliana* at three ontogenetic stages: four-leaf (*4LF*), six-leaf (*6LF*), and 1st-flower (*FLW*). Means that share a *letter* are not significantly different according to a Tukey–Kramer multiple comparisons test

Table 4 General linear model for the effects of growth and reproductive phenology on compensatory ability

Source	df	MS	F
Accession (A)	2	0.0549	3.24*
Ontogeny (O)	2	0.0178	1.05
A × O	4	0.2371	14.00****
Leaf mass	1	0.2651	15.65***
Stem mass	1	1.3843	81.71****
Days to bolt	1	0.1271	7.50**
Fruiting period	1	0.1534	9.06**
Leaf mass × A	2	0.2573	15.19****
Error	140	0.0169	

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$

stages did not differ significantly from each other in compensatory ability. Although H plants did not show a significant effect of ontogeny on compensatory ability, there was a consistent trend of increasing compensatory ability from the four-leaf to the 1st-flower stage.

Accessions cannot be rated for compensatory ability without reference to the ontogenetic stage at which plants were damaged. L compensated less than the other two accessions at the four-leaf stage, but MH compensated better than L and H at the 1st-flower stage (Fig. 1).

Because we had observed evident differences in shoot size among accessions, we repeated the above analyses using vegetative biomass (sum of stem, root, and leaf biomass) at harvest as a covariate. In this alternative model, both vegetative biomass and its interaction with ontogeny had significant effects on compensatory ability ($F_{1,148} = 25.17$, $P < 0.0001$, $F_{2,148} = 4.58$, $P < 0.05$, respectively), and the accession effect lost significance. The proportion of variance explained by the model increased with the addition of the covariate from 41 to 53%. This alternative model confirmed that the effect of ontogeny differs among accessions (accession-by-ontogeny effect: $F_{4,148} = 3.99$, $P < 0.01$), and indicated that differences in size and growth capacity explain a considerable proportion of the variation in compensatory ability between accessions.

In fact, ontogeny accounted for 54% of the variation in plant size at the time of feeding (as estimated by leaf area; data not shown), with plants having total leaf areas of 1.50 ± 0.10, 4.64 ± 0.18, and 10.51 ± 0.75 cm^2 at the four-leaf, six-leaf, and 1st-flower stages, respectively. An analysis of the effects of accession and ontogeny on compensatory ability using leaf area before feeding as a covariate revealed a highly significant effect of the leaf area-by-ontogeny interaction ($F_{2,142} = 7.87$, $P < 0.001$): compensatory ability increased with leaf area, but only for

plants at the four-leaf stage. Leaf area did not influence compensatory ability in plants at the six-leaf stage, and those at the 1st-flower stage had a negligible decrease in compensatory ability with increasing leaf area.

Effects of growth and reproductive phenology on compensatory ability

Stem and leaf mass at senescence, the time of bolting, the length of the fruiting period, and the interaction between leaf mass and accession were significant predictors of compensatory ability (Table 4). Accession and the interaction of accession and ontogeny were also significant factors, but the main effect of ontogeny disappeared with the addition of the growth and phenology variables. The equation took the form:

$$\omega = (-0.339 + X_0) + X_1 L + 2.042 S - 0.012 D + 0.008 F$$

where ω is compensatory ability, L and S are leaf and stem mass, respectively, D is days to bolting, F is the length of the fruiting period, $X_0 = -0.137$ for L at the four- and six-leaf stages, or -0.221 for MH at the six-leaf stage, and $X_1 = -3.217$ for H, or 0 otherwise.

We performed a multivariate ANOVA in order to examine the effects of accession and ontogeny on the continuous variables that explained a significant amount of variation in compensatory ability among plants according to the general linear model described above. We added root mass because allocation to roots has been linked with compensatory ability (Hochwender et al. 2000). We found significant overall effects of accession (Wilks' $\lambda = 0.2696$, $F_{10,398} = 36.85$, $P < 0.0001$) and ontogeny (Wilks' $\lambda = 0.4626$, $F_{15,549.75} = 11.81$, $P < 0.0001$), but not of their interaction (Wilks' $\lambda = 0.8210$, $F_{30,798} = 1.34$, $P = 0.1043$) on this set of variables. We proceeded to examine the univariate ANOVAs for the effects of accession and ontogeny. Accession H took less time to bolt than the other

