

Tielbörger, K., Petru, M. and Lampei, C. 2012. Bet-hedging germination in annual plants: a sound empirical test of the theoretical foundations. – *Oikos* 121: 1860–1868.

## Appendix 1

### Collection of supplementary data in the field

The study species were *Biscutella didyma*, *Bromus fasciculatus* and *Hymenocarpus circinnatus* (hereafter *Biscutella*, *Bromus* and *Hymenocarpus*); the study sites were representative of arid, semi-arid, Mediterranean and mesic Mediterranean climates and the study period for field data collection included six consecutive seasons from 2001-2002 to 2006-2007. In addition, there were rainfall manipulations (commencing in 2002-2003) at the semi-arid and Mediterranean site, which were used to supplement our data on the relationship between demographic response and precipitation. Five plots of 10 × 20 m each were subjected to 30% rainfall reduction and 30% rainfall increase each year in both sites, and selected demographic variables were measured within manipulated and control plots. For details of sites and rainfall manipulations see main text and Tielbörger and Petru (2010).

### Demography

Demographic data were obtained from several previous and ongoing studies within the GLOWA Jordan River project (<[www.glowa-jordan-river.de](http://www.glowa-jordan-river.de)>). Data included species-, site- and year-specific population size, survival probability of seedlings to seed set and fecundity. Most of the data were obtained as averages and from different sources each year. Therefore, the demographic data obtained in the field were not analyzed statistically.

### Population size (density) and seedling mortality

Density data were collected, in chronological order, by: H. Parag, C. Holzappel, M. Petru, Y. Talmon, H. Yaffe, E. Elazar and J. Metz. Average densities of annual plant species were counted twice each season. Seedling counts were conducted approximately two weeks after the major germination event at each site. Seedlings of all species were identified and counted

in 50 permanent quadrats per site and treatment. Quadrats were  $20 \times 20$  cm in size and were located randomly in an area of approx.  $200 \times 200$  m between shrubs. A second count in March to May each year recorded densities of reproductive plants. Average seedling survival was calculated for each species separately as the fraction of emerging plants surviving to seed set (Metz et al. 2010). Due to the high patchiness in species distribution, there were quadrats with no germination for some of the species, and the number of cases with survival data varied among species, sites and seasons. At the arid site, densities were extremely low, and data on seedling survival could be obtained in four years for *Biscutella* and only one year for *Bromus*.

## Fecundity

Seed production data were gathered from several sources and estimated with different methods. Estimates of the number of seeds produced per reproductive plant were obtained during a parallel experiment of our own (seasons 2002-2003 and 2003-2004, including rainfall manipulations) and in field experiments of colleagues (2001-2002, 2004-2005 and 2005-2006). Seeds were counted on plants in the field or plants were taken to a laboratory for counting under a stereo microscope (*Bromus*). Data from the 2001-2002 season for *Biscutella* and *Hymenocarpus* were provided by K. Schiffers and are based on 30 to 90 individuals collected at all but the arid site (Schiffers and Tielbörger 2006). Average number of seeds per plant ( $n = 50$ ) was provided by J. Nachreiner (Nachreiner 2005) for 2003-2004. These data included estimates of seeds per plant for *Biscutella* at the arid and mesic Mediterranean site, *Bromus* at the arid site and *Hymenocarpus* at the mesic Mediterranean site. In 2004-2005, no data were available for any of the study species. Data for all species, treatments and sites in 2005-2006 were provided by one of us (C. Lampei) and by C. Ariza. At the arid and mesic Mediterranean site, these data were based on random collections of at least 50 individuals. For the other sites, collections were not always complete and biomass data were used for calculating fecundity using seed number-biomass regressions obtained in a previous study (Nachreiner 2005). In 2006-2007, fecundity data was collected for 60 individuals per species, site and rainfall treatment (J. Metz and C. Ariza). The number of seeds produced per seedling ( $Y$ ) was calculated by multiplying site- and species-specific fecundity data with survival probabilities. Due to missing values for survival data at the arid site (when no seedlings emerged in the quadrats), fewer values were obtained for  $Y$  than for the original fecundity data. Table 1 in the main text shows sample sizes and results for the fitness-related variables.

