Rapid report

Experimental support of the stress-gradient hypothesis in herbivore–herbivore interactions

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Summary

• The stress-gradient hypothesis (SGH) postulates an increase in the frequency of positive species interactions at increasing amounts of stress. While the SGH has been extensively tested in plant–plant interactions along abiotic stresses, it remains unclear whether this hypothesis could apply to higher trophic levels, such as herbivores, along biotic stress gradients.

• To address this issue, we investigated how the interaction between two potato herbivores may change along a stress gradient created by an assortment of potato varieties with different tuber palatability. We used a tuber resistance trait as a measure for biotic stress and one herbivore as the facilitator to gain access to the tuber of the other herbivore.

• Our experiment revealed a switch from neutral to positive interactions with increasing stress, confirming for the first time the predictions of the SGH for herbivores. Moreover, the intensity of facilitation decreased at high stress levels, suggesting that benefits by the facilitating species were dampened in the most stressful environment.

• In view of the ubiquitous role played by positive interactions among herbivores, broadening our search image for facilitative effects among other plant enemies will allow a better awareness of the importance of the SGH in structuring plant communities.

Introduction

The stress-gradient hypothesis (SGH) states that environmental stress modulates species' nontrophic interactions, causing a shift from negative/neutral interactions to net positive interactions with increasing stress (Bertness & Callaway, 1994; Brooker *et al.*, 2008). In highly stressed environments, if one species ameliorates biotic or abiotic conditions, the frequency of facilitative interactions would increase and offset competition, thus leading to net positive interactions (Callaway, 2007). The SGH has received much attention and refinements from plant ecologists in recent years (Brooker, 2006; Sthultz *et al.*, 2007; Maestre *et al.*, 2009; Malkinson & Tielbörger, 2010; Anthelme *et al.*, 2012). A few experiments on variations in interactions among nonplant species, mostly sessile marine invertebrates, also provided a test of the SGH (Kawai & Tokeshi, 2007; Bulleri *et al.*, 2011; Silliman *et al.*, 2011). While the SGH has been extensively tested for low-trophic-level organisms (e.g. plant and particle filters) along abiotic stresses, it remains unclear whether this hypothesis could apply to higher trophic levels, such as herbivores, along biotic stress gradients.

We tested the application of the SGH to herbivores using two interacting potato tuber moths (*Tecia solanivora* Povolny and *Symmetrischema tangolias* Gyen; Lepidoptera: Gelechiidae; hereafter cited by their genus name) along a gradient of stress conditions represented by an assortment of potato varieties with different palatability characteristics. While the larvae of the specialist tuber-feeder *Tecia* are highly efficient burrowers, the generalist larvae of *Symmetrischema* (which attack leaves, stems, or tubers of the potato plants) are not (Dangles *et al.*, 2008). Owing to such differences in tuber penetration capabilities, it has been shown that the resource access by *Symmetrischema* can be ameliorated by the presence of *Tecia* (Mazoyer, 2007; Dangles *et al.*, 2009). Under natural conditions these moths feed on a wide array of potato varieties which differ in quality, in particular in epidermis thickness, a key barrier to potato moth larval feeding (Horgan *et al.*, 2010). Consequently, *Symmetrischema* may greatly benefit from the presence of *Tecia* when feeding on tubers with a thick epidermis

but not when feeding on tubers with a thin epidermis. We therefore hypothesized that *Tecia–Symmetrischema* interactions might follow the pattern predicted by the SGH, that is, that the intensity of facilitation between the two species should increase in response to increasing biotic stress, in our case an increase in epidermis thickness.

Materials and Methods

Resource quality gradient

We established a resource quality gradient by collecting tubers from 12 varieties of the potato *Solanum tuberosum* L. (Solanaceae), known by farmers and agricultural technicians to differ in their resistance to moth attack: *chaucha, coneja, fruit, gabriela, leona blanca, leona negra, limeña, puca shungo, supercecilia, superchola, suprema,* and *uvilla*. We confirmed that these varieties constituted a gradient of resource quality by measuring the thickness of tuber epidermis (periderm). For each potato variety, transverse slices of tubers taken from the eye buds (where neonates generally penetrate the tuber) were stained with 2% Safranin O (in 50% ethyl alcohol). The thickness of the tuber epidermis was determined by examination of at least 20 samples at ×100 magnification using compound light microscopy (Olympus BX41, Olympus, Tokyo, Japan).

