Back to Belgium Grants

Final Report

Name of the researcher	Filip Vandelook
Selection Year	2013
Host institution	Agentschap Plantentuin Meise
Supervisor	Steven Dessein
Period covered by this report	from 01/10/2013 to 30/09/2015
Title of the project	Germination ecology, an important factor in the response of plant species to climate change

1. Objectives of the proposal (1 page)

It is becoming clear that rapid climate change may severely impact biodiversity. Studies projecting future distributions of European plants suggest that between 8 and 30 % of the plant species may become extinct (Thomas et al. *2004 Nature*) and that more than half of the species could become vulnerable or threatened by 2080 (Thuiller et al. 2005 PNAS). Germination and seedling establishment are the crucial initial stages in a plants life cycle and therefore under severe selection (Donohue et al. 2010 Annu. Rev. Ecol. Evol. Syst.). It is expected that climate change will cause significant shifts in the sexual reproduction of plants, with some plants benefiting and others perishing (Walck et al. 2011 Global Change Biology). On the other hand, sexual reproduction may enable plants to cope with climate change by means of rapid adapation or dispersal.

With this project, which was framed in a Back to Belgium Grant, I studied how changed climate conditions impact plant performance, thereby focusing on early life cycle stages such as seed ripening, seed germination and seedling establishment. The results of the project will help us to understand how plants and ecosystems function and enable us to take appropriate conservation measures. The main plant species studied were submontane and boreal species that have a Belgian distribution area restricted to the High Ardennes ecological sector. This sector is potentially one of the regions most severely impacted by climate change in Belgium. The species restricted to this ecological sector have no possibilities for altitudinal migration, and we have no idea of the ability of these species to adapt to global warming. For the experiments described in [WP4] we also included species from other ecological sectors, as I outline below.

The study consisted of a dual approach, where I examined how plants respond to short term climate changes in a plastic way, which is especially relevant for perennial species with long adult life cycles, and by means of genetic changes, which applies mainly to annuals. Below I describe the progress that has been made in four Work Packages that were described in the project application. WP1 and WP2 deal with plastic responses of seeds to changed temperature conditions pre- (WP2) and post-dispersal (WP1). WP3 and WP4 are concerned with spatial (WP3) and temporal (WP4) genetic responses to climate change.

2. Methodology in a nutshell (1 page)

[WP1] Assess the temperature tolerance range for germination of wild species using thermal time models

In temperate climates where water is not limiting, germination is mostly a temperature dependent process. By testing germination at a range of temperature conditions it is possible to construct thermal time models which enable one to predict germination percentage and germination timing in function of temperature and time. I intended to construct such models for four perennial moorland species: *Meum athamanticum, Gentiana pneumonanthe, Vaccinium uliginosum* and *Comarum palustre*.

[WP2] Determine how climate change influences maternal effects and subsequent regeneration from seeds

An experiment was started to test how changed climate conditions during seed ripening of *Meum athamanticum* affect seed size at dispersal and subsequent germination. The experiment was set up in an isolated meadow near Elsenborn to avoid human interference. Fourteen 60 cm high Plexiglas open top chambers (OTCs) were placed around randomly selected *Meum athamanticum* individuals (Fig. 2), while fourteen control plots without OTC, but covering the same surface area were established as well. Air temperature and humidity was monitored in two OTCs and two control plots using data-loggers placed inside temperature radiation shields. The experiment was initiated in April 2014 and finished in August 2015.

[WP3] Determine quantitative genetic variation in Gentiana pneumonanthe *traits*

To assess the potential of *G. pneumonanthe* to adapt to changing climate conditions, *G. pneumonanthe* plants from about 35 populations across Europe are grown in a common garden. During two field trips in September and October 2014 fruits were collected from populations at the southern edge of the distribution range in Portugal, Spain and Italy, and at the northern edge of the distribution range in Denmark and Sweden. In February 2015 about 6000 seedlings were sown in Petri-dishes for germination. About 3020 seedlings from 35 populations were transplanted into pots in June 2015. During summer 2015, all accessions were grown in the Botanic Garden Meise and a series of plant traits measured

[WP4] Empirical evidence for adaptive responses of plant reproductive traits to recent climate change

An experiment has been set up to determine the extent of genetic change in five annual plant species over the past 25 years and to test whether short term evolution in response to climate change has occurred. This study has been set up with annual species, as they have shorter generation times and genetic changes are more likely to be detected. The Botanic Garden Meise has collections of seeds of *Thlaspi arvense, Cochlearia danica* and *Arenaria serpyllifolia* sampled in 1989. These seeds have been stored at -20°C and 15% relative humidity for almost 25 years and still show high viability. During spring and summer 2014 we resampled seeds of these species at exactly the same locations. To reduce maternal influences due to different conditions of seed ripening and differences due to seed storage, we decided to grow the first generation in common garden conditions. The F2 generation was grown in a control condition and in open top chambers.

