Institute of Landscape Ecology and Resources Management
Division of Landscape Ecology and Landscape Planning
Justus-Liebig-University Giessen

Arable Weeds in the Face of Global Climate Change

Inaugural Dissertation submitted to the
Faculty 09
Agricultural Science, Nutritional Science and Environmental Management
Justus-Liebig-University Giessen

for the degree of
Doctor agriculturae (Dr. agr.)

presented by

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Giessen, 2015

With permission from the Faculty 09 Agricultural Science,
Nutritional Science and Environmental Management,
Justus-Liebig-University Giessen
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“I declare that the dissertation here submitted is entirely my own work, written without any illegitimate help by any third party and solely with materials as indicated in the dissertation.

I have indicated in the text where I have used texts from already published sources, either word for word or in substance, and where I have made statements based on oral information given to me.

At all times during the investigations carried out by me and described in the dissertation, I have followed the principles of good scientific practice as defined in the ‘Statutes of the Justus Liebig University Giessen for the Safeguarding of Good Scientific Practice’.”

“It is not the strongest of the species that survives, nor the most intelligent, but the one that is able best to adapt and adjust to the changing environment in which it finds itself”

Leon C. Megginson (1963)

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This thesis is based on the following research articles:

1) Distinct germination response of endangered and common arable weeds to reduced water potential
A. Theresa Rühl, R. Lutz Eckstein, Annette Otte and Tobias W. Donath
Plant Biology (in press) DOI 10.1111/plb.12331

2) Future challenge for endangered arable weed species facing global warming: Low temperature optima and narrow moisture requirements
A. Theresa Rühl, R. Lutz Eckstein, Annette Otte and Tobias W. Donath
Biological Conservation 182 (2015) 262-269

3) Impacts of short-term germination delay on fitness of the annual weed *Agrostemma githago*
A. Theresa Rühl, Tobias W. Donath, Annette Otte and R. Lutz Eckstein
manuscript

Author's contribution:

In the three research articles being part of this multiple-paper thesis I performed the experimental work and had the main responsibility for data analysis and writing. The co-authors contributed to the study designs and gave valuable comments and criticism.

Chapter 1

Arable weeds in a changing world - Synthesis

Background

As a consequence of global human population growth, the importance of food production increases continuously. Since the global land area cannot be enlarged and there are multiple conflicting demands on landscapes, it is not possible to satisfy the increased demand for agricultural products with extended production areas. Agricultural intensification, which includes breeding of new crop varieties and rising inputs of pesticides and mineral fertilizers, represents one way to increase agricultural yields (Tscharntke *et al.* 2012). However, the conservation of biodiversity as the foundation of human well-being, which has become a focus of attention, is in conflict with agricultural intensification (Brussaard *et al.* 2010; Tscharntke *et al.* 2012).

With about 25 % of the land area being used for agriculture, Central Europe is characterised by arable landscapes (Eurostat 2013) and these are very important for biodiversity. As a kind of currency, measuring the benefits of biodiversity for humans, the concept of 'ecosystem services' was introduced (United Nations 1992). To supply ecosystem services, arable sites with their monocultures depend on the diversity of the associated weed flora as an important food source for insects and birds (Diehl *et al.* 2012; Marshall 2003; Gibson 2006; Storkey 2006). Since the weeds considerably contribute to the biodiversity of arable landscapes, they are considered as keystone species of European biodiversity (Albrecht 2003).

Since humans began to practice agriculture, many herb and grass species have been competing with the crops for the limited resources. With the spread of farming, beginning in the region of the Fertile Crescent, cultivated plants together with their typical weeds were introduced to Central Europe (Ellenberg and Leuschner 2010). Most arable weed species of Central Europe have their origin in the Mediterranean climate. Over time they were able to adapt to the climatic conditions in Central Europe, so that many species expanded their distribution area successfully into the oceanic climate zone (Hanf 1983). Obviously, arable weeds are very closely connected to agricultural management. Arable weeds are annuals, completing their life cycle within one or two years (Ellenberg and Leuschner 2010). Plant communities on arable sites are depending on crop rotation. There are characteristic weed communities of cereal crops and root crops (Hüppe and Hofmeister 1990; Oberdorfer 1983). Therefore, seeds of arable weed species show dormancy as an adaptation to inter-seasonal changes in agricultural management. On nutrient rich sites they are weak competitors due to high light requirements and low biomass (Schneider *et al.* 1994). Since this species group relies on open soil, it is dependent on anthropogenic land use in Central Europe (Hofmeister and Garve 2006). The co-evolution with the crops over thousands of years turned them into specialists for arable sites, closely adapted to the development of the crops and associated land use practices (Otte *et al.* 2006), e.g. timing and intensity of mechanical disturbance.

The agricultural methods, to which the weeds are adapted, remained nearly the same for hundreds of years. Since the time of Charlemagne (747 – 814) the European agriculture was dominated by the extensive management of the traditional three-field crop rotation (Hofmeister and Garve 2006). From the middle of the 20th century, agriculture changed fundamentally. Modern agriculture and intensive land use management with the application of herbicides and fertilizers, enhanced seed treatments, simplified crop rotation, and abandonment of marginal arable sites are the main causes for the continuous decline of arable weeds (Otte 1984; Sieben and Otte 1992; Albrecht 1995; Matson *et al.* 1997; Gerowitt 2003; Fried *et al.* 2008; Simmering *et al.* 2013) and a shift in the spectrum of the wild arable plant species (Sutcliffe and Kay 2000; Marshall *et al.* 2003, Hawes *et al.* 2010). Competitive, nitrophilous autumn germinating species and thermophilic weed species of crops like maize have benefitted from agricultural intensification (e.g. *Polygonum persicaria*, *Setaria viridis* and *Calystegia sepium*) (Bürger *et al.* 2014), while spring germinating species which are herbicide susceptible and less nitrophilous have decreased (Hawes 2010). Arable weeds indicating extreme site conditions suffer from levelling of arable sites (Meyer *et al.* 2013). Therefore, many typical species indicating nutrient-poor acidic or shallow chalky soils are listed in the Red Lists of Germany as endangered or threatened by extinction (e.g. *Arnoseris minima*, *Teesdalia nudicaulis*, *Galeopsis segetum*, *Adonis aestivalis*, *Caucalis platycarpos* and *Scandix pecten-veneris*). The long-term co-evolution with the crops turned them into specialists for traditionally managed arable sites. Since recent land use changes strongly affected environmental conditions on crop fields, arable weeds currently have an extremely high extinction risk and are one of the most endangered species groups in Europe (Kornas 1988; Meyer *et al.* 2013; Storkey *et al.* 2011).

Besides the changing land use, global climate change may present a further challenge to the adaptability of arable weeds (Thuiller *et al.* 2005; Dreesen *et al.* 2012). Differing climate scenarios predict a general increase of annual mean temperatures by 1.5 °C to 4 °C until the end of 21th century (IPCC 2013) and an increased frequency of extreme meteorological conditions (Walck *et al.* 2011). As a consequence, plants may be more often subjected to high temperatures and low soil moisture during the growing season in spring and summer (Knapp *et al.* 2008). Global warming already leads to an observable prolongation of the growing season (Walther *et al.* 2002). The arrival of migrant birds, leaf unfolding and budburst of trees occurs significantly earlier and farmers start sowing crops earlier in spring (Parmesan and Yohe 2003; Menzel *et al.* 2006; Kaukoranta and Hakala 2008).

Germination is a crucial stage especially for annual species. As temperature and water availability control germination of seeds (Baskin and Baskin 2001), changes in these factors may have major impacts on the establishment and survival of plant populations (Hedhly *et al.* 2008; Walck *et al.* 2011). Temperature is presumably an important local trigger allowing seedlings to germinate when the most favourable environmental conditions prevail (Cochrane *et al.* 2011). Consequently, due to an expansion of the growing season, some species may be able to germinate at an earlier date in the year. However, despite matching temperature, these seedlings may encounter unfavourable conditions with respect to diurnal temperature fluctuations or water availability because they germinated in the ‘wrong’ season (Cochrane *et al.* 2011; Mondoni *et al.* 2012). Additionally, higher temperatures combined with less precipitation may completely prevent germination and seedling emergence.

The Red List Status of arable species suggests that their adaptability towards recent changes in land use has reached its limit. This fact may not only be caused by agricultural intensification (e.g. application of herbicides and fertilizers, early stubble clearing, and enhanced seed treatments) but partly also by a mismatch between germination requirements and prevailing environmental conditions (Cochrane *et al.* 2011). If this is true, it is very likely that these species will be particularly sensitive to changes in temperature or water availability due to global climate change.

Objectives

Changes in human land use are always accompanied by the adaption of vegetation. Additional adaption processes are induced by climate change. For the development of successful conservation programs for decreasing arable weed populations it is essential to understand the response of endangered species to future changes of environmental conditions.

It is evident that reproductive traits play a major role for the persistence of the species group of annual weeds. Therefore, the objective of this thesis was to assess the plasticity and adaptability of arable weeds in the face of global climate change. To this end the impact of lower soil moisture (Chapter 2) and higher temperatures (Chapter 3) on the germination process of endangered and common arable weed species was investigated. Based on these studies, the effect of mean germination time, i.e. delayed germination of a few days, on plant fitness throughout the life-cycle was highlighted in a third experiment (Chapter 4).

Material and methods

The elasticity of arable weeds towards changes in temperature and water availability was assessed with a germination experiment using climate chambers. Results of the articles ‘Distinct germination response of endangered and common arable weeds to reduced water potential’ (Chapter 2) and ‘Future challenge for endangered arable weed species facing global warming: Low temperature optima and narrow moisture requirements’ (Chapter 3) are based on germination data of ten, respectively eight arable weed species, characterised as endangered and common species.

We chose five familial pairs of common and endangered arable weeds (Tab Syn-1). This ensured a phylogenetic balanced design and therefore a higher explanatory power of the results (Gitzendanner and Soltis 2000). The Red List Status refers to the Red Lists of threatened plant species in Germany (Ludwig and Schnittler 1996) and Hesse (BVNH 2008). The seeds for the experiment were obtained from a commercial supplier of autochthonous seeds (Rieger and Hoffmann GmbH, Blaufelden-Raboldshausen, Germany).

For both experiments, we used factorial designs to investigate the effects *species* nested in *status*, *water potential* and *temperature* on germination. To establish defined water potentials, we used the osmotic agent Mannitol. Water potentials of -0.3, -0.6, -0.9 and -1.2 MPa were prepared with Mannitol concentrations of 0.12, 0.24, 0.36 and 0.48 mol/L. Distilled water was used as a control for full water availability (0 MPa). For detailed information to performance and used germination variables please see the method parts of chapter 2 and 3.

Tab Syn-1: Study species with information to plant family and Red List Status.

Species	Family	Red List Status [a]
Anthemis arvensis L.	Asteraceae	common
Glebionis segetum (L.) Fourr.		endangered (3*)
Silene latifolia Poir.	Caryophyllaceae	common
Silene noctiflora L.		endangered (3*)
Daucus carota L.	Apiaceae	common
Bupleurum rotundifolium L.		endangered (1)
Papaver rhoeas L. [b]	Papaveraceae	common
Papaver argemone L. [b]		endangered (V*)
Campanula rapunculoides L.	Campanulaceae	common
Legousia speculum-veneris (L.) Chaix		endangered (3)

[a] Red List of threatened plant species in Germany, respectively Hesse (*): V = premonition list, 3 = threatened, 1 = threatened with extinction

[b] These species were not used for the experiment the second article (Chapter 3) is based on.

Data used in the article ‘Impacts of short-term germination delay on fitness of the annual weed *Agrostemma githago* L.’ (Chapter 4) is based on a split-plot design with three blocks to investigate the effect of *delayed germination* (delay of 1, 2, 3 and 7 days) with and without interspecific *competition* on the fitness of the annual weed *Agrostemma githago L.* (Fig Syn-1). To assess the development and fitness of the seedlings with differing delay of germination and competition treatments several variables were assessed. During growth, height and number of shoots of all *A. githago* individuals were recorded weekly. The plants were harvested after three months. During harvesting number of flowers and number of shoots were counted. Additionally, three capsules of every plant were collected to count seeds per capsule and to estimate seed mass. After drying plants at 60 °C for 24 h, biomass was weighed.

For all data sets the effects of single experimental factors and the factor combinations were assessed with multi-factorial ANOVAs. Subsequently, significance of differences between treatments was assessed by employing Tukey-HSD tests. All statistical analyses were carried out using the programme STATISTICA (ver. 10.0, Statsoft Inc., Tulsa, OK, USA).

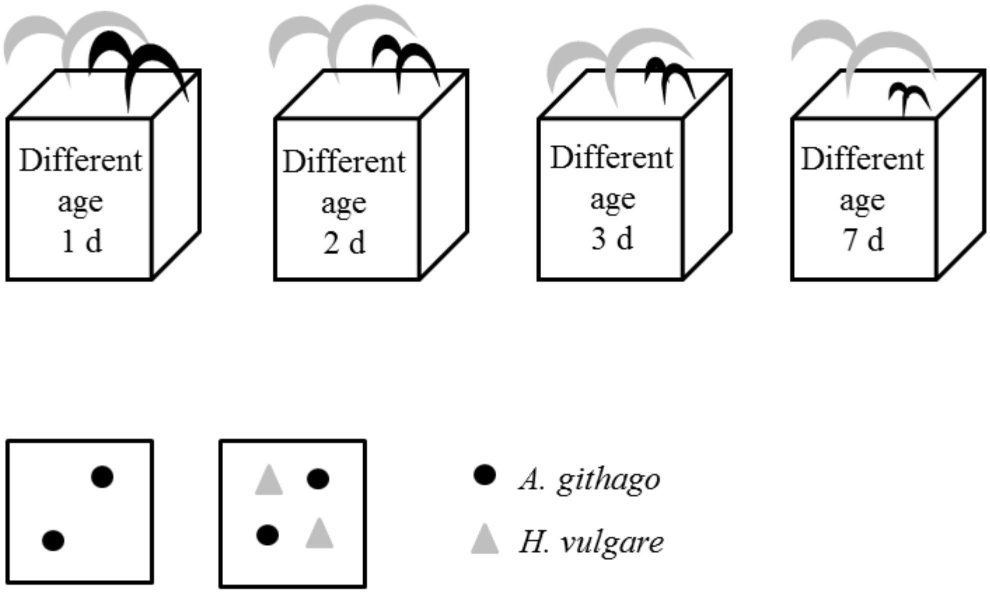

Fig Syn-1: Experimental design of the arrangement of *Agrostemma githago* plants with and without competition through *Hordeum vulgare*.

Main results and conclusions

Arable weeds are very closely connected to agricultural management. Long-term co-evolution with the crops turned them into specialists for arable sites, closely adapted to the associated land use practices (Otte *et al*. 2006). Currently, a shift in the spectrum of arable weed species has been observed (Sutcliffe and Kay 2000; Marshall *et al*. 2003). Some weed species successfully compete for light with the crops and benefit from high nutrient supply and thus increased in frequency (e.g. *Galium aparine*, *Convolvulus arvensis*, *Alopecurus myosuroides*). Whereas formerly widespread species like *Centaurea cyanus, Glebionis segetum* and *Legousia speculum-veneris* decreased in frequency because, they are less competitive on intensively managed arable sites (Otte *et al.* 2006). This shift is an ongoing process intensified by climate change induced land use change, e.g. new crop species and corresponding management systems due to a northward shift of cropping zones (Bindi and Olesen 2011). The Red List Status of many arable weeds shows that their adaptability towards recent changes in agriculture and land use has reached its limit (Meyer *et al*. 2013; Storkey *et al*. 2011). This thesis suggests that, additional to land use changes, climate change may further challenge them.

The results of **Chapter 2** of this thesis revealed a significant link between the reaction of arable weed species to water availability and their Red List status: At optimal water supply endangered species tended to germinate to a higher percentage than common arable weeds; however, this trend was reversed when water availability decreased. Thus, endangered arable weeds showed a stronger negative response to water stress during germination than common arable weeds. With decreasing water availability mean germination time of all investigated species increased. Furthermore, common and endangered arable species showed significant differences in mean germination time; the endangered species germinated 3.2 days earlier (21.83 %) than the common species. Additionally, the endangered species tended to germinate more synchronously. These results suggest that endangered species may be more negatively affected by global warming than common species. This is supported by the findings of **Chapter 3**: Endangered species germinated significantly less than the common arable weeds, except at very low temperatures (3 °C and 5 °C). The preference of endangered arable weed species for low germination temperatures was confirmed by their low optimal germination temperature (15.8 °C ± 0.4). In contrast, common species germinated at significant higher temperatures (optimal temperature 18.4 °C ± 0.2), had a significantly wider range of germination temperatures (endangered: 24 °C ± 3.5, common: 31 °C ± 0.5) and were also more flexible towards changes in water potential. Calculations based on response surfaces for three climate change scenarios indicated that endangered arable weed species may benefit less from climate warming than common species.

Intensive agriculture with repeated applications of herbicides, use of mineral fertilizers, early stubble clearing, narrow spacing of crop rows and breeding of highly competitive crop varieties leads frequently to comprehensive disturbances and a strong competition situation on arable sites. Additionally, climate change with higher temperatures and lower soil moisture during the vegetation period will cause stress for annual plants which have the challenge to complete their life cycle to maintain a viable population. Since there is a close relationship between water and temperature requirements for germination (abiotic on-site parameters) and timing of agricultural measurements (agrotechnical on-site parameters) both, climate and land use change together cause a selection and shift in the spectrum of arable weed species.

In the current situation species are at a disadvantage in terms of germination, when they show low temperature optima, narrow moisture requirements (cf. chapter 3) and a highly synchronous germination with an overall low total germination of the whole seed batch (cf. chapter 2). Seedling emergence of these species is closely related to a small time window of the year. The investigated endangered species are putting everything on one card with the effect that there is less potential within the plant population to recover after an event of disturbance during the vegetation period. In a study under field conditions, seedlings of *Bupleurum rotundifolium* and *Legousia speculum-veneris* only emerged in early spring (Otte 1996). In case of an event like spring drought, erosion after heavy rainfall or herbicide application during spring or early summer the populations of these species will be severely affected.

By contrast, species are in advantage when they produce seeds with high capability of germination (high total germination) and the ability to germinate in a broad range of temperatures and water potentials (cf. chapter 3). Under field conditions it could be demonstrated that, seedlings of *Anthemis arvensis* and *Papaver rhoeas* are able to emerge in spring, early summer and autumn (Otte 1996). The flexibility of these species is further supported by spreading the germination over a longer period of time (asynchronous germination, cf. chapter 2). Arable weed populations with these characteristics have a high potential for regeneration from disturbances without limitations by temperature or water availability within the whole vegetation period. As a result, they are able to produce new seedlings after disturbances very flexibly. Thus, flexibility with regard to timing of germination is an adaptation of successful arable weed species. Other studies about parameters explaining the change of frequency of arable weeds in Germany support these findings. Species with high optimal germination temperatures, a brought range of potential germination temperatures and high Ellenberg nutrient values recently increased in frequency (Otte *et al.* 2006) whereas species with narrow temperature optima tended to decrease (Otte 1994; 1996). The common weed *Viola arvensis,* as an example for successful adaptation to intensive agriculture, was able to expand its range of germination temperature. In addition with an increased production of seeds, *V. arvensis* is capable to react flexibly to disturbances and can use successfully temporal gaps between herbicide applications, tillage and harvest (Schubert *et al*. 2003). The results of the present studies of this thesis confirm these findings and additionally suggest that the observed pattern is enhanced by global climate change.

