

**Effects of water availability on the relationship between seed set and pollen received in
*Ipomopsis aggregata***

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Abstract

Plants systems rely on a variety of resources to survive and reproduce. The amount and quality of pollen a flower receives can determine not only the number of seeds that are produced, but also whether they will be viable or not. The purpose of the study looked at the relationship between the amount of pollen received by a flower and the number of seeds produced under varying watering conditions (wet and dry conditions) at the Rocky Mountain Biological Laboratory. The study consisted of 26 *Ipomopsis aggregata* to look at the relationship between water availability and the changes in the seed to pollen relationship. Our results suggested that water conditions was not a factor in the amount of seeds made per flower, which instead depended more on the amount of pollen received on the stigma.

Introduction

By the end of the 21st century, predicted climate changes include a 2–4.5°C increase in mean annual temperature and major shifts in precipitation patterns (IPCC 2012). Much of the evidence for impacts of climate change on organisms comes from experimental studies of the effects of temperature and precipitation on a target species or on related species with similar habitats (Walther et al., 2002; Parmesan and Yohe, 2003; Parmesan 2006). There is increasing evidence that climate changes will include extreme weather events (Easterling, et al. 2002); these extremes may cause strong effects on communities as abrupt shifts give little time for adaptation (Easterling et al. 2000). Additional work is needed to understand the consequences of climate change on individual organisms and communities.

Plant fitness can be affected by a large number of morphological and physiological traits that mediate interactions with local environmental conditions (Campbell and Wendlandt, 2013).

Here, I focus traits associated with plant reproductive success. Without enough pollen a plant may have reduced reproductive success (Ashman et al.2004). There is little known about how resources associated with local environmental conditions interact with the role of pollen in plant reproductive success, in particular how does the pollen dose response change with resource availability? For example, seed production may be most sensitive to the amount of pollen, or to the amount of water available, or respond to an interaction between water and pollen. The pollen dose response is the relationship between pollen load and seed set. Typically, in a pollen dose curve the number of seeds increases when the flower receives more pollen on its stigma up to some maximum number of seeds . Resources such as water could affect the pollen dose curve, and resources such as water may become even more variable with climate change, such that we see increased droughts in coming years.

In this study the relationship between the amount of pollen received by a flower and the number of seeds produced under varying watering conditions (wet and dry conditions) was investigated at the Rocky Mountain Biological Laboratory. The watering treatments were based on previous studies done on precipitation and soil moisture using *I. aggregata* (Campbell et al., 2010; Campbell and Wendlandt, 2013). We anticipated that seeds would be more resource-limited in dry conditions and more pollen-limited in wet conditions. We determined how the maximum seed set and the shape parameter of the pollen dose curve changed with water availability. There are at least three possible outcomes for how seeds change with pollen load. In the first possibility (Figure 1), the seed to pollen relationship is the same for wet and dry conditions until pollen receipt becomes high and water becomes a factor. When precipitation varies from wet to dry years then that changes the seed to pollen relationship. The second possibility (Figure 2) is that the pollen dose response is fundamentally different between dry and wet conditions,

regardless of the amount of pollen received. In the last case (Figure 3), water availability does not change the maximum seed set but does change the shape of the pollen dose curve at lower levels of pollen receipt. Having less pollen creates a crucial year for seed production because the amount of water does not matter as much as the pollen received by flowers. To test the changes in the pollen dose curve I used *Ipomopsis aggregata* (Pursh) V.E. Grant (Polemoniaceae, scarlet gila) a native species in Gothic, Colorado.

Methods

The project site was within the Rocky Mountain Biological Laboratory (RMBL) town site. *Ipomopsis aggregata* was used as the study system to look at the relationship between water availability and the changes in the seed to pollen relationship. This plant is native to the area and is a monocarpic species that only blooms once before dying. A total of 26 *I. aggregata* that were in the bud stage were collected along kettle pond road. The *I. aggregata* were re-potted in a circular pot (12.7 cm diameter) filled with the same soil where flowers were collected, and placed in a weather port near Gothic Research center. The pots were watered thoroughly and after 3 days 1t/gal of MiracleGro 20-20-20 fertilizer was added to each pot. All 26 *I. aggregata* were watered every one to two days for a week to ensure plant establishment.

