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Effects of bulbil mass and elevation on germination and leaf growth of *Persicaria vivipara* (L.) RONSE DECR. (Polygonaceae) across the Alps

by

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Synopsis: Plants living in alpine regions suffer from harsh environmental conditions such as low temperatures and short growing seasons. These conditions affect reproduction, germination rate and seedling performance. Species with broad ecological ranges may perform better at lower than on higher elevations. To investigate that, we selected *Persicaria vivipara* as a model species and collected bulbils from 7 different localities across the Alps on different bedrock and elevation. Bulbil mass, germination rates and leaf length of the young plants were determined. Five different hypotheses were tested: (1) the mass of the bulbils will be different according to the provenance, (2) bulbils from lower elevations germinate better than bulbils from higher elevations, (3) the mass of the bulbils has an impact on germination and development of leaves, (4) heavier bulbils produce longer leaves, (5) the successional gradient affects the rate of germination and the development of leaves. Bulbil mass resulted to be strongly correlated with provenance and leaf length was correlated with bulbil mas. However, germination did not depend on bulbil mass and provenance.

Keywords: Elevation gradient, germination rates, growth, provenance

1. Introduction:

Germination, growth and reproduction of plants in alpine and arctic regions are mostly influenced by temperature and short growing seasons (MOONEY & BILLINGS 1961; BILLINGS & MOONEY 1968; BLISS 1971; GUGERLI & BAUERT 2001; SCHWIENBACHER et al. 2011). The climatic conditions during seed development influence the germinability (LAINE et al. 1995). If temperatures fall below a critical point in summer or the growing season is not sufficiently long enough, the plant might not be able to produce germinable

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seeds. Therefore, plants in alpine regions possess different reproducing strategies (KÖRNER 2003). Alternatively to the production of seeds, asexual reproduction can be regarded as an ideal possibility to counter limited outcross pollination opportunities in high altitudes. The possibilities of clonal propagation are multiple (PRICE & MARSHALL 1999), including stolones, runners, rhizomes, layering of stems and agamospermy. Some of the alpine plants are 'viviparous' or 'pseudoviviparous', i.e. they produce ramets on the inflorescences of the mother plant, such as *Persicaria vivipara* (DIGGLE 1997) or *Poa alpina* (PIERCE et al. 2003).

For this study, *Persicaria vivipara* (Polygonaceae), a rhizomatous perennial plant, was chosen as model species because it is widely spread in alpine regions and representative for a common growth habit within these environments (DIGGLE 1997). Along glacier foreland chronosequences, *P. vivipara* follows immediately after the colonization by pioneer species such as *Saxifraga aizoides* and it is distributed in all successional stages along the glacier foreland chronosequence (ERSCHBAMER et al. 2012). Under these circumstances *P. vivipara* prefers vegetative reproduction: flowering stems have flowers in the upper parts and bulbils in the lower parts (GUGERLI & BAUERT 2001). Bulbils are propagules of the plant to ensure vegetative reproduction (WALCK et al. 2010) and increase in mass in accordance to increasing temperatures (TOTLAND & NYLÉHN 1998). When the plants have managed to establish successfully, growth and bulbil mass are higher at low altitudes because there the abiotic environment is more suitable whereas at high altitudes more severe conditions occur (TOTLAND & NYLÉHN 1998). It is assumed, that the mass of the bulbils decreases with increasing altitude and this might have an impact on germination. According to this hypothesis, we expected that the bulbils from lower altitudes are more vigorous and have a higher germination ability compared to those from higher altitudes. We therefore collected bulbils from 7 different locations including North-, Central- and South Alps. The main aim of this study was to show that intraspecific variation of germination prevails in relation to different altitudes as well as along different successional gradients, depending on the bulbil mass.

Following the hypothesis of heavier bulbils developed under more benign conditions at lower altitudes, we expected also that seedlings from these bulbils might have the potential to develop longer leaves compared to seedlings from bulbils collected at higher altitudes. We cultivated the seedlings for 30 days in order to validate this hypothesis.

All in all the following hypotheses were tested:

- the mass of the bulbils will be different according to the provenance
- bulbils from lower elevations germinate better than bulbils from higher elevations
- the mass of the bulbils has an impact on the germination and on the development of leaves
- heavier bulbils produce longer leaves
- the successional gradient affects the rate of germination and the development of leaves.

