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Population dynamics and functional traits of annual plants – a comparative study on how rare and common arable weeds persist in agroecosystems

par-von

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PREFACE

This thesis is part of a series of studies on diversity at the landscape scale, plant communities and populations in the agricultural landscapes of the Luberon area including recently the works of Affre, Barroit, Hill, Gerbaud, Roche, Véla, and Le Mire-Pecheux (Dutoit *et al*. 1999;Barroit *et al*. 2000;Gerbaud *et al*. 2001;Dutoit *et al*. 2001;Roche *et al*. 2002;Véla 2002;Dutoit *et al*. 2003;Affre *et al*. 2003;Le Mire Pecheux 2004;Gasc 2005;Dutoit *et al*. 2007).

This work was sustained and funded by the Parc Naturel Régional du Luberon, the Bayerisch Französische Hochschulzentrum, the Conservatoire Botanique National Méditerranéen d'Hyères and the Groupement de Développement Agricole du Sud Luberon, Ansouis. Research work was conducted at the Institut d'Ecologie et de Paléoécologie (Marseille) and at the Institut für Botanik (Regensburg). Through the first three years of the thesis the author was working half time as a consultant at ECO-MED (Marseille) involved in Environmental Impact Assessments and botanical surveys of high plant diversity sites in South Eastern France and Northern and Central Algeria and in the last year as assistant teacher at Université Aix-Marseille III.

The different chapters of this thesis are written as independent articles. Chapter 2 has been accepted in Annals of Botany (2009). The remaining chapters are in preparation for submission in international scientific journals. Therefore, we had to repeat some aspects in the 'Introduction' and 'Materials and Methods' section.

Marseilles, July 2009

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GENERAL INTRODUCTION

Biological diversity as a result of evolutionary and ecological processes always fascinated naturalists and lead to central theories in ecology and evolution (Darwin 1859;Janzen 1970;Connell 1978;Hubbell 2001;Mayr 2004). Facing the actual man-made mass extinction of species *i.e.* 100-1000 times the geological background rate (Pimm *et al.* 1995), a deepened understanding of the processes leading to maintenance of taxa and their populations through time is now crucial for the preservation of this diversity (Doak *et al.* 2002).

EXPLANATIONS FOR COEXISTENCE AND PLANT DIVERSITY - A MIRROR OF POPULATION PERSISTENCE

For plants, early studies on the maintenance of biological diversity stressed the importance of competition as the main limiting factor for diversity, a process working through 'competitive exclusion' and 'limiting similarity' (Gause 1934;MacArthur and Levins 1967;Hubbell 2005). It is astonishing that this limit was so long little criticised; even Darwin (1859) already claimed competition as a motor for the naissance of new species rather than a limit to its number. The abandonment of a stable and a-spatial view of competition was step-wise and among the first attempts to reconcile competition with the apparent diversity of earth's ecosystems was the consideration of disturbances as diversity maintaining processes, like in the 'intermediate disturbance hypothesis' (Connell 1978). More precise consideration of the resource usage in ecosystems generated a 'resource-ratio hypothesis' suggesting that as many species as different resources can coexist in a local community (Tilman 1985). Later the integration of spatiality and dispersal in competition models (Tilman 1994) showed that competitive exclusion is rather a limited process. Competition has also been shown to be of different nature according to environment (Ackerly 2004;Liancourt *et al.* 2005) and its opposite, *i.e.* indirect positive interactions have been shown as important in many plant communities (Michalet *et al.* 2006). Consequently, competition is far from being the main

limiting factor for diversity even for late successional states (Tilman 1994;Zobel and Pärtel 2008). However, the recent claims of 'recruitment limitation' *i.e.* the unavailability of suitable patches attributed at least partly to surrounding vegetation (Sanchez and Peco 2007) –in other words competition– highlights again why plants developed adaptations to detect temporally and spatially limited gaps with low levels of competition, often *via* enhanced germination under diurnally fluctuating temperatures (Thompson *et al.* 1977). This adaptation is known as 'gap detection'. It is thus reasonable to think about different processes triggering diversity of plant communities at a local level, which are related to maintenance of plant diversity.

