Germination asynchrony is increased by dual seed bank presence in two desert perennial halophytes

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<th><em>Botany</em></th>
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<td>cjb-2019-0071.R2</td>
</tr>
<tr>
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<td>Article</td>
</tr>
<tr>
<td>Date Submitted by the Author:</td>
<td>21-Jun-2019</td>
</tr>
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<td>Bhatt, Arvind; GORD, Bhat, N.R.; Kuwait Institute for Scientific Research Lozano-Isla, Flavio; Universidade Federal Rural de Pernambuco, Biology Gallacher, David; Zayed University Santo, Andrea; Universita degli Studi di Cagliari Batista-Silva, Willian; Universidade Federal de Viçosa, Plant Biology Fernandes, Denise; Instituto Federal Catarinense POMPELLI, MARCELO; Universidade Federal de Pernambuco, Botany;</td>
</tr>
<tr>
<td>Keyword:</td>
<td>desert, arid, recruitment, synchrony, perianth</td>
</tr>
<tr>
<td>Is the invited manuscript for consideration in a Special Issue?:</td>
<td>Not applicable (regular submission)</td>
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Germination asynchrony is increased by dual seed bank presence in two desert perennial halophytes

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\hspace{1cm}\\
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Abstract
Maintaining a viable seed bank throughout the germination season is considered very important for plant recruitment in desert environments, where environmental conditions are unpredictable. Fully matured *Seidlitzia rosmarinus* and *Halothamnus iraqensis* seeds were collected in December 2016, then April, June and September 2017 from both soil-surface and aerial seed banks. Both species were selected mainly by their capacity to rehabilitation of saline coastal sites. Germination was analyzed under two photoperiods (0 or 12 hours light per day) winged and dewinged perianth. *S. rosmarinus* had a shorter seasonal range in comparison with *H. iraqensis* (6 and 9 months) respectively and winged perianth presence reduced the germination rate of both species. The permanent winged perianth has significantly inhibited the germination in both species. The absence of perianth, the germination registered in December 2016 was mostly 100%, but declined to around 34 20% in September 2017. Seeds are thus more likely to germinate after scarification from wind mobilization, and do not require burial. Our results showed that seeds of both aerial and soil banks are transitory, and viable only during the winter months. Taken together, combination of aerial and soil seed banks has greatly facilitated germination asynchrony in their environmentally unpredictable desert habitat.

Keywords: desert; arid; recruitment; synchrony; perianth

1. Introduction
Populations that are adapted to unpredictable environmental conditions commonly rely on seed banks to improve the likelihood of successful recruitment within a season. Weather unpredictability means that seeds must remain viable throughout the season so they can germinate when conditions are favorable. However, plant mechanisms for maintaining a seed bank can reduce the success of habitat restoration efforts in arid systems (Pompelli et al. 2006; Liu et al. 2013; Miranda et al. 2014; Schroeder 2015; Bhatt et al. 2018; Bhatt et al. 2019a; Chen et al. 2018; Lozano-Isla et al. 2018).

Mechanisms to maintain a soil seed bank can include asynchronous timing of detachment from the maternal plant to replenish the soil seed bank through the season, and asynchronous timing of the release of seed dormancy. Thus, germination asynchrony
is increased through the use of both soil and aerial seed banks (Baskin and Baskin 2014; Gao et al. 2014). Seeds are exposed to different micro-environmental conditions in the aerial and soil seed banks (Lamont and Enright 2008). Germination synchrony was shown to decline when salinity was increased, and germination uncertainty also increased (Lozano-Isla et al. 2017). Release from aerial seed banks is highly interspecifically variable in desert habitats (El-Keblawy and Bhatt 2015). It can occur over several months or be triggered rapidly by environmental cues. Aerial seed banks can prolong seed viability during physical dormancy, provide protection from ground-level threats such as predation and temperature extremes (Lamont et al. 1991), and provide a mechanism for asynchronous timing of germination. Seed burial can provide similar benefits, but requires the presence of a burial mechanism.