Rarity in arable plant species is determined by a wide variety of factors. *Agrostemma githago* formerly was a widespread pernicious weed, very flexible towards temperatures and water availability. Today it is threatened by extinction. The main reasons for the extremely high extinction risk are improved seed cleaning techniques. This is a vivid example for the challenging situation of the species group of arable weeds facing recent changes in land use and current changes in climate. *Agrostemma githago* is able to germinate in a brought range of temperatures and water potentials but to establish successfully, this arable weed is relying upon continuous reintroductions from contaminated grain. As crop mimic it is adapted to simultaneous phenological development with the crops (Firbank and Watkinson 1986). In this case, timing of germination is more important than prevailing temperatures and water availability. The question arises here, when delayed germination has significant impacts on the fitness of plant individuals. Does a short-term germination delay of a few days already lead to ecologically relevant effects on plant fitness across the life-cycle (cf. **Chapter 4**)?

Two principal classes of adaptation to environmental change were described, in which selection acts to maximize fitness: adaptive phenotypic plasticity and bet hedging (Simons 2014). Successful arable weeds should have at least one of these abilities to stay flexible and be able to persist in agricultural landscapes. To cope with unfavourable growing conditions between vegetation periods, seed dormancy and the establishment of persistent soil seed banks are general characteristics of many arable weeds. Germination fluxes of weeds are highly controlled by dormancy release or dormancy induction strongly determined by water availability and temperature (Baskin and Baskin 2001). This represents a ‘large scale bet-hedging strategy’ to match the challenges of different years or growing seasons (Gremer and Venable 2014; Rees 1994; Simons 2011; Tielbörger and Valleriani 2005), like changes from winter to summer sown crops. Delayed germination may be a ‘short term bet-hedging strategy’ to cope with unfavourable conditions within one season, spreading the risk over a longer period of time. This species specific germination strategy is sacrificing fitness of individual plants in order to decrease the risk of a failure of the whole seed batch (Gremer and Venable 2014; Rees 1994).

The results of the study described in chapter 4 revealed a decrease of fitness for all investigated life history traits of the study species *Agrostemma githago*. Plants with delayed germination of seven days produced 25 % less shoots, 28 % less biomass, 16 % less flowers, 27 % lighter seeds and were 8 % lower as compared to control plants without delayed germination. A former study under field conditions showed similar decreases in the fitness parameter ‘seed number’ of different cohorts of several arable weed species within one growing season (Otte 1996). The first cohort produced considerably more seeds than following cohorts. Thus, the early emerged seedlings have a greater contribution to the reproduction success of the population.

Since *Agrostemma githago* is a highly adapted crop mimic which is only competitive as weed when it is able to develop simultaneously with the crops (by reintroduction with contaminated cereal seeds), delayed germination is not a successful risk spreading strategy. Similar to developed grain varieties *A. githago* is germinating as soon as seeds are in contact with water (Rühl, unpublished data). The missing flexibility towards timing of germination is the main reason for the high extinction risk of this species.

Risk spreading strategies may become more important in future because of increasing frequency of extreme meteorological conditions. Thus, higher temperatures and lower precipitation during the vegetation period will cause long periods of drought disrupted by events of extreme precipitation (IPCC 2013). As a consequence, plants may be more often subjected to higher temperatures and lower soil moisture during their whole life cycle (Knapp *et al*. 2008, Walck *et al*. 2011; Dreesen *et al*. 2012).

Germination is a crucial stage in the life cycle of plants, particularly for annual species. For the persistence of a plant population, germination requirements have to match the environmental conditions. This is especially important on arable sites, where the timing of agricultural measurements influences germination, seedling emergence, growth and reproduction, while the timing of germination *per se* influences the whole development of the weeds throughout the life cycle (Fig Syn-2). To find the best time for germination is a challenging task for an annual plant. Early emerged seedlings are especially threatened by differing environmental hazards like spring frost or drought, heavy rainfall events or agricultural measures. On the other hand they have advantages concerning interspecific competition of crops and intraspecific competition of individuals of the same species. The situation of competition influences life history traits like height, biomass and number of flowers, which are finally determining the reproduction success (Rees 1992).

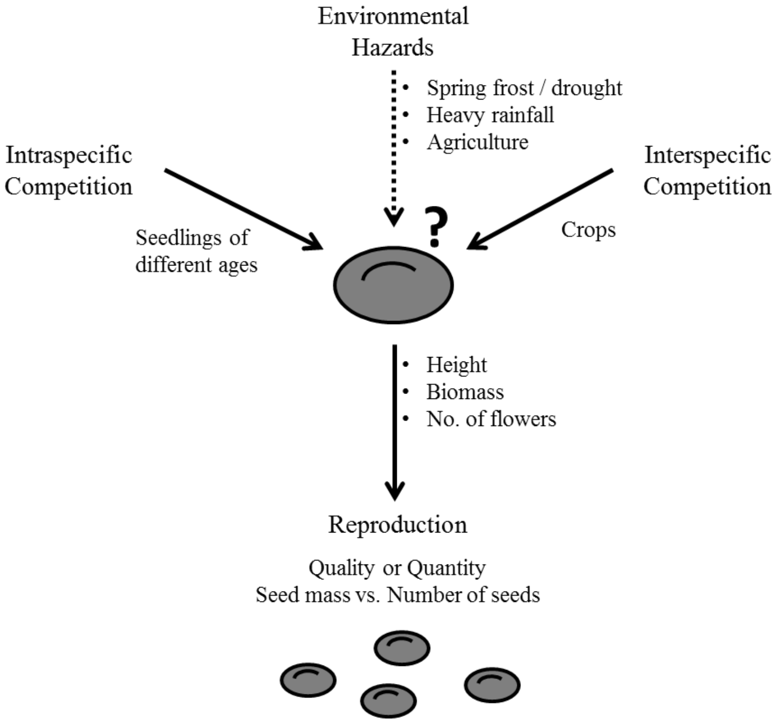

Fig Syn-2 Challenge of an annual: When is the best time for germination?

The distinct germination response of endangered and common arable weeds to changes in water potential and narrow temperature requirements suggest that the endangered arable weeds may be more negatively influenced by climatic changes than today's common species. Competitiveness of the common species may further increase under future climate conditions (cf. chapter 3). Low synchrony of germination combined with long mean germination time is a successful strategy of the common weed species to cope with unpredictable environmental conditions. As the results of chapter 4 show, there is a price to pay for this risk reducing germination strategy. The investigated endangered species show a different strategy, they germinate as soon as favourable conditions prevail and put everything on one card. Therefore they are more sensitive towards unpredictable short-term unfavourable site conditions and disturbances.

In former times it was an advantage to develop simultaneously with the crops. Synchronously seedlings of the weeds and the crops emerged directly after seedbed preparations early in the vegetation period. The probability of early emerged individuals of weeds to survive was relatively high; therefore they reached higher biomass and great fecundity. The mechanisation of agriculture and the development of herbicides led to highly efficient weed control and weeds are exposed more often to comprehensive disturbances than in traditional agriculture. The extreme specialisation and adaptation of these arable weed species to previous agricultural methods had brought them to the edge of extinction. Environmental change moves the average phenotype of well-adapted populations further from its optimal value and thereby reduces mean fitness (Bell and Collins 2008). The adaptability of arable weed species is not able to keep up with the pace of recent changes in land use. A similar development could be imminent in the scope of global climate change.

The successful adaptation to environmental changes requires a large population size and an exchange between populations for genetic variation (Bell and Collins 2008). To preserve the biodiversity in agricultural landscapes conservation efforts have to focus on arable weed species occurring in small and isolated populations. They may not be able to migrate fast enough to sites suitable under future climatic conditions. Climate change calls for support of rare arable weeds at the landscape scale. Accessibility of suitable sites and genetic exchange between populations will enable species to adapt to climate *and* land use change. Agro-environmental schemes for arable weeds should be explicitly intensified to come up from locally limited release for the endangered species to a denser grid of weed conservation sites which would enable migration. Political tools like the concept of ‘High Nature Value Farmland’ (PAN, IFAB, INL 2011) and the ‘Production Integrated Compensatory Measures’ (Litterski *et al.* 2008) of the European Union can be helpful to conserve rare and endangered arable weeds at the landscape scale. Another possibility for building a large-scale conservation grid are field margin strip programs (Marshall and Moonen 2002; Schumacher 1980) with focus on spontaneous vegetation at field margins without herbicides and fertilizers. These conservation measures would give arable weed species greater margins to face future climatic changes.

Perspectives

The ten weed species investigated in this thesis show exemplary the germination response patterns of the special species group of arable weeds. A former study classified 114 arable weed species in 12 differing germination types considering germination temperature and total germination (Otte 1996). This study revealed for example a ‘*Consolida regalis* type’ which is characterised by very low germination temperatures (3 to 15 °C) and an overall low total germination. This germination type contains exclusively species which are listed in the Red Lists of Germany as endangered: *Buglossoides arvensis, Bupleurum rotundifolium, Consolida regalis, Melampyrum arvense* and *Veronica praecox*. In contrast the ‘*Amaranthus retroflexus* type’ includes species with very high germination temperatures (20 to 35 °C) like the typical weeds of maize *Amaranthus chlorostachys*, *Amaranthus retroflexus* and *Chenopodium glaucum*, which showed recently increased distribution (Otte *et al.* 2006). Another category is the ‘*Veronica persica* type’, which is characterised by a brought range of germination temperatures (3 to 35 °C) and very high total germination. This category contains mainly common arable weeds like *Anthemis arvensis*, *Capsella bursa-pastoris*, *Chenopodium album* and *Sinapis arvensis* (Otte 1996). This classification into differing germination types depending on germination temperatures shows a possibility to arrange the 350 arable weed species of Central Europe (Schneider *et al.* 1994) in order to model the future development of populations of weed species under climatic changes. Since the flexibility of species specific germination requirements are partly reflected by the current Red List Status the presented results can be used to assess the risk potential of other arable weed species. A comparison with the described germination patterns of the endangered species *Glebionis segetum* (Compositae), *Silene noctiflora* (Caryophyllaceae), *Bupleurum rotundifolium* (Apiaceae) and *Legousia speculum-veneris* (Campanulaceae) enables to identify further species which are in disadvantage through climatic changes. For example *Bupleurum rotundifolium* can be considered to be representative for the species of the '*Consolida regalis* type' described by Otte (1996). Furthermore, currently common species which may experience a decreasing frequency under future climatic conditions can be identified. On the other hand, the germination patterns of the investigated common weeds can be used to assess the development of problem weeds of the future. A study using this approach was assessed recently to predict weed problems in maize cropping under future climatic conditions through species distribution modelling by combining occurrence data of weeds with high resolution climate, soil and land use data (Bürger et al. 2014).

The efficient method to assess the germination response to differing temperatures and water potentials, presented in this thesis, allows a screening of a larger set of species. Based on further germination data a geographical map assessing the risk of endangered arable weeds could be created. Using this map a model could be developed to predict the impact of different scenarios of changes in climate and land use. Additionally, the pedologic characteristics of arable sites must be taken into account, as soil types with a low water holding capacity will dry faster under conditions of low precipitation and therefore expectedly produce stronger stress (Edler et al. 2015). This approach would allow an identification of locations with high efficiency of conservation measures for endangered arable weed species at the landscape-scale.

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Chapter 2

Distinct germination response of endangered and common arable weeds to reduced water potential

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Plant Biology (in press) DOI 10.1111/plb.12331

Besides the predicted general warming, it is anticipated for Central Europe that the frequency of extreme meteorological conditions will increase. Thus, higher temperatures and lower precipitation during the vegetation period will cause long periods of drought, disrupted by events of extreme precipitation. If the Red List status of arable weeds is partly caused by a mismatch between germination characteristics and environment, it is very likely that these species will be particularly sensitive to changes in temperature and water availability due to climate change. The germination response of arable weeds to decreasing water potential is the subject of the present chapter.

Abstract

Arable weeds are one of the most endangered species groups in Europe. Modern agriculture and intensive land use management are the main causes for their dramatic decline. However, besides the changes in land use, climate change may further challenge the adaptability of arable weeds.

Therefore, we investigated the response pattern of arable weeds to different water potential and temperature regimes during the phase of germination. We expected that endangered arable weeds react more sensitive to differences in water availability and temperature than common arable weeds.

To this end, we set up a climate chamber experiment where we exposed seeds of five familial pairs of common and endangered arable weed species to different temperatures (5/15 °C, 10/20 °C) and water potentials (0.0 to -1.2 MPa).

The results revealed a significant relationship between the reaction of arable weed species to water availability and their Red List status. The effects of reduced water availability on total germination, mean germination time and synchrony were significantly stronger in endangered than in common arable weeds. Therefore, global climate change may present a further threat to the survival of endangered arable weed species.

Keywords: agro-environment scheme, biodiversity, climatic changes, endangered plant species, land use change

Introduction

Arable weeds are one of the most endangered species groups in Europe (Storkey *et al.* 2011). Once introduced with and evolved under agriculture, arable weeds are losing the contest for space and resources against crops after thousands of years of co-evolution (Ellenberg and Leuschner 2010). Modern agriculture and intensive land use management with the application of herbicides and fertilizers, enhanced seed treatments, simplified crop rotation and abandonment of marginal arable sites are the main causes for the continuous decline of arable weeds (Sieben and Otte 1992; Albrecht 1995; Matson *et al.* 1997; Gerowitt 2003; Fried *et al.* 2008; Simmering *et al.* 2013) and a shift in the spectrum of the wild arable plant species (Sutcliffe and Kay 2000; Marshall *et al.* 2003, Hawes *et al.* 2010).

Central Europe is characterised by arable landscapes, with about 25 % of the land area being used for agriculture (Eurostat 2013). Since the weed flora which accompanies crops may thus considerably contribute to the biodiversity of these landscapes, arable weeds are considered as keystone species of European biodiversity (Albrecht 2003).They represent important determinants of ecosystem functioning because they increase the compositional, structural and functional diversity of arable landscapes, otherwise dominated by only few crop species (Hawes *et al.* 2003).

In addition to recent changes in land use, global climate change may further challenge the adaptability of arable weeds (Thuiller *et al.* 2005; Dreesen *et al.* 2012). Current climate scenarios in Europe predict a general warming of 1.5 – 4 °C until the year 2080 compared to 1980 (IPCC 2013). Additionally, it is anticipated that the frequency of extreme meteorological conditions will increase. Thus, higher temperatures and lower precipitation during the vegetation period will cause long periods of droughts disrupted by events of extreme precipitation (IPCC 2013). As a consequence, plants may be more often subjected to higher temperatures and lower soil moisture during germination, seedling emergence, growth and reproduction (Knapp *et al.* 2008; Walck *et al.* 2011; Dreesen *et al.* 2012).

The increase in annual mean temperatures is accompanied by an extended growing season for plants in Central Europe. Events like the arrival of migrant birds, leaf unfolding and budburst of trees occur significantly earlier in the year (Parmesan and

Yohe 2003; Menzel *et al.* 2006). Higher spring temperatures let farmers in Finland already start sowing summer crops earlier in the season (Kaukoranta and Hakala 2008).

A successful germination is the first step of a plant to complete its life cycle. As temperature and water availability control germination of seeds, changes in these factors may have major impacts not only on germination *per se* (Ludewig *et al.* 2014; Rühl e*t al.* 2015) but also on the establishment and survival of plant populations (Baskin and Baskin 2001; Hedhly *et al.* 2008, Walck *et al.* 2011), especially in annual species.

Temperature as a local trigger has a major impact on plants, because it presumably allows seedlings to germinate when the most favourable environmental conditions prevail (Cochrane *et al.* 2011). The importance of temperature for the germination process and the corresponding vulnerability of plants in this life cycle stage are highlighted by numerous studies (e.g. Günter 1997; Akhalkatsi and Lösch 2001). Due to an earlier onset of the growing season, some plant species may be able to germinate at an earlier date in the year. However, despite matching temperature trigger, these seedlings may encounter unfavourable conditions with respect to diurnal temperature fluctuations (e.g. frost spells) or water availability because they germinated in the 'wrong' season (Mondoni *et al.* 2012). In addition, the fitness of plants in terms of germination success of their offspring may depend on the season of germination of the mother plant (Baskin *et al.* 2004; Mennan and Nguajio 2006).

A time shift in germination due to higher temperatures is only one possible influence of climate change. An opposing effect could result from higher temperatures in spring accompanied by less precipitation, which may completely prevent germination and seedling emergence. However, since approximately 90 % of arable weeds root in the upper 30 cm, many species, like spring annuals, avoid summer drought by early germination (Ellenberg and Leuschner 2010).

To establish successfully and sustain populations in the landscape, arable weeds have to deal with in many respects extreme site conditions. Germination strategies are crucial for the establishment of species in changing landscapes. The species-specific germination strategies of plant species and their response towards environmental factors like temperature and water supply during the first stage of their life cycle can be characterised by the variables 'germination time', 'synchrony' and 'total germination' (Baskin and Baskin 2001; Ranal and de Santana 2006). Differences in these germination variables may explain the decline of species as a response to environmental conditions that do no longer match germination requirements (Patzelt 1998; Schütz 2000). Even if this assumption is not appropriate for all plant species (e.g. Hölzel and Otte 2003), it is generally accepted that reproductive traits play a major role for the persistence of the species group of annual weeds. Since the introduction of agriculture, species composition on arable sites has always been changing through time. Species traditionally dominating arable sites are typically those species that spread their germination across time (asynchronous germination) to successfully avoid short periods of unfavourable site conditions (Ellenberg and Leuschner 2010). If the Red List Status of arable weeds is partly caused by a mismatch between germination characteristics and environment, it is very likely that these species will be particularly sensitive to changes in temperature or water availability due to climate change.

In this context, the setup of an effective conservation programme for endangered arable weeds and their preservation at the landscape level requires a detailed assessment of their possible future reaction to changing site conditions. Therefore, we investigated the response patterns of arable weeds to higher temperatures and lower moisture regimes during germination. We employed a multi factorial climate chamber experiment to study the impact of defined water potentials and diurnal temperatures regimes on germination of five familial pairs of common and endangered arable weed species.

We addressed the following research questions and hypotheses:

1) Do common and endangered arable weeds show specific response patterns to reduced water availability? Our hypothesis:

   Endangered species react more sensitive to changes in water availability than common arable weeds. Specifically, endangered species will show lower germination percentage and higher mean germination time and synchrony than common species.

2) Do common and endangered arable weeds show specific response patterns to different temperature regimes? Our hypothesis:

   Endangered species prefer cooler germination temperatures, while the common weed species are able to germinate at a wide temperature range.