A global historical and biogeographic view of local biodiversity generated a concept that relates pools of species at different spatial scales (Zobel 1997;Pärtel 2002). This 'species pool concept' emphasises that a regional pool of species limits the possible diversity-environment relations, and that regional diversity patterns can be explained by evolutionary history of the region (Pärtel 2002). It has recently been extended for the role of habitat productivity and plant diversity (Zobel and Pärtel 2008). At the same time this concept explicitly emphasises on the dispersal limitation of plant communities and the need to take dispersal processes on a local to regional scale into account in order to explain realistic changes of diversity in local communities (Zobel *et al.* 2006). Since the classical works on dispersal limitation by Harper (Sagar and Harper 1960;Begon *et al*. 1996), several studies on dispersal limitation showed its importance for the diversity of real communities (Tilman 1994;Ehrlen and Eriksson 2000;Poschlod and Biewer 2005;Poschlod *et al*. 2005). Dispersal has effects on both species richness in communities (Bonn and Poschlod 1998) as well as genetic diversity within species (Willerding and Poschlod 2002). Hence, dispersal *via* pollen or seeds are important processes for the maintenance of diversity at a local level, and traits related to these processes can give insight into persistence of local populations. Many open questions remain on how diversity

on smaller spatial and temporal scales are related to global processes and the importance of traits for local population persistence with a comparative approach can yield insights.

A functional trait based approach is a good opportunity to answer such questions (McGill *et al.* 2006). A functional trait is any morphological, physiological or ecological trait that can be triggered by ecosystem properties (response trait) or that has effects on the ecosystem or population dynamics (effect trait) (Gitay and Noble 1997). Detailed work on evolution of functional traits as a response to cyclic and a-cyclic disturbances such as herbivory (Diaz *et al.* 2007), summer drought (Espigares and Peco 1995), flooding (Stromberg *et al.* 2008) illustrate the high importance of specialised structures and finally the many idiosyncratic responses in vegetation. Dispersal in space (Zobel 1997;Bonn and Poschlod 1998;Zobel *et al.* 2006), time and regeneration niche (Grubb 1977;Kahmen and Poschlod 2008) have been identified to explain both, high diversity of at a first view, simple layered homogeneous ecosystems and unexpected low diversity of others. Reviews on the regeneration niche (Grubb 1977) and especially on germination ecology (Baskin and Baskin 1998) revealed the important diversification of regenerative strategies among plants of the same ecosystem. This trait based-approach opposes to neutral theory (Hubbell 2001) which assumes that environmental gradients and interspecific differences in traits are without effects on population dynamics. It proposes that simple time between emergence and extinction of taxa is sufficient to explain many observed diversity patterns. A major problem of the trait-based approach is the high number of putative traits and environmental factors to explain population dynamics (McGill *et al.* 2006), more rapid insight can thus come from simpler but complete systems.

In annual plants, there is no resting stage other than seeds. Therefore, temporal variability in habitat quality cannot be buffered by long living adults and together with other monocarpic plants, individual fitness (Metcalf *et al.* 2003), population persistence (Kalisz and McPeek 1993;Menges 2000) and community diversity (Facelli *et al.* 2005) depend highly on

persistence of seeds. This relative simplicity of annual plants and annual dominated plant communities make them ideal study models for testing hypotheses on the relative importance of different life stages for population growth and survival (Harrison and Ray 2002). Additionally, Venable and Brown (1993b) showed, using models on evolutionary stable strategies on dispersability in space and time, that perennial plants follow similar models than annuals. The remarkable difference is that the selective pressure on dispersal is less important for perennials than annuals as they also rely on adult persistence. It is thus likely that findings concerning the population dynamic function of seed dispersal in space and time from studies on annuals can successfully be generalised to the remaining plants.