3. Results (8-10 pages)

[WP1] Assess the temperature tolerance range for germination of wild species using thermal time models

Large differences in germination requirements have resulted in difficulties calculating thermal time models. During the past two years, germination of these species was tested at a range of temperature conditions to gain insight in the germination requirements. Seeds of *Comarum palustre* did not germinate at any of the conditions. The seeds are very dormant and further testing is needed to resolve requirements for dormancy break and germination. *Vaccinium uliginosum* seeds germinate best at daily fluctuating temperatures (23/9°C, 12h/12h) after several weeks at low temperatures (5°C; Fig 1). Seeds of *Meum athamanticum* require a long period at low winter temperatures (5°C) to germinate to about 70% (Fig 1). Seeds of four different *Gentiana pneumonanthe* populations germinate to high percentage when incubated at daily fluctuating temperatures of 23/9°C (Fig 1). However, seeds of *G. pneumonanthe* germinated faster after a few weeks at 5°C. These results show that all germination requirements are very species specific, which complicates the development of thermal time models. The experiments did provide insight in the requirements for dormancy break and germination for 3 out of 4 species tested. Further germination tests will be performed to more accurately describe germination requirements and to develop the thermal models.

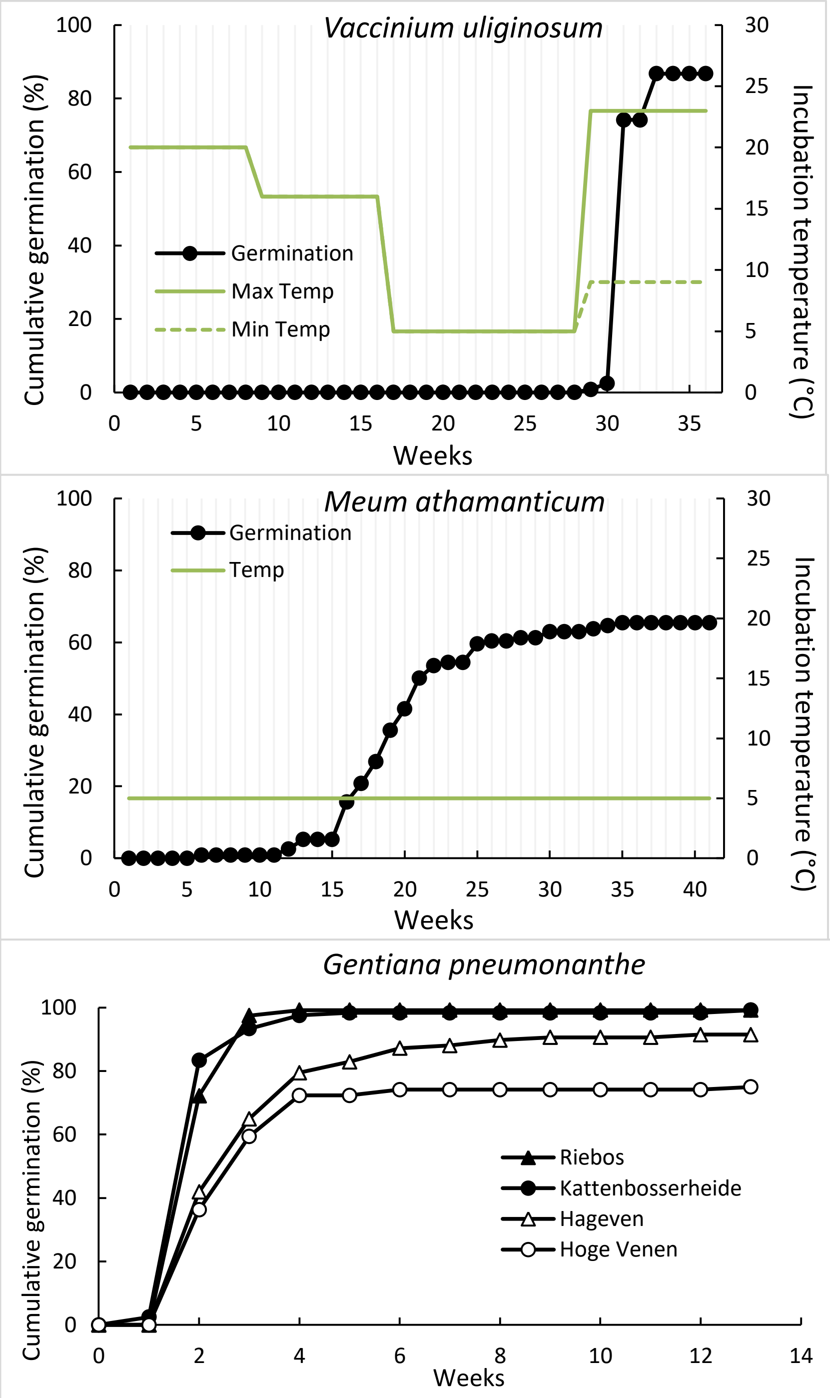

Fig 1. Cumulative germination of three species growing in the High Ardennes region in Belgian.