Materials and Methods

Study species

We chose five familial pairs of common and endangered arable weed species (Tab 1). This ensured a phylogenetic balanced design and therefore a higher explanatory power of the results (Gitzendanner and Soltis 2000). The Red List Status referred to the Red List of threatened plant species in Germany (Ludwig and Schnittler 1996) and Hesse (BVNH 2008). All species are typical arable weeds with an annual life cycle. Even potentially biennial species like *Silene latifolia*, *Daucus carota* and *Campanula rapunculoides* are considered as typical arable weed species in literature (e.g. Leuschner *et al.* 2013). Since their life cycle will be terminated through agricultural measures such as crop harvest or ploughing they are necessarily annual when they grow on arable sites. They will have to adapt their life-cycle in this extreme anthropogenic habitat and thus will depend strongly on seed germination for re-establishment under these conditions. The seeds for the experiment were obtained from a commercial supplier of autochthonous seeds (Rieger and Hoffmann GmbH, Blaufelden-Raboldshausen, Germany).

Experimental Design

We used a factorial experimental design to investigate the effects *species* (k=10) nested in *status* (k=2, common vs. endangered), *water potential* (k=5; 0, -0.3, -0.6, -0.9, -1.2 MPa) and *temperature* (k=2; 15/5 °C and 20/10 °C) on germination.

To establish defined water potentials, we used the osmotic agent Mannitol (D-Mannitol, Euro OTC Pharma GmbH, Bönen, Germany). Water potentials of -0.3, -0.6, -0.9 and -1.2 MPa were prepared with Mannitol concentrations of 0.12, 0.24, 0.36 and 0.48 mol/L. Distilled water was used as a control for full water availability (0 MPa). For each treatment combination five replicates were set up, resulting in a total of 500 experimental units.

In a Petri dish fifty seeds were placed on one layer of filter paper moistened with 3 mL of D-Mannitol solution or distilled water. To minimize evaporation, five Petri dishes were sealed together in a plastic bag. After 14 days of stratification at 3 °C in darkness in climate chambers (Rumed type 3401, Rubarth Apparate GmbH, Laatzen, Germany) the prepared Petri dishes were placed into climate chambers with fluctuating temperatures of 15/5 °C and 20/10 °C, with a 16-h day/8-h night cycle (Otte 1996). Seeds were controlled twice a week. Germinated seeds were counted and removed at the time when the radicle was observable. After five weeks, germination almost ceased and the experiment was finished.

Analysis

To assess different aspects of germination, we calculated for each experimental unit total germination (%), mean germination time (days) and synchrony of germination according to Ranal *et al.* (2006). Total germination is the percentage of all germinated seeds from the total number of seeds. Mean germination time in days ($\bar{t}$) is calculated as the weighted average of the time (days) to germination:

$$\bar{t} = \sum_{i=1}^{k} n_i t_i \Big/ \sum_{i=1}^{k} n_i$$

where t_i is the time from the start of the experiment to the i^{th} observation day, n_i is the number of seeds germinated at day i and k is the last day of germination.

Synchrony (Z) of germination of one seed with another seed in the same experimental unit is defined as:

$$Z = \sum Cn_{i,2} / N \qquad \mathrm{Cn}_{i,2} = \mathrm{n}_i(\mathrm{n}_i\text{-}1)/2 \qquad N = \sum n_i \left(\sum n_i - 1\right)/2$$

where $\mathrm{Cn}_{i,2}$ is the combination of the seeds germinated in i^{th} days. This unitless index ranges from 0 to 1. When all seeds germinate at the same time, then $Z = 1$. When at least two seeds germinated, but at different points of time, then $Z = 0$.

Before analysis, mean germination time was Box-Cox transformed whereas total germination and synchrony were arcsine-square-root-transformed to obtain normal distribution and variance homogeneity (Quinn and Keough 2002).

With the transformed dataset a single factor variance analysis (ANOVA) with the factor *family* was carried out to account for potential phylogenetic effects. All further calculations were performed with the residuals of this ANOVA. The effects of the single factors and the factor combinations of *species (status)*, *status*, *temperature* and *water potential* were assessed with a multi-factorial hierarchical ANOVA. Subsequently, significance of differences between treatments was assessed by employing a Tukey-HSD test. To avoid zero inflation and missing data, the water potential -1.2 MPa was excluded from the statistical analysis. All statistical analyses were carried out using the programme STATISTICA (ver. 10.0, StatSoft Inc., USA).

Tab 1 Study species with information to the plant family, the Red List Status, viability (%) and germination capacity (%) of the seeds.

Species	Family	Floristic Status [a]	Life Cycle [b]	Red List Status [c]	Viability [%] [d]	Capacity [%] [e]
Anthemis arvensis L.	Asteraceae	Archaeophyt	summer and winter annual	common	94 ± 2.83	65 ± 7.07
Glebionis segetum (L.) Fourr.		Archaeophyt	summer annual	endangered (V)	92 ± 5.66	52 ± 5.66
Silene latifolia Poir.	Caryophyllaceae	indigenous	perennial	common	88 ± 5.66	54 ± 5.66
Silene noctiflora L.		Archaeophyt	summer and winter annual	endangered (V)	90 ± 2.83	94 ± 8.49
Daucus carota L.	Apiaceae	indigenous	biennial	common	92 ± 5.66	63 ± 12.73
Bupleurum rotundifolium L.		Archaeophyt	summer annual	endangered (1)	98 ± 2.83	72 ± 8.49
Papaver rhoeas L.	Papaveraceae	Archaeophyt	summer and winter annual	common	88 ± 5.66	50 ± 8.00
Papaver argemone L.		Archaeophyt	summer and winter annual	endangered (V)	96 ± 5.66	58 ± 0.00
Campanula rapunculoides L.	Campanulaceae	indigenous	perennial	common	86 ± 2.83	87 ± 1.41
Legousia speculum-veneris (L.) Chaix		Archaeophyt	summer and winter annual	endangered (3)	94 ± 2.83	84 ± 2.83

[a] Floristic Status according to FloraWeb (2014)

[b] Life cyle according to Jäger and Werner (Ed.) (2005)

[c] Red List of threatened plant species in Germany, respectively Hesse (*): V = premonition list, 3 = threatened, 1 = threatened with extinction

[d] Viability tested with a tetrazolium chloride test with 25 seeds per replicate (n=2), mean ± SD

[e] Germination capacity tested in common garden, 50 seeds per replicate (n=2), mean ± SD

Results

The ANOVA revealed significant effects and interactions of *species* (S), *status* (St), *temperature* (T) and *water potential* (WP) on total germination, mean germination time and synchrony. The strong effect of water potential on germination was highlighted by the high proportion of total variance, particularly in comparison with the impact of temperature (Table 2).

Total germination

The results concerning total germination indicated a general response pattern of the arable species to differences in water availability: with decreasing water availability, germination of all study species declined. Furthermore, the data revealed a significant link between the reaction of arable weed species to water availability and their Red List status (Fig 1). At optimal water supply endangered species tended to germinate to a higher percentage than common arable weeds, however, this trend was reversed when water availability decreased. Thus, endangered arable weeds showed a stronger negative response to water stress during germination than common arable weeds. Total germination of the latter was significantly higher at water potentials of −0.6 and −0.9 MPa. A further decline of the water potential near the permanent wilting point resulted in very low levels of germination of all study species, irrespective of Red List Status (Fig 2).

The impact of temperature was more species specific. While at full water availability for example *Silene noctiflora* (5/15 °C= 89.6 %, 10/20 °C= 97.2 %) and *Anthemis arvensis* (25.2 %, 38.8 %) showed higher total germination at 10/20 °C, *Bupleurum rotundifolium* (74.8 %, 62.4 %) and *Papaver argemone* (22.4 %, 17.6 %) tended to germinate better at cool temperatures of 5/15 °C.

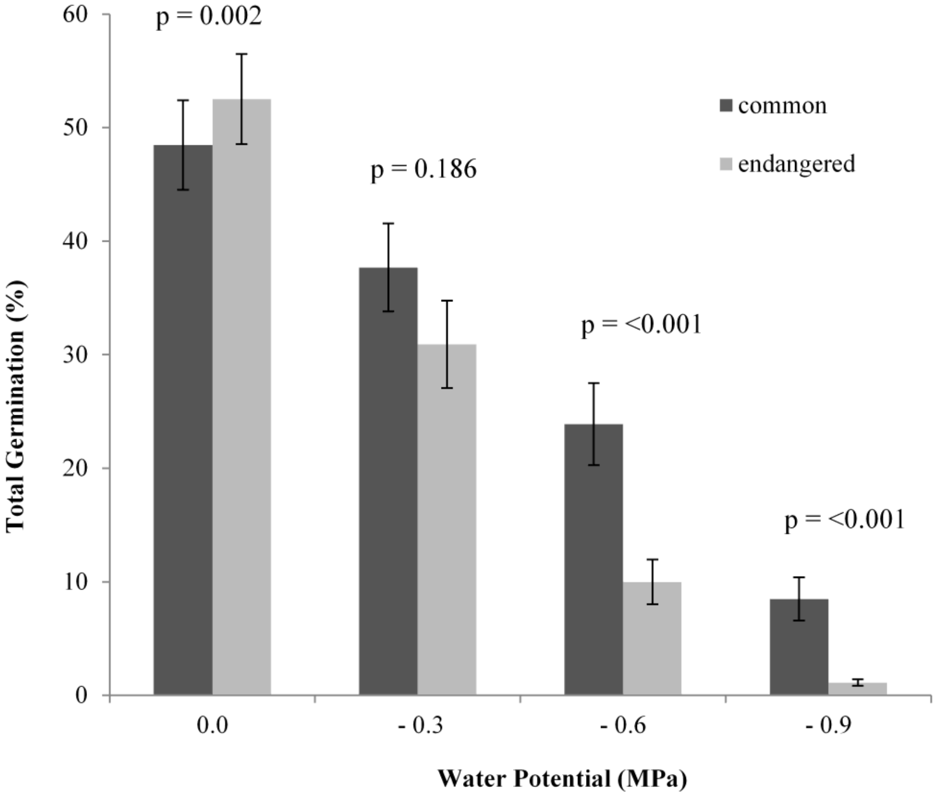

Fig 1 Effects of Red List Status and water potential (WP) on *total germination* [%]. The bars show the original data with standard error. P-values for comparisons between common and rare species (within each level of water potential) are from a Tukey HSD test using the residuals of the initial ANOVA for the factor *family* (see Methods).

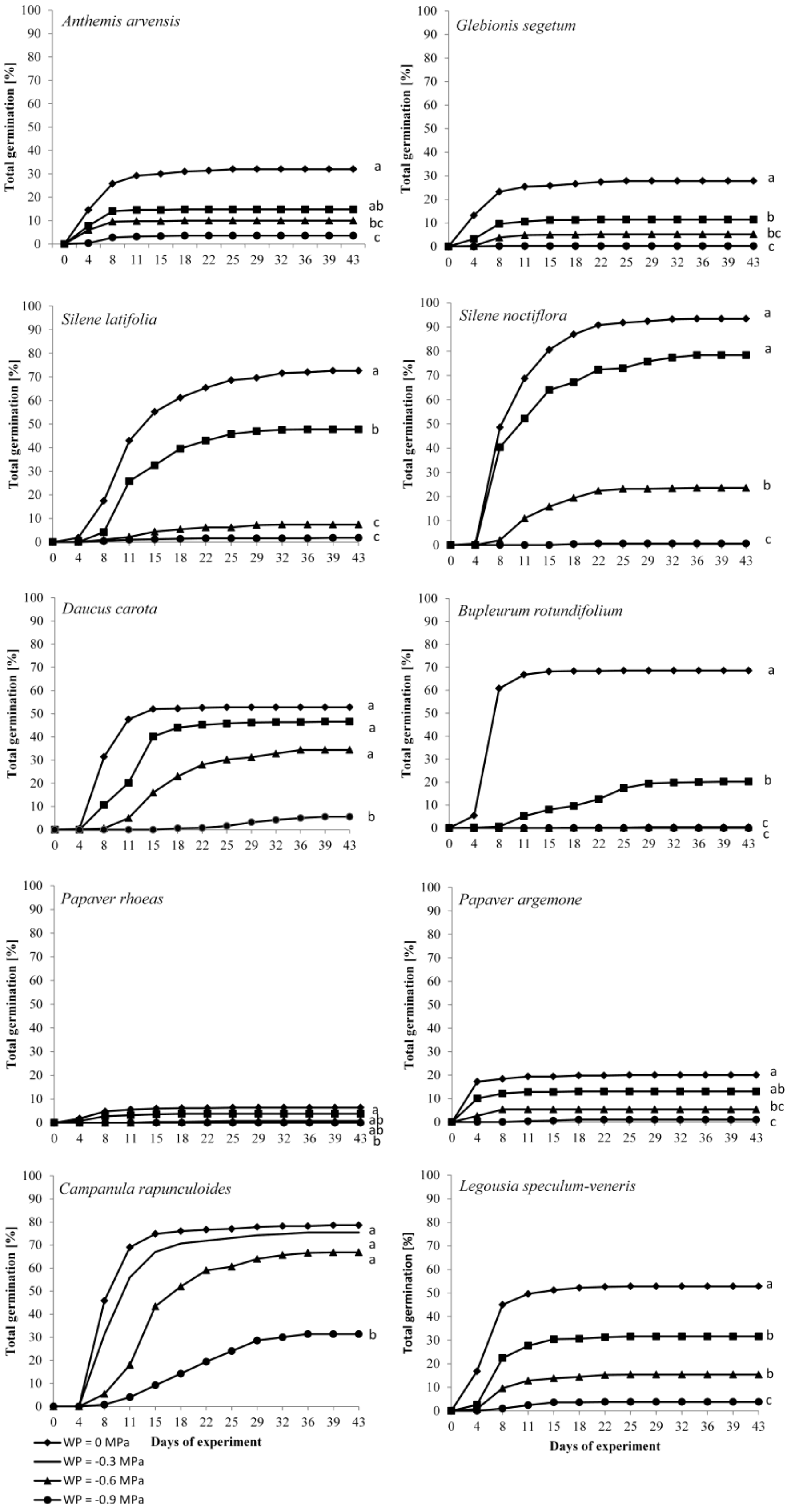

Fig 2 Total germination of the ten study species, separately for the four different water potentials. The two temperature treatments are pooled.

Tab 2 Effects of *species* (nested in status), *status*, *water potential* and *temperature* on total germination (%), mean germination time (days) and synchrony of germination; including water potentials 0, -0.3, -0.6, -0.9 MPa, df = degrees of freedom, F = variance ratio, p = error probability, vc (%) relative contribution of individual factors and their interactions to total variance. P-values <0.05 are written in bold.

	Germination (%)					**Mean germination time (d)**					**Synchrony**				
	df	***MQ***	***F***	***p***	**vc (%)**	**df**	***MQ***	***F***	***p***	**vc (%)**	**df**	***MQ***	***F***	***p***	**vc (%)**
Intercept	**1**	**0.00**	**0.0**	**1.000**		**1**	**0.75**	**57.8**	**<0.001**		1	0.17	1.7	0.192	
species (status) [S(St)]	**8**	**0.70**	**79.6**	**<0.001**	**12.6**	**8**	**0.29**	**22.7**	**<0.001**	**14.3**	**8**	**0.36**	**3.5**	**<0.001**	**6.6**
status [St]	**1**	**0.57**	**64.9**	**<0.001**	**1.3**	**1**	**0.62**	**48.3**	**<0.001**	**3.8**	**1**	**2.72**	**26.8**	**<0.001**	**6.2**
temperature [T]	**1**	**0.33**	**37.8**	**<0.001**	**0.7**	**1**	**1.32**	**102.1**	**<0.001**	**8.1**	1	0.03	0.3	0.611	0.1
water potential [WP]	**3**	**8.33**	**944.8**	**<0.001**	**56.1**	**3**	**1.45**	**112.5**	**<0.001**	**26.7**	3	0.24	2.4	0.073	1.6
S (St) x T	**8**	**0.16**	**18.2**	**<0.001**	**2.9**	**8**	**0.11**	**8.4**	**<0.001**	**5.3**	8	0.19	1.9	0.068	3.5
S (St) x WP	**24**	**0.30**	**33.9**	**<0.001**	**16.1**	**22**	**0.12**	**9.4**	**<0.001**	**16.3**	**21**	**0.25**	**2.5**	**<0.001**	**12.0**
St x WP	**3**	**0.33**	**37.8**	**<0.001**	**2.2**	**3**	**0.08**	**6.2**	**<0.001**	**1.5**	**3**	**0.66**	**6.5**	**<0.001**	**4.6**
St x T	1	0.01	0.9	0.352	0.0	1	0.01	0.5	0.466	0.0	1	0.07	0.6	0.422	0.2
T x WP	**3**	**0.03**	**2.9**	**0.037**	**0.2**	3	0.001	0.1	0.977	0.0	**3**	**0.32**	**3.2**	**0.026**	**2.2**
St x T x WP	3	0.01	1.5	0.209	0.1	**3**	**0.04**	**2.9**	**0.034**	**0.7**	3	0.02	0.2	0.872	0.2
S (St) x T x WP	**24**	**0.03**	**3.0**	**<0.001**	**1.4**	**19**	**0.03**	**2.4**	**0.001**	**3.5**	**16**	**0.27**	**2.6**	**<0.001**	**9.8**
error	320	0.01			6.3	250	0.01			19.7	228	0.10			53.1

Mean germination time

Similar to the results on total germination, the factor with the highest explanation power for mean germination time was *water potential*. In comparison to total germination, mean germination time was more species specific. Thus, the factor *species* explained 16.7 % of the variation. Independent of species or Red List Status high temperatures decreased mean germination time. At full water availability and 5/15 °C mean germination time was 10.3 days across all species and at 10/20 °C it was 7.9 days.

Our results clearly showed that mean germination time increased with decreasing water availability (Fig 3). In addition, common and endangered arable species showed significant differences in mean germination time (Tab 2). The endangered species germinated 3.2 days earlier (21.83 %) than the common species.

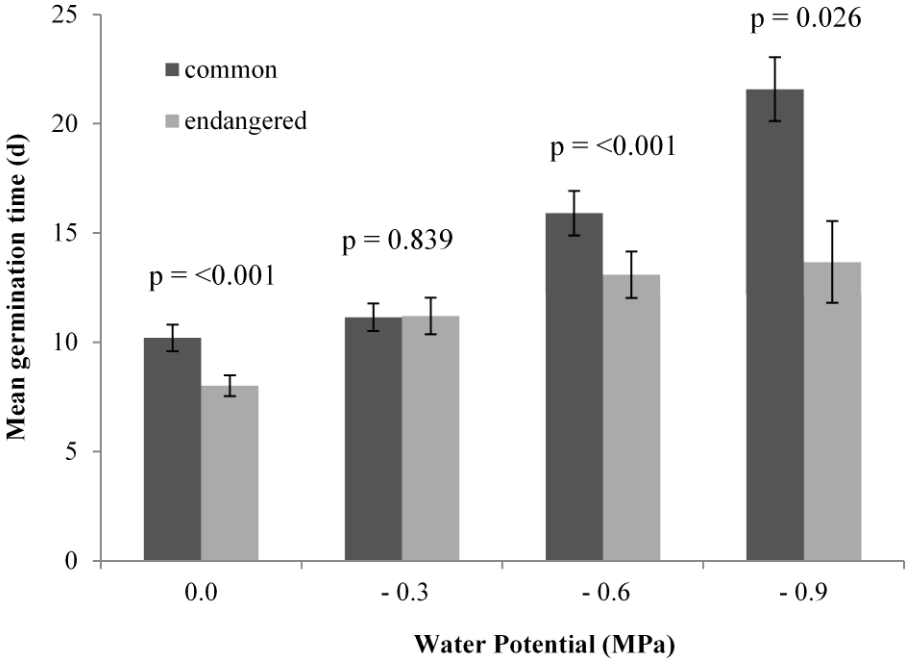

Fig 3 Effects of Red List Status and water potential (WP) on *mean germination time* [d]. The bars show the original data with standard error. P-values for comparisons between common and rare species (within each level of water potential) are from a Tukey HSD test using the residuals of the initial ANOVA for the factor *family* (see Methods).