2. Materials and methods:

2.1. Plant material:

Persicaria vivipara belongs to the family Polygonaceae and is also known as *Polygonum viviparum* or *Bistorta vivipara* (Fig. 1). Its habitus is erect, branchless and reaches a height of 5 to 25 cm (LAUBER et al. 2012) or even up to 50 cm (HEGI et al. 1981). The leaves are lance-shaped and reach a maximum size of 8 cm (LAUBER et al. 2012). The inflorescence is terminaly positioned and is developed as a spike. In the lower part bulbils, in the upper part whitish to rosy flowers are formed. In rare events *P. vivipara* can also form three-sided, dark brown seeds.

The range of the species *P. vivipara* extends from Arctic to northern Europe, Asia, the Alps, the Apennines, the Carpathian Mountains, Balkans, Caucasus Mountains, the mountains of southeast and central Asia as well as the Rocky Mountains (HEGI et al. 1981). In general it occurs in meadows, willow communities, mires, and in montane, subalpine and alpine grasslands (FISCHER et al. 2008; LAUBER et al. 2012).



Fig. 1: *Persicaria vivipara* fully in bloom. The bulbils are seen in the lower part of the inflorescence (Photo: Gabi Markl 2009).

2.2. Sample collection and sample sites:

Bulbils of *P. vivipara* were collected at 7 different locations (i.e. 7 bulbil populations), following longitudinal (North-, Central-, South Alps) as well as elevational gradients (1380-2400 m a.s.l.). The collection was carried out between July and August 2013 (Tab. 1). Three sample sites were located along a successional gradient in the Ötztal Alps: i.e. in the surrounding area of Obergurgl (1) and on the glacier foreland of the Rotmoosferner on a 156 years ice free (2) and a 43 years ice free moraine (3). The fourth site was situated at the Axamer Lizum, an area in the Stubai Alps. The fifth location was the Halltal, a small side valley of the Inntal in Tyrol. The surrounding area of the Zugspitze, the highest peak of Germany, was chosen as the sixth sample site. The seventh site was at Mt. Rosengarten in the Dolomites (Italy).

Table 1: Elevation, collection time and bedrock of the 7 sample sites of *Persicaria vivipara*.

Location	Elevation (m)	Collection time	Bedrock
Tyrol, Ötztal Alps, Obergurgl, Central Alps	1950-2000	03.08.13	Gneisses
Tyrol, Ötztal Alps, Central Alps Glacier foreland of the Rotmoosferner (156 years ice-free)	2280	30.07.13	Gneisses, micaschists with garnets and Hornblende, marble
Tyrol, Ötztal Alps, Central Alps, Glacier foreland of the Rotmoosferner (43 years ice-free)	2400	30.07.13	Gneisses, micaschists with garnets and Hornblende, marble
Tyrol, Hall-Valley, North Alps	1380	14.07.13	Wetterstein-Limestone
Germany, Zugspitze, North Alps	2209	02.08.13	Wetterstein-Limestone
Italy (Dolomites), Mt. Rosengarten, South Alps	2250	24.08.13	Schlern-Dolomite

Bulbils were collected randomly from more than 30 individuals per site. The bulbils were stored for approximately two months in paper bags in the fridge at +4°C.

2.3. Bulbil mass and germination experiment:

10 bulbils of each collection site were selected randomly. Measurement of bulbil mass was carried out by a superfine balance (SARTORIUS MC 210 P). Mean values and standard error were calculated per sample site. Bulbils which were used for setting up the bulbil mass were not the same as used in the germination experiment.

For the germination experiment 25 bulbils per sample site were set up with four replications. The bulbils were placed in Petri dishes on three layers of filter paper, using a soft sterile pincette. 4 ml distilled water was added with a dispenser (Dispensette III, BRAND). All Petri dishes were transferred to a growth chamber (SANYO, Growth cabinet MLR-350H) for 30 days. The growth chamber was kept at 25°C on daytime (16 h) and 10°C at night (8 h) with a light intensity of 20.000 lux and a humidity of 60%.

Every two days the germination of the bulbils was controlled by using a stereo microscope (OLYMPUS SD30). The bulbils were recognized as germinated when either the leaf or the root had reached half of the length of the bulbils (Fig. 2). Bulbils with strong black or white mildew infection were sorted out.