Watering Treatments

Soil moisture data described in two papers (Campbell, 2010; Campbell and Wendlandt, 2013, and Campbell, 2010) were used to mimic wet and dry years at RMBL. The first 10 *I. aggregata* had watering conditions that approximated a wet year (14-17% volumetric water content of the soil), and the 10 other *I. aggregata* received less water to achieve 7% volumetric water content.

The last six *I. aggregata* were pollen donors and were watered normally to ensure healthy flowers.

To quantify the wet and dry water treatments, volumetric water content (VWC) was taken daily using a soil moisture probe with 15.2 cm long probes. The VWC of each pot was taken to ensure water treatments were at 14-17% VWC (wet) and 7% VWC (dry). If pots were not at the required VWC reading then water was added to pots to increase VWC. The average of water per day added to pots was 43.3mL for the wet treatment. Pots in the dry treatment received 22mL of water per day, on average. The average of VWC per day shows that water treatments were near the target conditions of wet and dry (Figure 4).

Varying Pollen Level

To score the amount of pollen received by a stigma, anthers were emasculated during the bud stage so that the only pollen on the stigmas would be the outcross pollen that was applied. The six additional potted plants were used as pollen donors, and we randomized the six pollen donors (A-F) to provide outcross pollen. The outcross pollen was applied in different levels (high, medium, or low) to multiple flowers on each plant, alternating from wet to dry pots, and then the pollinated flower stigmas were left alone for 48 hours. Hand-pollinations were done in a specific way so that once pollination was done three flowers were pollinated having all three levels in one pot and each having a different donor. The hand-pollinations (total of 271) were done with a toothpick to deliver various amounts of pollen. For the low level the toothpick was barely touched to the anthers from pollen donors, for the medium level it was dabbed four times on to anthers, and for the high level it was swiped twice into anthers. A swipe collected more pollen around the surface area of the toothpick, whereas the four dabs only collected pollen on the tip of the toothpick. More pollen was visible on a swiped toothpick than on a dabbed one. The order in

which the three pollen load treatments were completed was randomized for each plant. Hand-pollinations were done during July 12-21, 2014, and after each one, the flower was left to stand alone for 48 hours to help ensure that pollen tubes had a chance to grow. This resulted in 271 hand-pollinations, divided among six different pollen donors and three pollen levels. After the 48 hours, stigmas were removed with forceps and each one was mounted on a microscope slide, stained with fuchsin gel, and covered with a cover slide. The fuchsin gel stained the pollen grains. Then with a microscope, pollen grains were counted at 100X magnification. To collect the fruit as they matured we waited ~3-4 weeks after pollination. Mature fruit would start to turn brown and would easily open up once given a gentle squeeze. The collected mature fruit were placed in labeled coin envelopes and left for a day to dry. Once the fruit was dry, then seeds were counted and recorded.

Data Analysis

To score the amount of pollen levels, the pollen dose curve was used to plot seeds against pollen. The pollen dose curve is a function explaining how seeds relate to pollen, which explains that when the amount of pollen increases the amount of seeds also increases up to a point. The function that is used is a non-linear function: $\text{seed} = (k * \text{pollen}) / (\beta + \text{pollen})$ where k is the asymptote representing the maximum seed set, and β is the number of pollen grains that produces half of the maximum seeds (saturation parameter; Campbell and Bischoff, 2013). Seeds were plotted vs. pollen. Procedure NLIN in the program SAS (SAS Institute Inc., Cary, NC) was used to estimate the parameters k and β in the dose response model. First, a model was fit to the entire data set. Second, the wet and dry treatments were analyzed separately to obtain separate parameter estimates. Finally, the two models were compared to test if using separate parameter estimates would significantly reduce the residual sum of squares.