Revegetation of arid habitats can be improved with an understanding of how species maintain a viable soil seed bank throughout the season (Clemente et al. 2007; Bastida et al. 2010). Studies of seed bank replenishment and seed viability duration therefore assist in conservation management. The role of aerial seed banks in desert halophytes is relatively understudied. Seeds stored aerially are exposed to different climatic factors that influence germination, including temperature, seed moisture content, relative humidity and scarification (Moncaleano-Escandon et al. 2013; Bhatt and Pérez-García 2016; Bhatt et al. 2019b). Germination is regulated primarily by light, temperature and moisture (Watt and Bloomberg 2012; Baskin and Baskin 2014; Elnaggar et al. 2018; Bhatt et al. 2019a; Savaedi et al. 2019).

Seidlitzia rosmarinus Bunge ex Boiss and Halothamnus iragensis Botsch. (Amaranthaceae) are perennial shrubs native to Bahrain, Jordan, Iran, Iraq, Oman, Kuwait, Qatar, Saudi Arabia and the United Arab Emirates (Freitag et al. 2009; Deymeh et al. 2012). Fruits of both species are enclosed by a winged perianth that remains attached to the mature fruit. Perianth wings facilitate wind dispersal of seeds (Burtt and Lewis 1954). Both species have C4-type photosynthesis, are tolerant of extreme temperatures and desiccation (Kadereit et al. 2003), and have strong sand binding ability (Supplementary Fig. S1). Seeds germinate easily and are highly salt tolerant (Hadi et al. 2007; Bhatt et al. 2019a), making them a good candidate for saline coastal site rehabilitation (Mahmoodi et al. 2013). In the present study, we hypothesized that (i)
germination rate will increase with scarification to remove winged perianths; (ii) germination rate will decline over time; and (iii) that seeds stored in aerial seed banks will exhibit higher germination rates than seeds in soil seed banks.

2. Materials and Methods

2.1. Seed collection and experimental conditions

Mature fruits were collected from Julaia, Kuwait (Seidlitzia rosmarinus 28°52'49"N, 48°16'32"E; Halothamnus iraqensis 28°53'29" N, 48°14'17" E) in December 2016 from aerial seed banks, followed by April, June and September 2017 from both aerial and soil seed banks. The fruits were cleaned and perianth wings removed from half the sample at the time of collection. All seeds reached maturity in December, hence aerial seed samples represented 0, 4, 6 and 9 months aerial storage of mature seeds, and soil seed samples of storage first aerially, then at the soil surface. Aerial seed banks diminished gradually and were unavailable for S. rosmarinus in September 2017. Seeds were collected from 20-25 individuals of each species to sample genetic diversity of the population. Germination experiments started one week after each collection. Atmospheric temperature, rainfall and relative humidity data were collected from the Sulaibiya meteorological station (Kuwait Institute for Scientific Research), using a datalogger (EM50G, Meter Washington, USA) throughout the experimental period (Fig. 1).

2.2. Germination analysis

A total of 6,400 seeds were germinated in incubators at a controlled temperature of 15/20°C (12/12 hours of night/day) in a full-factorial design. Factors were four 25-seed replicates for each of two species (S. rosmarinus, H. iraqensis), four seed collection dates (December, April, June, and September), two seed banks (aerial, and soil), two winged perianth states (winged, and dewinged), and two light regimes (0 and 12 hours of light daily). The temperature regime was selected to represent field conditions between December and March when there is a higher chance of rain (Omar et al. 2007). Seeds from both seed banks had been exposed to sunlight prior to collection. Seeds were placed in 9-cm diameter Petri dishes containing two sheets of filter paper (Whatman Nº 1). Light absence was achieved by wrapping petri-dishes in two layers of aluminum foil.
Germination was defined as the emergence of a radicle to ≥ 2 mm (Ranal and Santana 2006). Germination of the light treatments was assessed daily and dark treatments were assessed at the end of the 14-day experiment. After 14 days, ungerminated seeds were dissected under a binocular microscope to evaluate their viability for germination, where a white embryo indicated viable and turgid/brown non-viable (Baskin and Baskin 2014).

