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Supplementary Material

Improving weed management by targeting the seed ecology of blackberry (*Rubus anglocandicans*) in a biodiversity hotspot

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Moisture permeability of pyrenes

Methods

Moisture permeability (imbibition) was determined for three replicates of 30 dry pyrenes that were either untreated or heat-treated for one minute in boiling water. Pyrenes were placed onto filter paper in Petri dishes and stored in ambient laboratory conditions (23°C). Before the test was conducted all replicates were examined through X-ray image analysis to confirm that the seed filled the pyrene. During the imbibition period, moisture was applied through pipetting deionised water at every 12 h to ensure that moisture was not limiting during the imbibition period. Moisture uptake was measured hourly between 1 and 8 h, then at 12-, 24-, 36-, 48-, 56-, 84-, 96- and 178-h time points.

Results

After initial exposure to moisture, both fresh (control) and heat-treated *R. anglocandicans* pyrenes rapidly increased by at least 20–30% in pyrene weight. This can be attributed to their woody coat (endocarp, see Fig. 2) behaving like a sponge and absorbing moisture instantaneously. Also notable is that initial moisture uptake of heat-treated pyrenes during the first 8 hrs was much higher than for fresh pyrenes, resulting in greater initial mass (Fig. S1). After 48 h, however; both fresh and heat-treated pyrenes measured similar weights, having increased up to 34% in mass. After 1 week, overall weight had increased by 38% (Fig. S1).

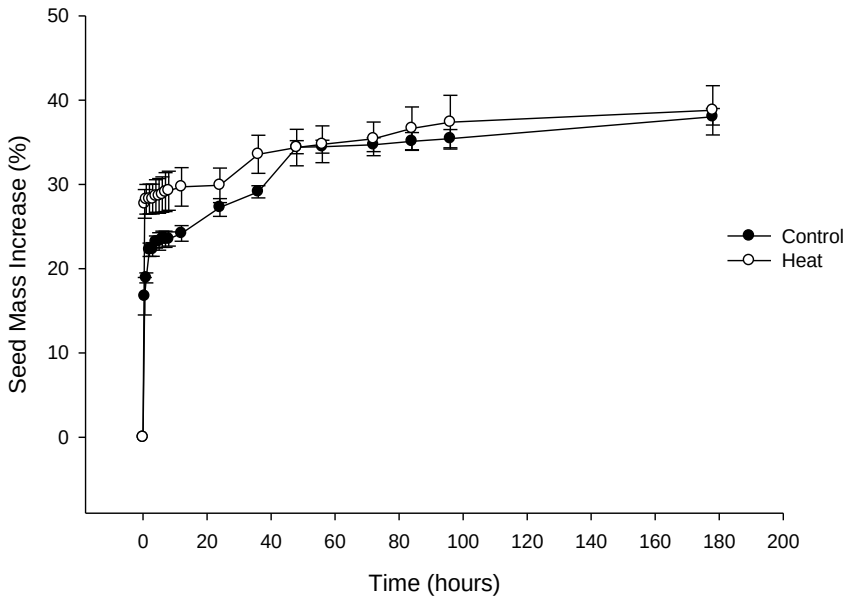


Fig. S1. Average pyrene mass increase with standard error of fresh (control) and heat-treated *Rubus anglocandicans* seeds over 1 week of moisture exposure.

Germination after long term immersion in water

Methods

To determine if simulated flooding conditions influenced seed germination by causing the endocarp to weaken and break down, pyrenes (0.6–0.7 g, ~180–270 pyrenes per treatment) were collected from two different sources, fresh fruit and fresh emu scat, in two different years, 2013 and 2015, then soaked for three different lengths of times (1 week, 1 month, 2 months). During the soaking phase, we drained the water once per week the water and added another 200 mL of

water to simulate the flushing out of any possible chemical inhibitors within the endocarp that would occur naturally during flooding. For the duration of the soaking, seeds were stored at room temperature (22–25°C) in a lab.

At the completion of the soaking period, six pyrenes were placed in each of six replicate Petri dishes per treatment combination and placed in incubators at either a constant 25°C (72 Petri dishes; i.e. 6 replicates of 2 seed sources collected in 2 years and soaked for 3 time periods) or fluctuating day–night temperatures that followed a 14:10 h day–night cycle (72 Petri dishes). In the latter case, the day temperature was set at 25°C and the night temperature was 10°C. Germination was recorded after 6 weeks and after 52 weeks.

Results

After 6 weeks, a few soaked seeds with endocarps intact (sourced from 2013 fruit and emu, 2015 fruit and emu) began germinating. Over the course of the entire trial which ran for 52 weeks, 3.94% (17 out of 432) of seeds with endocarps intact germinated in 25°C day–10°C night conditions, with a majority being seeds collected in 2015 from emu scats. No seeds with the endocarp intact (n = 432) germinated when held at a constant 25°C during the 52 weeks. Most of the trials has zero results and consequently it was not possible to carry out statistical analysis on the data.