[WP2] Determine how climate change influences maternal effects and subsequent regeneration from seeds

During the first growing season plants were monitored during spring and summer and seeds collected in early and mid-August. In the second growing season, the experimental site was visited more frequently to accurately monitor phenological processes such as flowering and seed set. Plant traits such as number of inflorescences, plant height and seed size were recorded during both growing seasons. In the course of the experiment several difficulties have arisen. In a few instances seeds grew above the OTC, thus limiting the effect of experimental warming. Therefore, in the second year the OTCs were placed on 14 cm high bricks to increase the height of the OTCs. The results of the subsequent phenological events such as flowering and seed set during the second growing season are shown in Fig. 3. It is clear that difference between control conditions and artificial warming are minor at first sight. In a subsequent analyses we will take the spatial configuration of the plots into account, as there were some obvious differences in micro-climatic conditions at the site due to shading by the surrounding trees. We did observe that *M. athamanticum* plants in OTCs were significantly higher than in control plots (Fig. 4), which may be related to the on average higher vegetation in the OTCs. No significant differences in leaf size were, however, observed between plants in OTCs and in control plots.To test whether an increase in temperature affected subsequent seedling emergence in field conditions, we collected seeds from plants grown in OTCs and in control plots and sew them in a common garden in Meise in autumn 2014. Seedling emergence was recorded weekly and the results are shown in Fig 5. Seeds from OTC plants germinated to a significantly higher percentage, but the germination time course was rather similar for the two conditions. The results will be further analysed and a manuscript of the results will be submitted for publication in the first half of 2016.

Fig 2. Spatial distribution of fourteen OTC chambers (white) and 14 control plots in a meadow near Elsenborn (blue).

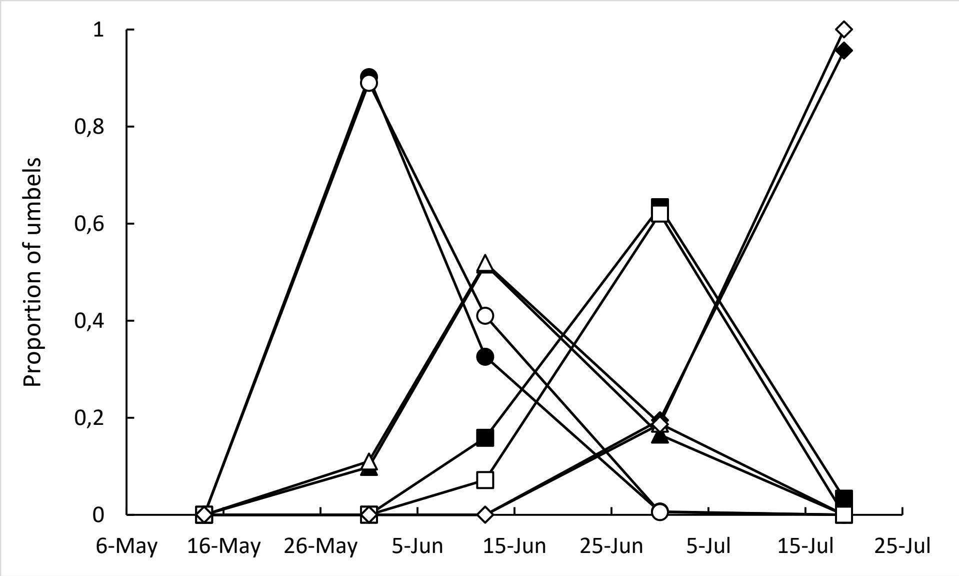

Fig 3. Proportion of umbels with closed flowers (circles), open flowers (triangles), finished flowers (squares) and green fruits (diamonds) for *Meum athamanticum* growing in OTCs (closed symbols) and control plots (open symbols).

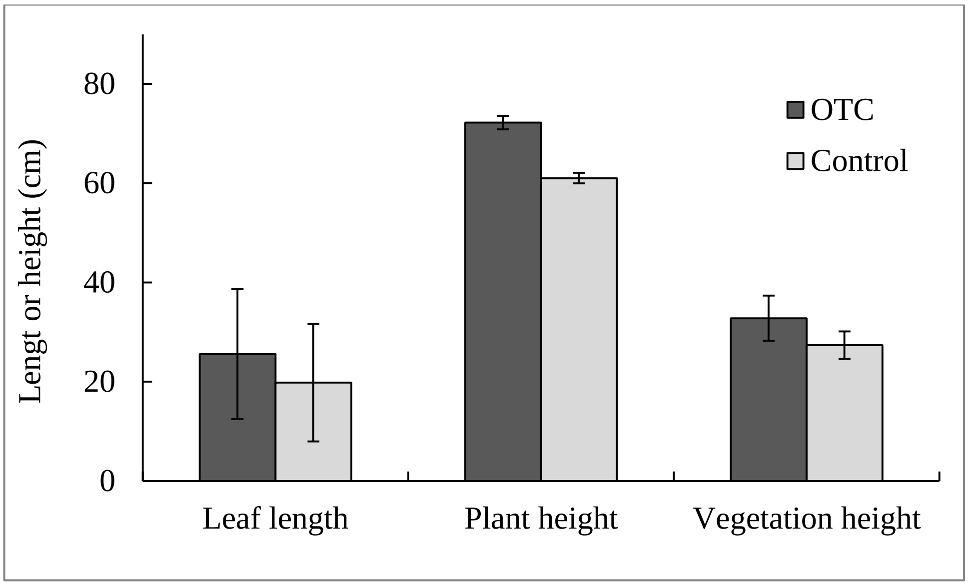

Fig 4. Length of the longest leaf, height of the tallest *Meum athamanticum* plant and height of the surrounding vegetation in OTC and control plots. Error bars denote 1SE