After the germination of the bulbils the seedlings were transferred into multipot plates with standard soil and placed in a greenhouse. The temperature in the greenhouse did not drop below 16°C, maximum temperatures were depending on outdoor conditions. Seedlings were watered regularly. After 30 days of plant growth the experiment was stopped and measurements of the leaf length by using scale paper (mm) were performed. Per individual only the longest leaf was measured.

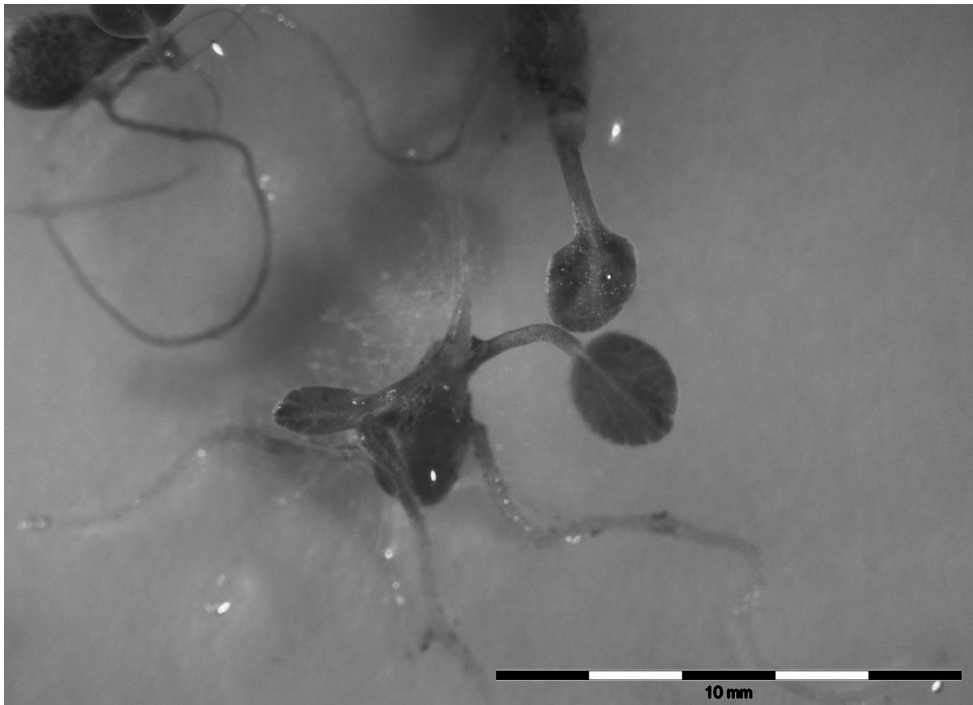


Fig. 2: Germinated bulbils of *Persicaria vivipara* (Photo: Thomas Sansone 2013)

2.4. Statistical analyses:

All statistical analyses were implemented with SPSS 18. The data were checked for normal distribution by applying the Kolmogorov-Smirnov-Test. A normal distribution was given for the germination rate as well as the bulbil mass, but not for the leaf length. Furthermore a Oneway-ANOVA was set up for all of the investigated parameters. The ANOVA test showed homogenous distributions for germination rate and bulbil mass. Only the variances of the leaf length did not show homogenous distribution. The homogenous variances of germination rate were tested for statistical significance using a Posthoc-Test by Bonferroni. Means of leaf length and bulbil mass were tested using the non-parametric Mann-Whitney U-Test.

For testing the relation between the factors germination rate and bulbil mass a regression was calculated. The same was done for bulbil mass and leaf length and also for germination rate and leaf length.

3. Results:

3.1. Mass of bulbils:

Bulbil mass of the 7 locations differed highly significantly ($p \leq 0.001$). The bulbils from the lowest site (Halltal) with a mean mass of 4.7 mg were significantly heavier compared to the bulbils of all other sites (Fig. 3). The lightest bulbils were those of the highest site (1971 moraine) with 1.4 mg. This confirms the hypothesis that the mass of the bulbils varies according to the provenance. The bulbil mass showed a strong positive correlation to the sampling altitude ($R^2 = 0.73$, Fig. 4).