Table 5 F-values and least squares means (SEs) for phenology and growth-related traits hypothesized to contribute to compensatory ability to herbivory, for the different accessions and ontogenetic stages at which plants were damaged

Trait	Accession				Ontogenetic stage				
	$F_{2,203}$	L	MH	H	$F_{3,203}$	Control	Four-leaf	Six-leaf	1st-flower
Days to bolting	48.0****	25.1^a (0.3)	25.9^a (0.3)	22.3^b (0.3)	26.6****	23.1^a (0.3)	26.9^b (0.3)	24.1^a (0.3)	23.8^a (0.3)
Fruiting period (days)	0.41	21.8 (0.6)	21.3 (0.6)	22.1 (0.6)	11.6****	24.8^a (0.7)	18.7^c (0.8)	21.2^{bc} (0.7)	22.0^b (0.7)
Leaf mass (g)	26.9****	0.0495^a (0.0048)	0.0895^b (0.0047)	0.0953^b (0.0049)	6.3***	0.0958^a (0.0054)	0.0611^b (0.0059)	0.0778^{ab} (0.0055)	0.0777^{ab†} (0.0053)
Stem mass (g)	161.7****	0.0996^a (0.0068)	0.2397^b (0.0067)	0.2598^b (0.0070)	36.8****	0.2626^a (0.0078)	0.1445^c (0.0084)	0.1886^b (0.0079)	0.2033^b (0.0076)
Root mass (g)	14.0****	0.2300^a (0.0113)	0.3060^b (0.0112)	0.3004^b (0.0116)	0.55	0.2852 (0.0128)	0.2652 (0.0140)	0.2774 (0.0130)	0.2873 (0.0126)

Overall effects of accession and ontogeny were highly significant (see text for details). Means with different letters are significantly different according to Tukey–Kramer multiple comparisons ($\alpha = 0.05$)

L Low resistance to *T. ni*, *MH* mid-high resistance to *T. ni*, *H* high resistance to *T. ni*

*** $P < 0.001$, **** $P < 0.0001$

† Significantly different from the control according to a Dunnett's test ($\alpha = 0.05$)

two accessions, and L accumulated significantly less leaf, stem and root biomass than the other accessions (Table 5). Leaf damage delayed bolting, although this effect was significant only for plants damaged at the four-leaf stage, possibly because many individuals that were damaged at more advanced stages had already bolted by the time they were damaged. Damage shortened the fruiting period, and this effect was more severe in plants damaged at the four-leaf stage, followed by those at the six-leaf and 1st-flower stages in that order (Table 5). Damage also reduced leaf and stem biomass, with the four-leaf stage showing a more pronounced effect again (Table 5).

Growth analysis

All accessions increased in leaf, stem, and root biomass over the three developmental stages. In terms of RGR, plants grew the fastest between the four-leaf and six-leaf stages (Fig. 2). H plants had the lowest RGR during that initial phase. RGR values from 1st-flower to senescence were similar for all accessions. L plants had their lowest RGR between the six-leaf and 1st-flower stages. We were unable to calculate an independent RGR for H plants between those stages because many H plants in the growth analysis group started flowering at the six-leaf stage.

Discussion

Our results indicate that tolerance in *A. thaliana* increases from the four-leaf stage to the 1st-flower stage. However, the ontogenetic changes in compensatory ability, a measure of the individual expression of tolerance, differed among accessions. Accession L undercompensated at all stages, but more severely so at the four-leaf stage; MH under-compensated significantly at the two earliest stages, but equally compensated at the 1st-flower stage; and H un-dercompensated at all stages, although with a trend for an increase in compensatory ability from the four-leaf to the 1st-flower stage.