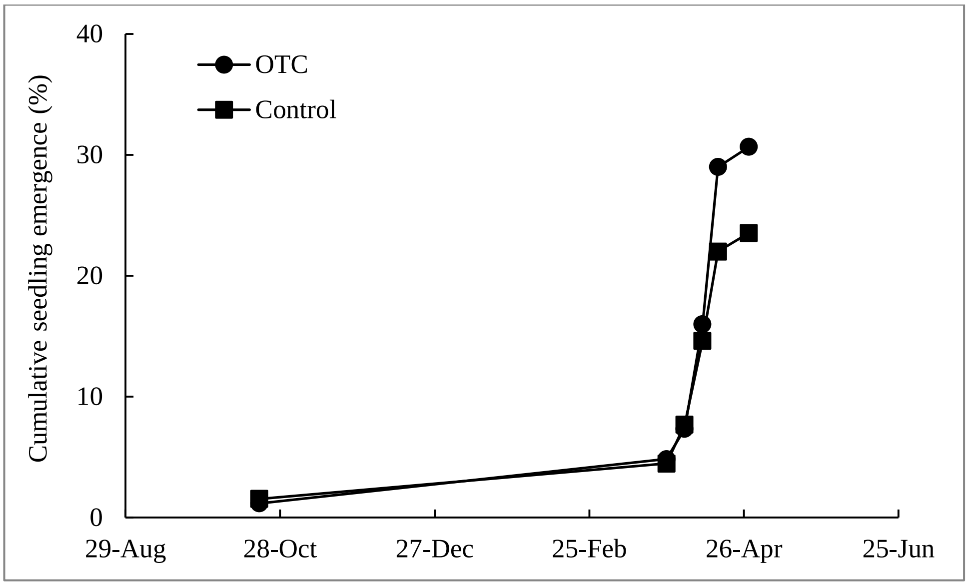

Fig 5. Cumulative seedling emergence of seeds from OTC plots and control plots sown in a common garden in Meise.

[WP3] Determine quantitative genetic variation in Gentiana pneumonanthe traits

Of the 3020 seedlings planted in spring 2015, 2712 were still alive at the end of August 2015. The mortality rate of 9.6% is fairly low. A total of 124 individuals had started flowering during the first year after planting, with one individual forming up to five flowers. On 10th June 2015 the number of leafs was counted for each plant (Fig. 6). Already at this early stage a large between population variation is evident, with the mean number of leafs per plant varying from about 6 leafs in population 13 to almost 15 in population 3. Also plant diameter measured on 26th August varied considerably between populations ranging from about 2 cm in population 13 to almost 6 cm in population 29 (Fig 7). It can also be noted that populations with on average larger plants also produced more flowering individuals (Fig. 8). More traits will be recorded in 2016 and flowering phenology can be recorded more accurately as only 4.5% of the individuals flowered during the first season. The experiment will be vastly expanded as we seek to add eastern European populations. In cooperation with Pieter De Frenne (UGent) a research project will be applied for, which should enable us to perform a molecular genetic analysis and to set-up additional experiments.