POPULATION DYNAMICS IN ANNUALS - WHICH TRAITS FOR LOCAL POPULATION PERSISTENCE?

To understand the importance of different factors such as soil seed mortality, competition and predation for population dynamics of annual plants it is helpful to have a look on the life cycle of an annual plant (Fig. I.1A). Let us imagine a population of 10 adult annual plants, each adult producing 200 viable seeds (a realistic value, *cf.* chapter 2). This results in 2000 individuals in the stage of seeds at the end of the growing season. It is obvious that the next generation would never consist of 2000 adult plants but rather of a limited number may be again only 10. There is not one single factor that limits the final number of adults (Fig. I.1A), mortality in different life stages finally very heavily reduces this number (Symonides 1983;Günter 1997;Silvertown and Charlesworth 2001). However, mortality is not equally distributed among life stages and has various reasons at the different life stages (Fig. I.1A) each of these is related to a set of traits (Fig. I.1B).

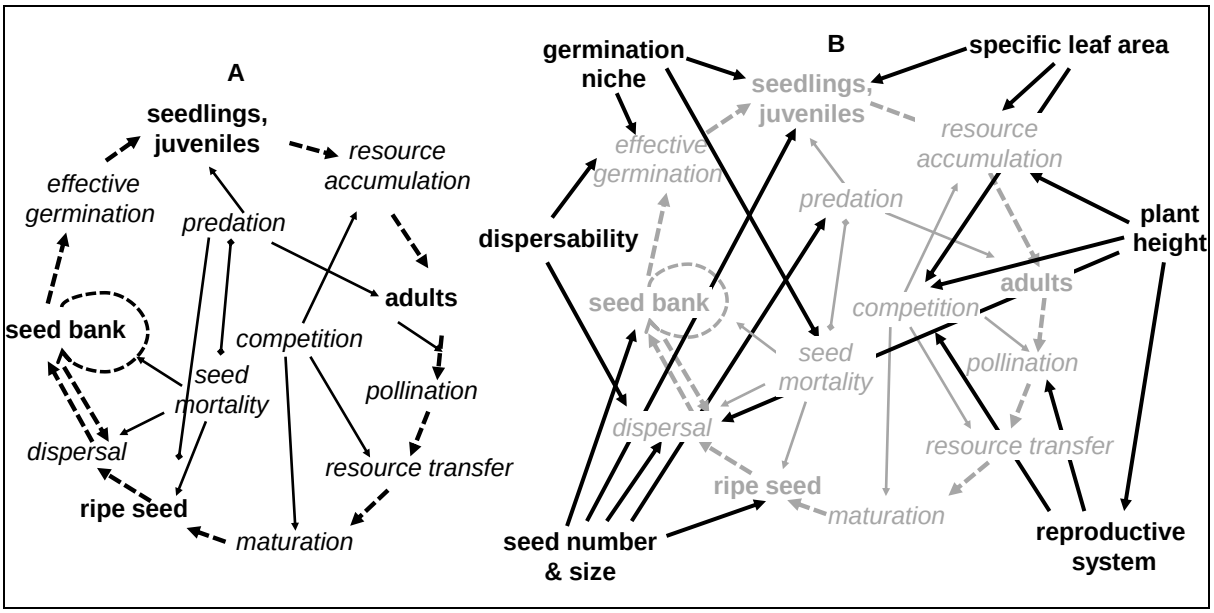


Fig. I.1: (A) Annual plant life stages and transitional processes, inside the circle: sources for mortality influencing population growth and extinction dynamics- note that environmental changes can influence all stages and processes reducing effectives; (B) Plant traits related to these life stages and processes -note that some 'traits' are also processes such as dispersability and that seed mortality is often considered as a 'trait' in form of seed bank persistence.

