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Ontogenetic changes in defence against a generalist herbivore in *Arabidopsis thaliana*

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Abstract Plant defence against herbivores includes resistance (avoiding damage) and tolerance (reducing the negative fitness effects of damage). As plants grow and increase their resource–garnering capacity, they should invest more in defence, but tradeoffs between plant functions may also change with ontogeny, thus complicating predictions on the simultaneous ontogenetic trajectories of resistance and tolerance. In a laboratory experiment, we used ten accessions of *Arabidopsis thaliana* that represent a gamut of resistance levels naturally found in the species to examine whether resistance and tolerance increase between an early vegetative stage and the fruit-ripening stage, and to test for tradeoffs between both defence modes. We measured constitutive resistance and tolerance to larvae of the generalist moth, *Trichoplusia ni*, and estimated costs of tolerance at both ontogenetic stages. We found an increase in constitutive resistance from the vegetative to the fruit-ripening stage, but not for tolerance. Accessions differed in tolerance, but not in resistance. Despite finding significant costs of tolerance at both ontogenetic stages, we found no tradeoff between resistance and tolerance. An examination of biomass partitioning at senescence revealed that the plants with a propensity to make roots were more tolerant to herbivory. Our results show that the ontogenetic trajectories of resistance and tolerance can be different, and even though costs of tolerance may be detected, these are not necessarily linked to a tradeoff with resistance. Lastly, the capacity of plants to grow a large shoot (mostly inflorescence) and make root tissue is linked to their ability to tolerate herbivory.

Keywords Cost of tolerance · Ontogeny · Resistance · Resource allocation · Tradeoffs

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Introduction

Plant defence against herbivores may occur through resistance, the ability to avoid damage by herbivores, or tolerance, the ability to reduce the negative effects of damage on fitness (Strauss and Agrawal 1999). Traits that confer resistance include trichomes, secondary compounds, tissue toughness, and mutualistic relationships with certain insects (Marquis 1991). The ability to translocate stored nutrients and carbohydrates and meristem availability have been proposed to favour tolerance (Strauss and Agrawal 1999; Stowe et al. 2000). Both resistance and tolerance traits require resources that otherwise could be used for

growth and reproduction—functions more directly linked to fitness—and therefore the construction, maintenance, and activation of defence traits are expected to have a fitness cost (Harper 1977; Simms and Rausher 1987; but see Koricheva 2002).

The purported costliness of defence traits entails not only the possibility of allocation tradeoffs between resistance and tolerance, but also ontogenetic changes in allocation to defence such that, past the seedling stage, older (larger) plants are expected to be better defended than younger (smaller) plants (Boege and Marquis 2005). Alternatively, a negative ontogenetic trajectory of defence has been predicted based on the idea that young plants may be under stronger selection for resistance than mature individuals (Swihart and Bryant 2001), an idea that perhaps applies more to perennials with a longer juvenile stage than annuals. Several empirical studies have found support for Boege and Marquis' prediction of a positive ontogenetic trajectory of defence in terms of resistance (Villamil et al. 2013; Barton 2014) and tolerance (Gedge and Maun 1994; Barton 2013). However, few studies have examined the ontogenetic trajectories of both resistance and tolerance (Boege et al. 2007; Du et al. 2008; Hoque and Avila-Sakar 2015). Moreover, there is no simple prediction as to whether both types of defence should increase with ontogeny or not (Boege et al. 2007). On one hand, older and larger plants should have access to more resources, which would allow them to invest more in defence (Boege and Marquis 2005). On the other, as older plants start investing in reproduction, fewer resources would be available for defence, which could cause or accentuate a tradeoff between allocation to resistance versus tolerance (Simms and Triplett 1994; Mauricio et al. 1997). For example, in *Raphanus sativus*, resistance decreases while tolerance increases with ontogeny (Boege et al. 2007), but in *Penstemon virgatus* only tolerance increases with ontogeny (Quintero and Bowers 2013).

At least partly, the difference in ontogenetic trajectories of resistance and tolerance among different species (and even populations of the same species) could be due to the particular mechanisms of resistance and tolerance and their costs. There is ample evidence that resistance is costly, and many mechanisms of resistance are well known (Dirzo and Harper 1982; Zangerl and Berenbaum 1997; Strauss et al. 2002; Sletvold et al. 2010; Cipollini et al. 2014). In

contrast, few studies have attempted to measure the costs of tolerance (Fornoni et al. 2004; Boege et al. 2007), and the mechanisms of tolerance are still largely unknown despite recent progress (Schwachtje et al. 2006; Siddappaji et al. 2013; Kleessen et al. 2014).