Results

We first compared the mean seed production per flower for plants in wet vs. dry conditions, which showed that the plants in the wet treatment made approximately 3 more seeds per flower than the plants in the dry treatment (Figure 5). However, a one-way analysis of variance did not detect a significant difference between the water treatments ($F_{1,14} = 0.88$, $P = 0.36$). The results show that we are seeing a slight trend in the direction we expected to see.

For both wet and dry treatments, the data showed saturating pollen dose curves, although there was substantial variability in seed production. The dry treatment pollen dose curve (Figure 6) had k estimated as 11.6 and β estimated as 33.9. For the wet treatment, k was estimated as 11.8 and β estimated as 24.6. (Figure 7). The two treatments did not differ significantly in the parameters according to comparison of residual least squares. In addition, we also used a quadratic regression to fit seeds as a function of pollen. The results of this analysis showed no interaction between treatment and the linear effect of pollen on seeds ($F_{1,138} = 0.2$, $P = 0.6231$) and no interaction between treatment and the quadratic effect ($F_{1,138} = 0.97$, $P = 0.3271$). In an analysis of a quadratic model with treatment taken out, the results showed that there was significant effect of the linear term (pollen) and the quadratic term (pollen square) on seeds (Figure 7).

Discussion

The study found no effect of water availability on the shape parameters of the pollen dose curve for the monocarpic perennial, *Ipomopsis aggregata*. Based on this result alone, we would conclude from these data that drier future conditions would not alter the relationship between

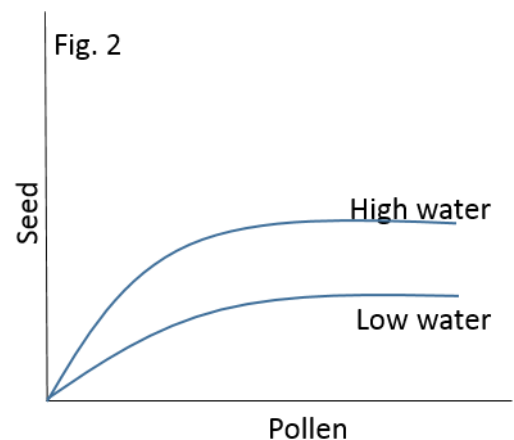
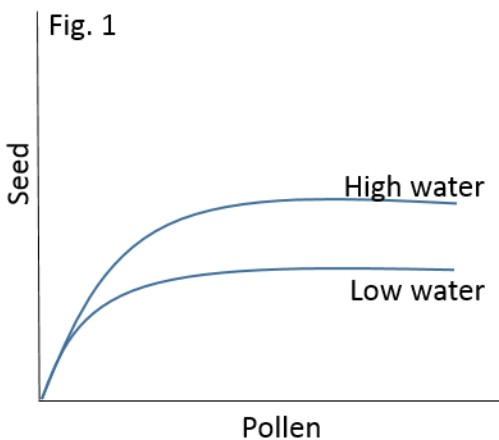
pollen receipt and seed production per flower for this species. However, there are some limitations to the current study.