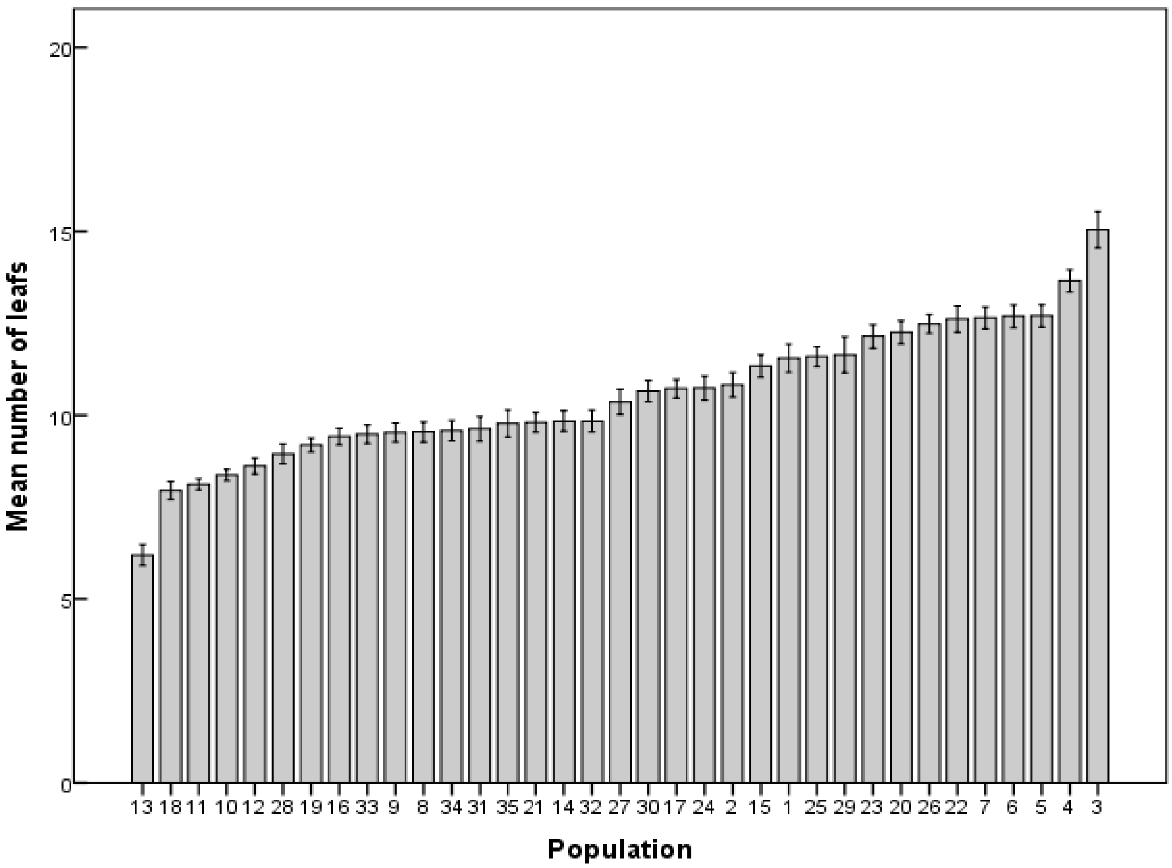

Fig 6. Mean number of leafs for 35 populations of *G. pneumonanthe* grown in a common garden. Error bars denote 1SE

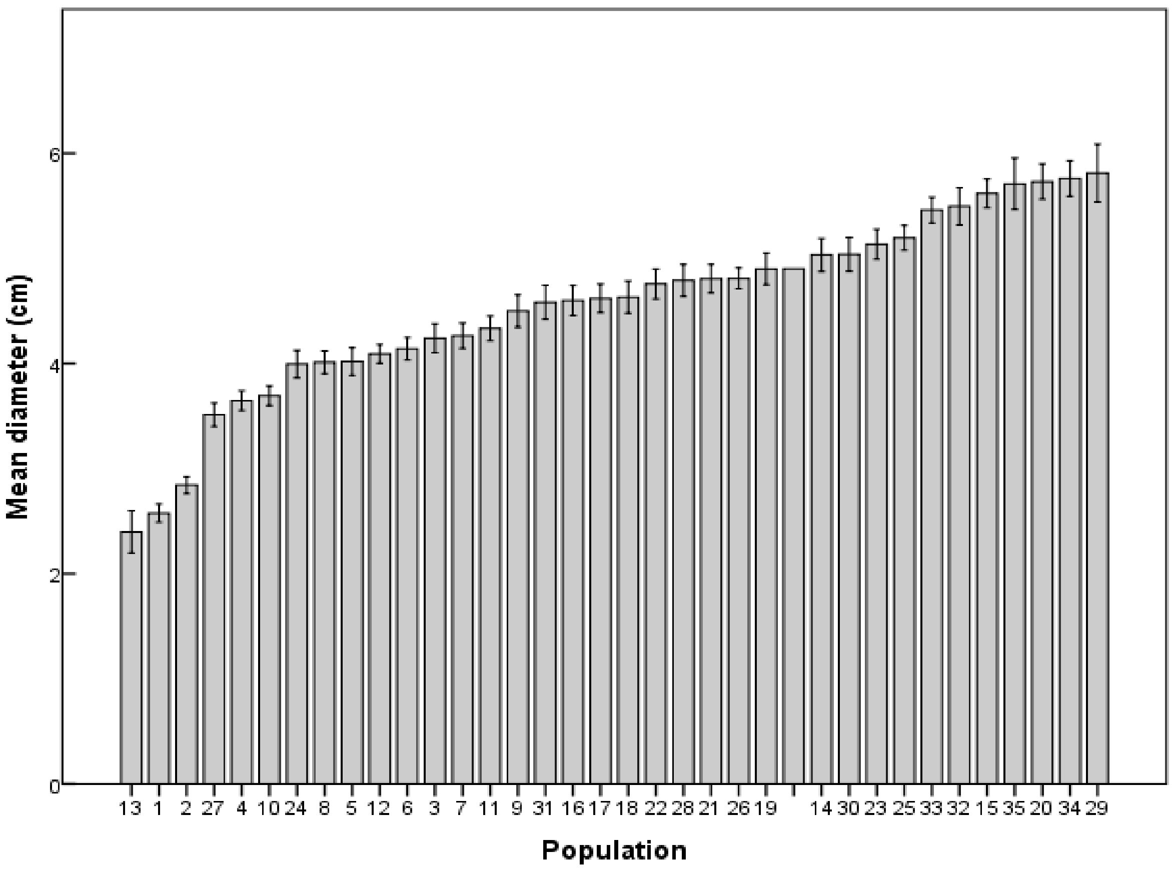

Fig 7. Mean plant diameter for 35 populations of *G. pneumonanthe* grown in a common garden. Error bars denote 1SE