SGH experiment

After confirming that our selection of potato varieties formed a resource quality gradient (see Supporting Information, Fig. S1), we placed potato moths (*Tecia*, the facilitator, and *Symmetrischema*, the beneficiary) in experimental containers with one of the 12 tuber varieties and measured whether interactions between the two moth species varied with litter quality. For each variety, our experimental design consisted of three treatments replicated 15 times; two single-species treatments with 20 individuals of *Tecia* and *Symmetrischema* and one two-species treatment (10 + 10 individuals) with both species. The constant total density of 20 individuals in each treatment allowed us to test for the effect of inter-specific interactions without density effects (see Dangles *et al.*, 2011). For each variety treatment, two tubers were inoculated with 20 moth eggs taken from permanent cultures in our laboratory (see Dangles *et al.*, 2009 for further details on laboratory colonies) and placed in a 250 ml plastic container covered with nylon mesh. Eggs were all of the same age, that is, laid within 24 h preceding the experiment. Egg and neonate mortality was checked every day using a stereomicroscope (Leica, MZ3). After pupation, tubers were dissected to assess larval mortality and pupae were weighed to the nearest 0.1 mg. All experiments were conducted under controlled conditions (i.e. in a room with 70 ± 10% relative humidity and 12 : 12 h, light : dark period).

Data analyses

The relative interaction index, RII (Armas *et al.*, 2004), was used to quantify the outcome of the interactions between *Tecia* and its beneficiary *Symmetrischema*. RII ranges from pure competition

(−1) to pure facilitation (+1) and is symmetrical *c.* 0. RII was calculated as follows:

$$ \text{RII} = \frac{P_{+N} - P_{-N}}{P_{+N} + P_{-N}} $$

where P_{+N} and P_{-N} represent the performance of *Symmetrischema* in the presence and absence of *Tecia*, respectively. RII was calculated with two performance variables: larval survival and total pupa biomass (10 individuals). While the first performance variable directly referred to the short-term benefit for *Symmestrischema* of holes made by *Tecia*, the second one, which is strongly related to female fecundity (Dangles *et al.*, 2009), allowed us to assess the relevance of facilitation by *Tecia* in the long term. We compared the survival (arcsine-transformed) and biomass in one-species vs two-species treatments at different stress levels using a two-way ANOVA. This analysis (interaction term) allowed us to test whether the effects of the presence of the facilitator varied according to stress levels.

To generate a stress–species interaction curve that could be used to graphically compare our results with existing SGH models (Kawai & Tokeshi, 2007), we calculated a value of relative resource quality stress by dividing, for each potato variety, its mean epidermis thickness by the highest mean epidermis thickness found in our study (*Puca* variety). To characterize the general form of stress–species relationships, we fitted data to linear, logarithmic, and peak functions (e.g. normal, lognormal, Weibull) that have previously been used in the literature (e.g. Kawai & Tokeshi, 2007; Fugère *et al.*, 2012). Based on r^2 values provided by TableCurve 5.0 (SYSTAT Software, Inc., San Jose, CA, USA), lognormal models always gave the best fit to our data.