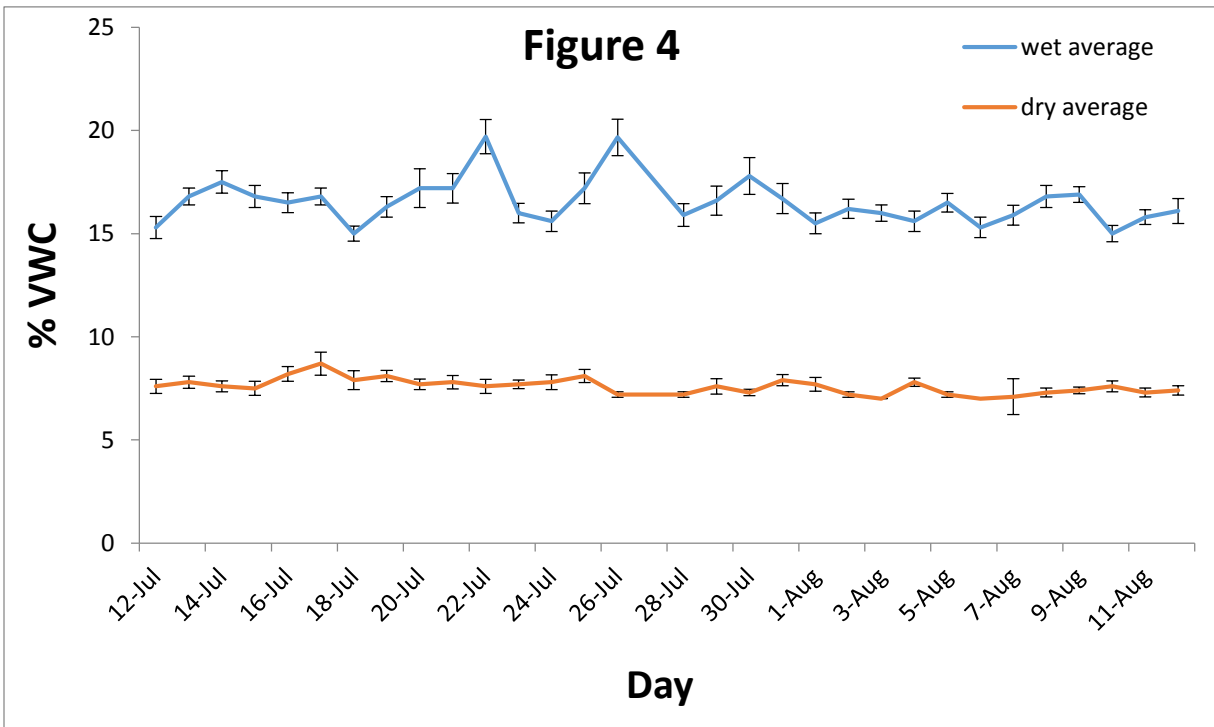
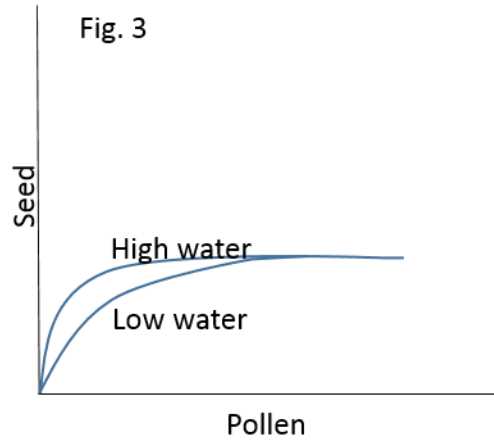
First, plants in the two treatments did not differ significantly in seeds per flower, although there was a trend for the wet treatment to produce about 3 more seeds per flower. The large number of seeds made by the plants in the dry treatment were surprising results since the plants within the treatment were in a stress condition as indicated by the flower and stems were wilting up along with less flowers being made. The lack of a strong effect of the watering treatment suggests that plants may need a longer time of exposure to drier conditions or a stronger reduction in water to produce strong changes in seed production for this species. However, we have not yet collected all of the fruits, so these results might change. In addition, we did not start the watering treatments until the middle of the growing season, had plants experienced altered water for a longer time, we may have observed stronger effects of the treatment.