Synchrony

The two main factors explaining most of the variation in synchrony were *species* and *Red List Status*. The synchrony in germination of the endangered species had an average of Z=0.58 (SE=0.05) and for common species of Z=0.35 (SE=0.03), i.e. in case of the common species a lower osmotic potential led to a shorter germination period than in case of the endangered species (Fig 4). Most of the variance of the variable *synchrony* was explained by the error term (53 %). This suggests that the synchrony of germination is strongly influenced by other factors not studied here. Therefore, the results of this germination variable have to be interpreted with caution.

The described results of *total germination* (%) and *mean germination time* (days) revealed a clear connection between the germination behaviour of the study species and their Red List Status. The response patterns of these germination variables showed that in endangered arable weed species germination was lower albeit faster than in the common species. Additionally the endangered species tended to germinate more synchronously.

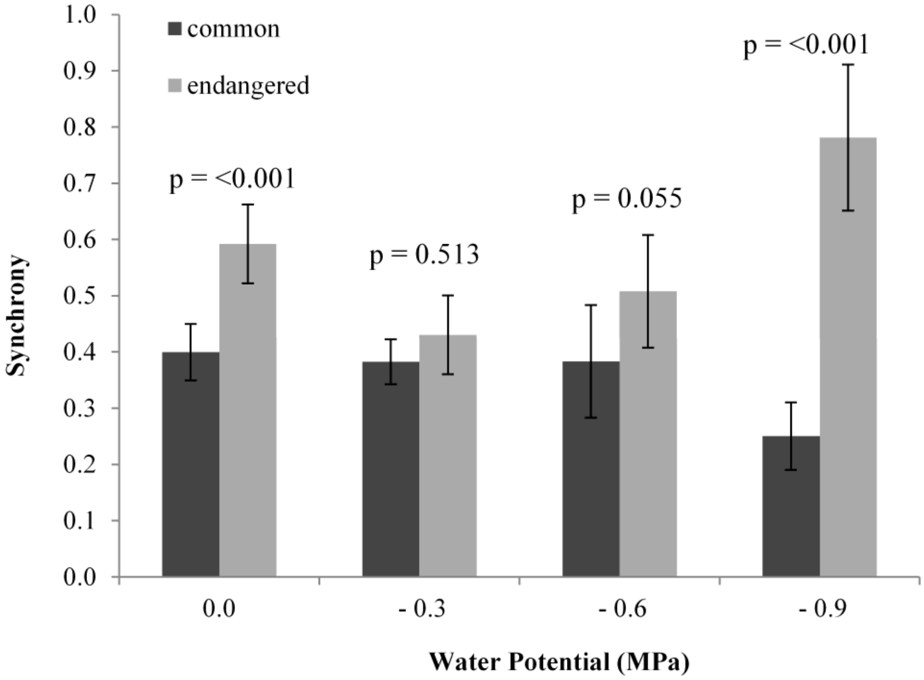

Fig 4 Effects of Red List Status and water potential (WP) on *synchrony*. The bars show the original data with standard error. P-values for comparisons between common and rare species (within each level of water potential) are from a Tukey HSD test using the residuals of the initial ANOVA for the factor *family* (see Methods).

Discussion

We investigated the response pattern of endangered arable weeds under different water supplies and different temperature regimes. The first aspect of interest was the specific response pattern of common and endangered arable weeds to reduced water availability. As expected, germination of all study species decreased and mean germination time increased with decreasing water availability. Furthermore, the data revealed a significant link between the reaction of arable weed species to water availability and their Red List status. This confirmed our first hypothesis. In response to declining water availability, there was a stronger decrease of germination of endangered species than of common species. In addition, the endangered arable weeds germinated faster and more synchronously. Thus, the germination peak was more pronounced and total germination showed a strong negative link to reduced water availability. The data confirm that endangered species often have smaller germination windows, more pronounced germination requirements and they show a lower plasticity. This is corroborated by a study demonstrating that common arable weeds through their germination syndrome, defined through several derived germination traits, may respond more flexible to abiotic stress than endangered species and even take advantage of changing conditions owing to global warming (Rühl *et al.* 2015). Since germination in endangered weeds takes place over a shorter period the risk for a complete failure of seedling establishment is higher than for common arable weeds, which spread this risk by germinating across a wider time window. Thus, common species are capable to establish even on sites characterised by unfavourable site conditions like short periods of drought, disturbance through tillage or herbicide application. These species are therefore traditionally predominant on arable sites and are able to persist under modern agriculture (Ellenberg and Leuschner 2010). This is in line with studies of Günter (1997), Patzelt (1998) and Schütz (2000) who showed that declining and endangered plant species possess inappropriate germination characteristics such as low germination rates or specific germination requirements in terms of light and temperature.

Our study was not able to show a significant connection between the temperature during germination and the Red List status of arable weeds. This suggests that the investigated annual species are able to react flexible to changes in temperature, which is in line with results of Otte (1994). Her extensive study on the germination requirements of 70 arable weeds revealed that the majority of the study species were capable to expand or shift their temperature range for germination as a response to changes in land use in the time period of 1948 to 1990. To investigate the future impact of increasing temperatures on endangered arable weed species, more differentiated temperature regimes than those selected in the current experiment might be necessary.

Still, our findings suggest that endangered arable weeds in Central Europe could be profoundly affected by the climatic developments in the future. Water availability not only interacts with the initial germination process, but also with the establishment of the seedling. Despite facilitative biotic interactions and distinct differences in species richness, canopy height or standing crop, survival and growth of seedlings strongly depended on water availability in grassland (Eckstein 2005) and dune systems (De Jong and Klinkhamer 1988). Additionally, a decelerated germination at low water potential leads to a reduced height of the seedling and a shorter radicle, which also translates to a higher vulnerability to drought (Akhalkatsi and Lösch 2001; Ruprecht *et al.* 2008).

It can thus be concluded that the extension of the growing season described by Menzel *et al*. (2006) probably has no positive influence on the occurrence of endangered arable weeds. The agronomic management measures are closely connected with the phenological development stages of the crops. Most of the arable weed species which were capable to adapt successfully to recent changes of land use will likely benefit from climatic changes leading to an extended growing season (Rühl *et al.* 2015). As the present study shows, common study species spread their germination over a longer time period, and additionally their total germination is higher than that of endangered species, at least at reduced water supply. The common species are less sensitive towards short-term unfavourable site conditions such as reduced water availability. Today common arable weeds are especially competitive species germinating in fall and are able to establish on nutrient-rich soils with high crop density (Hyvonen *et al*. 2003; Hawes *et al*. 2005; 2010). Thus, the establishment of arable weeds is not only connected with favourable conditions for germination but also for the completion of their life cycle (Otte *et al.* 2006). Especially the importance of the agricultural management was shown in numerous studies (Albrecht 2005; van Elsen 2000). Schubert *et al.* (2003) suggest that the common weed *Viola arvensis* adapted to modern land use management through extending its germination range with respect to temperature and with higher seed production. As a consequence, this species germinates in short periods between herbicide application, harvest and tillage to proliferate. Similar interactions between climate change and land use change with regard to the species richness of grassland are highlighted by Bütof *et al.* (2012).

The set-up of a conservation strategy for endangered species in the agricultural landscapes of Central Europe has to consider both, the impact of land use change and climate change. Climate change will further enhance the decline of several arable weed species initiated through land use changes. On the one hand it will – as our experimental results suggest – directly influence the fitness of weed populations, on the other hand it can be assumed that climatic changes will indirectly affect the arable weeds by climate change induced land use changes (e.g. new crop species and corresponding management systems due to a northward shift of cropping zones).

Our results highlight that endangered arable weed species are in particular sensitive to the effects of climate change. Especially changes of water availability during the germination period may lead to a total failure of seedling emergence. As the consequences of climate change are not avoidable, the conservation efforts have to focus on measures to compensate these negative effects. Conservation measures like ex-situ propagation in botanical gardens and reserves cultivated according to traditional agriculture have only local impact. Accordingly, the establishment of a nationwide network of conservation fields for arable weeds gives these species only punctual relief. In contrast, nature conservation programs at the level of federal states aiming at the establishment of field margins excluded from fertilisation and application of herbicides are quite successful in re-establishment endangered arable weed species, as they have already proven in the past (Marshall and Moonen 2002).

Especially species occurring in small and isolated populations call for appropriate conservation measures. In addition to impacts of land use (e.g. herbicide application, tillage), they are particularly vulnerable to deleterious climate change induced events on small spatial scale (e.g. drought, Thuiller *et al.* 2005). Since already in today's landscapes suitable habitats of arable weeds are rarely found near their current occurrence, only human support will ensure their spread to sites suitable under future climatic conditions (Pfeifer-Meister *et al.* 2013). For this purpose adapted conservation and restoration strategies are required to preserve arable weeds at the landscape level.

Acknowledgements

We thank Josef Scholz-vom Hofe, Lena Kretz and Laura Satkowski for their assistance in data collection in the lab. This work was funded by a graduate scholarship of the Justus Liebig University, Giessen. We confirm, to have no conflict of interest.

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Chapter 3

Future challenge for endangered arable weed species facing global warming: Low temperature optima and narrow moisture requirements

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Chapter 2 has shown that there is a link between the reaction of arable weed species to water availability and their Red List status. The results suggests that endangered species often have smaller germination windows, more pronounced germination requirements and show lower plasticity against reduced water availability. As drought and high temperatures are often co-occurring it is important to understand the impact and interaction of both stress factors on plants. The interaction of decreasing water potential and increasing temperature is the topic of the present chapter.

Abstract

As a result of the intensification of agriculture in Central Europe, many arable weed species have declined. Global climate change may further challenge the adaptability of arable weeds since plants may be more often subjected to higher temperatures and lower soil moisture during the germination period.

A climate chamber experiment analysed the response of four familial pairs of common and endangered arable weeds from Germany. To this end we used a large range of temperatures and water potentials to assess specific traits defining their germination requirements. Using a simple response surface approach, we predicted germination response under three climate change scenarios.

Results supported our expectation that endangered species, owing to their narrow germination requirements, may be more negatively affected by global warming than common species. Endangered species germinated significantly less than the common arable weeds, except at very low temperatures (3 °C and 5 °C). The preference of endangered arable weed species for low germination temperatures was confirmed by their low optimal germination temperature (15.8 °C ± 0.4). In contrast, common species germinated at significant higher temperatures (optimal temperature 18.4 °C ± 0.2), had a significantly wider range of germination temperature (endangered: 24 °C ± 3.5, common: 31 °C ± 0.5) and were also more flexible towards changes in water potential.

Calculations based on response surfaces for three climate change scenarios indicated that endangered arable weed species may benefit less from climate warming than common species.

Keywords: biodiversity, climate change, extinction risk, germination niche, rare plant species, temperature, water availability

Introduction

With about 25 % of the land area being used as crop fields, Central Europe is characterised by arable landscapes (Eurostat 2014), which are important for biodiversity. However, as a result of agricultural intensification, populations of arable weeds have declined dramatically during the last century (Sieben and Otte 1992; Storkey *et al*. 2011, Meyer *et al*. 2013; Simmering *et al*. 2013). Arable weeds are very closely connected to agricultural management. Long-term co-evolution with the crops turned them into specialists for arable sites, closely adapted to the associated land use practices (Otte *et al.* 2006). Since recent land use changes strongly affected environmental conditions on crop fields, many arable weeds currently have an extremely high extinction risk and weeds are one of the most endangered species groups in Europe (Meyer *et al*. 2013).

However, not all plant species are affected equally. In fact, a shift in the spectrum of species has been observed. Spring germinating species which are herbicide susceptible and less nitrophilous have decreased recently, while competitive, nitrophilous and autumn germinating species have benefitted from agricultural intensification (Hawes 2010). Competitive weeds that expand their distribution range efficiently use the good nutrient and water supply on arable fields despite a high density of the crop stands (Otte *et al*. 2006). Today, these few generalists are common in agricultural landscapes. At the same time many arable weeds are endangered or even threatened by extinction because of unfavourable environmental conditions (Storkey *et al*. 2011).

With respect to abiotic conditions, climate scenarios predict an increase of annual mean temperatures by 1.5 °C to 4 °C by the end of 21th century (IPCC 2013) and an increased frequency of extreme meteorological conditions (Walck *et al*. 2011). As a consequence, plants may be more often subjected to high temperatures and low soil moisture during the growing season in spring and summer (Knapp *et al*. 2008). Therefore, global climate change may present a further threat to the survival of arable weeds (Thuiller 2005; Dreesen *et al*. 2012).

Germination is a crucial stage especially for annual species. As temperature and water availability control germination of seeds (Baskin and Baskin 2001), changes in these factors may have major impacts on the establishment and survival of plant populations (Hedhly *et al*. 2008, Walck *et al*. 2011). Temperature is an important local trigger allowing seedlings to emerge when the most favourable environmental conditions prevail (Cochrane *et al.* 2011). Consequently, due to an expansion of the growing season (Menzel *et al.* 2006) some species may be able to germinate at an earlier date in the year. However, despite matching temperature, these seedlings may encounter unfavourable conditions with respect to diurnal temperature fluctuations or water availability because they germinated in the ‘wrong’ season (Cochrane *et al.* 2011, Mondoni *et al.* 2012). Additionally, higher temperatures combined with less precipitation may prevent germination and seedling emergence completely.

The Red List Status of arable species suggests that their adaptability towards recent changes in land use has reached its limit. This status may not only be caused by agricultural intensification but partly also by a growing mismatch between germination requirements and prevailing environmental conditions (Cochrane *et al.* 2011). If this is true, it is very likely that these species will be particularly sensitive to further changes in temperature or water availability due to global climate change. Endangered arable weeds showed significantly lower germination percentage in response to reduced water potential at two fluctuating temperatures and their germination occurred in a smaller time window (characterised by mean germination time and synchrony) as compared to common species (Rühl *et al.* in press). As drought and high temperatures are often co-occurring it is important to understand the impact and interaction of both stress factors on plants. Such information is essential to understand the response of endangered arable weed species to future changes in environmental conditions and for the development of successful conservation programs. Still, there is a dearth of studies that have addressed this topic.

The aims of this experimental study on arable weeds were (1) to compare germination of endangered and common arable weeds in response to a broad range of constant temperatures and water potentials, (2) to quantify the impact of different future climate scenarios on the germination success of endangered and common arable weeds and (3) to characterize and compare the germination syndrome of endangered and common arable weeds based on derived germination traits. To this end, we set up a multifactorial climate chamber experiment to study the impacts of temperature and water availability on germination of four familial pairs of common and endangered arable weed species.

Materials and Methods

2.1 Study species

We chose four familial pairs of common and endangered arable weed species (Tab 1) to ensure a phylogenetic balanced design and therefore a higher explanatory power of the results (Gitzendanner and Soltis 2000). The Red List Status of these species referred to the Red List of threatened plant species in Germany (Ludwig and Schnittler 1996). The seeds for the experiment were obtained from a commercial supplier of autochthonous seed material (Rieger and Hoffmann GmbH, Blaufeld-Raboldshausen, Germany).

2.2 Experimental Design

To investigate the effects on germination, we used a factorial experimental design with *species* (k=8) nested in *status* (k=2; common vs. endangered), *water potential* (k=5; 0, -0.3, -0.6, -0.9, -1.2 MPa) and *temperature* (k=8; 3, 5, 10, 15, 20, 25, 30, 35 °C).

The water potentials were manipulated by using Mannitol (D-Mannitol, Euro OTC Pharma GmbH, Bönen, Germany). Water potentials of -0.3, -0.6, -0.9 and -1.2 MPa were prepared with Mannitol concentrations of 0.12, 0.24, 0.36 and 0.48 mol l^{-1} (according to Ludewig *et al*. 2014). As a control, distilled water was used for full water availability (0 MPa). For each treatment combination five replicates were set up, resulting in a total of 1 600 experimental units.

In a Petri dish fifty seeds were placed on one layer of filter paper moistened with 3 mL of D-Mannitol solution or distilled water. To minimize evaporation, five Petri dishes were sealed together in a plastic bag. After 14 days of stratification at 3 °C in darkness in climate chambers (Rumed type 3401, Rubarth Apparate GmbH) the prepared Petri dishes were moved into climate chambers with constant temperatures of 3, 5, 10, 15, 20, 25, 30 and 35 °C with a 16-h day/8-h night cycle (Otte 1996). Seeds were checked for germination twice a week. Germinated seeds were counted and removed at the time when the radicle was observable. After five weeks germination almost ceased and the experiment was finished.

Tab 1 Study species with information on plant family, floristic status, life cycle, Red List Status, viability (%) and germination capacity (%) of the seeds.

Species	Family	Floristic Status [a]	Life Cycle [b]	Red List Status [c]	Viability [%] [d]	Capacity [%] [e]
Anthemis arvensis L.	Asteraceae	Archaeophyt	summer and winter annual	common	94 ± 2.83	65 ± 7.07
Glebionis segetum (L.) Fourr.		Archaeophyt	summer annual	endangered (V)	92 ± 5.66	52 ± 5.66
Silene latifolia Poir.	Caryophyllaceae	indigenous	perennial*	common	88 ± 5.66	54 ± 5.66
Silene noctiflora L.		Archaeophyt	summer and winter annual	endangered (V)	90 ± 2.83	94 ± 8.49
Daucus carota L.	Apiaceae	indigenous	biennial	common	92 ± 5.66	63 ± 12.73
Bupleurum rotundifolium L.		Archaeophyt	summer annual	endangered (1)	98 ± 2.83	72 ± 8.49
Campanula rapunculoides L.	Campanulaceae	indigenous	perennial*	common	86 ± 2.83	87 ± 1.41
Legousia speculum-veneris (L.) Chaix		Archaeophyt	summer and winter annual	endangered (3)	94 ± 2.83	84 ± 2.83

[a] Floristic Status according to FloraWeb (2014)

* under arable use maximum biennial

[b] Life cyle according to Jäger and Werner (Ed.) (2005)

[c] Red List of threatened plant species in Germany, respectively Hesse (*): V = premonition list, 3 = threatened, 1 = threatened with extinction

[d] Viability tested with a tetrazolium chloride test with 25 seeds per replicate (n=2), mean ± SD

[e] Germination capacity tested in a cold greenhouse, 50 seeds per replicate (n=2), mean ± SD

2.3 Total germination

First we calculated for each experimental unit (i.e. Petri dish) the total germination (expressed as %), as the number of all germinated seeds divided by the initial number of seeds per Petri dish (50 seeds). Before analysis, total germination was arcsine-square-root-transformed to improve normal distribution and variance homogeneity (Quinn and Keough 2002). With the transformed dataset a single factor variance analysis (ANOVA) with the factor family was carried out to account for potential phylogenetic effects ($F_{3,\ 1596} = 25.2$, $p = <0.001$). All further calculations were performed with the residuals of this ANOVA. The effects of the single factors and the factor combinations of *species* (nested in *status*), *status*, *temperature* and *water potential* were assessed with a multi-factorial hierarchical ANOVA. Subsequently, significance of differences between treatments was assessed by employing a Tukey-HSD. To avoid zero inflation and missing data, the water potential -1.2 MPa was excluded from the statistical analysis. All statistical analyses were carried out using the programme STATISTICA (ver. 10.0, Statsoft Inc., Tulsa, OK, USA).