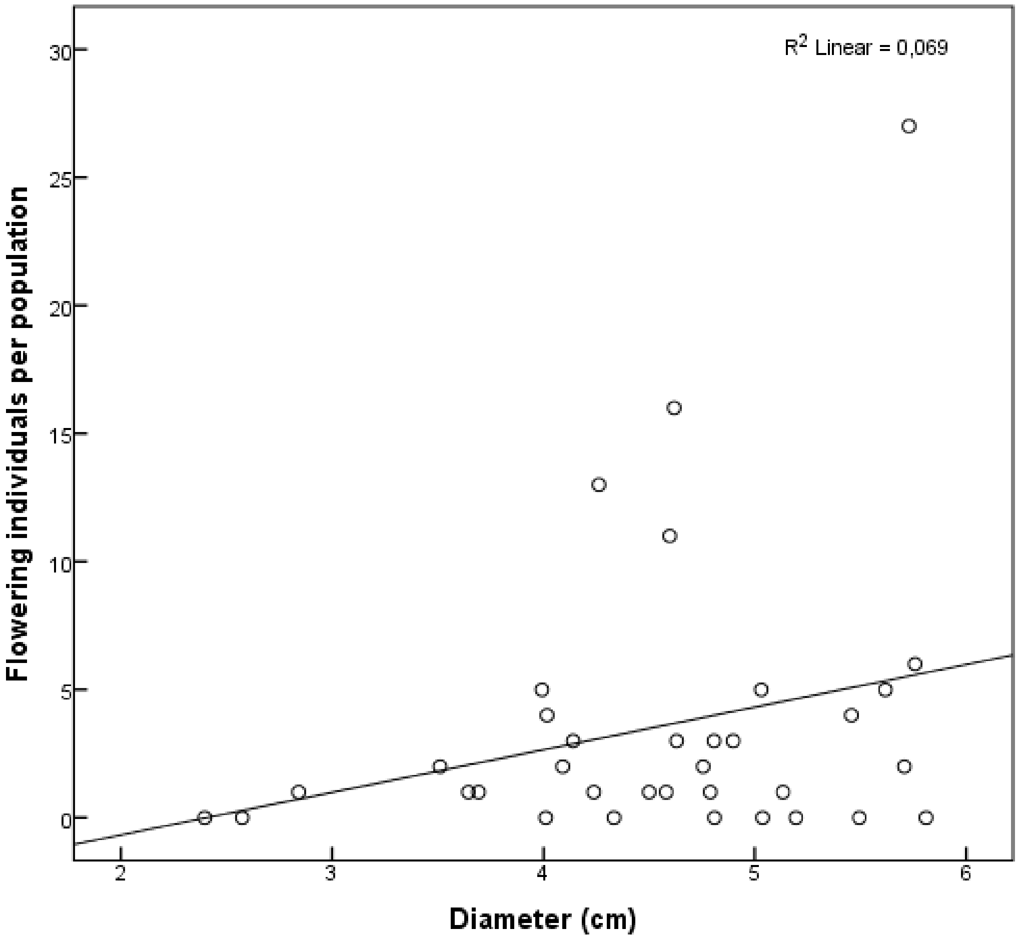

Fig 8. Relation between number of flowering individuals per population and mean plant diameter for each population.

[WP4] Empirical evidence for adaptive responses of plant reproductive traits to recent climate change

F2 plants of *Cochlearia danica*, *Thlaspi arvense* and *Arenaria serpyllifolia* were grown in a common garden under two different treatments. For each species half of the plants were grown in a control conditions, while the other half was grown in open top chambers which increased the mean temperature by about 2°C. For *A. serpyllifolia* and *C. danica*, two winter annual species, the diameter was measured in autumn at the end of the first growing season. For *A. serpyllifolia* we found a clear treatment effect on plant diameter (Fig. 9), but no difference between plants from stored and freshly collected seeds. Therefore it seems no genetic changes in response to changed climatic conditions have occurred in *A. serpyllifolia* over the past 25 years, at least for plant size. For *C. danica*, however, we found that plants from seeds collected in 2014 did not respond strongly to the different treatments (Fig 9). Plants from seeds collected in 1989 on the other hand grew much better in the OTC as compared to the control condition. This may be point to an evolution towards reduced plasticity in response to differing climatic conditions. For the summer annual *Thlaspi arvense* plants from stored and fresh seeds had a similar biomass, which was much higher in the OTC than in the control conditions (Fig 10). The number of leafs developed however was significantly higher in plants from the 1989 collection, as was the response to passive warmer in the OTC. Twelve *Thlaspi arvense* plants from the 1989 and the 2014 seed batch grown in control conditions were selected for measurement of photosynthesis rate. The measurements revealed that maximal photosynthesis was significantly higher in plants from the 2014 seed batch (Fig. 11), which is a potential genetic adaption to increased temperature over the past 25 years. Measurement of phenology and leaf traits are still ongoing and may provide additional interesting insights in genetic adaptations to recent climate change.

