

1 Classification: Biological Sciences, Ecology

2

3 **Parental environmental effects are common and strong, but unpredictable, in**

4 *Arabidopsis thaliana*

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30 Keywords: environmental stress, maternal effects, natural variation, phenotypic plasticity,
31 transgenerational effects

32

33 **Abstract**

34 The phenotypes of plants can be influenced by the environmental conditions experienced by
35 their parents. In some cases, such parental effects have been found to be adaptive, which has
36 led to much speculation about their ecological and evolutionary significance. However, there
37 is still much uncertainty about how common and how predictable parental environmental
38 effects really are. We carried out a comprehensive test for parental effects of different
39 environmental stresses in the model plant *Arabidopsis thaliana*. We subjected plants of three
40 *Arabidopsis* genotypes to a broad range of biotic or abiotic stresses, or combinations thereof,
41 and compared their offspring phenotypes in a common environment. The majority of
42 environmental stresses (16 out of 24 stress treatments) caused significant parental effects, in
43 particular on plant biomass and reproduction, with positive or negative effects ranging
44 from -35% to +38% changes in offspring fitness. The expression of parental effects was
45 strongly genotype-dependent, with some effects only present in some genotypes but absent,
46 or even in the opposite direction, in others. Parental effects of multiple environmental stresses
47 were often non-additive, and their effects can thus not be predicted from what we know about
48 the effects of individual stresses. Intriguingly, the direction and magnitude of parental effects
49 were unrelated to the direct effects on the parents: some stresses did not affect the parents but
50 caused substantial effects on offspring, while for others the situation was reversed. In
51 summary, parental environmental effects are common and often strong in *A. thaliana*, but
52 they are genotype-dependent and difficult to predict.

53

54 **Significance**

55 Stress experienced by plants can alter the phenotypes of their offspring. To understand the
56 ecological and evolutionary significance of such parental effects, we must know how
57 common and how predictable they are. In a large experiment with *Arabidopsis thaliana*, we
58 show that the majority of 24 environmental stresses cause significant, and often strong,
59 positive or negative parental effects. However, we also find that parental effects are genotype-
60 specific and unrelated to the direct effect of individual stresses, and that multiple stresses
61 often act in non-additive ways across generations. Thus, parental effects appear to be
62 common and strong, but difficult to predict. Our findings have important implications for the
63 study of plant responses to environmental change, and the design of stress experiments.

64

65 **Introduction**

66 Phenotypic variation is at the heart of ecology and evolution. The variation in phenotype that
67 we observe among individuals of the same species either reflects underlying genetic
68 differences, and thus the evolutionary potential of a species, or it results from plastic
69 responses to the environment, and could thus be related to a species' environmental tolerance.
70 A third source of phenotypic variation are parental effects, where the environmental
71 conditions of parents affect the phenotypes of their progeny (1, 2, 3). Parental effects are
72 somewhat peculiar in that they can generate patterns of resemblance among relatives that
73 would usually be considered evidence for underlying genetic variation, while in fact they
74 represent special cases of phenotypic plasticity that extend across generations. The biological
75 mechanisms that cause parental effects include simple nutritional effects such as differential
76 seed provisioning, but also physiological effects mediated by hormones, toxins or other
77 cytosol components, or even epigenetic mechanisms where differential DNA methylation or
78 chromatin changes are passed on to offspring (2, 4, 5).

79 Previous studies showed that parental effects can be ecologically important (e.g. 6, 7, 8)
80 and also influence evolution (e.g. 9; 10, 11, 12, 13). In particular the demonstration that some
81 parental effects are adaptive, with offspring thriving better in parental than non-parental
82 environments (e.g. 6, 14, 15, 16, 17, 18), triggered a debate to what extent parental effects
83 may be evolved mechanisms and a means of rapid adaptation to environmental change (e.g.
84 2, 12, 19, 20, 21, 22). However, despite great current interest in parental effects, many
85 important questions remain unresolved.

86 One of the key challenges in the study of parental effects is to understand how general
87 and how strong they really are. An increasing number of studies showed that parental effects
88 can be substantial, and that they can both increase or decrease offspring fitness (e.g. 6, 8, 23,
89 24, 25, 26, 14, 18, 27, 28, 29), but many of these studies tested a single environmental factor
90 on a single species, sometimes using only a single genotype (but see e.g. 17, 28, 30, 31, 32,
91 33). As a consequence, we still do not have a good idea of how widespread parental effects
92 are across different environmental factors, and how consistent they are across species and
93 genotypes. Given that non-successful tests for parental effects are more likely to end up in
94 file drawers, researchers sceptical of parental effects might suspect that studies as the ones
95 cited above merely represent 'freak' cases that cannot be generalized. Ultimately, the debate
96 can only be settled through comprehensive experiments that test for parental effects across
97 multiple species, genotypes and/or environmental factors.