The main purpose of this study was to assess simultaneously the ontogenetic trajectories of resistance and tolerance, to measure the cost of tolerance, and to explore the traits that confer tolerance to herbivory using the model plant, *Arabidopsis thaliana*. In particular, we asked (1) whether resistance and tolerance increase between the early vegetative and fruit-ripening stages; (2) if tolerance is costly at both stages; (3) if there is a negative correlation between resistance and tolerance, and, if so, whether it holds throughout ontogeny; and lastly, (4) whether certain patterns of biomass allocation among root, stem, leaf, and reproductive tissue favour tolerance.

Materials and methods

Study organisms

Arabidopsis thaliana (L.) Heynh (Brassicaceae) is a self-pollinating annual native to Europe and central Asia that grows in recently disturbed areas (Pigliucci 2002; van Poecke 2007). Plants for this study were F_2 progeny of seeds obtained from The Arabidopsis Biological Resource Center of The Ohio State University and propagated in our lab through selfing.

Trichoplusia ni Hubner (Noctuidae), the cabbage looper, is a common pest of many crop species, including some Brassicaceae (Ahuja et al. 2010), and it has been used previously in studies of resistance in *A. thaliana* (Jander et al. 2001). Third-instar larvae were used for resistance assays or placed to feed on plants to measure tolerance (see Electronic Supplementary Material for details).

Experimental design

To test for ontogenetic changes in resistance and tolerance, we grew 18 *A. thaliana* plants from each of ten accessions and randomly assigned six individuals from each accession to one of three foliar damage treatments: undamaged (U), four-leaf (L, plants were damaged when the length of the fourth true leaf

reached 1 cm), and first-fruit (F, plants damaged when the first fruit started to ripen, defined as having ten developing fruits on the main inflorescence for consistency across plants and accessions). Resistance and tolerance were tested at both the L and F ontogenetic stages (hereafter, ontostages). The ten accessions were chosen specifically because they span a broad range of resistance levels according to Jander et al. (2001; ESM Table 1) and because they represent part of the genetic variation for resistance found in natural populations (see Electronic Supplementary Material for details of growth conditions).

Resistance

We conducted a no-choice bioassay to estimate resistance by presenting single leaf discs to individual *T. ni* larvae. Leaf discs were cut from a freshly excised leaf from each test plant with a 6.25-mm-diameter cork borer (disc area = 30.68 mm^2). To reduce effects of leaf age on resistance (Jander et al. 2001; Barto and Cipollini 2005), we selected the first true leaf for L plants, and on F plants we selected a leaf that roughly corresponded in size and age (usually the fourth leaf, but sometimes up to the seventh). We starved third-instar *T. ni* larvae for 18–24 h and allowed them to feed on leaf discs for 40 min or until the entire disc was consumed. If larvae consumed the entire disc before 40 min, we recorded the time at which they finished it. At the end of the feeding period, the remaining disc area was measured with a scanner-based image analysis system (Winfolia©, Regent Instruments, Quebec, Canada). Resistance was estimated as

$$R = (A_i/A_{max})(T_i/T_{max})$$

where A_i is the leaf area remaining of the disc taken from plant i, A_{max} is the maximum leaf area remaining of any of the discs (the most resistant one), T_i is the time it took the larva on plant i to remove A_i, and T_{max} is the maximum time a larva took to feed on any leaf disc. Calculated in this manner, resistance takes values between zero (most susceptible) and one (most resistant). We take this as a measure of constitutive resistance since plants had not experienced any damage prior to the removal of the leaf for the bioassay. This operational measure of resistance is consistent with the definition of resistance as the ability of a plant to reduce the attack of natural enemies (Karban and Baldwin 1997), and includes all traits of a plant that may result in such reduction (secondary metabolites, trichomes, waxes and low nutritional quality; Fineblum and Rausher 1995).

Tolerance

The measurement of tolerance requires estimates of fitness at two or more levels of damage within a group of closely related individuals (Simms 2001). In the case of *A. thaliana*, each accession is a group of full siblings produced through selfing. Plants of each accession were either left undamaged or subjected to feeding by *T. ni* at the L or F stage. As a measure of individual fitness, we used total seed production, estimated for each individual as the product of the number of fruits produced and the mean number of seeds per fruit. For the latter, we counted the number of seeds produced by two ripe fruits haphazardly sampled from the main axis of its main inflorescence and divided it by two.