Early approaches like 'key factor analysis' (Podoler and Rogers 1975;Silvertown 1982) identify life stages contributing most to survival focussing on temporal variations of mortalities, however this approach is only meaningful for organisms without overlapping life stages. Later 'λ-contribution analysis' (Sibly and Smith 1998) integrated 'sensitivity' and 'elasticity' analysis - using contributions of absolute and proportional change of life stage transitions (De Kroon *et al*. 1986;Benton and Grant 1999) to identify life stages important for population growth and abundance. These temporally fine scale population dynamic measures are linked to coarser measures such as local population extinction rates and population turnover and are hence a key ingredient of population viability analyses (Menges 1990;Beissinger and McCullough 2002;Reed *et al*. 2002).

An important life history stage to understand population growth and persistence are seeds. Mortality in the seed stage can be caused by predation through animals (Abramsky 1983;Louda 1989;Hulme 1998;Moles and Drake 1999;Azcarate and Peco 2006) and infestation (predation) through fungi (Blaney and Kotanen 2001;Schafer and Kotanen 2003) including density dependent effects (Van Mourik *et al.* 2005). However, it is difficult to imagine

competition among resting seeds in the soil. Additionally, there can be losses due to dispersal into unsuitable habitats although this point is rarely addressed (Günter 1997). Further on, there can be considerable mortality due to fatal timing of germination at this stage (Baskin and Baskin 1989;Thompson 2000;Davis and Renner 2007). Traits triggering differences in mortality among species in this stage include therefore germination niche traits and seed number and size. At the seedling and juvenile stage, competition becomes important because of the limited carrying capacity of the habitat, but there is still an important part of predation in the mortality (Bonfil 1998;Leishman *et al*. 2000b;Coomes and Grubb 2003). Traits related to predation such as seed size still explain interspecific differences in mortality at this stage; but other traits related to competitive ability gain importance. These traits include again seed size (Coomes and Grubb 2003;Moles *et al*. 2004), but also specific leaf area (SLA) (Liancourt *et al.* 2005) and plant height (Tilman 1988), with however opposite relations according to environmental constraints (Ackerly 2004;Liancourt *et al*. 2005). Comparative analyses of which stage or stage transition are most important in determining the final number of adult annual plants all point on soil seed mortality and germination as most important (Symonides 1983;Günter 1997). Differences in fitness can appear in the reproduction of plants. These differences are bound to different fecundity among species according to environmental constraints, for example the dependence on pollinators (Gibson *et al.* 2006). Whenever seed number or seed size is involved in the differential performance at a particular stage, the fundamental trade-off between them has to be considered which suggest that they are equally effective for reproduction (see below for details, Jakobsson and Eriksson 2000). Finally, genetic diversity and related traits may also influence on all life stages because of the better performance of *e.g.* outbreeding (Charlesworth and Charlesworth 1987), which is an important aspect for perennials whereas in annuals autogamy is more frequent. All this shows, that there is a potential to deepen insights into causes for local population extinction rates and population turnover studying

them comparatively in a functional trait-based approach (McGill *et al.* 2006). Agro-ecosystems are characterised by a high diversity of annual plants, which enables the study of many aspects of their life history in relatively short time. In arable fields, there are also many unpredictable changes and disturbances so it is easier to study changes in population turnover, extinction rate and their relation to morphologic and life history traits.

OUTLINE OF THE THESIS

The points discussed above show the many open questions on the relation between species traits and population dynamics and the role of these traits for coexistence in communities. The main evidence in the field comes either from population ecology of single species or from comparative trait analyses of whole communities, but both approaches are still little linked (McGill *et al.* 2006). We therefore studied explicitly traits related to population ecology in detail for a set of species –numerous compared to population ecological studies, and limited for community ecology– to add an intermediate approach. The principal research questions from a fundamental and applied point of view of this thesis are: (i) What are the main determinants of annual plant diversity in agro-ecosystems and how is it influenced by changing land-use? (ii) Is there a consistent relation between soil seed mortality, seed production and effects in the community? (iii) What are the functional roles of germination and dormancy characteristics in the soil seed bank of annual plants? What is the role of other seed traits? (iv) Can differential soil seed mortality explain differences in population turnover and extinction dynamics among species? Which other traits are related to these differences? (v) What are the differences between locally abundant and scarce and between regional widespread and regional rare annual plant species?