98 Another fundamental question about parental effects is how predictable they are. For
99 instance, is the magnitude and direction of a parental effect related to (and thus predictable
100 from) the direct effect of an environmental stress on the parental generation? Intuitively, one
101 should expect that environmental factors with stronger effects on parents are more likely to
102 also affect their offspring, and that environmental factors with little or no effects on the
103 parents should neither affect their offspring. But is this really true? We are not aware of any
104 published study that has tested these simple but important assumptions.

105 Environmental change usually involves simultaneous changes in multiple
106 environmental factors (34, 35, 36, 37). Still, most previous studies on parental effects worked
107 with single environmental factors. We know, however, that the direct effects of multifactorial
108 environmental changes are often non-additive (e.g. 36, 38, 39, 40, 41, 42). It thus appears
109 critical to also compare the transgenerational effects of single versus multiple environmental
110 changes, to test the predictability of complex parental effects and assess the meaningfulness
111 of previous simplified studies. However, so far only few studies (e.g. 17, 23, 24, 43, 44)
112 tested for the parental effects of multiple simultaneous environmental changes.

113 Here we used the model species *Arabidopsis thaliana* to thoroughly assess the
114 generality and predictability of parental effects. We subjected multiple genotypes of *A.*
115 *thaliana* to a broad range of biotic or abiotic environmental stresses, or combinations of
116 these, altogether 24 different stress treatments, and then assessed phenotypic variation in the
117 offspring of these plants. Our experimental set-up allowed us to address the following
118 questions: (1) How common and how consistent are parental effects across different
119 environmental stresses and plant genotypes? (2) Can the direction and magnitude of parental
120 effects be predicted from the direct effects of environmental stresses on the parental
121 generation? (3) Are the parental effects of multiple simultaneous environmental stresses
122 additive or non-additive?

123

124 **Results and Discussion**

125 *Generality and consistency of parental effects*

126 Many of the studied abiotic or biotic environmental stresses, or their combinations, caused
127 significant parental effects in our experiment. The magnitude and direction of these effects
128 strongly depended on the treatment, plant genotype, and the measured plant trait (Table 1).

129 The strongest parental effects were on plant biomass and fruit production, where several
130 stresses experienced by mother plants increased or decreased the performance of their
131 offspring by 30-40% (Figure 1). For instance, exposure of mother plants to cold, mild heat or

132 shading transgenerationally increased biomass and reproduction by 20-35%, whereas intense
133 heat, or salt in combination with drought, had the opposite effect and decreased both biomass
134 and fruit production by similar amounts (Figure 1). The magnitudes of these effect sizes are
135 well within the range of what previous studies have reported for parental effects in *A.*
136 *thaliana* and other species (e.g. 6, 15, 16, 18, 26, 30, 45). Overall, 7 out of the 12 studied
137 stresses had significant transgenerational effects on plant biomass, and 5 out of 12 on plant
138 reproduction (Table 1). Thus, parental effects appear to be common in *A. thaliana*, and
139 elicited by a broad range of environmental stresses – with likely consequences for ecological
140 interactions and evolutionary trajectories (9, 10, 46).

141 Compared to plant biomass and reproduction, the growth rate and flowering time of
142 plants were much less subject to parental effects, with only few percent changes across
143 generations (Figure 1), and few individual stresses with significant transgenerational effects
144 (Table 1). Clearly, some plant traits are much less prone to parental effects than others,
145 possibly because they are under tighter developmental control. A good example is flowering
146 time, which is strongly differentiated among geographic origins (significant ecotype effects in
147 Table 1; see also 47), but it is hardly plastic across generations.

148 The three *Arabidopsis* ecotypes included in our study often differed in the degree and
149 magnitude of transgenerational effects (Figure 1; significant ecotype interactions in Table 1).
150 Sometimes the effects were even in opposite directions, resulting in non- or hardly significant
151 main effects of an environmental stress across ecotypes. For instance, drought and salt stress
152 had negative transgenerational effects (i.e. lower performance of offspring compared to the
153 offspring of control plants) on the *Col* ecotype, but positive effects on *Tsu*, and none at all on
154 *Sha* (Figure 1). Our results thus demonstrate substantial genetic variation for parental effects
155 among *Arabidopsis* ecotypes, which supports previous studies with *Arabidopsis* and other
156 plant species (e.g. 17, 29, 30, 48, 49, 50, 51; 52) that also found genotype-specificity of
157 parental effects. Compared to previous studies, our experiment included a much broader
158 range of environmental stresses, and it thus demonstrates that G x E effects are very common
159 across generations, just as they are for within-generation plasticity (53, 54).