Plants were subjected to foliar damage by placing a larva (starved as above) on each plant and allowing it to feed for 3 h or until approximately 75 % of the leaf area was removed. The leaf area excised for the resistance bioassays was considered in the estimation of leaf area damage. If necessary, more leaf area was removed with scissors until it reached ca. 75 %, as estimated by eye. Later, we measured the actual leaf area removed for each plant by means of image analysis of photographs of the rosettes taken before and after larval feedings (Nikon D90, Nikon Corporation, Tokyo, Japan). This method would have been inaccurate for F plants because of substantial overlap among their larger leaves and because cauline leaves do not appear in a photo of the rosette. Therefore, we estimated total leaf area before and after damage for each F plant from the lengths of all its leaves longer than 0.5 cm using the relation: leaf area = 0.1631 (length^2) + 0.064 (R^2 = 0.9672), obtained from a separate sample of plants. This method may have underestimated the total leaf area after damage, albeit only slightly because larvae removed entire leaves and when we adjusted the amount of leaf area removed, we clipped partially eaten leaves, thus leaving few partially damaged leaves on each individual. The proportion of leaf area damaged (a continuous

variable) was entered as a covariate in the analyses used to estimate tolerance (see below).

After senescence, three randomly selected plants per accession–ontostage combination were used to assess the relation between compensatory ability and biomass partitioning. We separated these plants into rosette leaves, cauline leaves, roots, and stems, and we dried and weighed those parts to measure biomass allocation to each part. Roots were extracted so as to minimize the attachment of soil particles. To examine plastic changes in biomass allocation in response to the damage suffered at a particular ontostage, we would have needed to harvest plants at different ontostages. Space limitations precluded us from growing the additional plants required for these harvests, and therefore the particular end-of-life pattern of biomass partitioning obtained for a plant reflects both its innate programme to allocate biomass to its different parts and its plastic response (if any) to damage suffered at a particular ontostage (depending on the treatment).

Data analysis

Resistance

We used a general linear model (GLM) to analyse the effects of accession, ontostage at which plants were damaged, and the accession–ontostage interaction on resistance.

Tolerance and compensatory ability

To estimate tolerance, we conducted a general linear model using seed production as fitness estimate and the proportion of leaf area damaged as a continuous explanatory variable (a covariate). The model included accession (A), ontostage (O), and the double and triple interactions with damage (D). A significant O × D interaction would indicate that tolerance varied with ontostage. While this analysis correctly captures the definition of tolerance as a norm of reaction, its power is limited by the number of accessions examined (a single estimate of tolerance is obtained per accession). Therefore, we also calculated individual compensatory ability as

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

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 (only individuals subjected to damage), and c is the control group (undamaged individuals) for a particular accession. We used medians ($\tilde{y}$) instead of means in order to reduce the influence of outliers. This measure reflects the level of tolerance of an individual plant in a particular accession-by-ontogeny combination to the mean level of leaf area loss experienced by plants in the damage treatment (Belsky 1986; Hochwender et al. 2000; Tucker and Avila-Sakar 2010). A value of zero for compensatory ability indicates equal compensation, while significant negative or positive deviations from zero indicate under- or overcompensation, respectively. We used a GLM to analyse the effects of accession and ontostage on compensatory ability.

Cost of tolerance and tradeoff with resistance

To estimate the cost of tolerance at each ontostage, we tested whether the covariance between tolerance (the slope in the relation between fitness and damage) and the Y-intercept (fitness in the absence of damage) was negative (Mauricio et al. 1997; Pilson 2000). To obtain the necessary slopes and intercepts, we conducted separate regressions of seed production on damage for each accession within each ontostage. Because this analysis produces a bias in the estimation of costs due to non-independence between the intercept and the slope, we performed the necessary correction (see Appendix B in Mauricio et al. 1997). We used a jackknife procedure to obtain standard errors for the corrected covariances (Sokal and Rohlf 1981). Since a cost of tolerance is indicated by a negative true covariance, we conducted a one-tailed t test to determine whether the covariance for each cohort was significantly less than zero using a type I error rate of 0.05.