2.4 Germination traits

For a characterisation of the germination syndrome of common and endangered species we calculated for every level of the factor water potential the following traits (according to Olff *et al.* 1994; Hölzel and Otte 2004; cf. Table 4) for each species: *Tmean* (°C), the mean temperature for germination, calculated as weighted average of total germination over all constant temperatures:

$$Tmean = \sum_{i=1} n_i t_i \Big/ \sum_{i=1} n_i \qquad \text{(eq.1)},$$

where n_i is the total germination at temperature i and t_i is the constant temperature i in °C. *T_Gmax(final)* (%), the maximum total germination, was defined as the highest total germination in any of the constant temperatures at the end of the experiment. *T_Gmax(8d)* (%) was the maximum total germination after 8 days in the climate chamber. *Tmax(final)* (°C) was the temperature at which *T_Gmax(final)* was reached and *Tmax(8d)* (°C) was the temperature at which *T_Gmax(8d)* was reached. *T_Amp* (°C) was the range between the lowest (*T_Low*) and the highest temperature (*T_High*) with at least 5 % total germination. Similar to these values we calculated for every constant temperature (k=8): *WP_Gmax(8d)* (%), i.e. the maximum total germination after 8 days across all water potentials, and

WP_Gmax(final) (%), i.e. the maximum total germination at the end of the experiment. Similarly, *WPmax(8d)* and *WPmax(final)* were the water potentials at which *WP_Gmax(8d)* and *WP_Gmax(final)* were reached, respectively. *WP_Amp* (MPa) was the range between the lowest (*WP_Low*) and the highest water potential (*WP_High*) with at least 5 % total germination. *WPmean* (MPa), the average water potential for germination, was calculated as weighted average of total germination over all water potentials:

$$WPmean = \sum_{i=1} n_i w_i \Big/ \sum_{i=1} n_i \qquad \text{(eq.2),}$$

where n_i is the total germination at water potential i and w_i is the water potential i in MPa. Some of the traits were excluded from further analyses since their values were zero for all species (cf. Tab 4).

A principal-component-analysis (PCA) was carried out to reveal the correlation structure of the various germination traits. To test for differences of the germination traits between common and endangered species, we conducted a multi-factorial ANOVA for each temperature-based value with the factors *status* (k=2; common vs. endangered) and *water potential* (k=4; 0, -0.3, -0.6, -0.9 MPa) and for each water potential-based value with the factors *status* (k=2; common vs. endangered) and *temperature* (k=8; 3, 5, 10, 15, 20, 25, 30, 35 °C). The principal-component-analysis (PCA) was calculated with PC-Ord (ver. 5.32, MjM Software, Oregon, USA).

2.5 Modelling

The results of the present germination experiment enabled us to predict the response both of common and endangered arable weed species under future conditions of temperature increase. To this end, we fitted quadratic surfaces to the measured germination data by ordinary least squares regression, and then replaced temperatures in the regression equations by data from Representative Concentration Pathway scenarios (RCP) for the end of the 21st century of the Intergovernmental Panel on Climate Change (IPCC 2013). Total germination was specified as dependent variable; temperature and water potential were specified as predictor variables. The regression models included quadratic terms and the interaction temperature x water potential to allow for non-linear relationships between dependent variable and predictor variables. To depict the differences in the germination response of endangered and common arable weeds the regression models were superimposed by subtracting total germination (%) of the endangered arable weeds from total germination (%) of the common arable weeds. The Statistica software package (ver. 10.0, Statsoft Inc., Tulsa, OK, USA) was used to build the regressions.

Results

3.1 Total germination

The ANOVA of germination percentage revealed significant effects of all experimental factors and their interactions (p < 0.001, Tab 2). The main effects *species*(*status*), *status*, *temperature* and *water potential* explained 62 % of the variation of the germination data. The effect *status* and its interactions accounted for 10 % of the total variance. *Water potential* (25.1 %) had a slightly stronger effect on total germination than *temperature* (18.3 %), but they range in a comparable order of magnitude.

Tab 2 Effects of *species* (nested in *status*), *status*, *water potential* and *temperature* on total germination (%); including water potentials 0, -0.3, -0.6, -0.9 MPa, df = degree of freedom, F = variance ratio, p = error probability, vc (%) relative contribution of individual factors and their interactions to total variance.

	df	**MQ**	**F**	**p**	**vc (%)**
Intercept	1	12.04	1864.0	<0.001	
species (status) [S(St)]	6	3.88	600.5	<0.001	12.7
status [St]	1	10.19	1578.6	<0.001	5.6
temperature [T]	7	4.81	744.2	<0.001	18.3
water potential [WP]	3	15.33	2373.6	<0.001	25.1
S (St) x T	42	0.63	98.2	<0.001	14.5
S (St) x WP	18	0.38	59.1	<0.001	3.7
St x WP	3	0.11	16.4	<0.001	0.2
St x T	7	0.90	139.0	<0.001	3.4
T x WP	21	0.37	57.9	<0.001	4.3
St x T x WP	21	0.10	15.3	<0.001	1.1
S (St) x T x WP	126	0.11	16.8	<0.001	7.5
error	1024	0.01			3.6

Red List status had a strong effect on total germination (%). Endangered species showed generally significantly lower germination than common arable weeds, except for the treatments with low temperatures (3 °C and 5 °C) and high water potentials (0 MPa and -0.3 MPa). In these cases germination of endangered species was equal or higher than that of common species (Fig 1). The difference between the germination response of endangered and common species demonstrates that pattern even more clearly. Subtracting total germination (%) of the endangered arable weeds from total germination (%) of the common arable weeds revealed that endangered species germinate better under cold conditions; while over a large range of temperature and water potential constellations common species are superior (Fig 2).

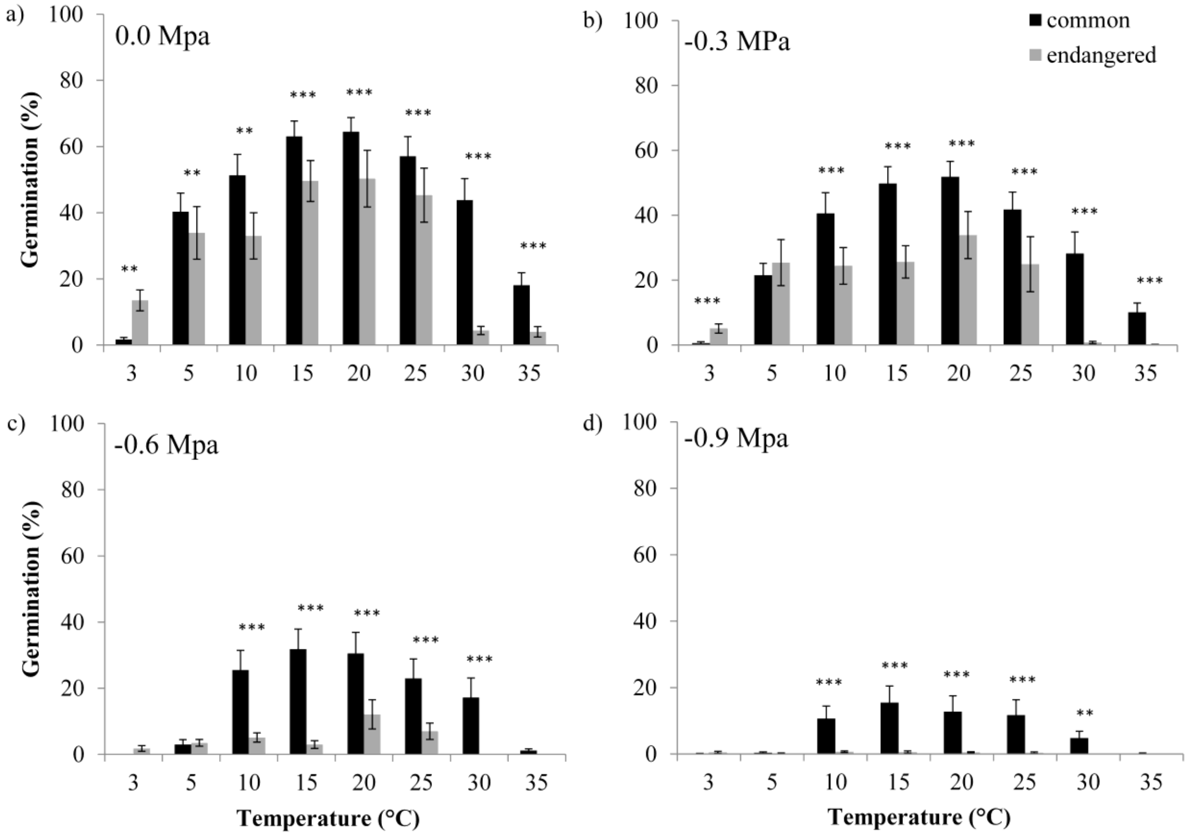


Fig 1 Effect of Red List status on total germination (%) of common and endangered arable weed species depending on temperature (°C) and water availability (MPa). Mean of the original data with standard error. Statistically significant differences within each temperature are marked with asterisks: *** level of significance α = 0.1 %, ** level of significance α = 1.0 %.

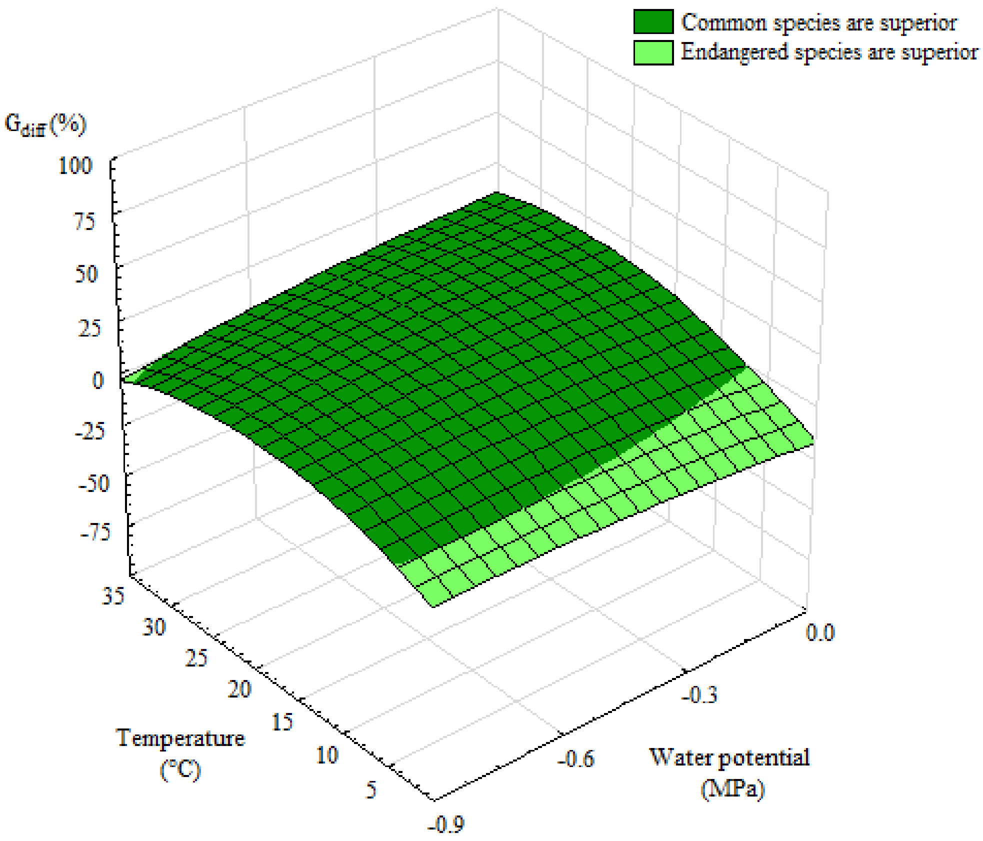


Fig 2 Differences in the germination response (G %) of common and endangered arable weed species to different temperatures (°C) and water potentials (MPa). Regression surfaces (see Methods) of endangered and common arable weeds were superimposed by subtracting total germination (%) of endangered from total germination (%) of common species.

3.2 Germination traits

The preference of endangered arable weed species for lower germination temperatures is confirmed by the mean temperature for germination (Fig 3). Common arable weeds in this study showed significantly higher mean germination temperatures at water potentials of -0.3 MPa ($p = 0.003$) and -0.6 MPa ($p < 0.001$). At full water availability, common species had a mean germination temperature of 18.4 ± 0.22 °C (mean ± SE), whereas endangered species had a mean germination temperature of 15.8 ± 0.4 °C. Additionally, the analysis indicated that endangered species showed a stronger decrease in the mean germination temperature with decreasing water potential than common species (Fig 3).

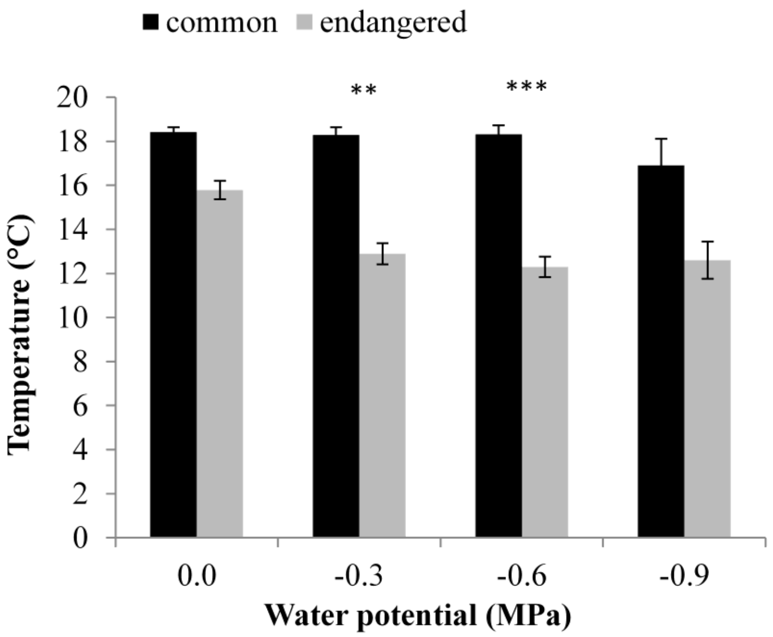


Fig 3 Mean optimal germination temperature (°C) at different water potentials (MPa) for endangered and common arable species.

The PCA revealed the correlation structures of the temperature-based and the water potential-based germination traits (Fig 4, Tab 3). There was a clear pattern for the temperature-based germination traits (Fig 4a). The first axis (explaining 58 % of the variance) grouped species with high (*T_Gmax(final)*) and rapid (*T_Gmax(8d)*) germination even at high temperatures (*Tmax(final), Tmax(8d), T_High*) on the left and species with low and slow germination on the right. The latter species are also characterised by lower mean germination temperatures (*Tmean*) and a narrower range of germination temperatures (*T_Amp*). Since decreasing water availability influenced all investigated species the aforementioned pattern is affected by the differing water potentials. High water potentials (WP0=0.0 MPa and WP1=-0.3 MPa) are concentrated in the lower left ordination space while the lower water potentials (WP2=-0.6 MPa and WP3=-0.9 MPa) are arranged towards top right. The impact of the water potential is reflected by the germination success of the investigated species (*T_Gmax(final)* and *T_Gmax(8d)*). This influence of decreasing water potential resulted in a diagonal arrangement in the ordination graph according to the Red List Status of species (Fig. 4a). Only *Silene noctiflora* deviated from this general pattern. Along axis 2, explaining 21 % of the variance, common and endangered species were arranged according to their preference of low germination temperatures (*T_Low*) and the temperature range for germination (*T_Amp*).

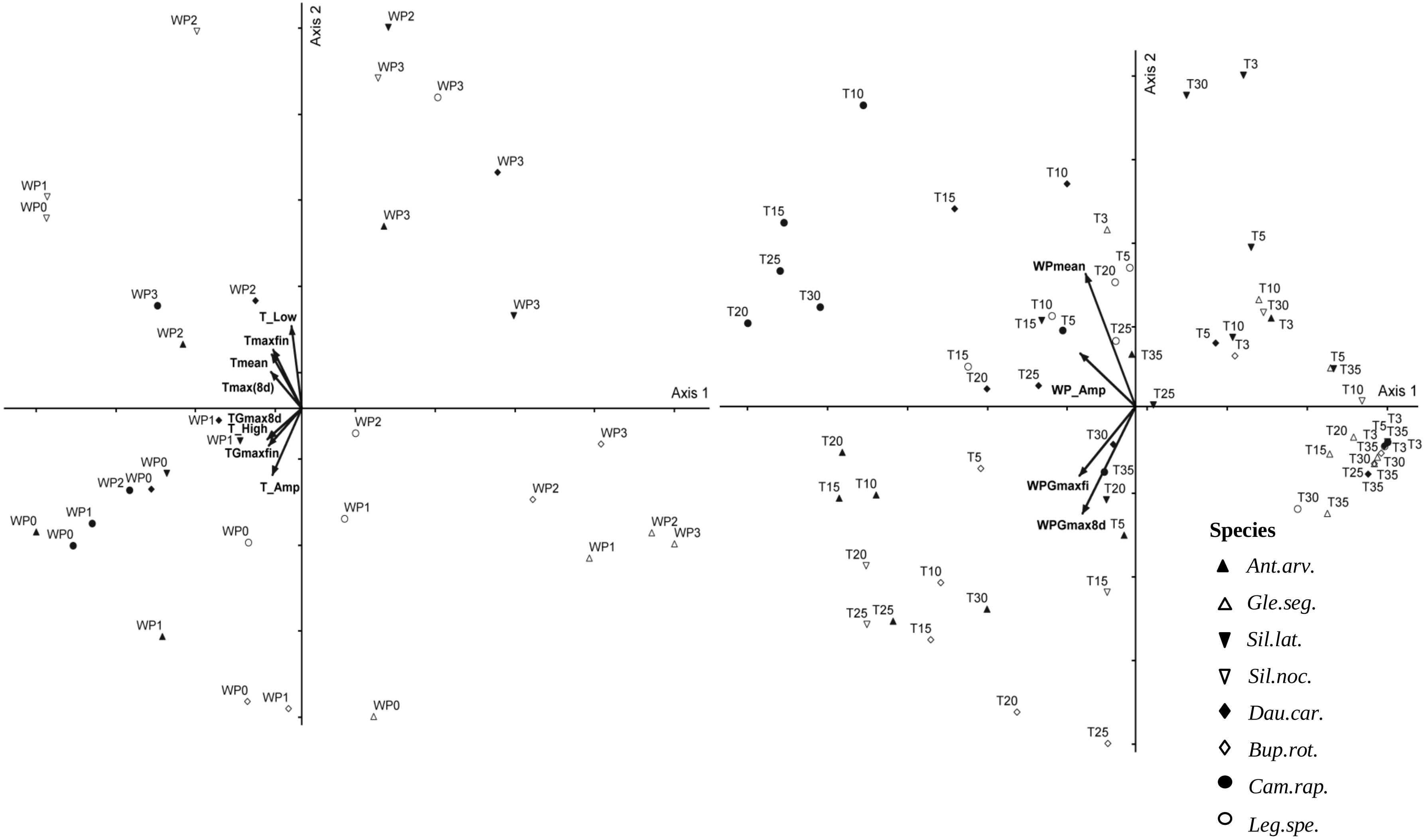
The PCA of the water potential-based germination traits demonstrated the impact of decreasing water potential in more detail (Fig. 4b). The first axis, explaining 80 % of the variance, separated the species with high and rapid germination (*WP_Gmax(8d)*, *WP_Gmax(final)*) and high flexibility for water potential (*WPmean*, *WP_Amp*) from those with low and slow germination and a narrow range of water potential for germination. Additionally, the impact of differing water potentials was clearly influenced by temperature. While all species were very similar at high and low germination temperatures (3 °C, 30 °C and 35 °C) they were clearly separated at mean temperatures by their flexibility towards decreasing water potential (*WPmean*) as the separation by axis 2 showed (explaining 13 % of the variance). Common species tended to be on top left side and endangered species were positioned on the down left side of the ordination diagram (Fig 4b).