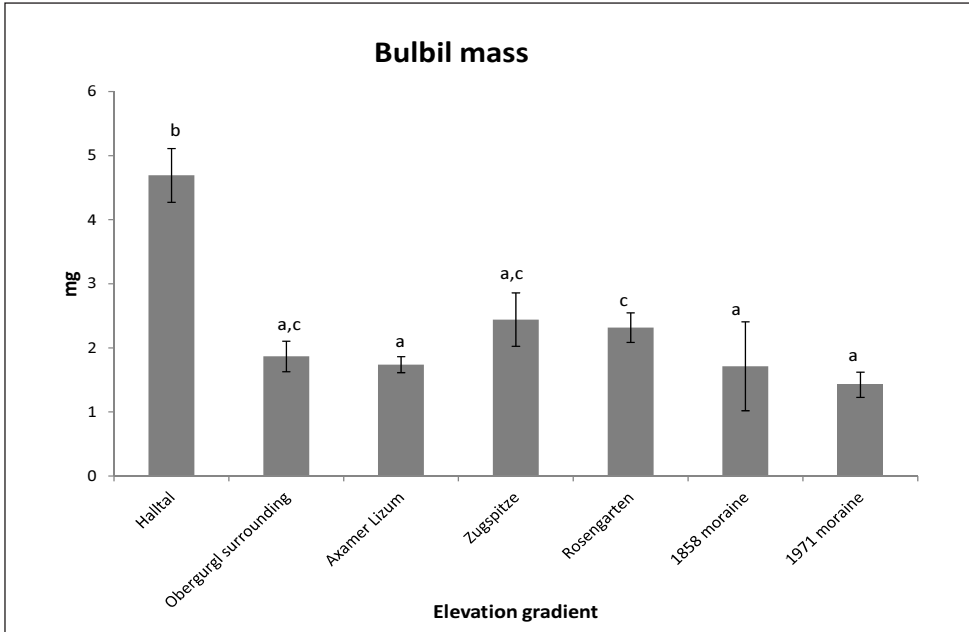


Fig. 3: Bulbil mass \pm standard error of *Persicaria vivipara* from 7 different locations (elevation increases from left to right). Different letters indicate significant differences.

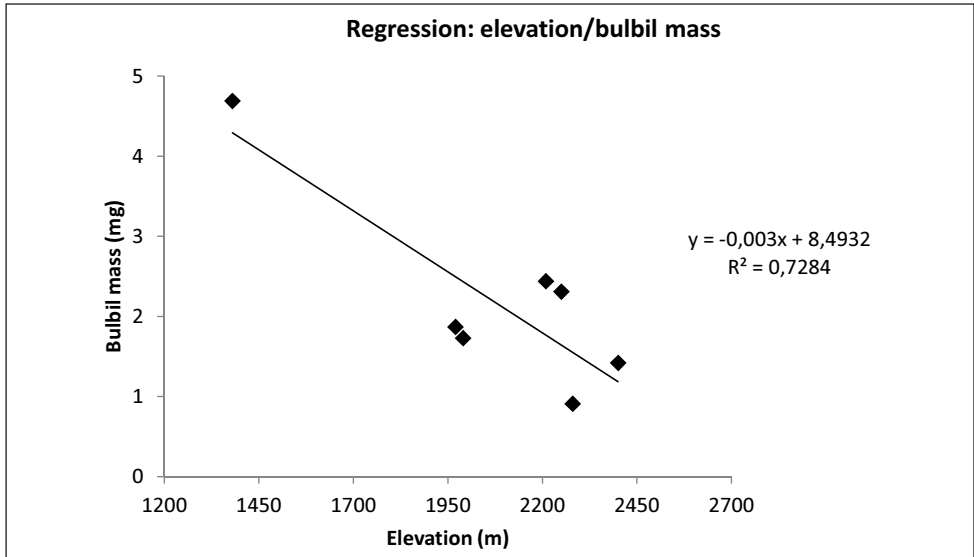


Fig. 4: Correlation between elevation (x axis) and mean bulbil mass (y axis). R^2 = coefficient of determination.