To test for tradeoffs between tolerance and resistance, we performed a Pearson correlation analysis between the median resistance of an accession and its tolerance at each ontostage. We also tested for correlations between the ontogenetic trajectories of resistance and tolerance by means of a regression analysis of the genotypic (accession) ontogenetic

changes in resistance ($\Delta R = R_L - R_F$) on those of tolerance ($\Delta T = T_L - T_F$; Boege et al. 2007).

Biomass partitioning in relation to compensatory ability

Since the allocation of biomass to different plant parts is usually correlated, to explore the patterns of biomass allocation that favour tolerance, we conducted a principal component analysis on root, stem, rosette, and cauline leaf biomass. We subsequently used the first two principal components (PC) in a GLM to elucidate the influence of these biomass components together with accession and ontostage on compensatory ability.

Results

Damage treatments removed 66.47 ± 6.86 % (mean ± SE) of the total leaf area on L plants and 75.21 ± 3.81 % on F plants. Seed production for each accession showed much variation, with some damaged plants exceeding seed production of undamaged controls (Fig. 1). Accessions CS20 and CS910 could not be damaged at the F stage due to asynchronous development with the larvae and, therefore, were removed from any analyses involving tolerance at that ontostage.

Resistance

Plants at the F stage were significantly more resistant than L plants (L: 0.14 ± 0.03, $n = 48$, F: 0.42 ± 0.04, $n = 41$, $F_{1,73} = 40.22$, $P < 0.001$). We found no significant effects of accession ($F_{7,73} = 0.92$, $P = 0.497$) or the accession-by-ontostage interaction ($F_{7,73} = 1.01$, $P = 0.434$). Notably, our estimates of resistance did not correlate with those of Jander et al.'s (2001) study (Pearson correlation between our median resistance estimate and Jander's score for each accession: $r = -0.04$, $P = 0.92$ at the L ontostage, $r = -0.12$, $P = 0.78$ at the F ontostage).

Tolerance and compensatory ability

Seed production decreased with damage, but we did not find differences in tolerance between ontostages, as indicated by the non-significant O × D interaction (Table 1). A preliminary analysis revealed a
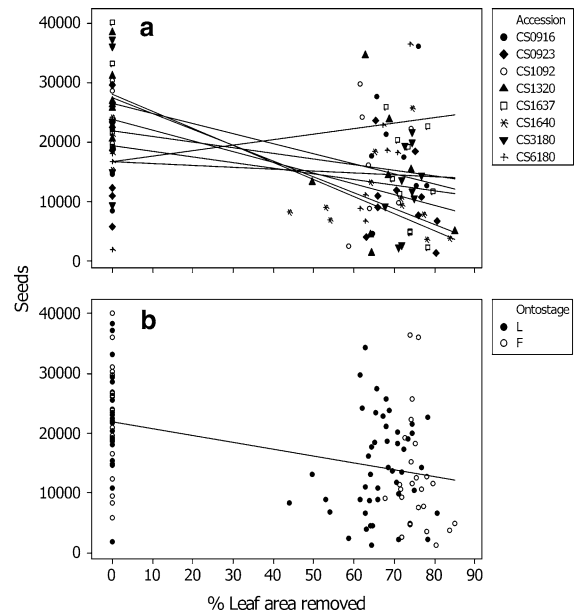

Fig. 1 Lifetime seed production of *Arabidopsis thaliana* plants subjected to different levels of foliar damage (percent leaf area removed). The same data are presented with different symbols by accession (**a**) or by ontostage at which plants were damaged (**b**). Lines depict the linear estimates for each accession (**a**), although the only one that was significantly different from the rest was that for CS916, the only line with a positive slope. The effect of ontostage was not statistically significant, and therefore only one linear estimate is shown (**b**)

marginally significant effect of accession on tolerance whereby CS916 tended to fully compensate. Four plants that produced over 45,000 seeds (all others produced fewer than 40,000) seemed to inflate within-accession variation, thus obscuring variation among accessions. A reanalysis without these data revealed significant variation in tolerance among accessions (significant accession-by-damage effect), due primarily to CS916, which overcompensated (Table 1; Fig. 1). Consistent with these results, compensatory ability did not differ significantly between ontostages, but varied significantly among accessions (Table 1), and CS916 clearly overcompensated.