Germination in relation to light

Methods

A preliminary trial was conducted to determine the light requirements to promote germination, using seeds sourced from emu scats in 2013. The endocarp was removed in the light and the bare seeds were transferred to Petri dishes that were then wrapped in foil. There were seven seeds per Petri dish and six Petri dishes per replicate. The covered dishes and the controls (uncovered dishes) were placed in an incubator for one month. A second trial consisted of removing the endocarp under an infrared light to avoid any possible light triggers required for germination. A third set of seeds were exposed to approximately one second of ambient laboratory light. Petri dishes were wrapped with foil and placed in an incubator. Petri dishes were exposed one month later, and proportion germinated was recorded.

Results

Germination still occurred when seeds were kept in the dark. There was no statistical difference from a control sample of seeds (66%) between seeds that had their endocarp removed under red-light conditions (‘no light’) (77%), were exposed to a second of light (50%) or had their endocarp removed in the light (71%). Seeds which germinated had elongated stems which grew up to 10 cm and had small, light-coloured cotyledons. After 1 month of growth in the dark, dishes were exposed to light in the cabinets for another week and seedlings resumed growth as per normal, with cotyledons greening and radicles growing.

Seed-burial trial

Methods

For pyrenes used for the seed burial trial, initial preparation was undertaken before the seeds were placed into mesh bags. Prior to counting out seeds for the experiments, the pyrene samples where sieved (2-mm diameter aperture) to remove very small pyrenes, sand and other small foreign material, passed through a Kimseed Vacuum Separator set on ‘5’ to remove sand, pyrene and foreign material that were less dense than most of the ‘visually healthy’ brown pyrene, and then the samples were hand sorted to remove any pyrenes that were black and any other remaining foreign matter. The seeds were counted using a Contador seed counter (Graintec Scientific) set with vibration = 30 and sensitivity = 1.97. Based on manually

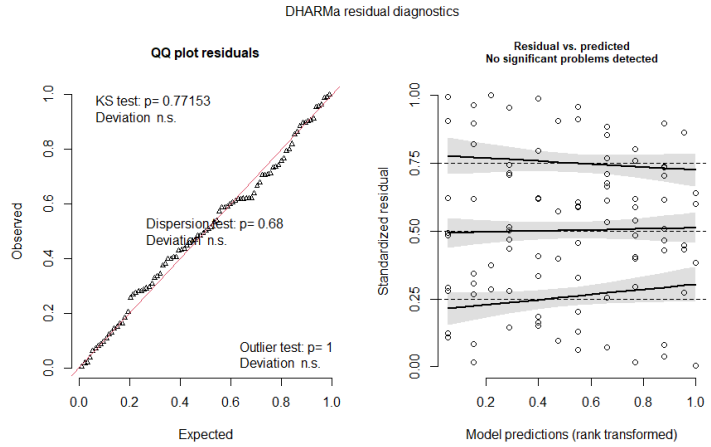
recounted subsamples the inaccuracy of the seed counter = 0.89 ± 0.23% (mean ± s.e., *n* = 40) of the target number (200).

Analysis

We used the glmmTMB package (ver. 1.1.2.3, see https://cran.r-project.org/package=glmmTMB; Brooks *et al*. 2017) in R (ver. 4.1.0, R Foundation for Statistical Computing, Vienna, Austria, see https://www.r-project.org/) to analyse the relative frequency of alive rather than dead seeds, and account for potential zero-inflation, using the beta-binomial distribution to deal with overdispersion.

```
bb.modA = glmmTMB(cbind(num.alive40, num.dead40) ~ decline + elev + seed.source +
                  decline:elev + decline:seed.source + elev:seed.source +
                  decline:elev:seed.source +
                  (1 | site/elev) , family= 'betabinomial',
                  zi=~decline,
                data=bb)

summary(bb.modA)

 Family: betabinomial  ( logit )
Formula:          cbind(num.alive40, num.dead40) ~ decline + elev + seed.source +
    decline:elev + decline:seed.source + elev:seed.source + decline:elev:seed.source +      (1
| site/elev)
Zero inflation:                                  ~decline
Data: bb

     AIC      BIC   logLik deviance df.resid
   576.7    619.7   -271.3    542.7       76

Random effects:

Conditional model:
 Groups    Name        Variance  Std.Dev.
 elev:site (Intercept) 2.112e-10 1.453e-05
 site      (Intercept) 2.078e-10 1.442e-05
Number of obs: 93, groups:  elev:site, 8; site, 4

Overdispersion parameter for betabinomial family (): 11.5

Conditional model:
                                       Estimate Std. Error z value Pr(>|z|)
(Intercept)                             0.07320    0.21351   0.343  0.73171
declineDecl                            -1.14643    0.38179  -3.003  0.00268 **
elevLow                                -0.49427    0.29789  -1.659  0.09707 .
seed.source2013-Fruit                   0.93161    0.45770   2.035  0.04181 *
seed.source2014-Emu                    -0.45162    0.30355  -1.488  0.13680
declineDecl:elevLow                    -0.30492    0.61270  -0.498  0.61872
declineDecl:seed.source2013-Fruit      -1.62050    0.75751  -2.139  0.03242 *
declineDecl:seed.source2014-Emu         0.07698    0.51217   0.150  0.88052
elevLow:seed.source2013-Fruit          -0.26551    0.59619  -0.445  0.65608
```

```
elevLow:seed.source2014-Emu                 0.12403    0.43334   0.286  0.77470
declineDecl:elevLow:seed.source2013-Fruit   1.89367    1.03593   1.828  0.06755 .
declineDecl:elevLow:seed.source2014-Emu    -0.38541    0.89215  -0.432  0.66574
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Zero-inflation model:
            Estimate Std. Error z value Pr(>|z|)
(Intercept)  -2.4047     0.5255  -4.576 4.75e-06 ***
declineDecl   1.1819     0.6792   1.740   0.0818 .
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
>
```