We study these questions in five corresponding chapters:

1 – Locating plant diversity in structured habitats – practices, soil types and history drive vineyard vegetation

2 – Can seed persistence be explained by germination parameters and seed traits? – Experimental evidence from cereal weeds

3 – The seed bank longevity index revisited - limited reliability evident from a burial experiment and database analyses

4 – Is there an effect of soil seed mortality and seed production on local population dynamics in annual plants? – the case of rare cereal weeds

5 – Comparison of traits between rare and common cereal weeds and implications for conservation

The following paragraphs of the introduction review in more detail scientific background and concepts of the thesis, and we present here methods and the study system. After each chapter, we use transition chapters to discuss the results in the frame of the thesis and to introduce following main chapter. In the conclusion, we replace the findings in a more general context, combining evidence from the main chapters and the introduction, evaluate their importance, show the limits and point out important future questions to resolve.

Theories, concepts and state of knowledge

STORAGE EFFECT AND BET HEDGING

Evolutionary models for species in temporally variable habitats predict that germination is delayed to spread the risk of no reproduction in bad years, a phenomenon called 'bet hedging'. The main prediction of bet hedging, *i.e.* the higher the risk the lower yearly germination percentages has been elucidated by Venable (2007). Population persistence in annual plants has also generated concepts to understand coexistence of species which would exclude each other by competition, leading to the 'storage effect' (Chesson and Warner 1981;Warner and Chesson 1985). The storage effect promotes coexistence under three conditions: (i) the species differ in their responses to temporal changes of the environment, *e.g.* germination; (ii) the strength of competition correlates to these changes and (iii) there is a life stage that buffers population growth and decline, *e.g.* a persistent soil seed bank (Chesson and Warner 1981;Warner and Chesson 1985;Levine and Rees 2004;Facelli *et al*. 2005). Several studies show the applicability of the model (Bonis *et al*. 1995;Cáceres 1997;Facelli *et al*. 2005). The first condition (species differ in their responses to temporal changes) is almost generally the case, with however spatially and temporally varying degrees. Nevertheless, it seems difficult to examine whether the second condition (competition correlates to these changes) really is different from the first, *e.g.* when annuals do not germinate in reaction to drought, they also will not enter in competition. We thus have doubts whether it is necessary to keep this condition to explain the diversifying effect, *i.e.* maintaining diversity in natural systems. There are simpler approaches to study population dynamics, which elucidate that only conditions (iii) and (i) may be sufficient for population persistence (Silvertown 1982;Kalisz and McPeek 1993;Günter 1997;Menges 2000;Adams *et al*. 2005). In figure 2, we summarise the storage effect that promotes the coexistence of two species with different responses to temporal changes ('good' *versus* 'bad'

years), different levels of competition and a seed bank for the subordinate species. This seed bank is the buffer during years with little reproduction, seed predation or high mortality. Additionally, bet hedging predicts that species with infrequent years of effective reproduction have a larger seed bank than regularly reproducing species.