Cost of tolerance and tradeoff with resistance

A significant cost of tolerance was detected at both ontostages: the corrected mean covariance between tolerance and fitness in the absence of damage was significantly less than zero ($\alpha = 0.05$, one-tailed tests,

Table 1 General linear models for the effects on seed production (square root transformed) and compensatory ability of accession, ontostage at which plants were damaged, and damage level (seed production only)

Source	Seed production				Compensatory ability			
	DF	Adj MS	F	P	DF	Adj MS	F	P
Accession (A)	7	2031	1.65	0.131	7	1.0182	3.68	0.002
Ontostage (O)	1	366	0.30	0.587	1	0.2611	0.94	0.335
Damage (D)	1	19,145	15.60	0.000	–	–	–	–
A × O	7	817	0.67	0.700	7	0.2033	0.73	0.643
A × D	7	2757	2.25	0.038				
O × D	1	7	0.01	0.942				
A × O × D	7	551	0.45	0.869				
Error	86	1228			69	0.2767		
Total	117				84			

Tolerance estimates are derived from the model on seed production

Adj MS adjusted mean square for unbalanced designs
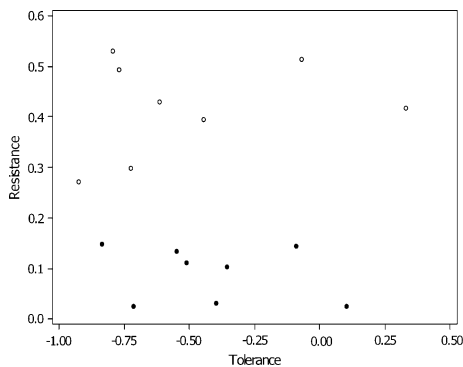
Fig. 2 Relation between resistance and tolerance measured at the four-leaf (*closed circles*) or first-fruit (*open circles*) stage of development in eight accessions of *Arabidopsis thaliana*

$SS_{xy} = -6.01$ at the L stage, and $SS_{xy} = -4.90$ at the F stage).

We did not find a significant negative correlation coefficient between resistance and tolerance at either ontostage (Pearson's $r = -0.234$, $P = 0.562$ at the L stage, and $r = 0.257$, $P = 0.539$ at the F stage, $n = 8$ in both cases; Fig. 2). Similarly, we did not find a significant correlation between the ontogenetic trajectories of resistance and tolerance ($F_{1,6} = 1.67$, $P = 0.243$).

Biomass partitioning in relation to tolerance

PC1 and PC2 explained 93 % of the cumulative variation among the biomass variables and were therefore retained for further analyses (Table 2).

Table 2 Eigen values, proportion of variance, and cumulative variance explained by the principal components of the biomass variables; and component loadings of the first two principal components

	PC1	PC2	PC3	PC4
Eigen value	3.0043	0.7139	0.1908	0.0911
Proportion of variance	0.751	0.178	0.048	0.023
Cumulative variance	0.751	0.930	0.977	1.000
Component loadings				
Variable	PC1			PC2
Cauline	0.526			−0.284
Rosette	0.530			−0.264
Stem	0.557			−0.079
Root	0.363			0.918

PC1 had positive loadings for all biomass categories, but especially for shoot biomass, and was interpreted to represent the "shoot biomass" of the plant (Table 2). PC2 had a strong positive coefficient for root, moderately negative coefficients for leaf biomass, and a slightly negative coefficient for stem. Thus, we denoted PC2 "root priority", since high PC2 components represent more biomass allocated towards root and less to aerial biomass.

The general linear model for compensatory ability as a function of accession, ontostage, and the two principal components and their interactions revealed that shoot biomass (PC1) contributed towards compensatory ability (coefficient = 0.57; Table 3). Root

Table 3 General linear model for the effects of accession, ontogenetic stage at which plants were damaged, and shoot biomass (PC1) and root priority (PC2) on compensatory ability

	DF	Adj MS	F	P
Accession (A)	7	0.3334	2.98	0.026
Ontogeny (O)	1	0.0112	0.10	0.755
Shoot biomass (PC1)	1	1.6820	15.04	0.001
Root priority (PC2)	1	0.0924	0.83	0.374
A × PC1	7	0.1834	1.64	0.182
A × PC2	7	0.1738	1.55	0.207
O × PC1	1	0.0004	0.00	0.952
O × PC2	1	3.5414	31.66	0.000
Error	20	0.1119		
Total	46			

Adj MS adjusted mean square for unbalanced designs

priority (PC2) also contributed towards compensatory ability, but only for plants at the L stage, as indicated by the significant O × PC2 interaction term (coefficient = 0.38, significantly greater than zero, $T = 5.63$, $P < 0.001$).