## Additional traits

Seed size was obtained in a study by J. Nachreiner (Nachreiner 2005). In this study, ripe seeds of 50 individuals of each species were collected randomly at each site for counting and weighing. Plant size was estimated in the same study by measuring the height of the 50 selected adult plants per site at the time of seed set. Seed survival is one of the most difficult traits to measure. This trait was not determined quantitatively, but qualitative estimates were provided by J. Kigel, who has studied seed dormancy of two of the species (*Hymenocarpus* and *Bromus*) for eight successive years. Competitive ability (competitive response) for *Biscutella* and *Hymenocarpus* was estimated in the field with neighbour removal experiments at the three wetter sites (Schiffers and Tielbörger 2006). In addition, greenhouse experiments (target-neighbour design) were conducted by C. Ariza between 2003 and 2006 for all three focal species and all four origins. Both experiments confirmed that at all sites, *Hymenocarpus* is a superior competitor, and that *Bromus* is the least competitive among the focal species. There were no differences among ecotypes from each site in competitive response ability (C. Ariza unpubl.), i.e. the competitive ranking can be regarded as a species-specific trait.

## Rainfall data

Long-term climate data were obtained from the Israeli Meteorological Service for the years 1950-2005 (1951-2005 for the arid site) and from own measurements between 2001 and 2011. Meteorological sites are next to our field sites in the arid and semi-arid climate, and no more than 10 km away from our sites in the Mediterranean and Mesic Mediterranean climate. The data for the years 2001-2011 did not differ more than 1% from measurements conducted directly at our field sites, confirming that the data were suitable for evaluating annual variation in rainfall along the studied gradient.

## Estimation of crucial demographic rates

For evaluating fitness variation and risk of reproductive failure, we calculated an estimate of  $Y$ , the seed yield per germinating seedling (Eq. 1 in main text), by multiplying the average number of seeds produced per reproductive plant with the average probability of seedling survival. Seed yield was calculated for each species, site and annual rainfall (including the manipulations) separately. Due to many missing values in seed survival estimates (e.g. no data for *Bromus* in the arid site);  $Y$  was pooled across years and treatments for subsequent

analyses, i.e. each dependent data point represented an estimated average per species, site, year and treatment.

We then tested for a positive relationship between  $Y$  and annual rainfall by means of regression with normal error distribution (note that single points in the regression are means of several values for seed production x survival). Since seed production should follow a sigmoid behaviour in response to rainfall, we tested for the significance of fecundity-rainfall relationships by fitting an s-shaped curve ( $Y = e^{b_0 + (b_1/rainfall)}$ ). Alternative models explained much less variation in the fecundity data. When relationships were significant ( $p < 0.05$ , Table A1) we used the coefficients and the residual error obtained from the nonlinear regressions for estimating the probabilities of reproductive failure of an individual plant in each site in three steps. When the regression was not significant the sample mean and the standard deviation of the mean were taken instead of the curve parameters. First the probability of reproductive failure ( $p^{fail}$ ) at a given rain level was estimated from a normal distribution with mean =  $e^{b_0 + (b_1/rainfall)}$ , and the standard deviation = error mean squares, as the area under the curve from  $-\infty$  to 1. In a next step, a normal distribution was determined to best fit the distribution of rain levels from the rainfall data described above, and the site specific parameters (mean, SD) were used to model the probability for each rain level between zero and maximum rainfall ( $max_{rain}$ ) to be observed ( $p^{rain}$ ). Finally, an approximate numerical double integration (one million steps) was applied, by multiplying the vectors of  $p^{fail}$ ,  $p^{rain}$  and the integration interval  $h$ , to estimate  $q$ , the probability of reproductive failure for an individual plant in each site. Since the numerical integration is only approximate, we further calculated the maximum error ( $err^{int}$ ) that may result from restricting the integration of  $p^{rain}$  to the interval between zero and  $max_{rain}$ . This was achieved by estimating the area under the normal curve for the intervals  $-\infty$  to zero and  $max_{rain}$  to  $+\infty$ . This is an upper limit for  $err^{int}$  as both, rain values below zero and rain values approaching  $+\infty$  are unrealistic.