Model simplification was then carried out using an information theoretic approach to remove uninformative model predictors (Table S1).

Final best model summary:

```
bb.mod.final = glmmTMB(cbind(num.alive40, num.dead40) ~ decline + elev + seed.source +
                   (1 | site/elev) , family= 'betabinomial',
                 zi=~1,
                 data=bb)

> summary(bb.mod.final)
 Family: betabinomial  ( logit )
Formula:          cbind(num.alive40, num.dead40) ~ decline + elev + seed.source +      (1 |
site/elev)
Zero inflation:                                ~1
Data: bb

     AIC      BIC   logLik deviance df.resid
   571.5    594.3   -276.7    553.5       84

Random effects:
```

```
Conditional model:
 Groups    Name        Variance  Std.Dev.
 elev:site (Intercept) 3.161e-09 5.623e-05
 site      (Intercept) 3.531e-12 1.879e-06
Number of obs: 93, groups:  elev:site, 8; site, 4

Overdispersion parameter for betabinomial family (): 9.16

Conditional model:
                    Estimate Std. Error z value Pr(>|z|)
(Intercept)           0.6621     0.2415   2.741 0.006116 **
declineDecl          -1.5422     0.2282  -6.757 1.41e-11 ***
elevLow              -0.5013     0.1844  -2.718 0.006562 **
seed.source2013-Emu  -0.5697     0.2589  -2.200 0.027791 *
seed.source2014-Emu  -0.9145     0.2542  -3.597 0.000322 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Zero-inflation model:
            Estimate Std. Error z value Pr(>|z|)
(Intercept)   -1.954      0.424  -4.609 4.04e-06 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

Table S1. Model comparison of the drivers of variation in seed survival of *Rubus anglocandicans* pyrenes buried for 5 years in mesh bags at four field sites in south-western Western Australia.

Model rank	Fixed effects	k	AICc	ΔAICc	Akaike weight
1	Decline + Seed source + Elevation	9	573.66	0.00	0.611
2	Decline + Seed source	8	575.91	2.24	0.199
3	Decline * Seed source + Elevation	11	577.01	3.35	0.114
4	Decline * (Seed source + Elevation)	12	579.49	5.83	0.033
5	Decline + Elevation	7	580.37	6.71	0.021
6	Seed source + Elevation	8	582.56	8.90	0.007
7	Decline	6	583.50	9.84	0.004
8	Seed source	7	583.55	9.89	0.004
9	Decline + Seed source + Elevation + Decline : Seed source + Decline : Elevation + Seed Source : Elevation	14	584.29	10.63	0.003
10	Decline * Seed source * Elevation	16	584.81	11.15	0.002
11	Elevation	6	589.61	15.94	0.000
12	none (intercept only)	5	591.99	18.33	0.000

Akaike's information criterion with correction for small sample bias (AICc), number of model parameters (k), change in AICc value between ranked models (ΔAICc) and probability that the model is the best fit to the data, out of the set of models tested (Akaike weight). All models included random intercepts for site and for elevation nested within site, as well as a model intercept term to accommodate significant zero-inflation. The full model was specified as: glmmTMB(cbind(num.alive40, num.dead40) ~ decline * Elevation * seed.source + (1 | site/Elevation), family = 'betabinomial', zi=~1, data=bb).

References

Brooks ME, Kristensen K, van Benthem KJ, Magnusson A, Berg CW, Nielsen A, Skaug HJ, Mächler M, Bolker BM (2017) glmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. *The R Journal* **9**, 378–400. doi:10.32614/RJ-2017-066