Common species germinated at significantly higher temperatures than endangered species (*Tmean*, *Tmax(8d)* and *T_High*) and they had a significantly wider range of germination temperature (*T_Amp*). Additionally, common arable weeds were more flexible than endangered species towards changes in water potential (*WP_Amp*, *WPmean* and *WP_Gmax(final)*).

Tab 3 Correlation between factor axes of PCA ordination and a) temperature based and b) water potential based germination traits. Correlation values greater than 0.5 are shown in bold.

a)	Axis 1	Axis 2	Axis 3	b)	Axis 1	Axis 2	Axis 3
Eigenvalue	4.65	1.67	0.79	Eigenvalue	3.22	0.52	0.17
Variance (%)	58.09	20.92	9.81	Variance (%)	80.46	12.87	4.11
Tmean	**-0.77**	0.46	0.30	*WPmean*	**-0.84**	**0.50**	0.22
T_Gmax(final)	**-0.86**	-0.33	-0.17	*WP_Gmax(8d)*	**-0.89**	-0.40	0.13
T_Gmax(8d)	**-0.88**	-0.27	-0.11	*WP_Gmax(final)*	**-0.93**	-0.26	-0.02
Tmax(final)	**-0.74**	**0.51**	0.06	*WP_Amp*	**-0.94**	0.20	-0.31
Tmax(8d)	**-0.79**	0.32	0.42				
T_Low	-0.26	**0.72**	**-0.63**				
T_High	**-0.86**	0.27	-0.26				
T_Amp	**-0.74**	**-0.58**	-0.01				

Fig 4 Graphs of the PCA-Ordinations of the a) temperature based germination traits (water potentials: WP0=0.0, WP1=-0.3, WP2=-0.6, WP3= -0.9 MPa) and b) water potential based germination traits of the investigated arable weeds (temperatures: T3=3, T5=5, T10=10, T15=15, T20=20, T25=25, T30=30 and T35=35 °C). Filled symbols = traits of common species, unfilled symbols = traits of endangered species.



Tab 4 List of the germination traits and their units. Temperature-based traits were obtained for each level of the factor *water potential* (factor levels = 4) and water potential-based traits for each level of the factor *temperature* (factor levels = 8) for each species. Indicated are means with standard error for water potential 0.0 MPa for the temperature-based and for 20°C for the water potential-based traits. Germination traits with significant difference (ANOVA, $p \leq 0.05$) between common and endangered species are shown in bold.

temperature-based	**common**	**endangered**	**water potential-based**	**common**	**endangered**
Tmean **[°C]**	**18.4 ± 0.2**	**15.8 ± 0.4**	***WPmean*** **[MPa]**	**0.25 ± 0.06**	**0.16 ± 0.03**
T_Gmax(8d) [%]	73.0 ± 11.9	66.0 ± 17.6	*WP_Gmax(8d)* [%]	71.0 ± 10.9	53.5 ± 21.7
Tmax(8d) **[°C]**	**21.3 ± 1.25**	**14.5 ± 4.5**	*WPmax(8d)* * [MPa]	0.0	0.0
T_Gmax(final) [%]	78.0 ± 11.0	70.5 ± 16.4	***WP_Gmax(final)*** **[%]**	**75.5 ± 9.8**	**53.5 ± 21.7**
Tmax(final) [°C]	21.3 ± 1.3	12 ± 5.1	*WPmax(final)* * [MPa]	0.0	0.0
T_Amp **[°C]**	**31 ± 0.5**	**24 ± 3.5**	***WP_Amp*** **[MPa]**	**0.75 ± 0.19**	**0.38 ± 0.14**
T_Low [°C]	4.5 ± 1.0	6.5 ± 5.7	*WP_Low* [MPa]	-0.75 ± 0.19	-0.38 ± 0.14
T_High **[°C]**	**35 ± 0.0**	**30 ± 2.0**	*WP_High* * [MPa]	0.0	0.0

* germination traits were not further used for analyses because values always were zero

3.3 Germination and global warming: the IPCC scenarios

Regression analysis of the germination data resulted in the following model equations describing the response of total germination to temperature and water potential:

$$f\,(wt)_{common} = 12.9 + 47.8\,w + 4.5\,t + 13.8\,w^2 + 0.2\,wt - 0.1\,t^2$$

(eq.3)

$$f\,(wt)_{endangered} = 26.0 + 64.8\,w + 1.6\,t + 25.8\,w^2 - 0.5\,wt - 0.1\,t^2$$

(eq.4),

where *w* = water potential and *t* = temperature.

The IPCC (2013) warming projections for the end of the 21st century vary between an increase of 1.5 °C (‘likely to exceed’, models RCP4.5, RCP6.0, RCP8.5), 2 °C (‘likely to exceed', models RCP6.0, RCP8.5) and 4 °C ('unlikely to exceed', models RCP2.6, RCP4.5, RCP6.0 and 'about as likely as not to exceed', model RCP8.5).

To estimate the future germination potential of common and endangered arable weed species under increasing temperature conditions we assumed an average spring temperature in Germany of 7.7 °C (DWD 2014). As three possible future scenarios we used the three stages of the IPCC warming projections: 1.5 °C = scenario I, 2 °C = scenario II and 4 °C = scenario III.

Field capacity of soils lies between -0.006 and -0.03 MPa, depending on the soil type (Scheffer and Schachtschabel 2002). Thus, further assuming an average of -0.018 MPa for spring, when the majority of arable weeds germinate, results in a total germination of 32.5 % (eq.3) and 30.6 % (eq.4) for common and endangered species, respectively, in the reference period. The difference between common and endangered species increases with increasing temperature. Scenario III with an increase of temperature in spring of 4 °C would lead to a total germination of 40 % of the common weeds and 32 % of the endangered weeds (Tab 5).

Tab 5 Modelled total germination (%) of common and endangered arable weeds for the reference period 1961-1990 and three possible future scenarios.

IPCC (2013)	1961-1990	scenario I	scenario II	scenario III
Spring temperature	7.7°C	9.2°C	9.7°C	11.7°C
Common arable weeds	32.5	35.6	36.5	39.8
Endangered arable weeds	30.6	31.3	31.5	32.1

Discussion

Our results revealed clear differences in germination syndromes between endangered and common arable weed species. Common arable weeds showed a significantly higher flexibility towards changes in water potential than endangered species (Fig 1). Ludewig *et al*. (2014) emphasize that the combination of germination requirements for temperature and water potential probably allows seeds to avoid warmer periods during which limiting water availability owing to increased evaporation is more likely to occur. Analogously, the high flexibility common arable weeds show towards water potential during germination in the present study may enable them to germinate also under (short-term) limited water availability in spring. To minimize the potential risk of a complete failure of all seedlings, common arable weeds possess an asynchronous germination strategy with a significantly longer mean germination time than endangered arable weeds (Rühl *et al.* in press).

Endangered species germinated significantly less and showed a narrower and lower range of germination temperatures than common arable weeds. Only at very low temperatures did these differences disappear (Fig 2). This is also confirmed by the significantly lower optimal temperature for germination of endangered species compared to common species (Fig 3). A tendency to lower germination temperatures of rare arable weeds was also found by Otte (1994): species with a strong commitment to cold germination temperatures and to short periods for seedling emergence strongly decreased from 1948 to 1991, whereas the distribution of species with a wide temperature range for germination increased. Successful arable weeds like *Stellaria media* and *Chenopodium ficifolium* showed an expansion of their temperature amplitude to higher temperatures (Otte 1994). Similar flexibility of the temperature amplitude was observed for *Viola arvensis*, which was able to adapt to intensive land use management through increased seed production and an expansion of the temperature amplitude for germination (Schubert *et al.* 2003). This enabled the species to take advantage of temporal gaps between herbicide application, harvest and tillage for successful germination. Thus, germination temperature plays a major role for the successful establishment of arable weed species (Otte 1996). Currently successful arable weeds show high adaptability to changing environmental conditions (Otte *et al.* 2006). However, adaptation is only possible to a species-specific extent and rate (Baker 1974). In arable weeds, endangered species are characterised by an unfavourable genetic structure, i.e. high differentiation between populations and low genetic diversity (Brütting *et al.* 2012). This may indicate that relatively recent threats such as habitat loss and fragmentation have reduced the evolutionary potential that will be needed for adaptation in the face of climate change.

The relation of germination success to water potential and temperature, described in the presented model (Tab 5), suggests that the higher germination flexibility of common species enables them to take advantage of increasing temperatures during their germination period. Germination flexibility is enhanced by high optimal germination temperatures and wide temperature amplitudes since these are the characteristic germination traits of species that expanded their distribution area during the last decades (Otte *et al*. 2006). Endangered species germinate better under cold conditions, while over a large range of temperature and water potential constellations common species have an advantage (Fig. 2). The significant differences concerning the germination traits *Tmean* [°C], *Tmax(8d)* [°C] and *T_High* [°C] revealed a different position and the significant differences in *T_Amp* [°C] revealed a different size of the amplitude of germination temperature between common and endangered species. As a consequence, the Red List status of arable weeds seems to be at least partly related to their specific germination requirements. Under future climatic conditions the complex relationship between temperature and soil moisture may lead to further decrease of endangered species, since they are more likely to be replaced by more flexible common arable weeds, which are able to deal successfully with increasing temperatures. The importance of optimal temperature or temperature amplitude for germination and its relations to the Red List status are not only evident for arable weeds, but also for other plant species. Also for endangered species of the typical flora of floodplain-meadows a correlation between Red List status and germination requirements for temperature was observed (Hölzel and Otte 2004). There was a significant trend in endangered species towards higher temperature requirements and delayed, asynchronous germination; what is disadvantageous under subcontinental conditions of flood-meadows (Hölzel and Otte 2004).

It is generally accepted that a high competitive ability enables species to occupy sites with conditions close to their physiological optimum (Gurevitch *et al*. 2002; Ellenberg and Leuschner 2010). Since rare or endangered species are often much less competitive, they are more likely occupy sites at the edge of their physiological amplitude (Ellenberg and Leuschner 2010). Why have endangered arable weed species only narrower germination requirements and how did they survive in the past? Arable weeds are very closely connected to agriculture. The strong adaptation of morphological and ecological traits due to the co-evolution with the crops made them specialists for arable land use practices (Barrett 1983; Kornas 1988; Otte *et al*. 2006). The ongoing land use change induces a shift of site conditions away from those endangered species are adapted to. This specific adaptation is the reason for their high extinction risk (Storkey *et al.* 2011).

Conclusions for conservation

The complex interaction between arable management and germination requirements of arable weeds highlight that a change in only one of these factors or both simultaneously will accelerate the decline of many arable species in today's landscapes (Otte 1994). With the Convention on Biological Diversity (United Nations 1992) the global community has committed itself to manage the conservation and restoration of biodiversity. To increase biodiversity of agricultural landscapes and to stop the further decline of arable weed species, conservation measures for arable weeds should be explicitly intensified. Agro-environmental schemes like the German program '100 fields for biodiversity' (Meyer 2010) need to be expanded in order to come up from local release for the endangered species to a denser grid of weed conservation sites which would enable migration of arable weed species. Political tools like the concept of 'High Nature Value Farmland' (PAN, IFAB, INL 2011) and the 'Production Integrated Compensatory Measures' (Litterski *et al.* 2008) of the European Union can be helpful to conserve rare and endangered arable weeds at the landscape scale. Another possibility for building a large-scale conservation grid are field margin strip programs (Marshall and Moonen 2002) with focus on spontaneous vegetation at field margins without herbicides and fertilizers instead of seeded flower stripes. These conservation measures would give arable weed species greater margins to face future climatic changes. Accessibility of suitable sites and genetic exchange between populations will enable species to adapt to climate and land use change.

Acknowledgements

We thank Klemens Ekschmitt, Philipp Kraft, Kristin Ludewig and Gabriel A. Schachtel for helpful discussions and comments on the manuscript and the data analysis. Josef Scholz-vom Hofe, Laura Satkowski and Lena Kretz are gratefully acknowledged for their assistance in data collection in the lab. This work was funded by a graduate scholarship of the Justus-Liebig-University, Giessen. We confirm to have no conflict of interest.

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Appendix 1 Effects of status (St) and water potential (WP) on a) temperature based and b) water potential based germination traits.
df = degrees of freedom, F = variance ratio, p = error probability

a)

	Tmean				*T_Gmax(final)*				*Tmax(final)*				*T_Gmax(8d)*				*Tmax(8d)*			
	df	MQ	F	p	df	MQ	F	p	df	MQ	F	p	df	MQ	F	p	df	MQ	F	p
Intercept	1	**7876.7**	**332.8**	**<0,001**	1	**68450.0**	**87.4**	**<0,001**	**1**	**7075.6**	**128.0**	**<0,001**	1	**49141.1**	**65.7**	**<0,001**	**1**	**8972.3**	**221.9**	**<0,001**
St	1	**177.1**	**7.5**	**0.012**	1	722.0	0.9	0.347	1	250.0	4.5	0.042	1	1128.1	1.5	0.231	1	**287.1**	**7.1**	**0.014**
WP	3	8.0	0.3	0.798	3	**5899.7**	**7.5**	**0.001**	**4**	**51.5**	**0.9**	**0.459**	3	**5939.5**	**7.9**	**0.001**	**3**	1.4	0.0	0.991
St x WP	3	5.0	0.2	0.889	3	109.0	0.1	0.936	4	16.5	0.3	0.877	3	62.5	0.1	0.968	3	12.1	0.3	0.826
error	24	23.7			24	783.4			30	55.3			24	748.4			21	40.4		

	T_Amp				*T_Low*				*T_High*			
	df	MQ	F	p	df	MQ	F	p	df	MQ	F	p
Intercept	1	**6868.1**	**88.4**	**<0,001**	**1**	**1485.2**	**62.4**	**<0,001**	**1**	**14742.0**	**298.5**	**<0,001**
St	1	**502.1**	**6.5**	**0.019**	**1**	0.1	0.0	0.961	1	**648.3**	**13.1**	**0.002**
WP	3	**547.1**	**7.0**	**0.002**	**3**	**57.7**	**2.4**	**0.100**	**3**	**328.0**	**6.6**	**0.003**
St x WP	3	17.2	0.2	0.880	3	11.7	0.5	0.693	3	56.2	1.1	0.357
error	20	77.7			18	23.8			20	49.4		

b)

	WPmean				*WP_Gmax(final)*				*WP_Gmax(8d)*				*WP_Amp/WP_Low*			
	df	MQ	F	p	df	MQ	F	p	df	MQ	F	p	df	MQ	F	p
Intercept	**1**	**1.2**	**133.8**	**<0,001**	**1**	**111556.0**	**127.6**	**<0,001**	**1**	**71289.0**	**95.9**	**<0,001**	**1**	**9.3**	**98.4**	**<0,001**
St	**1**	**0.1**	**15.8**	**<0,001**	**1**	**3969.0**	**4.5**	**0.038**	1	2652.3	3.6	0.065	**1**	**0.6**	**6.6**	**0.015**
Temp	7	0.0	2.0	0.080	7	3659.4	4.2	0.001	**7**	**4215.9**	**5.7**	**<0,001**	7	0.2	2.0	0.085
St x Temp	7	0.0	0.8	0.577	7	502.7	0.6	0.773	7	394.5	0.5	0.807	7	0.1	0.9	0.504
error	43	0.0			48	874.4			48	743.5			35	0.1		

Chapter 4

Impacts of short-term germination delay on fitness of the annual weed *Agrostemma githago* (L.)

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manuscript

Chapter 2 and Chapter 3 have revealed a distinct germination response of endangered and common arable weeds to decreasing water availability and increasing temperatures. Additionally significant differences between mean germination time and synchrony of endangered and common arable weeds could be demonstrated. Timing of germination is especially critical to plant performance in habitats like arable fields which are subject to frequent disturbances. Since it is unclear whether small deviations in germination date translate into fitness differences in the course of the life cycle, the aim of the present chapter was to quantify the effects of short germination delays on plant performance.

Abstract

Time of seedling emergence is an important step in the life-cycle of annual plants because it may determine subsequent performance and success. Timing of emergence is especially critical to plant performance in habitats like arable fields which are subject to frequent disturbances. Within-season variation in timing of germination of only a few days is typically for many arable weeds. However, since it is unclear whether such small deviations in germination date translate into fitness differences in the course of the life cycle, the aim of this paper was to quantify the effects of short germination delays on plant performance. We conducted a multi-factorial experiment to study the impact of delayed germination (1, 2, 3 and 7 days) in combination with competition on the fitness of the arable weed species *Agrostemma githago* (L.). We expected that delayed germination significantly reduces fitness in terms of several life history traits and that the decrease of fitness is higher in the presence of competition.

The results revealed a decrease of fitness for all investigated life history traits. Plants with delayed germination of seven days produced 25 % less shoots, 28 % less biomass, 16 % less flowers, 27 % lighter seeds and were 8 % lower as compared to control plants without delayed germination. Competition through barley pronounced this pattern and then fitness traits showed a sharp decrease from three to seven days of germination delay. Thus, early emerged seedlings have biologically significant fitness advantages over later emerged seedlings of the same species.