160 In summary, we find that parental effects are common and strong, but genotype-
161 specific, in *Arabidopsis thaliana*. Because of this genotype-specificity, and their effects
162 particularly on fitness-related traits, we should expect parental effects to influence selection
163 and evolution of the species.

164

165 *Effects on parental versus offspring generation*

166 Having demonstrated parental effects of a broad range of environmental stresses, we next
167 asked if the direction and magnitude of these cross-generation effects was related to the
168 within-generation effects of the different stresses. Intuitively, we expected that negative
169 transgenerational effects would be caused by environmental stresses that also have negative
170 effects on the same trait in mother plants, and vice versa. We found that this was the case for
171 some environmental stresses. For instance, the combination of short intense heat with
172 continuing mild heat significantly decreased the biomass of both mother plants and their
173 offspring (Figure 2). However, there were also cases where within- and across-generation
174 effects were in opposite directions. For instance, high light intensity increased the growth of
175 mother plants, but it decreased offspring biomass, and for mild heat it was vice versa (Figure
176 2). There were also cases where stress treatments affected mother plants but not the offspring,
177 e.g. for salt addition or intense heat, which strongly decreased the biomass of parents but had
178 no effects across generations (Figure 2). Most interestingly, we observed also cases where the
179 direct, within-generation effects of stresses were almost zero, but there were significant
180 transgenerational effects. Examples are cold and drought, which did not at all affect the
181 mother plants in our experiment, but they both strongly increased offspring biomass (Figure
182 2). Environmental stresses with strong direct impacts but no parental effects have been
183 reported previously (e.g. 17, 25), but we are not aware of any previous studies that have
184 shown the opposite. Altogether, because of the diversity of within- versus across-generation
185 responses, there was no relationship between the stress responses of mothers and offspring in
186 our experiment ($R^2=0.038$, $P = 0.358$). While a discussion of the biological mechanisms
187 underlying these diverse results is beyond the scope of this paper, an important take-home
188 message is that the direction and magnitude of parental effects cannot be predicted from the
189 parental responses to an environmental stress, and that sometimes seemingly ineffective
190 environmental changes may nevertheless cause strong parental effects.

191

192 *Parental effects of multiple simultaneous environmental stresses*

193 Environmental change is usually multifactorial (36, 37). It is therefore important to
194 understand interactions between multiple drivers of environmental change, and their potential
195 non-additive effects on organisms (e.g. 35, 38, 39, 41, 42). Our experiment allowed us to
196 address these questions for parental effects of a broad range of environmental stresses on *A.*
197 *thaliana*. We found that for 8 out of the 12 combinations of environmental stresses there were
198 significant statistical interactions in their effects on plant biomass and/or fruit number (Table
199 1), indicating non-additivity of stresses when occurring in combination. For instance, high

200 light intensity and pathogen infection caused negative parental effects on plant biomass when
201 tested individually, but in combination they increased the biomass of offspring plants (Figure
202 1C). Positive parental effects of cold and shading turned into a negative effect when the two
203 stresses were combined, and while drought and salt caused positive or neutral parental
204 effects, their combination caused the strongest negative parental effect on plant biomass
205 observed in our experiment (Figure 1C). In addition to the general interactions between
206 environmental stresses, we also found several significant three-way interactions between two
207 stresses and plant genotype (Table 1), i.e. the non-additivity of multiple stresses depended to
208 some degree on the plant genotype. Our results corroborate the findings of the few previous
209 studies that tested for transgenerational effects of multiple stresses (17, 24, 43) and that found
210 similar non-additive effects. They clearly show that the non-additivity – or context-
211 dependency – of multiple environmental stresses is another challenge for predicting parental
212 effects, particularly under realistic conditions.

213

214 *Conclusions*

215 In summary, our study demonstrates that parental effects strongly influence the growth and
216 reproduction of *Arabidopsis thaliana*, and that many different environmental stresses can
217 cause such parental effects. This is an important result also because we urgently need to
218 understand the mechanisms by which plants respond to global environmental changes, and
219 besides phenotypic plasticity (55, 56, 57) and longer-term adaptation (e.g. 58), parental
220 effects might be another, somewhat intermediate, facet of plant responses. We also found that
221 parental effects were strongly genotype-dependent, that effects of multiple stresses were often
222 non-additive, and that there was no relationship between the within- and across-generation
223 effects of environmental stresses. Thus, parental effects in *A. thaliana* are complex and
224 difficult to predict, and we should be cautious with generalizing from simple studies with
225 single plant genotypes and/or only few individual environmental stresses. From all we know
226 about the ubiquity of G x E interactions, it seems likely that the situation is similar also for
227 parental effects in other plant species. A thorough understanding of parental effects in plants
228 will therefore be possible only with large experiments that include multiple plant genotypes
229 and multiple, interacting environmental drivers.