## Results of regressions

The calculated relationship between seed yield and annual rainfall for the long-term meteorological dataset is depicted in Fig. A1; data points from field measurements are superimposed. The relationships were highly significant ( $p < 0.001$ ) for *Biscutella* and *Bromus*, while *Hymenocarpus* did not show any relationship of fecundity with rainfall (Table A1). Therefore, we did not calculate potential seed production, and risk of reproductive failure was assumed equal for all sites for that species (note that this must be treated with

caution, as this species does not occur below a certain rainfall and thus does show negative response to water limitation).

Table A1. Nonlinear regressions with observed seed yield as dependent variable and annual rainfall (2001-2007) as independent variable. Regression equations ( $Y = e^{b_0 + (b_1/rainfall)}$ ) were used to calculate the probability of reproductive failure for an individual plant in each site when the regression was significant ( $p \leq 0.001$ ), i.e. for *Biscutella* and *Bromus*.

	<i>Biscutella</i>	<i>Bromus</i>	<i>Hymenocarpos</i>
$r^2$	0.344	0.494	<0.001
$F$	17.17	21.52	0.001
$p$	0.001	<0.001	0.97
$b_0$	4.3	3.03	0.46
$b_1$	-915.5	-133.3	32.0

*Bromus* had no detectable risk of reproductive failure in the semi-arid and Mediterranean sites. *Biscutella* faced a high risk at the arid site and a decreasing trend of risk with increasing rainfall (Table 1 main text and Table A2). Risk was low for *Hymenocarpos* and similar for increasing annual precipitation. Note that despite using the mean as model for the relationship between  $Y$  and rainfall, the values for *Hymenocarpos* were not identical due to differences in rainfall probability distributions among sites.