Introduction

The species-specific germination strategies of plants triggered by environmental factors like temperature, light and water supply are crucial for the establishment of species in changing landscapes (Schütz 2000; Baskin and Baskin 2001). Time of seedling emergence is an important step in the life-cycle of plants because it may determine subsequent performance and success (Harper 1977; Otte 1995; Weiner 1990). Perennial plant species do not need to spread the emergence risk temporally because they are more independent than annuals from temporal environmental variation due to their iteroparous reproduction (Rees 1996). However, in perennials early emergence is often related to higher fitness and fecundity in terms of seedling recruitment, survival, height, biomass and number of flowers (Cook 1986; De Luis et al. 2008; Verdú and Traveset 2005). Germination differences of 15 days have even been detectable three years later in the perennial *Viola blanda* (Cook 1986). For annuals the effect of early germination is not that clear. For example, early germinated seedlings of the winter annual *Collinsia verna* produced more fruits than later germinated seedlings (Kalisz 1986). Similarly, for subterranean clover, a delay in emergence of five days resulted in a reduction of biomass of about 50 % (Black and Wilkinson 1963). A more complex pattern was found for the summer annual *Tagetes micrantha.* Seedlings that emerged at the beginning of the season had lower probabilities of survival than seedlings emerging later in the season. On the other hand, those early seedlings that survived showed higher fecundity than seedlings emerging at the end of the season (González-Astorga and Núñez-Farfán 2000). The same pattern was found for *Heterosperma pinnatum*: early emergence resulted in greater mortality, but seedlings which germinated early and survived attained greater size and produced more seeds (Venable *et al.* 1987).

In regularly disturbed habitats timing of seedling emergence is especially critical to plant performance (Quintana *et al.* 2004). In arable fields a time window for seedling establishment is opened by cultivation, which reduces competition for resources. Especially within crop fields early seedling emergence may be advantageous to avoid increasing competition for resources by the crops (Dyer *et al.* 2000). However, early emerged seedlings may have a higher risk of mortality due to different hazards like spring drought, erosive rainfall events or further agricultural measurements (Jones and Sharitz 1989). Species characteristic of arable sites germinate very quickly (short mean germination time) to take advantage of periods where environmental conditions are favourable (Otte *et al.* 2006). On the other hand, arable weeds are also characterised by their ability to spread their germination across time (asynchronous germination) to avoid periods of unfavourable site conditions (Ellenberg and Leuschner 2010).

Many studies have focussed on ‘mean germination time’ as a variable to quantify the germination response of plants to various environmental factors (Arnold *et al.* 2014; Cristaudo *et al.* 2014; Dyer *et al.* 2000; Funk *et al.* 2014; Loydi *et al.* 2015; Ludewig *et al.* 2014; Rühl *et al.* in press; Zhao *et al.* 2014). In some cases differences in mean germination time of a few days (e.g. 2 to 4 days) between species or individuals of the same species were statistically significant. However, it remains unclear whether such small differences in mean germination time translate into significant and ecologically relevant effects on plant fitness across the life-cycle.

Some studies investigated the effects of delayed germination in the range of several weeks or months on life history traits like growth, fecundity and survival (González-Astorga and Núñez-Farfán 2000; Kelly and Levin 1997; Rice 1990), whereas studies including short germination delays of a few days are scarce (but see Black and Wilkinson 1963). Therefore, we did a multi-factorial experiment to study the impact of delayed germination (in the range of one to seven days) on the fitness of the annual arable weed species *Agrostemma githago* (L.).

We addressed the following hypotheses:

> (1) Delayed germination in the range of one to seven days significantly reduces fitness (expressed through different vegetative and regenerative traits).
>
> (2) The decrease of fitness is higher in the presence of competition.

Materials and Methods

Experimental Design

We used a split-plot design with three blocks to investigate the effect of *delayed germination* (factor levels k = 4, delay of 1, 2, 3 and 7 days) with and without interspecific *competition* (k = 2) on the fitness of an annual plant. For this experiment the autumn-germinating annual weed *Agrostemma githago L.* was used. *A. githago* as a competitive, opportunistic weed with a crop mimic strategy (Barrett 1983) and large seeds is a suitable model species representative for a number of other weeds such as *Centaurea cyanus*, *Avena fatua*, *Bromus sterilis* and *Bromus arvensis* (Otte *et al.* 2006). The short after-ripening period and a lack of chilling requirements of the seeds enable the species to germinate at any time of year. Seeds germinated in autumn overwinter and complete their life cycle in the following summer, while seedlings emerged in spring behave as summer annuals (Firbank and Watkinson 1986). *A. githago* is native to the eastern Mediterranean area. Until the introduction of improved seed-cleaning techniques the species was a pernicious weed (Thompson 1973). Today, *A. githago* is endangered by extinction in Germany (Ludwig and Schnittler 1996) because it relies on continuous reintroduction from contaminated grain. Since the species occurs in cereal crops, we selected barley (*Hordeum vulgare L.*) as competitor for the experiment.

The experiment was done in pots (16 x 16 cm surface area) in a passive greenhouse in summer. First, seeds of *A. githago* and barley were sown separately into seed trays. Barley was sown three days later than the weed because it germinates very quickly and synchronously. Six days after sowing, the first seedlings of *A. githago* appeared. During one week, emerging seedlings were marked with their day of germination. The different experimental combinations were planted eight days after the first seeds germinated. For each pot one seedling of the earliest day of germination (day 0, control plant) was planted together with one seedling with delayed germination (delay of 1 day, 2 days, 3 days and 7 days), i.e. increasingly smaller initial plant size. For the competition treatment, half of the pots received two individuals of barley. All treatment combinations were replicated 15 times. In total, 240 plants of *A. githago* were grown in 120 pots.

Pots were arranged in three blocks in the greenhouse. Within each block, interspecific competition represented the main-plot factor, i.e. pots with barley were separated from pots without barley. Within each main-plot, pots of the germination delay treatment were placed randomly.

To assess the development and fitness of the seedlings with different delay of germination several variables were assessed. During growth, height and number of shoots of all *A. githago* individuals were recorded weekly. The plants were harvested after three month. During harvesting number of flowers and number of shoots were counted. Additionally, three capsules of each plant were collected to count seeds per capsule and to estimate seed mass. Biomass was estimated after drying plants at 60 °C for 24 h.

Analysis

The difference between the two *A. githago* individuals within one pot was calculated as a response ratio (eq.1) for each investigated trait:

$RR = x_t/x_c$ (eq.1)

where x_t is the value of the target plant with delayed germination (1, 2, 3 or 7 days) and x_c is the value of the control plant (day 0) without germination delay within the same pot.

The calculated response ratios were used as dependent variable to test the effects of delayed germination and competition. Data of ‘height’ and ‘seed mass’ were box-cox transformed before analysis to improve normality and variance homogeneity (Quinn and Keough 2002). Effects of the single factors and the factor combinations of *block*, *delay* and *competition* for the vegetative traits ’number of shoots’, ’height’, ’biomass’ and the regenerative traits ‘number of flowers’, ’seeds/capsule’ and ‘seed mass’ were assessed with a split-plot ANOVA. The factor *block* was considered random. Subsequently, significance of differences between levels of fixed factors was assessed through a Tukey-HSD test. All statistical analyses were carried out using the programme STATISTICA (ver. 10.0, Statsoft Inc., Tulsa, OK, USA).

Results

The experiment revealed a clear impact of delayed germination of one to seven days on the investigated life history traits of *Agrostemma githago* (Tab 1). The single factor *delay* showed significant effects on the studied traits ‘height’, ‘no. of shoots’, ‘biomass’, ‘no. of flowers’, ‘seeds/capsule’ and ‘seed mass’.

The single factor *competition* had significant effects on the vegetative trait ‘height’ ($p = 0.016$). Whereas, the *delay* x *competition* interaction (D x C) had significant effects on the generative traits ‘no. of flowers’ ($p = 0.027$) and ‘seeds/capsule’ ($p = <0.001$).

All life history traits showed a decrease with increasing delay of germination (Fig 1 and 2). Plants with delayed germination of seven days produced 25 % less shoots, 28 % less biomass, 16 % less flowers, 27 % lighter seeds and were 8 % smaller than control plants in the same pot that had germinated on day 0. This pattern was even more pronounced with additional competition through barley (Tab 2).

For all life history traits, a sharp decrease between three and seven days could be observed under additional competition through barley, resulting in a significant difference between the groups with 1 and 7 days germination delay. In contrast, without competition through barley there were no significant differences in the investigated traits in response to germination delay, except for the generative trait ‘seed mass’.

Tab 1 Effects of *block*, *germination delay* and *competition* on vegetative traits (height, number of shoots, biomass) and on regenerative traits (no. of flowers, seeds per capsule and seed mass), df = degrees of freedom, F = variance ratio, p = error probability.

				height ^{a}		**no. of shoots**		**biomass**		**no. of flowers**		**seeds/capsule**			**seed mass ^{a}**	
	effect	**error term**	**df**	**F**	**p**	**F**	**p**	**F**	**p**	**F**	**p**	**F**	**p**	**df**	**F**	**p**
Intercept	fixed	Block	1	**29.8**	**<0.001**	**10841.5**	**<0.001**	**1367.2**	**<0.001**	**2595.4**	**<0.001**	**5249.3**	**<0.001**	1	**195.9**	**<0.001**
block [B]	random	B x C	2	**23.4**	**0.041**	1.5	0.407	3.9	0.202	1.5	0.402	0.4	0.720	2	0.2	0.800
Competition [C]	fixed	B x C	1	**61.0**	**0.016**	3.8	0.189	3.8	0.190	0.6	0.528	5.6	0.141	1	1.9	0.303
B x C	random	error	2	0.1	0.909	0.0	0.959	0.1	0.887	0.2	0.856	0.9	0.427	2	1.1	0.348
delay [D]	fixed	error	3	**7.0**	**<0.001**	**4.1**	**0.008**	**4.6**	**<0.001**	**3.1**	**0.032**	**3.2**	**0.027**	3	**14.9**	**<0.001**
D x C	fixed	error	3	1.6	0.186	2.3	0.083	2.1	0.106	**3.2**	**0.027**	**6.5**	**<0.001**	3	1.4	0.261
error			108											107		

^{a} data box-cox transformed

Tab 2 Effect of delayed germination in percent. Shown are the means with standard error of the differences (%) between plants with and without germination delay within one pot. The superscripts are the results of a Tukey HSD-test between levels of the interaction of delay x competition (i.e. one column corresponds to one Tukey HSD-test, level of significance α = 5 %).

with competition	**Delay (d)**	**height**	**no. of shoots**	**biomass**	**no. of flowers**	**seeds/capsule**	**seed mass**
	1	-3.6 (± 1.4) ^{ab}	-9.2 (± 7.1) ^{a}	-13.1 (± 6.1) ^{a}	1.3 (± 8.3) ^{a}	-2.3 (± 6.2) ^{a}	5.9 (± 6.7) ^{c}
	2	-5.8 (± 3.1) ^{ab}	-11.8 (± 5.8) ^{a}	-15.5 (± 4.2) ^{a}	-11.1 (± 4.8) ^{a}	5.1 (± 3.0) ^{a}	-2.6 (± 3.4) ^{bc}
	3	-14.5 (± 3.4) ^{ac}	-29.3 (± 7.6) ^{ab}	-30.4 (± 8.4) ^{ab}	-26.5 (± 5.6) ^{ab}	-14.0 (± 8.2) ^{ab}	-8.7 (± 6.6) ^{abc}
	7	-23.2 (± 4.8) ^{c}	-54.1 (± 11.0) ^{b}	-57.3 (± 11.6) ^{b}	-51.7 (± 10.8) ^{b}	-37.4 (± 7.9) ^{b}	-35.5 (± 4.7) ^{d}
without competition							
	1	-3.8 (± 1.5) ^{ab}	-12.4 (± 4.3) ^{a}	-14.4 (± 4.3) ^{a}	-14.6 (± 3.9) ^{ab}	-9.0 (± 5.9) ^{a}	-5.9 (± 3.2) ^{abc}
	2	-1.7 (± 2.0) ^{b}	-21.4 (± 5.8) ^{ab}	-21.9 (± 5.6) ^{a}	-19.7 (± 5.5) ^{ab}	-3.9 (± 5.8) ^{a}	-7.6 (± 6.9) ^{abc}
	3	-8.2 (± 1.8) ^{ab}	-24.8 (± 5.3) ^{ab}	-29.0 (± 4.2) ^{ab}	-24.7 (± 5.5) ^{ab}	5.6 (± 5.4) ^{a}	-19.9 (± 2.6) ^{abd}
	7	-8.0 (± 1.9) ^{ab}	-24.5 (± 11.5) ^{ab}	-28.1 (± 13.1) ^{ab}	-15.6 (± 18.1) ^{ab}	2.4 (± 5.7) ^{a}	-27.3 (± 7.9) ^{ad}

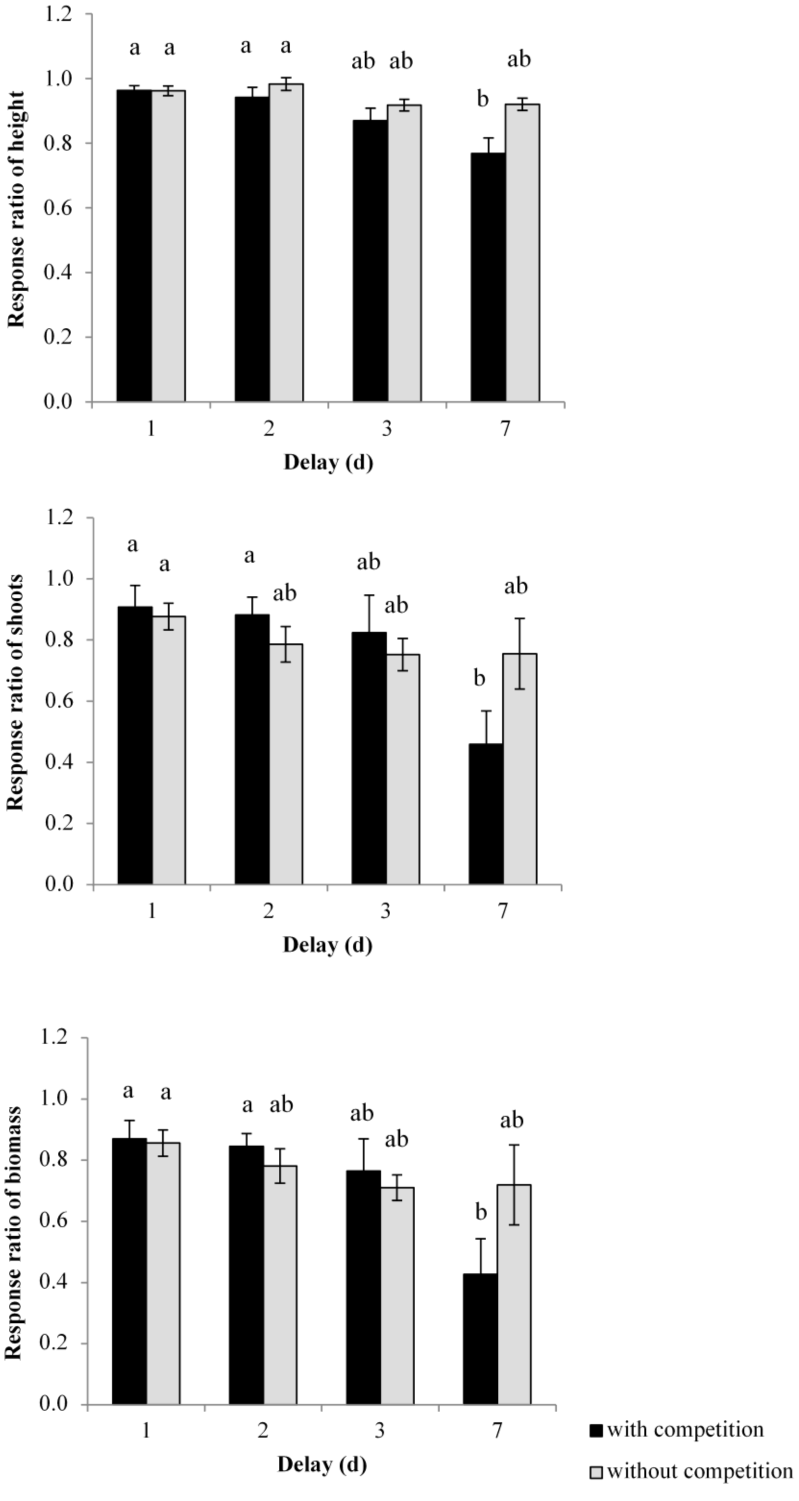

Fig 1 Effect of germination delay on the vegetative traits *height* (cm), *number of shoots* and *biomass* (g). Shown are the means with standard error of the response ratios of plants with and without germination delay within one pot. Response ratio > 1 implies an increase and < 1 implies a decrease for the trait of the target plant compared to control plant. Letters above bars show results of the comparison of the effects of the interaction *delay x competition* by means of a Tukey HSD-test (level of significance α = 5 %).

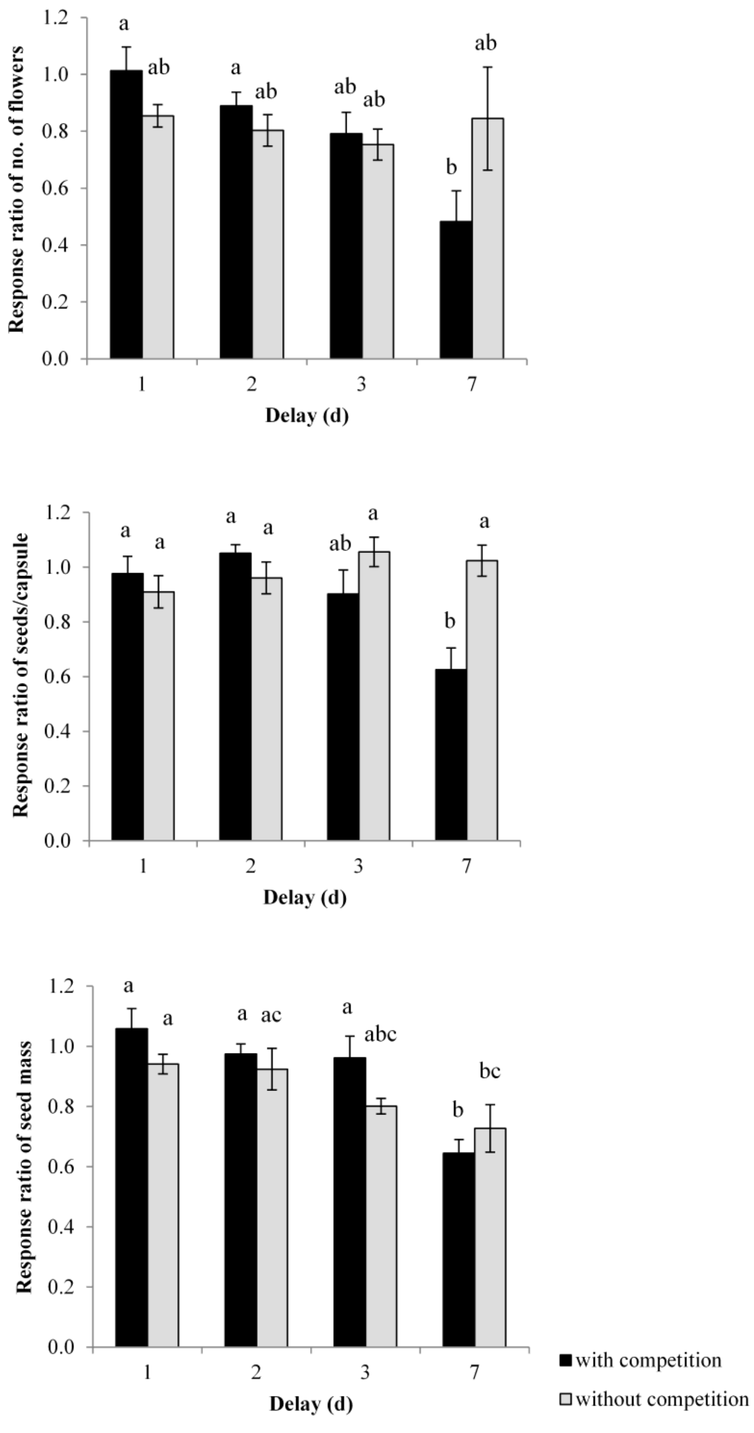

Fig 2 Effect of germination delay on the regenerative traits *number of flowers*, *seeds/capsule* and *seed mass* (g). Shown are the means with standard error of the response ratios of plants with and without germination delay within one pot. Response ratio > 1 implies an increase and < 1 implies a decrease for the trait of the target plant compared to control plant. Letters above bars show results of the comparison of the effects of the interaction *delay x competition* by means of a Tukey HSD-test (level of significance α = 5 %).