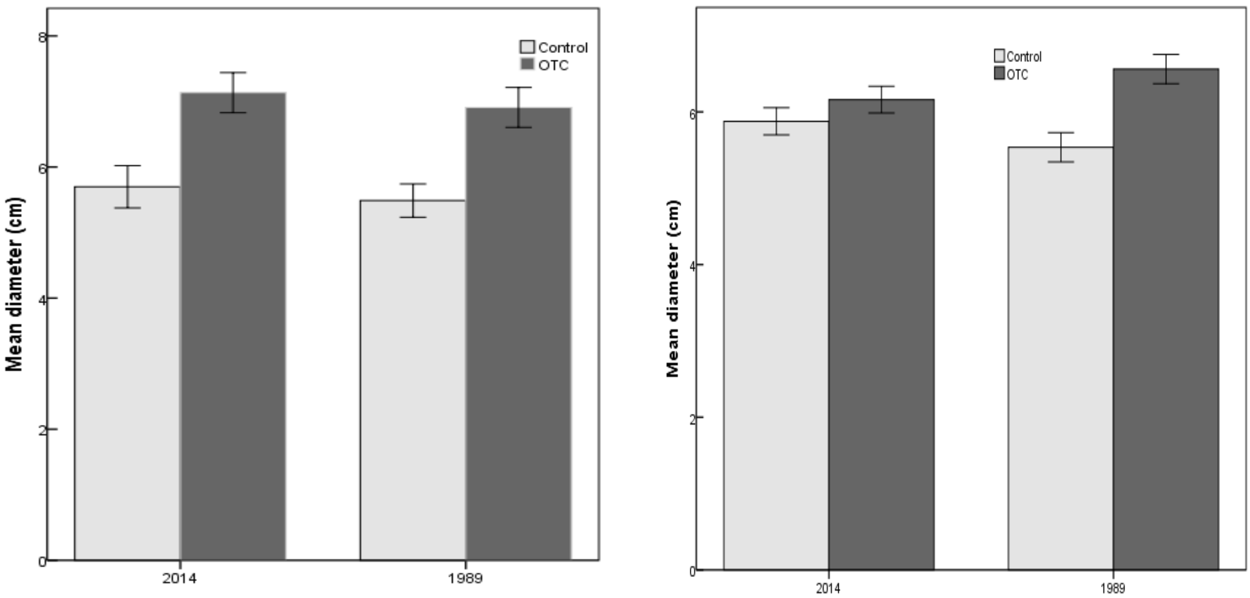

Fig 9. Mean diameter of *Arenaria serpyllifolia* (left) and *Cochlearia danica* (right) collected in 1989 and 2014 and grown in a common garden in open top chambers and in control conditions. Error bars denote 1SE.

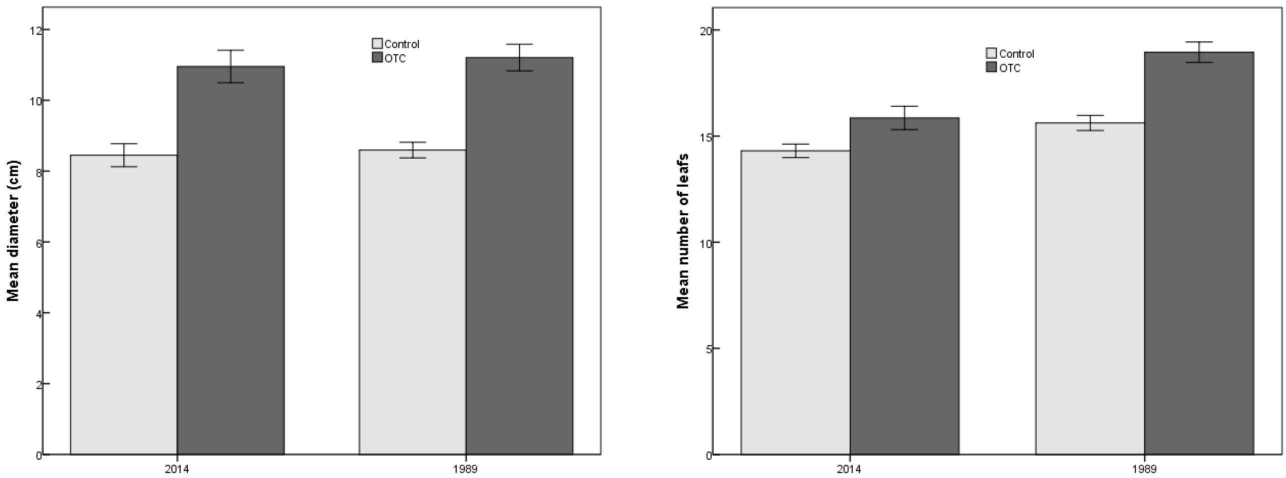

Fig 10. Mean diameter (left) and mean number of leafs (right) of *Thlaspi arvense* collected in 1989 and 2014 and grown in a common garden in open top chambers and in control conditions. Error bars denote 1SE.