230

231 **Methods**

232 *Plant material*

233 *Arabidopsis thaliana* is an annual species from open or disturbed habitats of the northern
234 hemisphere. Because of its small genome size, predominant selfing and rapid life-cycle the
235 species is a popular model species in plant biology as well as ecological and evolutionary
236 genetics and genomics (59, 60). In our study we worked with three ecologically and
237 geographically distinct genotypes of *A. thaliana*, the common laboratory strain *Col-0*
238 (Versailles Center ID 168AV), the *Sha* genotype (VC ID 236AV) originating from Tajikistan
239 and the *Tsu-0* genotype (VC ID 91AV) from Japan. All three genotypes are frequently used in
240 genetics and plant biology, and have served as parents for populations of recombinant inbred
241 lines. The same seed batch was used in all four experimental locations (see below).

242

243 *Parental generation*

244 We subjected the plants to 12 different individual biotic and abiotic parental stress treatments,
245 plus 12 pairwise combinations of these stresses, resulting in a total of 24 different stress
246 treatments. For logistic reasons, the 24 treatments were distributed across four different labs
247 (henceforth referred to as “locations”) in Bern, Hohenheim, Nijmegen and Vienna. In Bern,
248 we tested the effects of light stress, heavy metal, pathogens, and all pairwise combinations of
249 these. In Hohenheim, we tested the effects of cold treatment, shading and leaf removal
250 (simulated grazing), and their combinations. In Nijmegen we tested the effects of drought,
251 salt stress and jasmonic acid (simulation of herbivore attack), and their combinations, and in
252 Vienna we tested two different kinds of heat stress, as well as the effects of low nutrients, and
253 their pairwise combinations (see next section for more details on the treatments).

254 At each location, we grew the plants in temperature-controlled growth chambers under
255 the same standardized temperature and daylength conditions (16/8h light/dark, 21°C/16°C),
256 and we further minimized location differences by growing plants in the same pots (7 x 7 cm)
257 and substrate (Einheitserde ED 63T) everywhere. We stratified seeds on wet filter paper at
258 4°C for three days and transplanted seedlings to individual pots. All plants were bottom-
259 watered twice a week throughout the study. Sixteen days after sowing, we started the parental
260 stress treatments, with six treatments (see above) plus a control treatment in each location,
261 and seven replicates per treatment and genotype, i.e. 147 plants per location and 588 plants
262 overall. Where possible, treatments were terminated when the plants started to bolt.

263 To estimate the growth rates of plants under different experimental conditions, we
264 measured the rosette diameter of each plant at 16, 20, 24, 28 and 32 days after sowing, fitted
265 a power function to each plant’s data, and used the parameter *b* as a measure of

266 growth rate. Throughout the experiment, we continuously monitored plant phenology and
267 recorded the date of first flowering (= first petals visible) of each plant. The plants were
268 harvested sequentially, each at the same developmental stage when approximately one third
269 of the siliques had reached maturity. We harvested each plant aboveground, counted its fruit
270 number, and placed it in a paper bag for drying and after-ripening at room temperatures. After
271 14 days we collected the seeds from the paper bags, dried the remaining biomass at 70°C for
272 24 hours and weighed it. We pooled the seeds of all replicate plants per genotype and parental
273 treatment and used these to establish the offspring generation (see below).

274

275 *Parental treatments*

276 We experimentally subjected the parental plants to 12 different environmental treatments: (1)
277 **Light stress** was imposed by increasing light levels from approximately 250 $\mu\text{mol m}^{-2} \text{s}^{-1}$ in
278 the control environment to 450 $\mu\text{mol m}^{-2} \text{s}^{-1}$ in the treated plants. (2) **Heavy metal stress** was
279 created by adding 5 ml of a 8 mMol solution of CuSO_4 to each treated pot every second day,
280 with the last addition at day 28 after sowing. (3) For **pathogen infection** we sprayed the
281 plants four times (starting at day 16 after sowing, and then every third day) with a water
282 solution containing 8×10^8 bacteria of *Pseudomonas syringae* pv. *tomato* DC3000 per ml.
283 The *P. syringae* DC3000 strain is strongly virulent and causes disease symptoms in
284 *A.thaliana*. (4) **Cold stress** was imposed by regularly subjecting plants to 16 h of 4° C
285 temperature during one week (16 h cold followed by 8 h at 21° C; a total of 112 h of cold). To
286 keep plants at long-day conditions, the 16 h cold were divided into 8 h at light and 8 h at dark
287 conditions. (5) **Shading** was created by growing plants under a shading filter foil (122 Fern
288 Green; Lee Filters, Andover, UK) that reduced light by 50% and lowered the red:far red ratio
289 to 0.2. The plants were kept shaded until the control plants began to flower. (6) **Leaf**
290 **removal** was applied by cutting off all cotyledons, which at this time represented 50% of the
291 leaf area, at day 16. 20 days later we repeated the treatment and again cut 50% of the leaf
292 area of each plant. (7) **Drought stress** was created by not watering the treated plants unless
293 they showed signs of wilting, whereas all other plants were watered regularly. (8) To create
294 **salt stress**, we added a 4g/L NaCl solution at day 16 and after that treated plants twice a week
295 with a 8 g/L NaCl solution until day 30. (9) **Jasmonic acid** was applied by spraying treated
296 plants with a 0.5 mM jasmonic acid solution (Cipollini et al. 2002) and control plants with a
297 mock treatment of 0.5% ethanol every second day starting at day 16 days after sowing. (10)
298 **Low nutrient stress** was created by transplanting plants into a nutrient-poor substrate
299 (Huminsubstrat N3, Neuhaus, Germany) instead of the standard substrate used for all other