2.3. Experimental design and statistical analysis

Germination rate, mean germination time (MGT), germination synchrony and uncertainty were calculated by the following formulae (Laboriau and Pacheco 1978; Lozano-Isla et al. 2019):

\[
\text{Germination Rate} = \left( \frac{\sum_{i=1}^{k} n_i}{N} \right) \times 100 \quad (1)
\]

\[
\text{Mean Germination Time} = \frac{\sum_{i=1}^{k} n_i t_i}{\sum_{i=1}^{k} n_i} \quad (2)
\]

\[
\text{Synchrony} = \frac{\sum_{i=1}^{k} C_{n1,2}}{N}, \text{where } C_{n1,2} = \frac{n_i (n_i - 1)}{2} \text{ and } N = \frac{\sum n_i (\sum n_i - 1)}{2} \quad (3)
\]

\[
\text{Uncertainty} = \sum_{i=1}^{k} f_i \log_2 f_i \quad \text{where } f_i = \frac{n_i}{\sum_{i=1}^{k} n_i} \quad (4)
\]

where \( n_i \) is the number of seeds germinated on the \( i \)th day; \( t_i \) is the number of days from the beginning of the experiment to the \( i \)th observation; \( N \) is the total number of seeds in each replicate (\( N=25 \)); \( k \) is the last day of germination evaluation (\( k=14 \)); \( f_i \) is the relative frequency of germination.

The germination synchrony was initially proposed to evaluate the degree of overlap of flowering individuals in a population (Primack 1985). When synchrony is equal to 1, seed germination occurs at the same time, whereas synchrony near 0 denotes that at least two seeds complete the germination process at different times. The germination uncertainty is an adaptation of the Shannon index (Shannon 1948) that evaluates the uncertainty associated with the relative distribution of the frequency of germination. When the uncertainty values are low, the germination frequency must be higher since this index evaluates the degree of dispersion of the germination.
All germination parameters were used to perform principal component analysis with the PCA function of the Minitab 18.1.0.0 (Minitab LLC, Pennsylvania State University, PA, USA). The summary function of PCA was used to calculate the proportion of the variance of each germination parameter explained by each principal component. For hierarchical clustering, Pearson’s correlations were used to compare the similarities between all seed procedures using the “cor” function of the R stats package, and the complete linkage method and the Euclidean distance measure were used for hierarchical clustering with the R index in the Minitab 18.1 (Minitab LLC, Pennsylvania State University, PA, USA). All other calculations, statistical analyses and the generation of graphs were performed with Germina R package (Lozano-Isla et al. 2017, 2019). The analysis of variance (ANOVA) was performed to evaluate the differences between the factors, and the means compared using the Student-Newman-Keuls test ($p < 0.05$).

3. Results

3.1. Winged perianth removal

Seeds with removed perianths exhibited greater germination rates regardless of other factors, though the effect was more pronounced in *H. iraqensis* (Fig. 2). Germination rate was approximately 100% in dewinged seeds collected in December 2016. Germination rate declined throughout the season for all factors (Fig. 2).

3.2. Effect of light

Light seems to be necessary for germination in both studied species (Table 1, Fig. 4). When light was coupled with seed bank, collect time or seed procedure, light did not show significant interaction in *S. rosmarinus*. For *H. iraqensis*, light proved to be necessary to improve germination, both in isolated form and when coupled with other seed procedures or collection times. However, in some cases germination in darkness tended to be higher than those germinated in the light; however, this effect was not significant (Fig. 4D). The effect of seedbank type or seed procedure became more evident when we divided all the treatments into four groups: (i) winged seeds, (ii) dewinged seeds, (iii) aerial seed bank and (iv) soil seed bank (Supplementary Fig. S2). Thus, independent of the studied species, germination speed was in the following decreasing order:
dewinged seeds > aerial seed bank > soil seed bank > winged seeds (Supplementary Fig. S2). In all four groups, germination reached a maximum percentage in the 5th day after sowing, but the accumulated germination was nine times higher in dewinged seeds compared with winged seeds, while germination of the aerial seed bank was twice that of the soil seed bank.

3.3. Interaction between collection time and light

The interaction between collection time and light was significantly different in both species (Table 1). Collection time significantly decreased germination in all of the treatments here analyzed (Fig. 4). Dewinged seeds of *S. rosmarinus* germinated in the darkness showed the greatest drop over time (95.3% \( r = -0.99 \)), followed by germinated seeds of *H. iraquensis* germinated in the light (86.6% \( r = -0.97 \)), seeds of *S. rosmarinus* germinated in the light (84.4% \( r = -0.95 \)) and seeds of *H. iraquensis* germinated in the dark (80%, \( r = -0.93 \)). In the winged seeds the germination drop was less pronounced over time due to low germination regardless of treatment or species. The minimal influences of light in the germination suggest that seeds burial is indeed unnecessary. Finally, the combination of both aerial and soil seed banks greatly facilitated germination asynchrony, therefore improving recruitment in the unpredictable desert habitat.