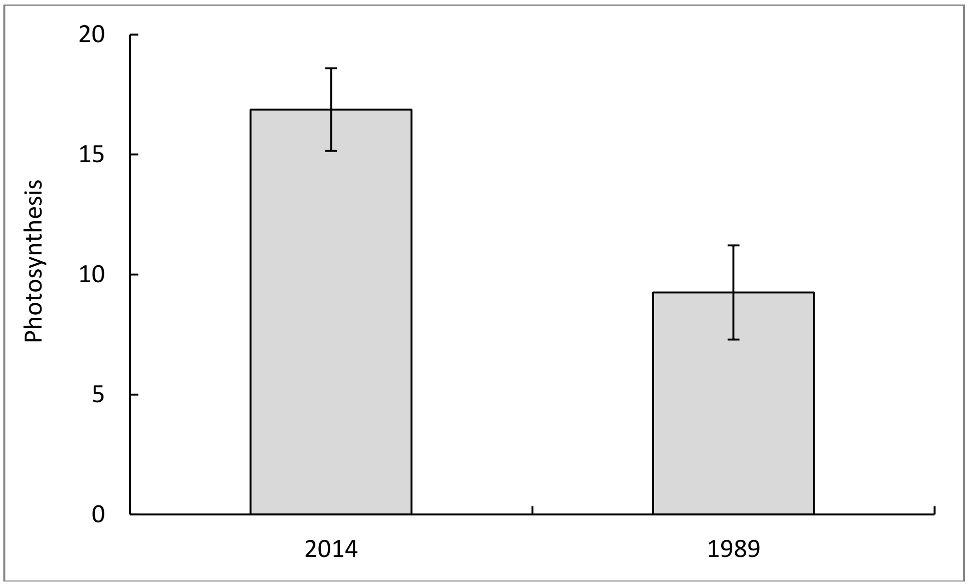

Fig. 11 Average photosynthesis for 12 plants grown from the 1989 and 2014 seed batches. Error bars denote 1SE

4. Valorisation/Diffusion (including Publications, Conferences, Seminars, Missions abroad...)

Much time has been devoted to setting up the experiments and acquiring the required infrastructure. During the past two years I gathered an extensive amount of data from several large scale experiments. My priority for the following year will be to write up the data, to valorise the research results and to write project applications. These data will also be presented at two congresses abroad. I also initiated joint research projects with the scientific staff of the Botanical Garden Meise, thus facilitating my integration once the Back to Belgium has finished.

Peer review publications

Parsons, R.F., **Vandelook, F** and Janssens ,S.B. 2014 Very fast germination: additional records and relationship to embryo size and phylogeny. Seed Science Research 24: 159-163.

Filip Vandelook, Steven B. Janssens and Diethart Matthies (2015) Ecology and evolution of seed mass in the Central European flora. *Journal of Evolutionary Biology*, Resubmitted IF 3.232

Steven B. Janssens, **Filip Vandelook**, Edmond De Langhe, Ines Vandenhouwe, Brecht Verstraete, Erik Smets and Rony Swennen (2015) Tales of the unexpected: historical biogeography of Musaceae reveal interesting insights in the evolution of tropical Southeast Asia. *New Phytologist*, In Press IF 7.672

Approved project applications

Leopopld III-Fonds: *Hypseocharis* species in Bolivia: looking for the origin of *Geraniums* and *Pelargoniums*. 1600 euro for travelling expenses.

FWO short stay abroad. 2600 euro.

Lecture

Vandelook, F. 2014 What do seeds think of climate change? Agentschap Plantentuin Meise. *Presentation of the Back to Belgium project to the scientific staff of the Botanic Garden Meise.*

Promoter/co-promoter of bachelor student

- Co-promotor Bachelor student Marischa Iglé-Anders (Uni Marburg) Die Angepasstheit der Keimung von europäischen und amerikanischen Populationen der wilden Möhre (Daucus carota) an Klimabedingunen

Jury member of a thesis at PhD level

- Jury member PhD Robert Gruwez (UGent) Sexual reproduction of common juniper (*Juniperus communis*) in the face of global change.

Travels abroad

- From 23-09-2014 to 29-09-2014 and from 6-10-2014 to 9-10-2014 Field trip to Portugal, Spain, France, Germany, Denmark and Sweden to sample fruits of *Gentiana pneumonanthe* as outlined in WP3.
- From 16-02-2015 to 14-3-2015 One month expedition to the tropical Andes in Cochabamba, Bolivia.
- On 29-07-2015 Visit to the Millennium Seed Bank, Kew

5. Future prospects for a permanent position in Belgium

My contract in the Botanic Garden Meise has been prolonged for one year, which allows me to finalize the back to Belgium project. I have integrated well in the research program of the Botanic Garden Meise and I'm involved in multiple joint research projects.

6. Miscellaneous