3.2. Germination rate:

The bulbils of the different sampling sites had a highly significantly different ($p \leq 0.001$) germination rate. Those from the Zugspitze showed the highest germination rate with a mean rate of 83%, whereas those from the Axamer Lizum had the lowest rate of 21% (Fig. 5). There

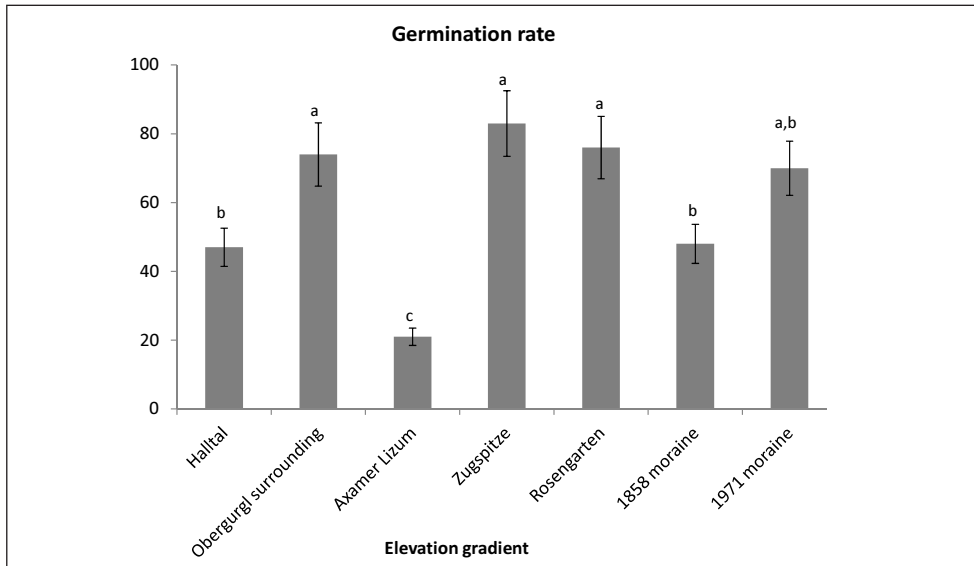


Fig. 5: Germination rate \pm standard error of *Persicaria vivipara* bulbils from 7 different locations (elevation increases from left to right). Different letters indicate significant differences.

is no correlation between provenance and germination rate ($R^2 = 0.0005$). The hypothesis that bulbils from lower elevations germinate better than bulbils from higher elevations cannot be confirmed.

3.3. Leaf length:

The population from the lowest site (Halltal) developed highly significantly longer leaves compared to all other ones (Fig. 6). The mean leaf length of this population amounted to 15.2 mm, being 3.2 times higher than the mean leaf length of the other individuals. The lowest leaf length was determined at the 1858 moraine (2 mm), being significantly lower than that of the 1971 moraine (4.2 mm).

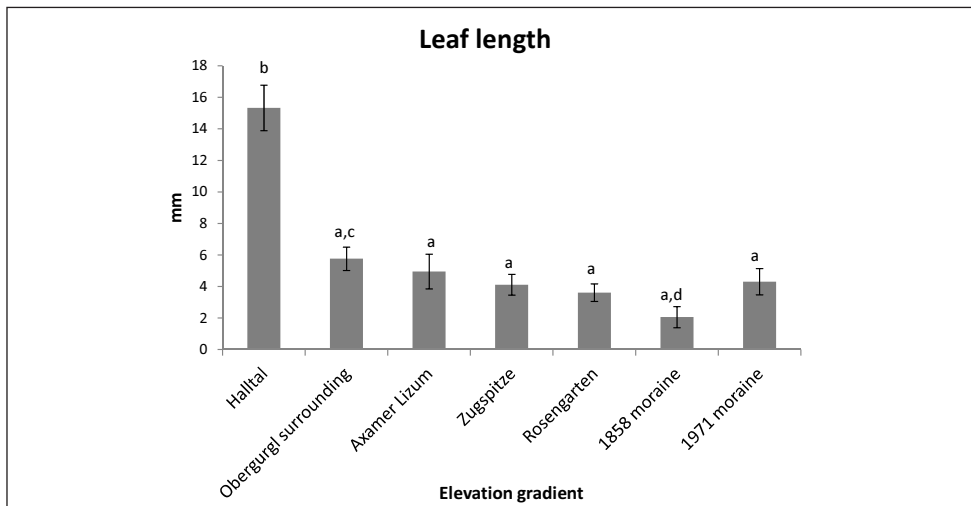


Fig. 6: Mean leaf length \pm standard error of *Persicaria vivipara* from 7 different locations (elevation increases from left to right). Different letters indicate significant differences.