Low levels of tolerance in younger plants may relate to their relatively constrained ability to acquire resources from the environment, but also to the greater allocation priority of growth (Boege 2005; Boege and Marquis 2005; Mole 1994; Tiffin 2002). An increase in tolerance from earlier to later stages of development has been reported as a general pattern for crop plants (Bardner and Fletcher 1974), and also for other cruciferous plants (Boege et al. 2007; Gedge and Maun 1994), grasses (del Val and Crawley 2005), several dicotyledonous grassland species damaged during the cotyledon stage (Hanley and Fegan 2007), an understory shrub of the tropical dry forest (Boege 2005), and Scots pines (Hodar et al. 2008). However, squashes are
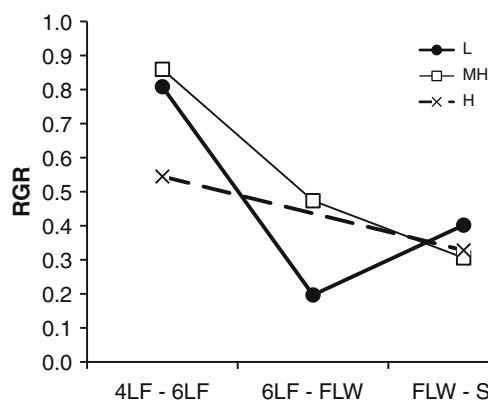
Fig. 2 Whole plant mean relative growth rate (*RGR*; g g^{-1} day^{-1}), for three accessions of *Arabidopsis thaliana*, measured for three intervals: four-leaf to six-leaf (*4LF–6LF*), six-leaf to 1st-flower (*6LF–FLW*), and 1st-flower to senescence (*FLW–SEN*). RGR for accession H was not calculated separately for the 6LF–FLW interval because the majority of those plants started flowering at the six-leaf stage

more tolerant to herbivory at earlier developmental stages (Du et al. 2008). In *A. thaliana* tolerance to the removal of young leaves was greater at the flowering than at the bolting stage, but in contrast to our findings, plants at the six-leaf stage were equally tolerant to those in flower (Barto and Cipollini 2005).

Greater tolerance at the 1st-flower stage could be achieved through the translocation of stored resources from stem to reproductive tissue elicited by the combined demand of resources from the developing reproductive tissue and the loss of foliar tissue to herbivores. Alternatively, it could be explained by the lower fitness value of the older (rosette) leaves at a time at which cauline leaves are already contributing most of the C (and presumably the transpiration that drives mineral nutrient acquisition) needed for the production of reproductive tissue (Barto and Cipollini 2005; Earley et al. 2009).

The inter-accession variation in ontogenetic patterns of compensatory ability found in this study is consistent with the finding of significant genetic variation for tolerance to rabbit herbivory among recombinant inbred lines of *A. thaliana* (Weinig et al. 2003). This variation among genetic lines suggests genotypic differences either in the kind of physiological mechanisms that allow plants to compensate for damage, or in the timing with respect to development at which these mechanisms operate.

Indeed, the accessions we used may differ in several ways related to herbivory (Strauss and Agrawal 1999; Trumble et al. 1993). For example, final vegetative biomass was an important component of variation in compensatory ability among accessions. Greater biomass is associated with tolerance through: (1) resource acquisition—plants that garner C or nutrients more efficiently become larger, which in turn allows them to grow more (Mooney et al.

1984); (2) storage—larger plants have more storage tissue (Stevens et al. 2008); and (3) growth (before damage) and re-growth (after damage), which allows plants to generate more resource-garnering and storage tissues (Bazzaz et al. 2000).

Growth rate has been proposed as a trait that can enhance or decrease tolerance to herbivory (Hilbert et al. 1981; Marshall et al. 2008; Maschinski and Whitham 1989; Wise and Abrahamson 2005). Putting together the results from the feeding experiment with those of growth analysis, we found an overall inverse relation between tolerance and relative growth rate, a result consistent with the mathematical model of Hilbert et al. (1981). Interestingly, Barto and Cipollini (2005) found a decrease in RGR from the vegetative to the flowering stage in both of the ecotypes they examined. The greater tolerance displayed by plants in flower may be due to rosette leaves: (1) contributing less of the resources needed by the developing reproductive tissues, and (2) consuming less of the resource pool because of their slower growth. Thus, some of the variation in tolerance among accessions could result from inherent differences in growth rate among them.