Discussion

In this study, we found that resistance, but not tolerance, increased from an early vegetative stage (four-leaf) to a late reproductive stage (first-fruit) in *A. thaliana*. We also detected significant costs of tolerance at both ontogenetic stages, but we did not find a tradeoff between resistance and tolerance. We found no evidence of an association between the ontogenetic trajectories of tolerance and resistance.

The increase in resistance from the vegetative to the reproductive stage seen in our study supports the idea that, through growth, plants increase their capacity to acquire resources for the several functions they must perform, including defence (Boege and Marquis 2005). The lack of a similar ontogenetic trajectory for tolerance in our study was unexpected given previous results from our lab using a different set of accessions (Tucker and Avila-Sakar 2010), but the result is consistent with a meta-analysis that reports no change in tolerance with ontogeny (Barton and Koricheva 2010). Shunting resources to produce flowers and fruits or to increase resistance at the first-fruit stage could have reduced the pool of

resources needed to compensate (and be more tolerant) throughout a prolonged reproductive period, although the absence of significant tradeoffs between resistance and tolerance suggests that the observed increase in resistance did not cause a more severe draw of resources away from tolerance. Alternatively, a decrease in foliar nitrogen content with age could have decreased the palatability of leaves for herbivores. The difference in ontogenetic trajectories between resistance and tolerance contrasts with that observed for *Raphanus sativus* (Boege et al. 2007) in that resistance in *A. thaliana* increases with ontogeny instead of decreasing, while tolerance does not change appreciably. However, the evolutionary implications are similar: greater tolerance at the fruiting stage has little or no fitness benefits and thus has not been fixed through natural selection in these accessions.

Resistance–tolerance tradeoffs and the cost of tolerance

Notably, the absence of a tradeoff between resistance and tolerance at either ontostage is unexpected given the significant costs of tolerance at both ontostages. Insufficient variation in resistance among accessions could hamper the detection of its covariation with tolerance. However, within each ontostage there was almost a two-fold variation in resistance between the least- and most susceptible accessions. Thus, a doubling of investment in resistance did not result in a tradeoff with tolerance.

Our results agree with a meta-analysis that found no significant negative tradeoffs between resistance and tolerance for eight non-crop species (Leimu and Koricheva 2006), and with findings from more recent studies, two of them on *A. thaliana* and one on *A. lyrata* (Mauricio et al. 1997; Weinig et al. 2003; Barton 2008; Manzaneda et al. 2010; Muola et al. 2010; Puentes and Ågren 2013), but not with findings that show a decreasing tradeoff with ontogeny for a different set of accessions of *A. thaliana* grown in a greenhouse (Hoque and Avila-Sakar 2015). Thus, while there may be an adaptive peak for plants at intermediate levels of resistance and tolerance (Núñez-Farfán et al. 2007), as shown for *Datura stramonium* (Solanaceae) and *Cucurbita pepo* ssp. *texana* (Du et al. 2008; Carmona and Fornoni 2013), it is also possible that tradeoffs have not been detected in this and other studies because of the particular

ontogenetic stages studied or because of growth conditions that may mitigate such tradeoffs.

A variable pool of resources may also preclude the occurrence of a simple tradeoff between resistance and tolerance (Reekie and Avila-Sakar 2005). Resource mobilization and changes in the rate of carbon and nutrient acquisition may occur in response to the depletion of such resources by particular sinks, including resistance traits. Resources needed for current tissue construction could decrease as an immediate consequence of resource investment in resistance traits. However, the temporary scarcity of resources created by such investment in resistance could also stimulate resource uptake by source tissues, thereby enhancing future growth and thus counterbalancing the negative fitness effects of such use of resources.

Lastly, the distinction between induced and constitutive resistance is important when considering tradeoffs (Kessler and Baldwin 2002). Accessions in this study were selected for variation in resistance as determined by Jander et al. (2001). However, much of the resistance measured in that study might have been induced rather than constitutive because larvae were left on plants for up to 3 days, at which point resistance was scored. In contrast, by measuring the proportion of leaf area not consumed after a short period (maximum 40 min) using leaf discs taken from plants that had not experienced damage, we measured constitutive resistance. This could explain the lack of correlation between our resistance measurements and those in Jander et al. (2001). More importantly, our use of constitutive resistance measurements may also explain the lack of correlation with tolerance, since constitutive resistance traits have been reported to correlate less with tolerance than induced resistance traits (Strauss et al. 2003). It must be noted that our use of an operational definition of resistance precluded us from figuring out which resistance traits and thus what resources were in demand for resistance. Given the lack of a tradeoff with tolerance, it would seem that different sets of resources were being used for resistance and tolerance.