Results

Egg survival in experimental containers was high (mean ± SD for all potato varieties combined = 98.2 ± 1.5% for *Tecia* and 97.9 ± 1.8% for *Symmetrischema*). The 12 potato varieties that we used had epidermis thicknesses ranging from 0.069 ± 0.012 (*Fruit*) to 0.187 ± 0.021 mm (*Puca*, see Fig. S1). The effect of the facilitator species *Tecia* on *Symmetrischema* performance showed three distinct phases along the food quality stress (Fig. 1). For potato varieties with thin epidermis (< 0.110 mm), *Symmetrischema* neonates could penetrate the tubers without the presence of *Tecia* and neither larvae survival nor pupa biomass differed significantly between two-species and one-species treatments (Table 1). When feeding on tubers with intermediate epidermis thickness (0.110–0.143 mm), individuals of *Symmetrischema* performed significantly better in the presence of *Tecia* than alone, in terms of both survival and biomass (Table 1). For potato varieties with thickest epidermis (> 0.170 mm), *Tecia* still significantly facilitated the performance of *Symmetrischema*, but at a lower magnitude than at intermediate stress levels (Table 1). These three phases along the food quality stress gradient led to a significant positively peaked (lognormal) relationship between resource quality stress and the interaction between *Tecia* and *Symmetrischema* as measured with the RII index (Fig. 1). Our analyses further confirmed that the effect of *Tecia* on
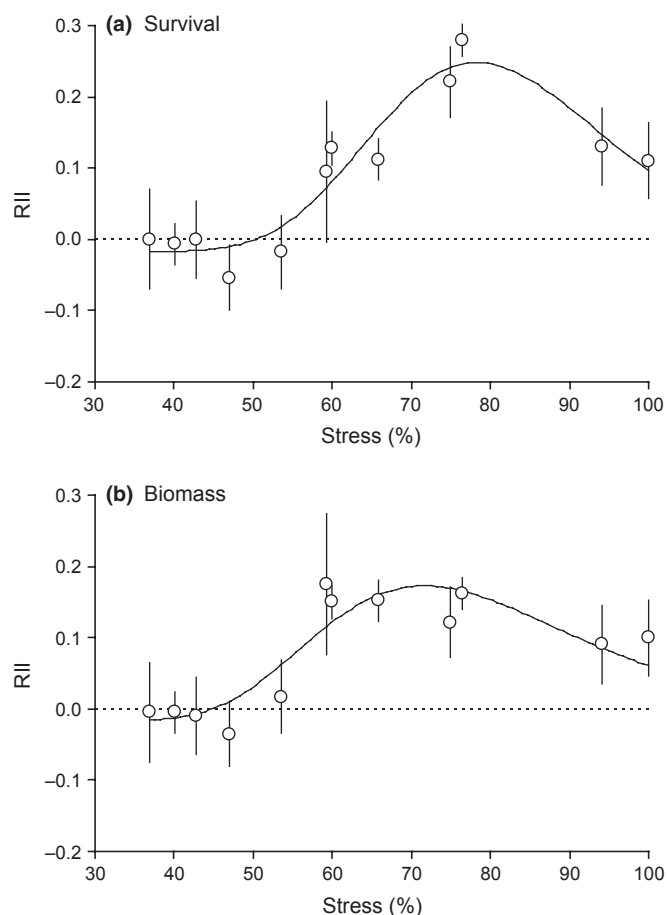
Fig. 1 Relationship between resource quality stress and herbivore species interactions (measured by the relative interaction index (RII); see the Materials and Methods section) for 12 potato varieties differing in epidermis thickness. The RII was calculated using two performance measures: (a) survival and (b) biomass of the beneficiary species *Symmetrischema tangolias* facilitated by the species *Tecia solanivora*. The black dotted line indicates net neutral interactions. The dark line represents the best-fit relationship, a lognormal function. (a) $r = 0.908$, $F = 26.377$, $P < 0.001$; (b) $R^2 = 0.909$, $F = 17.485$, $P < 0.001$. Model selection error bars are standard deviations of 15 replicate experimental units.

Symmetrishema survival and biomass significantly varied across stress levels (two-way ANOVA, 'stress × insect treatment': $F_{10,2} = 8.123$, $P < 0.001$, and $F_{10,2} = 7.639$, $P < 0.001$, respectively).