In contrast to the germination rate the mean leaf length showed a high correlation to elevation (Fig. 7): The lower the sampling site, the longer leaves were produced. Leaf length was not significantly correlated to germination rate ($R^2 = 0.05371$). However, there was a strong correlation between leaf length and bulbil mass ($R^2 = 0.8391$, Fig. 8).

4. Discussion:

It turned out that bulbil mass and leaf length differed significantly with elevation, i.e. bulbils from lower elevations had a higher mass and the seedlings produced longer leaves. The Halltal population had about three times longer leaves and more than two times heavier bulbils than all the others. Thus, bulbil mass seems to be a good predictor for plant performance. At lower elevations there are more and better resources available because the soil layers are better evolved and have, as a result, a higher water-capacity. Already WOOKEY et

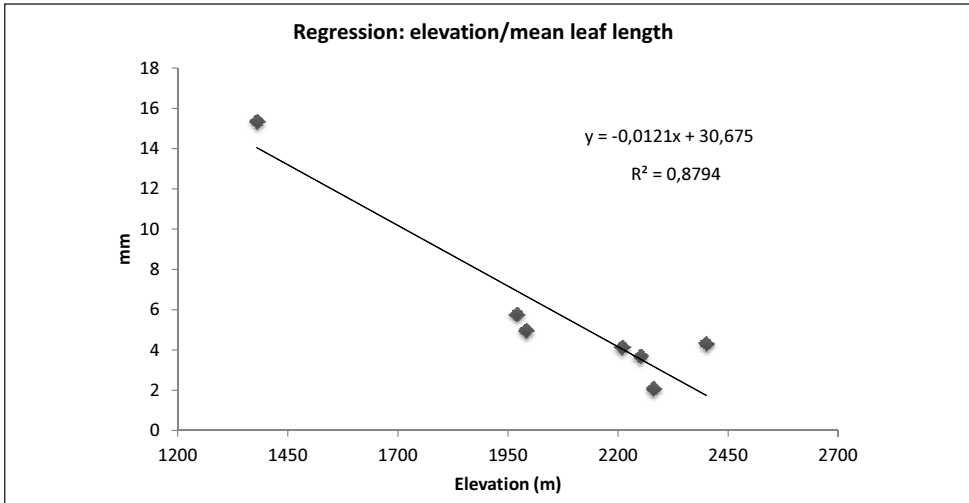


Fig. 7: Correlation between sampling altitude (x axis) and mean leaf length (y axis). R^2 = coefficient of determination.

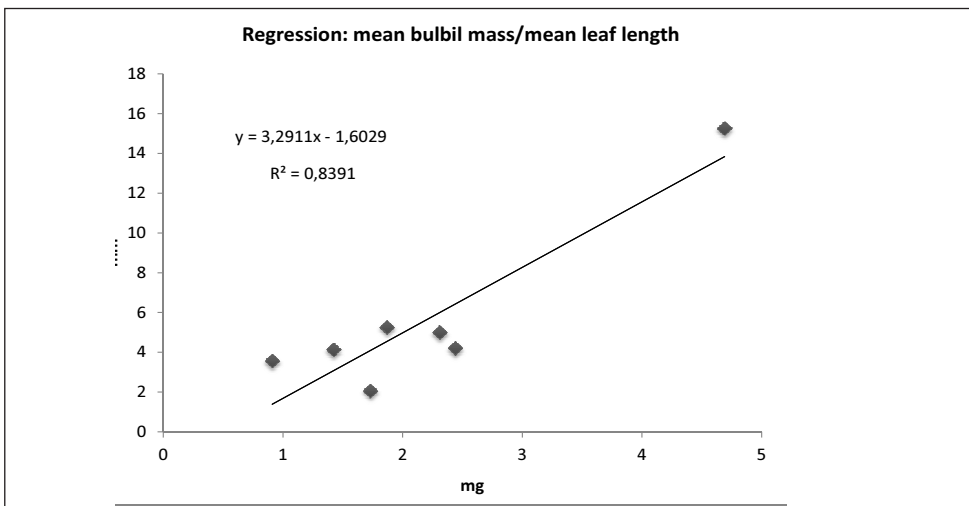


Fig. 8: Correlation between mean bulbil mass (x axis) and mean leaf length (y axis). R^2 = coefficient of determination.