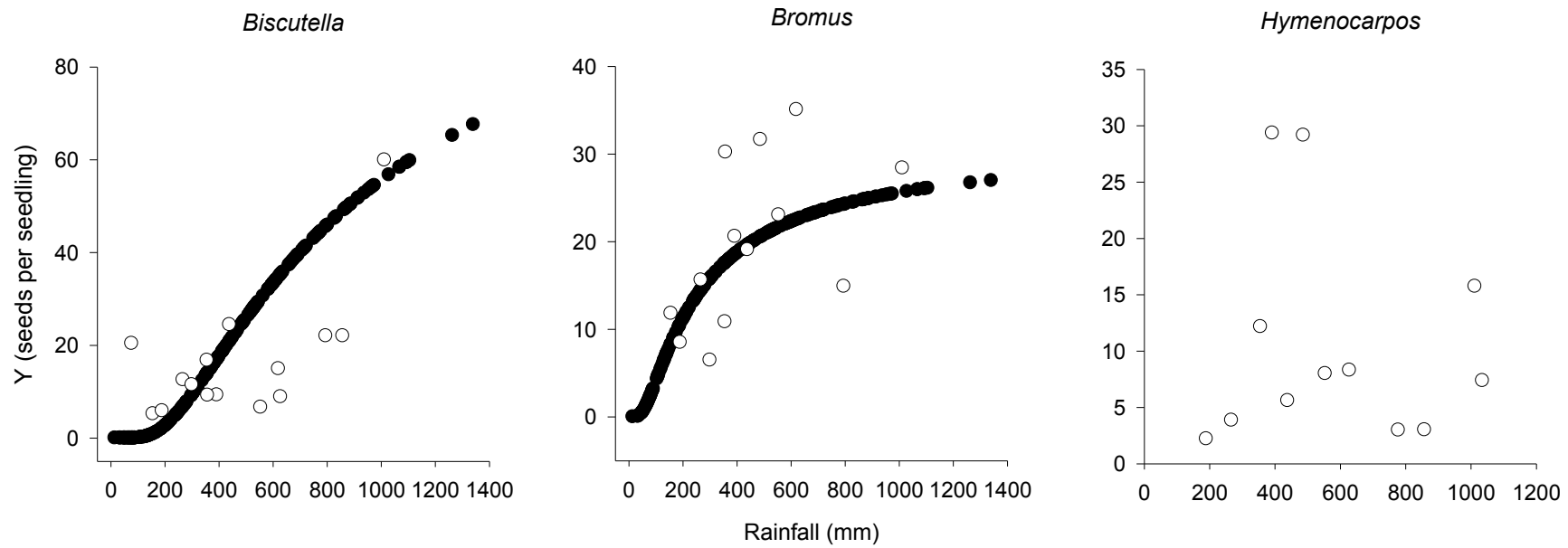
Our field data confirm a strong qualitative match between observed and the estimated risk of reproductive failure within species. For example, average seed yield per *Biscutella* seedling was zero in one year at the arid site, and increased markedly towards the wetter sites. However, the  $q$  values of *Hymenocarpos* are rather low, and are, with the exception of the arid site, where the species does not occur, intermediate compared to the values of *Biscutella* and *Bromus*. On the one hand, this result does not confirm the expectation of high  $q$  values for this species that arises from its strong seed dormancy. On the other hand, the slow decrease of  $q$  with higher mean annual precipitation demonstrates, that even in humid environments the probability of reproductive failure does not converge to zero for this species. However, these interpretations need to be taken with caution, since the sample mean can not be considered a good model for the relationship between  $Y$  and rainfall. Especially, in the arid site the estimates of  $q$  are most likely too low, as this species fails to grow under arid conditions.

Altogether, the qualitative estimates of  $q$  are highly useful for illustrating species- and site-specific differences in the risk of reproductive failure. Furthermore, estimates of demographic rates unbiased by environmental effects were qualitatively similar (see greenhouse experiment, Appendix 2).

Table A2. Site-specific risk of reproductive failure ( $q$ ) and maximal integration errors ( $err^{int}$ ) for the three focal species calculated from field measurements of seed yield and seedling survival, and long-term meteorological records (see above for details of calculations). The integration error changes only between sites, since it results from integrating over the rain probabilities.

site	<i>Biscutella</i>		<i>Bromus</i>		<i>Hymenocarpus</i>	
	$q$	$err^{int}$	$q$	$err^{int}$	$q$	$err^{int}$
<i>A</i>	0.4867	0.0303	0.1208	0.0303	0.0871	0.0303
<i>SA</i>	0.3859	0.0100	0.0049	0.0100	0.0780	0.0100
<i>M</i>	0.2706	0.0008	0.0006	0.0008	0.0756	0.0008
<i>MM</i>	0.1480	0.0007	$\approx 0$	0.0007	0.0653	0.0007

Figure A1. Relationship between the number of seeds produced per seedling and annual rainfall for the three focal species. Dark circles are data calculated for the period of 1950-2011, based on regressions obtained from field data (open circles). Regressions were not significant for *Hymenocarpus* (Table A1) and thus no calculated data was superimposed.



## Appendix 2

### Performance of species in an artificial aridity gradient in the greenhouse

The field estimates obtained above have the advantage of being measured under ‘natural’ conditions. However, they might be biased by maternal environment effects on demographic rates (Ariza et al. unpublished data) and limited by the unpredictability of rainfall in the field, i.e. we can not actively manipulate the range of rainfall under which we measure the demographic rates. Therefore, it is desirable to use inbred lines and a controlled environment for testing whether the suggested species-specific differences in demographic rates are indeed genetically determined. We grew plants of *Bromus* and *Biscutella* in an artificial rain gradient under controlled conditions to confirm that the qualitative patterns in demographic rates are the same as for the field data.