Besides differences in final vegetative biomass, accessions differed in leaf, stem and root biomass (Tucker 2008). At first glance, increased leaf biomass should contribute to tolerance through increased assimilation. However, *Arabidopsis* plants grown under elevated CO_2 (purportedly having an increased C assimilation) were recently shown to be less tolerant than those grown at ambient CO_2 (Lau and Tiffin 2009), thus indicating that the timing of increased assimilation with respect to ontogeny is crucial for tolerance. Similarly, stems may contribute to tolerance by providing additional photosynthetic area directly or through the cauline leaves they support, which, in turn, provide continuous assimilation during the reproductive stage of plants (Mooney et al. 1995; Stevens et al. 2008; Tiffin 2000). In fact, it was shown recently that the inflorescence contributes 36–93% of the lifetime C gain in *Arabidopsis* (Earley et al. 2009), depending on the accession. This partly explains the positive association between stem biomass and compensatory ability, and the differences among accessions found in our study, since most of the stem mass of *Arabidopsis* corresponds to the inflorescence, and accessions vary in their inflorescence:rosette size ratio. Stem mass could also be important because of its correlation with meristem availability, which seems crucial to tolerance in monocarpic, rosulate plants such as *Ipomopsis* (Paige et al. 2001), but is also important in trees and shrubs (Lehtila and Larsson 2005; Pratt et al. 2005).

In contrast to previous studies of ontogenetic changes in tolerance (Barto and Cipollini 2005; Boege et al. 2007; del Val and Crawley 2005; Gedge and Maun 1994; Hanley and Fegan 2007), our study used real herbivores for the

application of damage treatments. Preference for older leaves has been reported for the plant–herbivore system as the one we used (Jander et al. 2001). We observed the same trend, although no evident differences among accessions, and only a slight trend to a greater proportion of younger leaves attacked at the 1st-flower stage (Avila-Sakar and Tucker, unpublished data). Given the greater fitness value of young leaves, such pattern of preference would favor an ontogenetic pattern of tolerance opposite to the one we found (but significant leaf-age-dependent effects of damage on fruit mass were found only at the bolting stage; Barto and Cipollini 2005).

Accessions also differed in the duration of their pre-reproductive and reproductive periods (Tucker 2008). Longer pre-reproductive periods contribute to tolerance through increased accumulation resource-garnering and storage tissue (Lau and Tiffin 2009; Marshall et al. 2008), while longer reproductive periods allow plants to recover from losses in seed production caused by herbivory-induced decreases in resource intake. Extending the reproductive period towards the end of the growing season should allow greater compensation than extending it towards the beginning, which shortens the time available for accumulation of resource-garnering and storage tissue (Lau and Tiffin 2009; Marshall et al. 2008). Contrary to this expectation, plants in our study achieved greater compensatory ability by extending their reproductive period at the expense of the pre-reproductive period.

While we did not find any indication of a negative correlation between resistance and tolerance levels, nor was our experiment designed to test for it, the fact that different accessions (each one taken to express a single, constant level of resistance) may display different levels of tolerance to herbivory at different ontogenetic stages decreases the likelihood of finding a simple negative correlation between resistance and tolerance to herbivory. Future studies of resistance-tolerance tradeoffs should attempt to control for the ontogenetic stage at which plants are damaged, and the age of the leaves damaged. It is also possible that resistance varies with ontogeny, in which case both resistance and tolerance should be measured at several ontogenetic stages.

Lastly, we found no support for the hypothesis that tolerance would decrease at the onset of reproduction because of a diversion of resources to the construction of reproductive tissues. As discussed above, we see the opposite trend: overall, tolerance was greatest at the 1st-flower stage. Perhaps flower production is less costly than we anticipated, especially compared to fruit and seed development (Harper 1977; Snow and Whigham 1989; Stephenson 1981). Unfortunately, logistical constraints precluded us from including damage treatments after the 1st-flower stage.

In conclusion, our study found that tolerance to herbivory in *Arabidopsis* varies with ontogeny, and that the exact pattern of variation in compensatory ability differs among accessions. Whether this result applies to natural populations adapted to different local environments remains to be tested. Further studies using more accessions or natural populations, and focusing on the rates of growth of the different plant organs, plant architecture, resource translocation after herbivory, and the physiological mechanisms underlying these processes, are needed before we can unravel the traits that allow plants to compensate for herbivore damage at different ontogenetic stages.

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