300 plants. (11) **Short intense heat** stress was created by moving plants for 24 h to a 37°C
301 growth chamber at day 16 and then back to control conditions, whereas for the (12)
302 **prolonged mild heat** treatment plants were moved to a 30°C growth chamber for 10 days,
303 starting at day 16. For the combination of the two heat treatments, the plants were first moved
304 to the 37° chamber for 24 h and then to the 30°C chamber for another nine days.

305

306 *Offspring phenotyping*

307 To test for the effects of parental stress treatments, or their combinations, on offspring
308 phenotypes, we used the seeds collected from the parental generation to grow offspring of all
309 genotypes and parental treatments in a common greenhouse environment. Using the same
310 protocols for germination and growth and the same pots and substrate as for the parental
311 generation, we grew 10 replicate plants per genotype and treatment (= a total of 24 x 3 x 10 =
312 720 plants) in a greenhouse with a 16/8 h light/dark cycle and temperatures of 27/16°C
313 (day/night). The plants were arranged in a fully randomized order and watered regularly. We
314 measured the same phenotypic traits as in the parental generation: growth rate, aboveground
315 biomass, flowering time, and fruit number.

316

317 *Statistical analyses*

318 The parental generation data were analysed through linear models in which we tested the
319 effects of stress treatments, plant genotype, and their interactions, on the growth rate,
320 aboveground biomass, fruit production and flowering time of plants. We carried out these
321 analyses separately for each of the four locations.

322 For the offspring generation data, we first examined how large the differences between
323 the four parental locations were, in spite of our efforts to standardize conditions. A two-way
324 ANOVA testing for location and genotype effects among the control plants only showed that
325 there were still large differences among locations ($P < 0.001$ for all traits), and we therefore
326 decided to also analyse the offspring data separately for each location. We used similar linear
327 models as for the parental generation analyses, testing for the effects of parental treatment
328 and genotype, and their interactions, in each location. If the main effect of parental treatments
329 was significant, we additionally tested a series of contrasts comparing each treatment
330 combination to the control group, to identify which specific parental treatments had
331 significant effects on the offspring. We first ran these analyses across all genotypes and then,
332 since genotype by treatment interactions were significant in most cases, also separately for
333 each genotype.

334 To test for a relationship between the magnitude and direction of parental and offspring
335 stress responses, we calculated the cross-genotype % change caused by each treatment when
336 compared to the respective control plants. We did this for the parental and offspring data and
337 then used linear regression to test for a relationship between the two.

338

339 **Acknowledgements**

340 This work, as part of the European Science Foundation EUROCORES Programme
341 EuroEEFG, was funded by the Swiss National Science Foundation (SNF grant 31EE30-
342 131171 to O.B. and M.F.), the German Research Foundation (DFG grant SCHM 1354/4-1 to
343 K.S., the Austrian Science Fund (FWF I489 to Ortrun Mittelsten Scheid, and FWF I3687 to
344 R.G.), and the Dutch Research Council NWO grant to J.O.). V.L. acknowledges support from
345 the Czech Science Foundation 20-00871S and RVO 67985939. We are extremely grateful to
346 Vincent Colot and Ortrun Mittelsten Scheid for their intellectual contributions to this study.

347

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502 **Figure 1.** Effect sizes of parental effects of different environmental stresses, or their
503 combinations, on *Arabidopsis thaliana* plants. The values are % differences (mean \pm SE) in
504 performance between the offspring of treated parents and the offspring of control parents.
505 Note that the parental generation was grown in four different experimental locations. The
506 coloured squares indicate the significance levels (from contrasts) of parental effects for
507 individual genotypes (red spectrum = negative effects; blue spectrum = positive effects).