In winter 2006/2007, ten seed families of each population of *Biscutella* and *Bromus* were raised in an artificial rain gradient, which consisted of 12 irrigation treatments in a full factorial design. The rainfall gradient included the range of possible rainfall amounts experienced in the field (9.5 to 857 mm), and the lowest irrigation levels mimicked extreme drought conditions resulting in reproductive failure. The seed families were obtained by separately collecting seeds of individual plants in the field in 2005 and raising (inbred) offspring of these families under homogeneous greenhouse conditions in 2005/2006. Plants obtained from the seeds were grown in pots of 9 × 9 × 9cm in size filled with a 1:1 mixture of sand and nutrient-poor potting soil, following an established protocol used in previous studies (Ariza and Tielbörger 2010, Tielbörger and Petru 2010, Lampei and Tielbörger 2010). 100 ml OsmocotScott® slow release fertilizer (15N - 9P-5.9K) was added to each 40 l of substrate. The plants were irrigated according to the protocol established for the gradient and plants were thinned to one in each pot after no further emergence was observed.

In a first step, survival rates were calculated per treatment for each species, and in a second step they were multiplied with individual fecundity to obtain an estimate for the average number of seeds that is produced per seedling at a certain level of water availability. Fecundity was measured for each plant at the end of the season (May 2007) by harvesting the plants and counting the number of seeds.

### Data analysis

One outlier (one rainfall treatment) was removed from the data as the deviation could be traced back to an edge effect in the experiment. Namely, plants in that treatment were shaded from irrigation during seed set by tall plants in neighbouring treatments, which led to a strongly reduced fecundity in both species.

For each species a logistic curve was fitted with the equation:

$$y = \frac{\alpha}{1 + \exp[(\beta - x)/\gamma]}$$

where  $\alpha$  is the horizontal asymptote  $x \rightarrow \infty$ ,  $\beta$  is the x-value of the inflection point and  $\gamma$  is a scale parameter.

The results show that *Bromus* produces many more seeds per seedling than *Biscutella* (Fig. A2), and two of three curve parameters differ significantly between the two species (asymptote:  $t = 4.28$ ,  $DF = 764$ ,  $p < 0.001$ ; inflection point:  $t = 1.18$ ,  $DF = 764$ ,  $p = ns$ ; scale parameter:  $t = 3.19$ ,  $DF = 764$ ,  $p < 0.001$ ). This shows that the curves have the same shape (inflection point is at same value of  $x$ ) but they differ significantly in scale, i.e. *Bromus* produced more seeds than *Biscutella* under any given water availability. From these curves the minimal precipitation needed to yield the crucial demographic rate (we took this as 0.5 seeds produced per seedling) can be estimated (28.42 mm for *Biscutella* and 0.001 mm for *Bromus*). We then calculated the fraction of years with reproductive failure defined as the probability to produce less than 0.5 seeds using the long-term meteorological records. This risk was 0.93 for *Biscutella* in the arid site, and zero for the other sites and for *Bromus* in any of the sites. These results clearly demonstrate that *Bromus* is better adapted to produce seeds under arid conditions. However, survival in the greenhouse was overestimated compared to the field, because evapotranspiration is much smaller and other limiting factors such as locally unfavourable soil conditions, sudden heat spells, or competition with neighbours were excluded. Therefore, though these estimates are unbiased by maternal effects, we used them only as additional qualitative confirmation of species- and site specific differences in reproductive failure, i.e. we conclude that risk is highest towards the more arid sites and higher for *Biscutella* than for *Bromus*.

Figure A2. Nonlinear regressions of seed number produced per seedling (i.e.  $Y$  in Eq. 1 - genotype means per irrigation treatments) against artificial irrigation, for each species averaged across populations. The means at each irrigation level (*Biscutella*: black dots, *Bromus*: empty circles) are plotted together with their standard error.