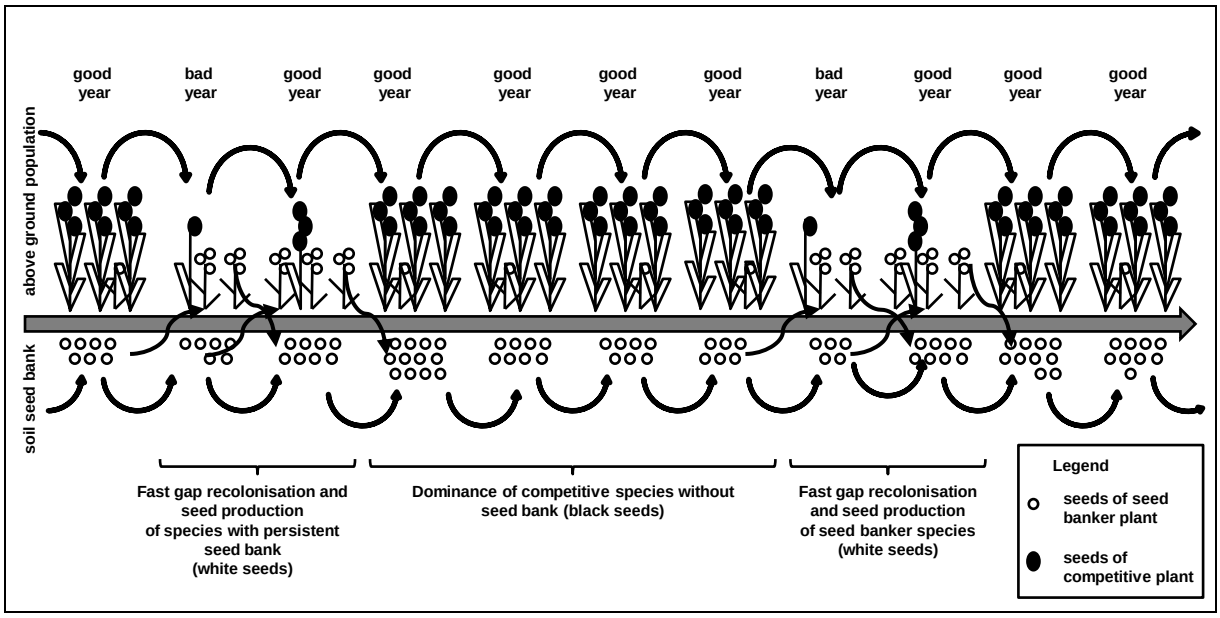


Fig. I.2: Schematic view of the storage effect.

At the same time, studies on life cycles of annual plants (Silvertown 1982;Günter 1997) were the first to identify 'key factors' of population size changes in plants such as seed mortality, seedling mortality and fecundity which are also a key for plant population persistence. Subsequently, models on evolution and population dynamics of annual plants have given additional insight into what factors are important for population dynamics, *e.g.* the buffered population growth due to seed bank development (Venable 1989;Kalisz and McPeek 1993;Pake and Venable 1995;Pake and Venable 1996).

FUNCTIONAL TRAITS

Plant functional traits are any measurable morphological, physiological, phenologic, chemical and ecological parameter of an individual plant or species (Violle *et al.* 2007). There is a long tradition in community and population ecology to analyse species characteristics in relation to their environments (Weiher *et al*. 1999;Poschlod *et al*. 2000). In the last years, these

analyses became more current and explicitly focussed on traits, and definitions have been refined, and a common methodology has been formed (Weiher *et al*. 1999;Lavorel and Garnier 2002;Violle *et al*. 2007;Kleyer *et al*. 2008). Violle *et al.* (2007) clarified the distinction between traits (*e.g.* 'plant height') and attributes (*e.g.* 'smaller than 10 cm') and Lavorel & Garnier (2002) between functional response (*e.g.* tall species increase in the community after fertilizing') and functional effect, (*e.g.* 'communities with many tall species lead to lower diurnal temperature fluctuations at the soil surface'). A difference is made between traits that are easy 'soft' or difficult 'hard' to measure.