508

509 **Figure 2.** Relation of offspring biomass production responses to parental treatments with
510 responses of parents to the treatments. The responses are % changes in biomass production of
511 plants experiencing treatment (or offspring of parents of the treatments) in comparison to
512 control plants (or offspring of control parents). Individual treatments are highlighted.

513

Table 1. Results of ANOVA testing for parental effects of individual stresses, or their combinations, on the growth and fitness of three genotypes of *Arabidopsis thaliana*. Since the parental generation was grown in four different locations, the data were analysed separately for each. Effects significant at $P < 0.05$ are highlighted.

	D.f.	Biomass		Growth rate		No. of fruits		Flowering time		
		F	Pr(>F)	F	Pr(>F)	Dev.	Pr(>Chi)	Dev.	Pr(>Chi)	
Bern	Light (L)	1	0.1	0.789	0.3	0.570	17	0.481	0.322	0.370
	Metal (M)	1	11.3	0.001	4.5	0.035	453	<0.001	0.028	0.791
	Pathogen (P)	1	1.0	0.326	0.0	0.986	2	0.797	3.47	0.003
	Ecotype	2	1.6	0.211	6.8	0.001	424	0.002	31.25	<0.001
	L : M	1	0.4	0.518	0.7	0.390	1	0.884	0.61	0.217
	L : P	1	24.3	<0.001	1.5	0.228	507	<0.001	0.377	0.332
	M : P	1	0.4	0.533	0.1	0.760	26	0.388	0.064	0.690
	L : Ecotype	2	1.1	0.324	1.0	0.372	201	0.056	5.736	0.001
	M : Ecotype	2	12.7	<0.001	0.5	0.632	628	<0.001	0.638	0.451
	P : Ecotype	2	2.7	0.073	0.5	0.635	255	0.026	0.666	0.436
	L : M : Ecotype	2	1.0	0.365	0.4	0.665	115	0.192	3.293	0.016
	L : P : Ecotype	2	2.2	0.117	0.2	0.813	103	0.226	0.396	0.610
	M : P : Ecotype	2	1.0	0.386	1.4	0.244	34	0.609	0.129	0.852
	Residuals	176					8846		108.5	
Hohenheim	Clipping (C)	1	28.4	<0.001	1.5	0.219	641	<0.001	0.693	0.234
	Low Temp. (T)	1	0.6	0.428	0.2	0.620	2	0.850	0.032	0.798
	Shade (S)	1	7.7	0.006	0.7	0.417	355	0.007	0.534	0.296
	Ecotype	2	8.5	<0.001	2.6	0.081	269	0.062	50.6	<0.001
	C : T	1	0.1	0.741	0.6	0.441	24	0.478	0.048	0.754
	C : S	1	0.4	0.520	1.5	0.227	12	0.616	0.973	0.159
	T : S	1	37.5	<0.001	1.1	0.286	1281	<0.001	0.246	0.478
	C : Ecotype	2	31.3	<0.001	0.4	0.658	1334	<0.001	0.346	0.702
	C : Ecotype	2	1.9	0.151	0.1	0.870	72	0.476	0.613	0.535
	S : Ecotype	2	12.3	<0.001	0.2	0.839	649	0.001	3.542	0.027
	C : T : Ecotype	2	0.1	0.949	1.8	0.162	32	0.719	0.307	0.731
	C : S : Ecotype	2	0.4	0.641	1.1	0.350	23	0.791	2.981	0.048
	T : S : Ecotype	2	0.3	0.767	0.7	0.483	119	0.291	0.436	0.640
	Residuals	197					14670		145.5	
Nijmegen	Drought (D)	1	4.1	0.045	6.1	0.015	42	0.360	1.826	0.177
	Jasmonic acid (Ja)	1	21.3	<0.001	6.0	0.015	618	<0.001	0.438	0.508
	Salt (S)	1	5.0	0.027	0.1	0.750	178	0.061	0.224	0.636
	Ecotype	2	26.7	<0.001	0.7	0.494	1758	<0.001	38.47	<0.001
	D : Ja	1	5.8	0.017	0.4	0.523	155	0.080	0.81	0.368
	D : S	1	27.3	<0.001	0.1	0.814	925	<0.001	0.714	0.398
	Ja : S	1	5.3	0.023	0.8	0.359	150	0.085	0.177	0.674
	D : Ecotype	2	7.3	0.001	2.9	0.057	444	0.012	1.396	0.498
	Ja : Ecotype	2	8.5	<0.001	0.8	0.444	271	0.069	3.796	0.150
	S : Ecotype	2	1.6	0.205	1.6	0.197	166	0.195	1.294	0.524
	D : Ja : Ecotype	2	9.7	<0.001	0.2	0.844	658	0.002	4.143	0.126
	D : S : Ecotype	2	11.0	<0.001	1.0	0.371	1289	<0.001	0.641	0.726
	Ja : S : Ecotype	2	2.8	0.061	1.1	0.333	151	0.225	2.619	0.270
	Residuals	175					15841		127.1	
Vienna	LowN (N)	1	2.8	0.094	1.4	0.233	64	0.212	0.095	0.648
	Long Heat (Lh)	1	0.0	0.848	2.3	0.132	0	0.926	0.543	0.275
	Short heat (Sh)	1	13.6	<0.001	0.7	0.394	384	0.002	0.069	0.698
	Ecotype	2	22.1	<0.001	1.8	0.163	1010	<0.001	58.65	<0.001
	N : Lh	1	9.3	0.003	0.3	0.571	188	0.033	0.272	0.440
	N : Sh	1	23.6	<0.001	0.1	0.748	281	0.009	0.787	0.189
	Lh : Sh	1	5.8	0.017	0.4	0.510	85	0.153	2.309	0.024
	N : Ecotype	2	0.9	0.416	0.1	0.917	134	0.198	2.475	0.066
	Lh : Ecotype	2	6.3	0.002	1.0	0.388	455	0.004	3.844	0.015
	Sh : Ecotype	2	4.4	0.014	0.5	0.589	224	0.067	1.339	0.231
	N : Lh : Ecotype	2	0.6	0.533	0.6	0.555	10	0.888	0.448	0.612
	N : Sh : Ecotype	2	9.3	<0.001	0.3	0.760	299	0.027	0.257	0.754
	Lh : Sh : Ecotype	2	2.6	0.078	0.7	0.485	344	0.016	0.268	0.745
	Residuals	194					12337		148.7	