Discussion

There are many influences determining success or failure of plant reproduction from seeds (Eckstein and Donath 2005; Fay and Schultz 2009; Schütz 2000; Walck *et al.* 2011). The time of germination and seedling emergence play major roles for further plant performance. Especially for autumn-germinated seedlings of perennials or winter annuals, which have to survive the unfavourable winter period, larger, and thus more vigorous seedlings have an advantage (Leishman *et al.* 2000; Schmiede *et al.* 2013). Similarly, seedlings that emerge early in spring are advantageous when competing with crops (Black and Wilkinson 1963, De Luis *et al.* 2008; Dyer *et al.* 2000). On the other hand, these seedlings are especially threatened by environmental hazards like spring drought or spring frost and agricultural measurements (Jones and Sharitz 1989; Storkey *et al.* 2010). A study about germination strategies of arable weeds suggests that a prolonged germination time within the vegetation period (lower synchrony of germination and higher mean germination time) is advantageous in highly variable environments like arable fields (Rühl *et al.* in press). Within-season spread of germination over a period of several days may be a response to short-term unfavourable conditions during the germination period of plant species (Ludewig *et al.* 2014).

Against this background the purpose of our study was to examine the effects of short term germination delays in combination with competition on plant fitness, ignoring potentially fatal environmental hazards. Our study demonstrates that a germination delay of only a few days leads to significantly decreased fitness, which is consistent across several vegetative and reproductive life history traits. Furthermore, the results showed that the decrease of fitness is considerable, amounting to up to 25 % without competition and >50 % with competition. Thus, early emerged seedlings have statistically and biologically significant fitness advantages over later emerged seedlings of the same species, if they meet favourable conditions for growth. In the case of additional competition our results revealed that even a short germination delay in the range of three to seven days means a decrease of fitness across different traits along the life-cycle.

The vegetative traits ’number of shoots’, ’height’ and ’biomass’ decreased with increasing delay of germination (Fig. 1). At germination delay of seven days the biomass of *A. githago* under competition of barley decreased by 57 %. This is in line with a former study about the impact of seedling emergence time of subterranean clover, where a germination delay of five days led to a reduction of about 50 % in final biomass (Black and Wilkinson 1963). Other studies demonstrated that early emerged seedlings grew higher than later ones but these studies addressed germination delays of several month or weeks (De Luis *et al*. 2008; Rice 1990; Quintana *et al*. 2004). In our experiment the investigated plants that germinated seven days later and grew together with barley were about 23 % smaller at the end of their life cycle compared to the controls. *Agrostemma githago* adjusts its height growth to the height of the cereals. It is one mechanism of the crop mimic strategy to cope with competition for light (Barrett 1983). The fact that a reduction in height was found in *A. githago*, which is an opportunistic weed with respect to plant height and considered as competitive ruderal strategist (Klotz *et al*. 2002), indicates that a significant effect of a relatively short germination delay on biomass and canopy height may be a general response.

The reproduction traits ‘numbers of flowers’, ’seeds/capsule’ and ‘seed mass’ were influenced in a similar way by delayed germination of only a few days. Under conditions of competition, a delay of seven days resulted in nearly 52 % less flowers, these flowers produced 37 % less seeds and these seeds showed 36 % lower seed mass (Tab 2). Other studies found the same general pattern, i.e. that early emerged seedlings were more fecund than later ones, for annual and perennial species with delayed germination of several weeks or months (González-Astorga and Núñez-Farfán 2000; Kelly and Levin 1997; Rice 1990). Since in monocarpic species reproductive traits are strongly correlated with biomass, this response is not unexpected (Sletvold 2002).

Despite clear fitness advantages of early germination, highly synchronous early germination may not necessarily be beneficial for plant populations in highly variable environments since a certain amount of persistent (dormant) seeds in the soil seed bank or germination delay may be mandatory to survive for example annual changes of agricultural measures (Kornas 1988; Rees and Long 1992) or unfavourable abiotic conditions. Therefore, selection for early germination seems to be counterbalanced by forces selecting for some degree of temporal germination spread under field conditions. Since ungerminated seeds of *A. githago* do not persist in the soil, it has adopted a crop mimic strategy (Barrett 1983), relying upon continuous reintroductions from contaminated grain with the next sowing (Firbank and Watkinson 1986). However, without seed dormancy, a long mean germination time and low synchrony may represent, at small temporal scales, another species-specific germination strategy for risk reduction to bridge short-term unfavourable environmental conditions (Rees 1994; Venable and Brown 1988).

Delayed germination thus appears to be a bet-hedging strategy (Gremer and Venable 2014; Rees 1994). Bet-hedging traits are expected to evolve under conditions of unpredictable environmental variance (Simons 2011). To avoid the risk of a failure of the whole seed batch, species accept lower fitness of the late-emerged seedlings (Childs *et al*. 2010; Gremer and Venable 2014). Several studies of perennial and annual species showed that early emergence resulted in greater mortality due to various hazards at the beginning of the season like spring drought or heavy rainfall events, but seedlings which germinated early and survived the seedling stage were more robust, attained greater size and produced more seeds (González-Astorga and Núñez-Farfán 2000; Quintana *et al*. 2004; Venable *et al.* 1987). Delayed germination expressed as long mean germination time and low synchrony of germination within one growing season seems to represent a promising strategy to cope with this challenging situation (Rühl *et al*. in press). As the current study showed there is a price to pay for this flexible strategy of delayed germination, i.e. decreased fitness through smaller plant sizes and lower off-spring production. In the case of *A. githago*, the results suggest additionally that there is a threshold for the effect of germination delay on fitness in the range of three to seven days. Plants with delayed germination beyond this threshold are not able to successfully utilise the crop mimic strategy because the developmental advantage of the cereals cannot be caught up by the weed.

Acknowledgements

We thank Josef Scholz-vom Hofe for the assistance in data collection in the lab and the greenhouse. This work was funded by a postgraduate scholarship of the Justus Liebig University, Giessen. We confirm to have no conflict of interest.

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Summary

Since humans began to practice agriculture many herb and grass species have been competing with the crops for the limited resources. This species group relies on open soiled sites; therefore it is dependent on anthropogenic land use in Central Europe. Most arable weed species of Central Europe have their origin in the Mediterranean climate. With the spread of farming, beginning in the region of the Fertile Crescent, typical weeds were introduced together with the cultivated crops into Central Europe. Because of co-evolution over thousands of years weeds adapted their life cycles to the development of crops. Additionally weeds were able to adapt to the climatic conditions in Central Europe and many species expanded their distribution area successfully into the oceanic climate zone.

The agricultural measurements, to which the weeds are adapted, remained nearly the same for thousands of years. But from the middle of the 20th century, agriculture changed fundamentally. Modern agriculture and intensive land use management with the application of herbicides and fertilizers, enhanced seed treatments, simplified crop rotation, and abandonment of marginal arable sites caused a dramatically decline of arable weeds and a shift in the spectrum of the wild arable plant species. The specific adaptation to arable fields is the reason for their high extinction risk. Today arable weed species are one of the most endangered species groups in Central Europe.

Besides changing land use, global climate change may present a further challenge to the adaptability of arable weeds. Differing climate scenarios predict a general increase of annual mean temperatures and an increased frequency of extreme meteorological conditions. As a consequence, plants may be more often subjected to high temperatures and low soil moisture during the growing season in spring and summer. Germination is a crucial stage especially for annual species. As temperature and water availability control germination of seeds, changes in these factors may have major impacts on the establishment and survival of plant populations. For the development of successful conservation programs it is essential to understand the response of endangered species to future changes in environmental conditions.

In this context, the presented studies investigated the response patterns of arable weeds to changes in temperature and water availability during germination. The results revealed a distinct germination response of endangered and common arable weeds. At optimal water supply endangered species tended to germinate to a higher percentage than common arable weeds, however, this trend was reversed when water availability decreased. Thus, endangered arable weeds showed a stronger negative response to water stress during germination than common arable weeds. In addition, endangered arable species germinated in a narrower time window, e.g. shorter mean germination time and higher synchrony of germination. The endangered species place everything on one card, while the more common species spread their germination over a longer time period. Therefore they are less sensitive towards short-term unfavourable site conditions and disturbances.

Based on comprehensive collection of data over a broad range of temperatures (3 – 35 °C) combined with several water potentials (0.0 – 1.2 MPa) germination response of endangered and common arable weeds could be modelled for differing climate scenarios. Less flexibility to decreasing water availability of the endangered species and a significant lower mean germination temperature (15.8 °C ± 0.4) compared to the common species (18.4 °C ± 0.2) suggests that the endangered arable weed species will be more negatively affected by global warming than common species.

The results of the study about the impact of the mean germination time on plant fitness show that the common weed species are following a bet-hedging strategy. They are sacrificing fitness of individual plants in order to decrease the risk of a failure of the whole seed batch. Timing of germination has a significant influence on plant fitness across the life-cycle. At delayed germination of seven days individuals of the target species *Agrostemma githago* had 54 % less shoots, 23 % less height, 57 % less biomass, 52 % less flowers, 37 % less seeds per capsule and 36 % lighter seeds.

To set-up a conservation strategy for endangered species in the agricultural landscapes of Central Europe it has to be considered that climate change will further enhance the decline of several arable weeds initiated through land use changes. As the consequences of climate change are not avoidable, the conservation efforts have to focus on measures to compensate negative effects. To increase biodiversity of agricultural landscapes and to stop the further decline of arable weed species, conservation measures for arable weeds should be explicitly intensified. It is necessary to move away from local release of endangered species to a denser grid of weed conservation sites, which enables migration of arable weed species. Conservation measures on landscape-scale would give arable weed species greater scope to face future climatic changes.

Zusammenfassung

Seitdem der Mensch Ackerbau betreibt konkurrieren eine Vielzahl von Kräutern und Gräsern mit den Kulturpflanzen um die verfügbaren Ressourcen. Ackerwildkräuter sind auf offenen Boden angewiesen und in Mitteleuropa daher eng an die anthropogene Landbewirtschaftung gebunden. Die meisten Ackerwildkräuter haben ihren Verbreitungsschwerpunkt im mediterranen Raum. Mit der Verbreitung des Ackerbaus, ausgehend von der Region des Fruchtbaren Halbmondes, wurden neben den Kulturpflanzen auch deren typische Unkräuter und Ungräser in Mitteleuropa eingeführt. Im Laufe der Jahrtausende fand eine Koevolution statt und die ursprünglich wild vorkommenden Arten passten ihren Lebenszyklus dem der Kulturpflanzen an. Auf den künstlich offen gehaltenen Ackerflächen passten sich die Arten darüber hinaus den klimatischen Bedingungen Mitteleuropas an, so dass viele Arten heute ihr Verbreitungsareal bis in die subozeanische Klimazone ausweiten konnten.

Die Ackerbaumethoden an die sich die Ackerwildkräuter angepasst hatten blieben über Jahrtausende sehr ähnlich. Doch ab Mitte des 20. Jahrhunderts änderte sich die Landbewirtschaftung grundlegend. Die Intensivierung der Landwirtschaft mit dem Einsatz von Herbiziden und Mineraldünger, verbesserter Saatgutreinigung, Vereinfachung der Fruchtfolgen, frühem Stoppelumbruch und der Aufgabe von Grenzertragsstandorten führten zu einem stetigen Rückgang der Ackerwildkräuter auf den ackerbaulich genutzten Flächen. Auf Grund ihrer starken Anpassung an die traditionelle Landwirtschaft sind die Ackerwildkräuter heute eine der am stärksten gefährdeten Artengruppen Mitteleuropas.

Zusätzlich zu der veränderten Landnutzung wird auch der Klimawandel die Anpassungsfähigkeit der Ackerwildkräuter auf die Probe stellen. Neben einer allgemeinen Erwärmung werden auch Extremwetterlagen in Zukunft häufiger erwartet. Außerdem soll sich der Trend zur jahreszeitlichen Ungleichverteilung des Niederschlags fortsetzen. Zukünftig wird mit mehr Niederschlag im Winter und weniger im Sommer gerechnet. Dies kann für die Pflanzen Mitteleuropas bedeuten, dass sie bei höheren Temperaturen und geringerem Niederschlag während der Vegetationsperiode ihren Lebenszyklus abschließen müssen.

Ackerwildkräuter sind meist annuelle Arten und der Keimungsprozess ist eine entscheidende Phase im Leben dieser Pflanzen. Da Temperatur und Wasserverfügbarkeit diesen Prozess maßgeblich steuern, können Änderungen dieser Faktoren große Auswirkungen auf die Keimungsrate und damit auf die Etablierung von Pflanzenarten haben. Um gefährdete Pflanzenarten auf Landschaftsebene schützen zu können ist eine Prognose ihrer Reaktion auf sich verändernde Standortbedingungen sinnvoll.

Vor diesem Hintergrund wurde in dieser Arbeit untersucht, wie Ackerwildkräuter auf Veränderungen von Temperatur und Wasserverfügbarkeit während des Keimungsprozesses reagieren. Die Ergebnisse zeigen, dass es in diesem Zusammenhang einen signifikanten Unterschied zwischen ungefährdeten und gefährdeten Arten gibt. Bei voller Wasserverfügbarkeit keimten die untersuchten seltenen Arten tendenziell mehr als die häufig vorkommenden Wildkräuter. Dieses Verhältnis kehrte sich um, sobald Wasserstress simuliert wurde. Die seltenen Ackerwildkräuter reagierten mit einer deutlich stärkeren Abnahme der Gesamtkeimung auf abnehmendes Wasserpotential als die häufigen Arten. Darüber hinaus keimten die seltenen Arten in einem engeren Zeitfenster, d.h. die mittlere Keimungsdauer ist kürzer und die Synchronität der Keimung ist höher als bei den ungefährdeten Arten. Damit setzen die seltenen Arten alles auf eine Karte, während die häufigeren Arten ihre Keimung über einen größeren Zeitraum streuen und so das Risiko des Verlusts einer gesamten Samengeneration durch kurzfristige Störungen verringern.

Durch eine umfassende Datenaufnahme über eine breite Temperaturspanne (3 – 35 °C) bei verschiedenen Wasserpotentialen (0,0 – 1,2 MPa) konnte darüber hinaus die Keimungsreaktion von seltenen und häufigen Ackerwildkräutern unter verschiedenen Klimaszenarien modelliert werden. Auf Grund geringerer Flexibilität gegenüber abnehmender Wasserverfügbarkeit und einer signifikant geringeren optimalen Keimungstemperatur der seltenen Arten (15,8 °C ± 0,4) im Vergleich zu den häufigen Arten (18,4 °C ± 0,2) lässt sich darauf schließen, dass die heute seltenen und gefährdeten Arten weniger vom prognostizierten Klimawandel profitieren werden als die häufigen Ackerwildkrautarten.

Die Ergebnisse des Versuchs zur Bedeutung der mittleren Keimungsdauer zeigen, dass die häufigen Ackerwildkrautarten einer Bet-Hedging Strategie folgen. Sie nehmen eine geringere Fitness von später gekeimten Pflanzenindividuen in Kauf um das Risiko der

Gesamtpopulation zu verringern. Denn der Keimungsverlauf hat einen maßgeblichen Einfluss auf die Fitness einer Pflanze während ihres gesamten Lebenszyklus. So zeigten die Individuen der Versuchspflanze *Agrostemma githago* bei einem Keimungsverzug von sieben Tagen 54 % weniger Triebe, 23 % geringeres Höhenwachstum, 57 % weniger Biomasse, 52 % weniger Blüten, 37 % weniger Samen pro Kapsel und um 36 % leichtere Samen.

Bei der Entwicklung von Schutzkonzepten für gefährdete Arten der Agrarlandschaften Mitteleuropas muss daher berücksichtigt werden, dass der Klimawandel den durch den Landnutzungswandel hervorgerufenen Rückgang vieler Ackerwildkräuter bzw. die Verschiebungen im Artenspektrum weiter verstärken kann. Da die Folgen des Klimawandels für die Ackerwildkräuter nicht direkt aufzuhalten sind, sollte der Fokus der Schutzbemühungen auf Maßnahmen liegen, die die negativen Auswirkungen des Landnutzungswandels ausgleichen. Das Ziel dieser Bemühungen sollte die Erhaltung der gefährdeten Arten auf Landschafsebene sein. Bei kleinflächigen und isolierten Vorkommen von Ackerwildkräutern besteht die Gefahr, dass diese bei Änderung der lokalen klimatischen Bedingungen kaum auf dann geeignete Standorte ausweichen können. Nur durch eine möglichst flächendeckende Förderung dieser Arten kann dem regional unterschiedlichen Ausmaß der klimatischen Veränderung und dem damit verbundenen unterschiedlichen Anpassungsdruck auf die Vegetation begegnet werden.

Acknowledgement

I would like to thank the Justus-Liebig-University Giessen for providing me financial support with the graduate scholarship and Prof. Annette Otte for supporting me since the bachelor programme and for giving me the possibility to write this doctoral thesis within her working group. For a friendly working environment and inspiring conversation I want to thank all members of the working group of the department for landscape ecology and landscape planning. In particular I want to thank Tobias Donath, Lutz Eckstein and Joseph Scholz-vom Hofe for their great support and the constant encouragement. Last but by far not least, I want to express my gratitude to my family members, especially Patrick, Luise and Johanna, for their patience with me in all respects.