3.4. Multivariate analysis

Lastly but not less important, the principal component analysis (PCA) was also performed to explore more deeply the contribution of changes in seed-germination attributes in both *S. rosmarinus* and *H. iraquensis* under different seed collection times and seed procedures (winged and dewinged; Fig. 5). This analysis confirmed that the dominant source of variation in the combined dataset was the differential contribution of seed germination over time and seed characteristics where our results showed a clear separation in *Seidlitzia rosmarinus*. Three main groups were separated according to a Euclidian similarity analysis (~75%, Fig. 5A). The PCA analysis showed a clear separation between time of harvesting and the winged and dewinged seeds. From our results we can identify a first group (April-dewinged and December-dewinged, light green) is promoted by higher synchrony and germination as main variable that contributed to the
separation from other groups. In addition, a second group (September-dewinged, April-winged, June-dewinged, and December-winged) is mainly promoted by lower germination and uncertainty. Additionally, a third group (September-winged, June-winged) was separated from these because the presence of perianth wing that depressed germination. On the other hand, PCA analysis of *H. iraqensis* (Fig. 5B) shows four main groups (Euclidian similarity analysis, ~76%) where PC1 and PC2 contributes by 63.4% and 31.4% of the variance. December-dewinged and April-dewinged was formed by two distinct groups, indicating the strongly time influences on germination characteristics in both species. The third group was formed by June-dewinged and September-dewinged and a lastly, the group four was formed by September-winged, April-winged and June-winged. Synchrony and germination was the main parameter that contributed substantially to the separation December-dewinged from other treatments, while uncertainty and MGT contributed by separation April-dewinged (2nd group) and September-dewinged and June-dewinged (3rd group). The perianth wings of *S. rosmarinus* had a major depressive effect on the germination parameters.

The aerial and soil seed banks comparison (Fig 5C-D) showed three groups. Irrespective of species, the aerial seed bank formed distinct groups from soil seed bank. In *S. rosmarinus* germination, uncertainty and synchrony contributed by separated aerial-dewinged from others, while MGT contributed by separated aerial-winged. The PCA analysis confirms the results presented in Fig. 3, where the seeds in the soil seed bank, independently of presence of perianth wing shows lower germination, that allowed dividing this group from others.

**Discussion**

This study has shown that *S. rosmarinus* and *H. iraqensis* produce seeds that remain viable within the season of production only, and seed viability declines gradually through the season. Nevertheless, asynchrony of germination is achieved through the use of both aerial and soil seed banks. The high germination rate of aerial seeds with perianth removed indicates that neither species exhibits seed dormancy other than the mechanical barrier of the perianth wings. Seed maturation naturally occurs in the winter months when the success of seedling establishment is most likely, negating the need for
seasonal realignment or the maintenance of a seasonally persistent seed bank. This timing is common for halophytic seeds in the Arabian desert (El-Keblawy et al. 2013; Bhatt et al. 2016b; Bhatt et al. 2017a,b; Bhatt et al. 2016b). Seed bank viability declines throughout the season, similar to that reported for other desert halophytes such as *Haloxylon salicornicum*, *Salsola imbricata* and *S. vermiculata* (Niane et al. 2013; Bhatt et al. 2017a,b).

*H. iraqensis* maintained both aerial and soil seed banks throughout the study, but the seed bank of *S. rosmarinus* was established later in the season and the aerial seed bank was exhausted earlier. Duration of aerial seed banks is species-specific (Bastida et al. 2010; El-Keblawy et al. 2015; El-Keblawy and Bhatt 2015). The high relative humidity measured in December 2016 might have contributed to the decay of seed viability. The higher relative humidity of aerial seed banks has a direct influence on seed moisture (Sun 2002; Colville 2017), resulting in an accelerated seed metabolic rate (Akowuah et al. 2012; Moncaleano-Escandon et al. 2013; Chdananda et al. 2014) that can reduce seed viability if metabolic reserves are depleted too quickly (Moncaleano-Escandon et al. 2013; Lozano-Isla et al. 2018). In this study, seed viability in aerial seed banks remained high until April and then declined dramatically to June, whereas soil seed bank viability had already declined to low levels by April. These results indicate that the aerial seed bank is important for maintaining seed viability throughout the winter/spring period, and for replenishing the soil seed bank during this period.