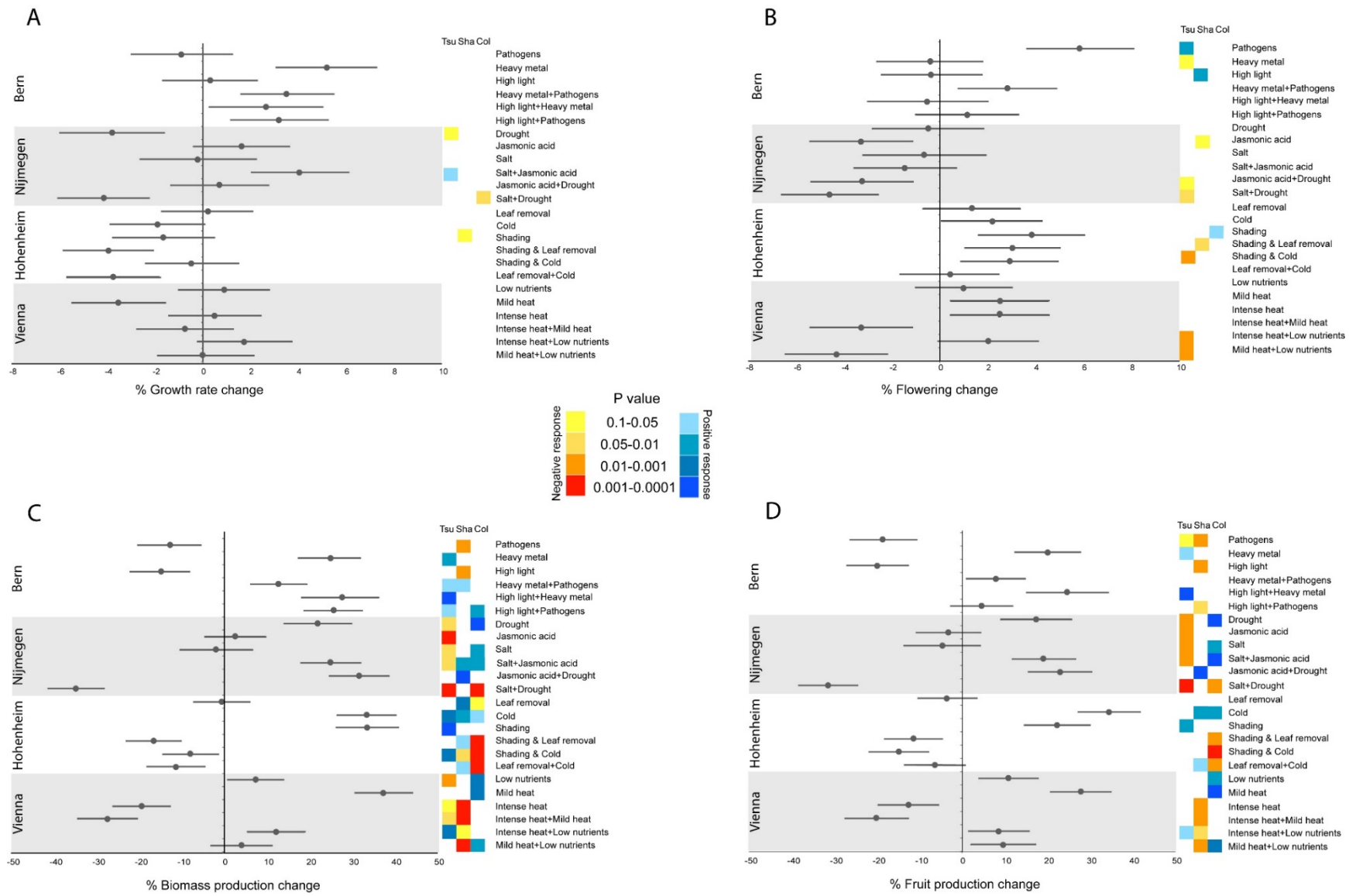


Figure 1