The study of morphological, physiological, phenologic and demographical traits gives insight how environmental conditions determine species composition. Ecological filters (Harper 1977) are understood to select taxa with a specific combination of attributes, *i.e.* values of a trait, (Lavorel *et al.* 1997). They can also enhance coexistence in diversifying other traits (Grime 2006). *In fine*, the trait based approach aims at predicting species composition and it can help to recognise extinction threats for species (Thompson 1994;Kahmen 2004;Bekker and Kwak 2005;Smart *et al*. 2005;Ozinga *et al*. 2008;Römermann *et al*. 2008). Simple single trait analysis connecting directly environmental conditions to functional traits (Peco *et al.* 2005) contrast with complex statistics involving classification into *a priori* functional groups (Lavorel *et al*. 1999;Kleyer *et al*. 2008).

THE SEED SIZE-SEED NUMBER TRADE-OFF: A CENTRAL GRADIENT IN COMPARATIVE PLANT ECOLOGY

In analysis of functional traits, it is important to consider trade-offs, because they add constraint to the trait-environment relationship. An ecological and evolutionary trade-off represents a compromise between two factors that cannot be optimised simultaneously because of limited resources or time. The most widely acknowledged trade-off in ecology is the one between number and size of offspring (Fig. I.3). For plants, this means that seed size

and seed production cannot be maximised at a time, and are hence related by a trade-off (Shipley and Dion 1992;Jakobsson and Eriksson 2000;Turnbull *et al*. 2000).