al. (1994) found that *Persicaria vivipara* reacts highly sensitive to nutrients. It is also obvious that season length and temperature have a high influence on the bulbil mass of *Persicaria vivipara*. The growing season is much longer at lower elevations. This leads to a better bulbil development (TOTLAND & NYLÉHN 1998). Warming experiments showed that *Persicaria vivipara* developed heavier bulbils under increased temperatures (WOOKEY et al. 1994; TOTLAND & NYLÉHN 1998; GUGERLI & BAUERT 2001). However, our results support only partly this finding. The observed trend of a higher bulbil mass at Zugspitze

(North Alps) and Rosengarten (South Alps) may probably be correlated with the higher precipitation at the margins of the Alps. *Persicaria vivipara* typically occurs on slightly moist, humus-rich alpine grasslands and snow beds (HEGI et al. 1981; FISCHER et al. 2008). These requirements partly explain the very low bulbil mass on the 1971 moraine where humus and water content of the substrate are scarce (SCHWIENBACHER & KOCH 2010).

When the bulbils were sampled, the Halltal collection had already bulbils with leaves. This advanced growth on the mother plants offered a clear advantage for the leaf lengths. However, germination was not found to be favoured by high bulbil mass. Our hypothesis about a correlation between elevation and germination rate could not be confirmed. Bulbil mass seems not to be a predictor for the germination rate. Many abiotic and biotic factors can lead to better or worse germination, for example soil components, structure and moisture, the existence of safe sites, inter- and/or intraspecific competition (SCHWIENBACHER et al. 2011). In our case, dormancy (BASKIN & BASKIN 1998) might have been an important factor too, especially for the Halltal and the Axamer Lizum bulbils. Bulbils which developed already leaves on the mother plant (Halltal) had to stop their growth when collected and stored in the fridge at 4°C. Thus, they became dormant. The germination rate of Axamer Lizum bulbils was extremely low (21 %) and significantly different from all other provenances. Viability might have been reduced due to unfavourable conditions during bulbil development or seed collection. During the germination experiment, there was a high fungal infection on many bulbils of this provenance and they were removed from the Petri dishes. Consecutive experiments (Brigitta Erschbamer, unpubl. data) showed that a high quantity of bulbils germinated also despite mildew infestation. In our experiment the infected bulbils probably were removed too early.

Equally low germination rates (21 %) to that of the Axamer Lizum bulbils were found also by ERSCHBAMER & PFATTNER (2002) for bulbils collected in the Dolomites. In contrast, SCHWIENBACHER et al. (2012) obtained high germination rates (> 90 %) for bulbils from the Rotmoos glacier foreland.

The bulbils of the calcareous provenances at the high altitudes showed the best germination. This partly confirms the pH-effects highlighted by TOTLAND & NYLÉN (1998). The authors pointed out that 'pH *per se*' does not influence plant growth but the availability of nutrients is higher with higher pH-values. However, neither the results of Halltal (calcareous site) nor those of Obergurgl (siliceous site) are in line with this hypothesis. Our results suggest that probably complex factors interact. Therefore, the environmental conditions and the biotic interactions for each collection site should be known in order to facilitate interpretations.

Considering the successional gradient, we found divergent results. The bulbils of the 1971 moraine had a significantly higher germination rate than those of the 1858 moraine and more or less the same rate as those of Obergurgl. The reason remains quite unclear. The 1971 moraine showed the lowest bulbil mass. Nevertheless germination of these bulbils was more or less equal to the other calcareous provenances. This might be an effect of the higher pH on this site (SCHWIENBACHER & KOCH 2010).

In general, the summer 2013 was relatively dry and hot during the ripening of the bulbils. It may be hypothesized that at the 1858 moraine dispersal of the bulbils started very early and the collected ones were the least viable and immature ones which were left on the mother plants. However, without phenological observations and measurements of the site conditions comparisons are difficult to draw.

5. Conclusion:

Bulbil mass was found to be a good predictor for the performance (i.e. leaf length) of *Persicaria vivipara*. In contrast, bulbil mass cannot be used as predictor for germination rates.

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