Discussion

The present study showed that interactions between two insect herbivores became more positive with increasing stress, and then slightly decreased at higher stress levels. This relationship corresponds to what was previously described as a 'facilitation only, positively peaked' relationship (Kawai & Tokeshi, 2007). It is not exactly in line with the relationship found in two other studies testing the SGH in animals (Kawai & Tokeshi, 2007; Fugère *et al.*, 2012), but it agrees with the general and most up-to-date view of plant ecologists that the importance and/or intensity of facilitative effects may reach an asymptote or even decline at high environmental stresses, probably because facilitators are less successful in ameliorating stress factors and promoting the survival of beneficiary species under very severe environmental conditions (Michalet *et al.*, 2006; Maestre *et al.*, 2009; Holmgren & Scheffer, 2010). In our study, the facilitator species *Tecia* had difficulty penetrating the tubers of the two potato varieties with very thick epidermis (the survival of *Tecia* neonates was only 45 ± 12 vs $86.9 \pm 9\%$ when fed with other potato varieties), thereby poorly ameliorating the access of the tuber for *Symmetrischema*. Nevertheless, its overall better ability than *Symmetrischema* in penetrating potato epidermis (better stress tolerance) is certainly the key to its facilitative effects, as observed for plants (e.g. Liancourt *et al.*, 2005). Note that the absence of competitive interactions at low stress values, as described in the initial SGH model (see Callaway, 2007), was explained by the fact that neither moth species competed for resources at the tested fixed larval densities (see Dangles *et al.*, 2009). It is therefore possible that herbivore–herbivore interactions could completely match the predictions of the SGH (a shift from negative to positive interactions along the stress gradient) when herbivores are found at high densities on their host plants and interfere with each other for feeding. Because switches in species interactions may be strongly density-dependent (Bulleri *et al.*, 2011) it is also possible that the effect of the facilitator on the beneficiary may change when the facilitator is present at a different density.

Unlike plants and sessile organisms, most herbivores are mobile and it is likely that the majority of positive interactions between facilitator and a beneficiary animal species would occur during specific life stages (Kaplan & Denno, 2007), thereby questioning the importance of these interactions in the long term. Our study reveals that even when positive interactions occur over a limited portion of the whole animal life (i.e. when moth neonates penetrate

Table 1 Mean performance (larva survival and total pupa biomass) $\pm$ SD of the beneficiary species (*Symmetrischema tangolias*) in the absence or presence of the facilitator species *Tecia solanivora* for three levels of food quality stress

	Survival (%)		Biomass (mg)	
	Beneficiary alone	Beneficiary and facilitator	Beneficiary alone	Beneficiary and facilitator
Low stress	85.3 ± 4.5	84.4 ± 5.8	2.04 ± 0.23	1.98 ± 0.17
Intermediate stress	55.2 ± 6.5	$75.4 \pm 3.5^*$	1.25 ± 0.13	$1.66 \pm 0.19^*$
High stress	35.5 ± 4.3	$45.3 \pm 3.1^*$	0.82 ± 0.34	$1.02 \pm 0.11^*$

Stress: low (thin tuber epidermis; < 0.010 mm); intermediate (tuber epidermis ranging from 0.110 to 0.143 mm); high (thick tuber epidermis, > 0.170 mm).
*Significant facilitation by *Tecia*, that is, significant differences between one-species and two-species treatments (two-way ANOVA, species treatment effect, $P < 0.01$).

their host tuber), facilitation persisted for a much longer time and had a significant impact on the beneficiary's fitness (through increased pupa biomass). This confirms the results of a recent study by Karban *et al.* (2012), who showed that facilitation between two herbivorous insects may persist over time even through indirect interactions that are nontrophic.

While most of the published examples of facilitation involve plants, and marine invertebrates to a lesser extent (Bruno *et al.*, 2003; Bulleri *et al.*, 2011), numerous examples of positive interactions between herbivores have been reported (Denno & Kaplan, 2007; Kaplan & Denno, 2007; Dangles *et al.*, 2009, 2011). These studies dealing with facilitation and with interactions between animals (and particularly herbivores) have historically been poorly integrated to the SGH, although (as our study reveals) they can allow the SGH to be refined and its relevance expanded in natural communities. As a further step, integrating the role of plant-mediated response to herbivore attacks (which did not occur in our study system, as tubers were separated from the plant) would provide a more complete picture of the role of the SGH in plant–herbivore communities along stress gradients. In view of the need to increase our knowledge of the mechanisms by which anthropogenic stressors affect interconnected populations and communities (Bellard *et al.*, 2012), this study therefore provides new insights into the importance of the SGH in predicting the response of plants, and the vast community of organisms that interact with them, in an increasingly stressed world.

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Supporting Information

Additional supporting information may be found in the online version of this article.

Fig. S1 Periderm thickness of the 12 potato varieties used for the stress-gradient hypothesis (SGH) experiment.

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