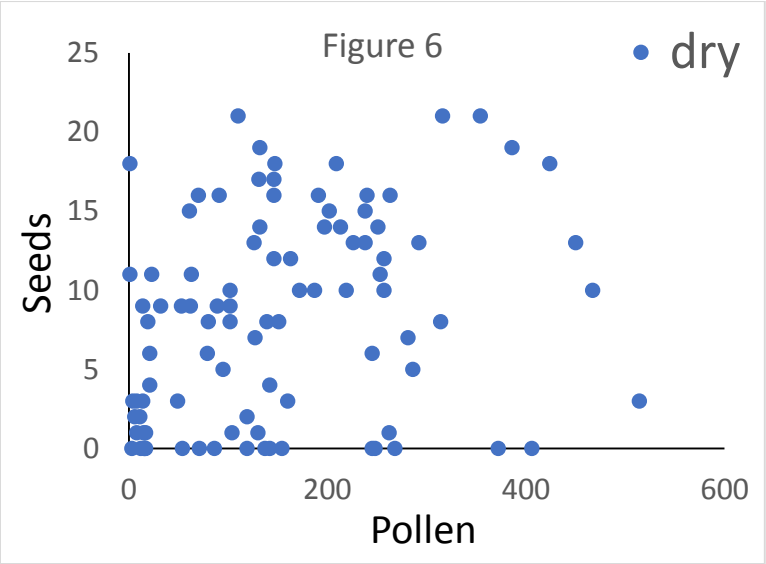
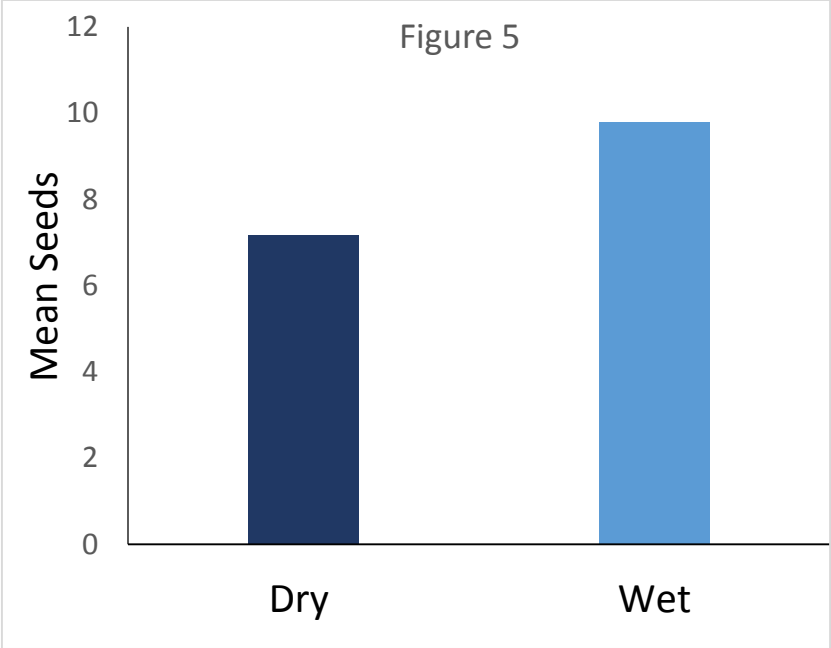
Second, our data to create pollen dose curves showed that increasing pollen did increase seed production per flower up to a maximum when more pollen is added, but showed no significant difference with different watering conditions. Significant difference between watering conditions may not show in the results because all fruits have not been collected and later fruit collected may be the difference between treatments results. We could also compare our pollen dose response curve from potted plants to that of plants in natural settings. In a prior study, flowers in natural meadows were receiving fewer than 100 pollen grains even when exposed for 3 days, and in comparing that result with our pollen dose response curve, seeds were still increasing at that point with more pollen grains. As a result, plants under natural conditions are usually pollen-limited, with some flowers not receiving pollen needed for maximum seed set.

Thus, it remains unclear whether altering water resources in the field might have different effects on plants than observed in our potted plant study.