Both species have persistent perianths that inhibit germination rate, as has also been observed in *Salsola affinis*, *S. ikonnikovii*, *S. rubescens*, *S. schweinfurthii*, *S. vermiculata*, and *Haloxylon salicornicum* (Wei et al. 2007; Xing et al. 2013; El-Keblawy et al. 2015; Bhatt et al. 2017a,b). Intact wings were reported to act as mechanical barrier to radicle emergence, hinder water absorption, and retain high concentrations of abscisic acid and chloride ions (Wei et al. 2007; El-Keblawy et al. 2013; Xing et al. 2013). With wings removed, seeds germinated faster and achieved significantly higher germination percentages in both species. These results imply that the presence of wings acts only as a physical barrier for radicle emergence, since wing removal did not appear to increase water imbibition, which is a mechanism for activating germination inhibitors within the seed. Similar results were obtained for other halophytic species, including *Salsola affinis*,

Another physical barrier has been reported in Prosopis juliflora seeds, where integuments inhibited germination rate and increased mean germination time (Miranda et al. 2011). Data were similar to the present study, in that the physical barrier reduced the germination rate to almost zero and increased asynchronization. For our two study species, light is beneficial but not essential for germination, indicating that burial by wind would reduce germination slightly (Table 1). Previous studies have reported that a requirement for light was induced after seed burial (Milberg and Andersson 1997).

Mean germination time was short for both species, with little chance for asynchronous timing when measured in days. Seed viability declined over time, and a germination rate of zero was recorded for winged seeds collected in June. This might be caused by an increasing rigidity of the perianth but is more likely due to a general decline in seed viability. Germination asynchrony is therefore provided by differential temporal release from the aerial seed bank, that replenishes the soil seed bank with viable seeds during the winter / spring season.

In S. rosmarinus the presence of the perianth wing permits the separation of September-winged and June-winged from April-dewinged and December-dewinged. Therefore winged and dewinged seeds have different germination conditions, where higher germination percentage and lower uncertainty were mainly responsible for separating these groups. Synchrony and MGT were responsible to group the four treatments in one only group. In H. iraqensis four groups were formed independently of winged or dewinged seeds. However, higher germination percentage and synchrony contributed to separate December-dewinged from April-dewinged, while higher MGT and uncertainty contribute to group September-dewinged with June-dewinged. In this species the presence of perianth wings was the main characteristic to group all winged seeds in only one group. The same way that perianth wings contributed to separate the aerial and soil seed bank groups. In both species, because the aerial seed resulted in a higher germination percentage, higher synchrony and higher uncertainty, the aerial seed bank showed different groups from the soil seed bank; the MGT contributed to separating the aerial-winged and aerial-dewinged. In the seed soil bank neither seed germination
parameters contributed to separate soil-winged from aerial-dewinged, perhaps due to seed age and granivory. A differential analysis on different parts of the seed tissue can alter the physiological role of the perianth in these species, in addition to their known ecological functions. Abscisic and gibberellic acids may play a key role in the establishment of these plants under arid conditions (Nambara et al. 2010; Vishal and Kumar 2018).

In conclusion, this study has showed our study shows that *S. rosmarinus* and *H. iraqensis* produce seeds mainly during winter and spring that remain viable only within these two seasons, thus producing transitory aerial and soil seed banks. Additionally, in addition seeds require scarification to remove perianth wings which has improved the seeds germination rate. In our understanding, these results is consequence of a evolutionary adaptation where, after strong wings the perianth is removed and increasing the germination rate, which are sometimes accompanied by rains. Asynchrony of germination is achieved through asynchronous release from the aerial seed bank, thus providing viable seeds for potential germination throughout the winter / spring season.