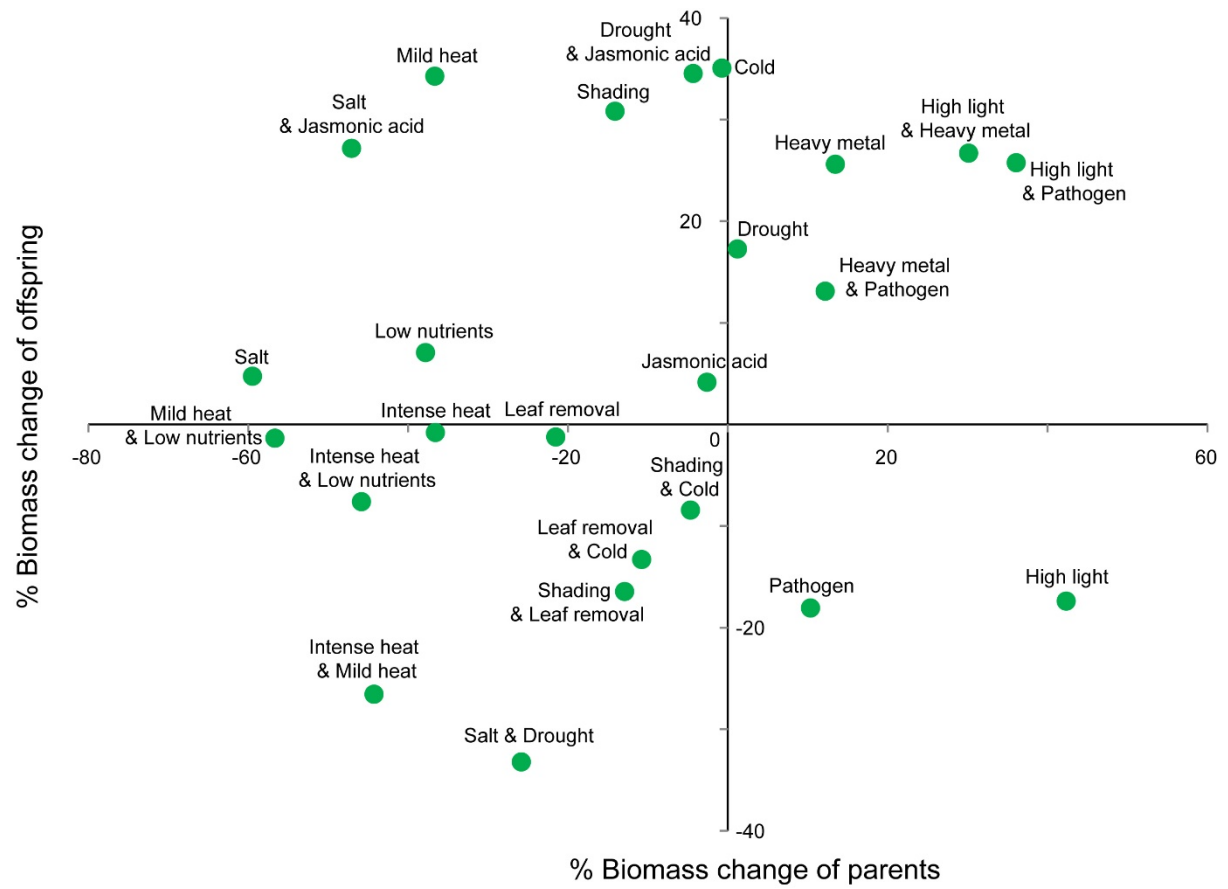


Figure 2