The data can help with more understanding on the relationship between pollen and seed set but there is a lot more to be learned. Future studies may include repeating the experiment to make sure that the finding in this experiment are the same or if they change, using alternative watering regimes than the two treatments imposed in our study, treating plants earlier in the growing season, or over multiple growing seasons, treating naturally occurring plants under field conditions, and using larger sample sizes.







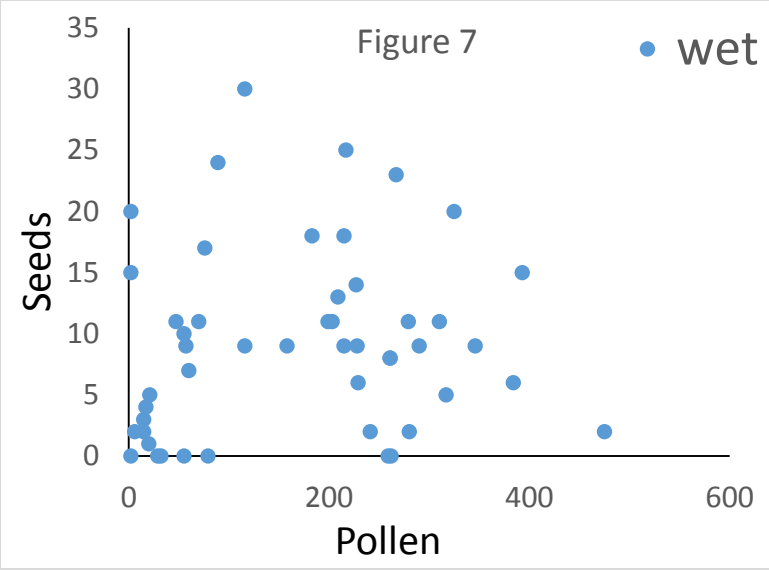
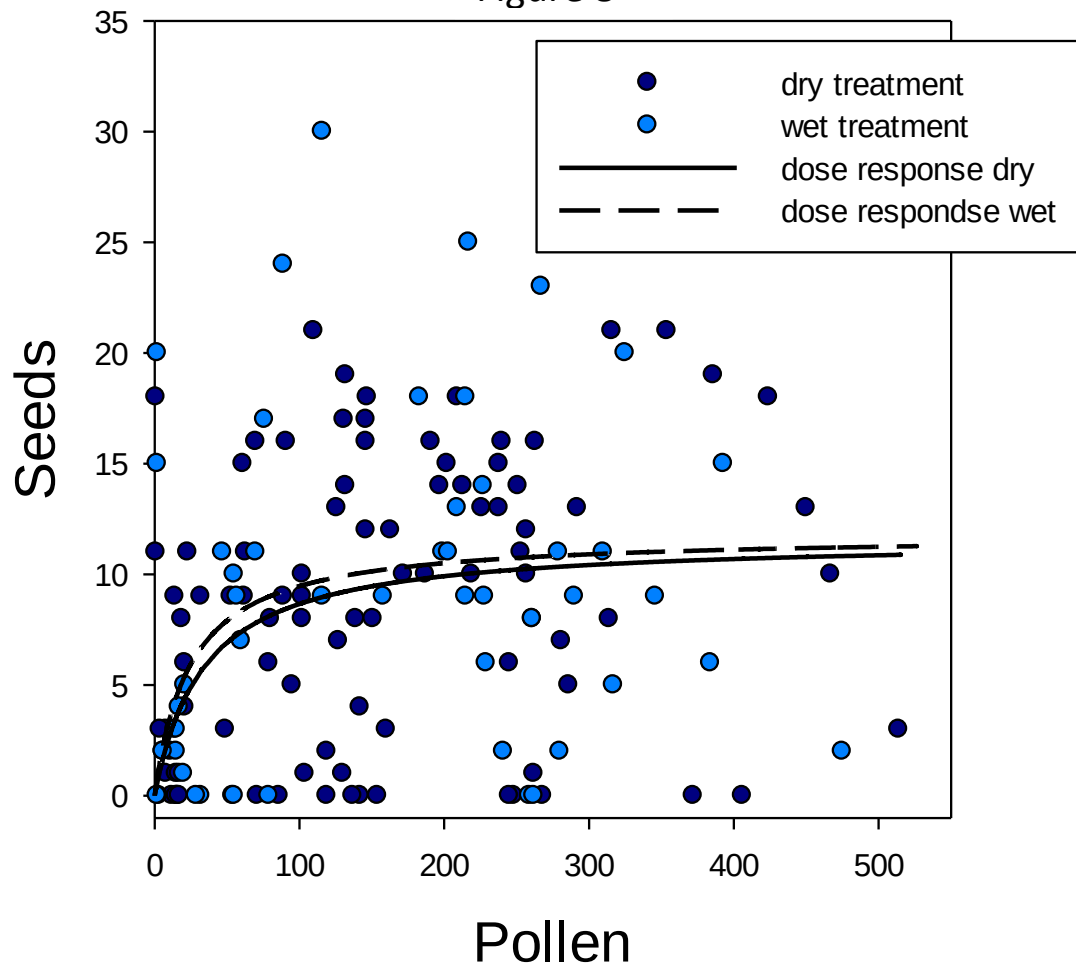