**Acknowledgements**

This work was made possible through financial support from Kuwait Institute for Scientific Research (KISR) to AB and NRB. We also thank the scholarships granted by the Brazilian Federal Agency for Support and Evaluation of Graduate Education (CAPES-Brazil) to WBS.
References


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**Figure captions:**

**Figure 1.** Minimum and maximum air temperature (solid lines) and relative humidity (dashed lines), and monthly rainfall (solid columns) from Sep 2016 to Dec 2017. Collection dates (Dec, Apr, Jun and Sep) are indicated by grey vertical bars.

**Figure 2.** Germination (A-B), mean germination time (MGT; C-D), synchrony (E-F), and uncertainty (G-H) of *Seidlitzia rosmarinus* (left panels) and *Halothamnus iraqensis* (right panels), sampled in four different times and winged (full bars) or dewinged (open bars). Each value represents the means (± SE) of four replicates. Different upper case letters denote significant differences between sample time in each winged or dewinged seeds and lower case letter denote significant differences between winged and dewinged in each sample time. Asterisks = not determined.

**Figure 3.** Germination (A-B), mean germination time (MGT; C-D), synchrony (E-F), and uncertainty (G-H) of *Seidlitzia rosmarinus* (left panels) and *Halothamnus iraqensis* (right panels), sampled in two seed banks and winged (full bars) or dewinged (open bars). Each values represent the means (± SE) of four replicates. Different upper case letters denote significant differences between seed bank in each winged and dewinged seeds and lower case letter denote significant differences between winged and dewinged seeds in each type of seed bank. Asterisks = not determined.

**Figure 4.** Germination of winged (A, B) and dewinged (C, D) of *Seidlitzia rosmarinus* (A, C) and *Halothamnus iraqensis* (B, D) seeds sampled in four different times germinated in 12 hours light day$^{-1}$ (dark bars) or 0 hours light day$^{-1}$ (gray bars). Each values represent the means (± SE) of four replicates. Different upper case letters denote significant differences between sample time in each light treatment and lower case letter denote significant differences between 12 hours light day$^{-1}$ and or 0 hours light day$^{-1}$ in each sample time.

**Figure 5.** Principal analysis component (PCA) of *Seidlitzia rosmarinus* (A, C) and *Halothamnus iraqensis* (B, D) through the seed collection (months) and seed procedure (winged and dewinged). C and D panels are comparing both, aerial and soil seed bank. In A and B the points denote December (triangle), April (diamond), June (square) and September (circle). In C and D the denote soil (square) and aerial (circle). In all images, empty points denote winged seeds and filled points denote dewinged seeds.
Figure 1.
Figure 2.
Figure 3.
Figure 4.
Figure 5.
Table 1. Analysis of variation for germination rate in *Seidlitzia rosmarinus* and *Halothamnus iraquensis*, testing seed collection month, seed bank type (aerial, soil), perianth wing status (intact, removed) and light during germination (0, 12 hours / day).

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<td>17.69</td>
<td></td>
<td></td>
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<tr>
<td><em>Halothamnus iraquensis</em></td>
<td></td>
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</tr>
<tr>
<td>Seed collection month (M)</td>
<td>3</td>
<td>21,857</td>
<td>7,286</td>
<td>742.72</td>
<td>2e-16 ***</td>
</tr>
<tr>
<td>Seed bank type (B)</td>
<td>1</td>
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<td>5,553</td>
<td>566.13</td>
<td>2e-16 ***</td>
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<tr>
<td>Perianth wing (P)</td>
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<td>43,214</td>
<td>4,405.34</td>
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<tr>
<td>Light (L)</td>
<td>1</td>
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<td>914</td>
<td>93.20</td>
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<tr>
<td>M : L</td>
<td>3</td>
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<td>204</td>
<td>20.76</td>
<td>0.0565 ns</td>
</tr>
<tr>
<td>M : B</td>
<td>2</td>
<td>58</td>
<td>29</td>
<td>2.97</td>
<td>0.0565 ns</td>
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<tr>
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<td>17,447</td>
<td>5,816</td>
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<td>174</td>
<td>17.77</td>
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<tr>
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<tr>
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<td>58</td>
<td>29</td>
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<tr>
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<tr>
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<tr>
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<td>Residuals</td>
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<td>824</td>
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<tr>
<td>Coefficient of Variance (%)</td>
<td>15.12</td>
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</table>

*P ≤ 0.05; ** P ≤ 0.01; *** P ≤ 0.001; ns, not significant*