Our study found costs of tolerance at both the early vegetative and the fruit-ripening ontogenetic stages. Of the few studies that have attempted to measure costs of tolerance, costs have been detected in *Datura stramonium* (only in certain location; Fornoni et al. 2004), *Arabis perennans*, and *A. drummondi* (only when not in competition with grass; Siemens et al.

2003), in *Asclepias syriaca* (Hochwender et al. 2000), and in a different set of accessions of *A. thaliana* (but only at an early vegetative stage; Hoque and Avila-Sakar 2015). Two studies found marginally significant costs of tolerance to apical meristem damage, one of them in *A. thaliana* (Tiffin and Rausher 1999; Weinig et al. 2003). Several studies have failed to find a cost of tolerance, including a field study of insect damage on *A. thaliana* (Mauricio et al. 1997; Stowe 1998; Honkanen and Jormalainen 2005; Boege et al. 2007; Rotundo and Aguiar 2008; Manzaneda et al. 2010; Hakes and Cronin 2011; McNutt et al. 2012). Thus, it seems that growing conditions, type of herbivore, habitat, and particulars of the experimental methods can affect the expression and detection of costs of tolerance. In fact, the lack of a tradeoff between resistance and tolerance in our study may have facilitated the detection of costs of tolerance, since the cost of simultaneously investing in resistance and tolerance should be greater than the cost of investing alternatively in resistance or tolerance. The generality of this pattern remains to be seen as the costs of tolerance to herbivory are measured for more species.

Patterns of resource allocation that favour tolerance

Our analyses of biomass partitioning revealed a general association between a propensity to grow more and tolerance. Notably, the three components of shoot biomass (stem, cauline leaves, and rosette leaves) contributed more or less equally, and about 1.5 times more than root biomass to tolerance, a result consistent with previous findings in a different set of *Arabidopsis* accessions (Tucker and Avila-Sakar 2010). Most of the stem biomass corresponds to the inflorescence. Therefore, stem biomass may contribute to tolerance together with the biomass of cauline leaves through their contribution to photosynthesis. In fact, the inflorescence contributes 36–93 % of the lifetime C gain of an *Arabidopsis* plant (Earley et al. 2009). Moreover, larger inflorescences support more fruits and seeds and have more meristems, which could contribute to tolerance.

We also found an association between root mass (PC2) and tolerance in the early vegetative stage that disappeared by the fruit stage. This finding indicates that roots have key roles in responses to shoot herbivory (Nalam et al. 2013), especially at a time

when the inflorescence is unavailable to contribute to tolerance because it has not been produced. Unfortunately, since biomass measurements were taken at senescence, we cannot tell whether the greater allocation to root growth in the more tolerant plants was a constitutive propensity to allocate more resources to roots or a greater induced re-allocation to root mass elicited by damage. Interestingly, an association between allocation to roots and tolerance has also been found in *Plantago major*, although not *P. lanceolata*, both naturalized perennials in Hawaii (Barton 2013). Roots may be important for tolerance in *Arabidopsis* because of mechanisms similar to those recently discovered in *Nicotiana* and tomato, whereby carbon- and nitrogen-rich molecules are transported from the shoot to the root in response to damage and later sent back to the shoot to supply for the construction of tissues, ultimately resulting in tolerance (Korpita et al. 2013).

In conclusion, our study found support for the prediction that defence should increase from an early vegetative stage to a late reproductive stage (Boege and Marquis 2005), but this was true only for constitutive resistance and not for tolerance. Thus, it seems that *A. thaliana* has evolved to be quite tolerant at a stage of high vulnerability to herbivory, but as they grow and become more resistant, greater tolerance does not contribute more to fitness, especially given its cost. Still, more populations and other species should be examined to assess the generality of this pattern, using both generalist and specialist herbivores. Our study adds to the handful that have found significant costs of tolerance, possibly due, in part, to the investments of resources in both tolerance and resistance traits, as evidenced by the lack of a tradeoff between resistance and tolerance. Lastly, our results show that both the ability to grow large rosettes and a large inflorescence and the propensity to make roots are associated with tolerance.

Conflict of interest The authors declare that they are aware of no conflicts of interest regarding funding or conduction of this study.

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