Figure 8



2014 *Ipomopsis aggregata* research and collection sites



Literature Cited

- Ashman, T., Knight, T., Steets, J., Amarasekare, P., Burd, M., Campbell, D. R., Dudash, M. R., Johnston, M. O., Mazer, S. J., Mitchell, R. J., Morgan, M. T., Wilson, W. G. 2004. Pollen limitation of plant reproduction: Ecology and evolutionary causes and consequences. *Ecology*, **85**: 2408-2421.
- Campbell, D. R., Wendlandt, C. 2013. Altered precipitation affects plant hybrid differently than their parental species. *American Journal of Botany*, : 1322-1331.
- Campbell, D. R., Bischoff, M. 2013. Selection for a floral trait is not mediated by pollen receipt even through seed set in the population is pollen-limited. *Functional Ecology*, **27**: 1117-1125.
- Campbell, D. R., Wu, C., Travers, S. 2010. Photosynthetic and growth responses of reciprocal hybrids to variation in water and nitrogen availability. *American Journal of Botany*, **97**: 925-933.
- Easterling, D.R., Meehl, G.A., Parmesan, C., Changnon, S.A., Karl, T.R. & Mearns, L.O. (2000) Climate extremes: Observations, modeling, and impacts. *Science*, **289**, 2068-2074.
- IPCC (2012) Summary for policymakers. *Managing the Risks of Extreme Events and Disasters to Advance Climate Change Adaptation: A Special Report of Working Groups I and II of the Intergovernmental Panel on Climate Change* (ed. by C.B. Field, V. Barros, T.F. Stocker, D. Qin, D.J. Dokken, K.L. Ebi, M.D. Mastrandrea, K.J. Mach, G.-K. Plattner, S.K. Allen, M. Tignor and P.M. Midgley), pp. 1-19. Cambridge University Press, Cambridge, UK.

- Parmesan, C. & Yohe, G. (2003) A globally coherent fingerprint of climate change impacts across natural systems. *Nature*, **421**, 37-42.
- Parmesan, C. (2006) Ecological and evolutionary responses to recent climate change. *Annual Review of Ecology Evolution and Systematics*, pp. 637-669.
- Walther, G.R., Post, E., Convey, P., Menzel, A., Parmesan, C., Beebee, T.J.C., Fromentin, J.M., Hoegh-Guldberg, O. & Bairlein, F. (2002) Ecological responses to recent climate change. *Nature*, **416**, 389-395.
- Walther J., Jentsch A., Beierkuhnlein C., Kreyling J. 2013. Ecological stress memory and cross stress tolerance in plants in the face of climate extremes. *Environmental and Experimental Botany*, **94**: 3– 8.