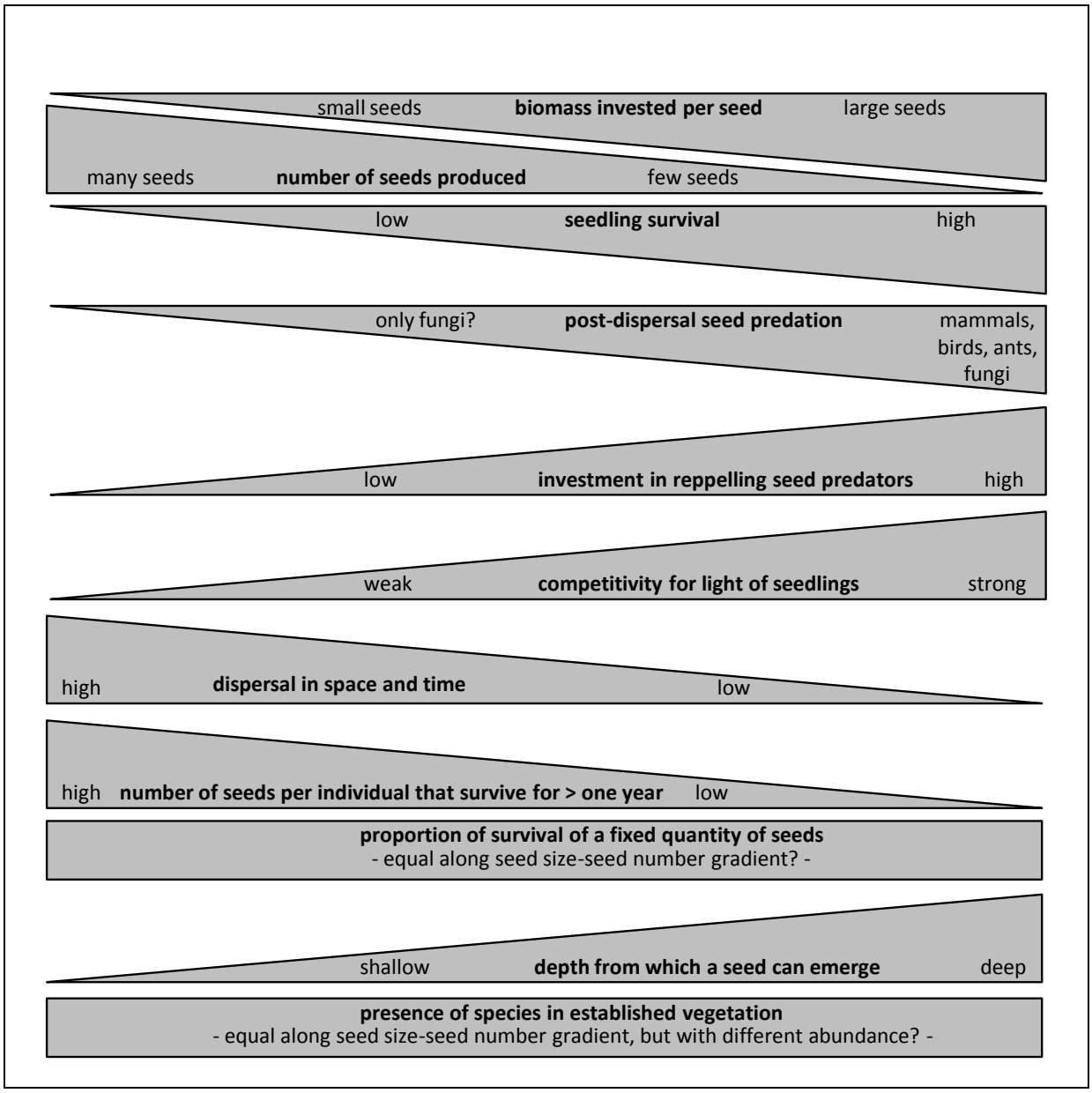


Fig. I.3: Existing and assumed hypothesis on seed size, seed number and related gradients of processes and traits

This trade-off implies that resources for seed production are limited and that a given quantity can be invested into either many small seeds or few large ones. It is clear that whenever other factors (size, survival) are constant, having a higher number of seeds yields higher chances of establishment and a higher fitness of the mother plant. Seed production influences seed rain (Jackel and Poschlod 1994), and high seed production enhances

dispersal efficiency (Tackenberg *et al*. 2003;Poschlod and Biewer 2005;Bruun and Poschlod 2006). It has also been suggested that high seed production increases seed bank persistence (Thompson 2000) and larger seeds can emerge from deeper burial depth (Grundy *et al.* 2003) and that therefore dormancy or the reaction *e.g.* to light in germination is not equal among different sized seeds (Milberg *et al*. 2000;Jurado and Flores 2005). However, after seeds are dispersed, the seed predation is higher for large seeds and hence a higher investment is done in repelling substances and structures (Louda 1989). On the other hand, the large range of seed sizes within communities implies that many small seeds are equally efficient for reproduction as few large seeds (Leishman *et al.* 2000b). This should be so even if there might be some differences in species density that are correlated to seed size (Murray *et al.* 2005). There are hence advantages in producing few large seeds. Few large seeds compensate for their lower number at other life stages, beginning with the seedling (Leishman *et al*. 2000b;Moles *et al*. 2004). This includes a higher seedling survival of large seeded species (Leishman *et al*. 2000b;Moles *et al*. 2004), which can be due to a higher survival to partial damage or higher competitivity of larger seeded species.

In this context, the crucial question is to know at which moment differences in size of seeds becomes important for function: great differences in seed number between large and small seeded species exist at dispersal. However, at the end, an equal number of reproductive adult plants become established. The short discussion above and figure I.3 show that most mechanisms that compensate larger seeds for their lower number act *after* germination, at the seedling stage (McGinley *et al*. 1987;Louda 1989;Jakobsson and Eriksson 2000;Leishman *et al*. 2000b;Coomes and Grubb 2003;Moles *et al*. 2004;Pizo *et al*. 2006;Bladé and Vallejo 2008). Consequently, seeds in the seed bank are not equally effective for establishment according to their size; indeed germinating species in gaps are not necessarily correlated to later established plants (Hillier *et al.* 1990). A major question persists therefore if seed bank

persistence estimates based on seedling counts from soil seed samples can give an accurate perception or if it is biased towards higher soil seed persistence for many small seeds.

GERMINATION CONDITIONS AND GERMINATION NICHE

The timing of germination is crucial for fitness of annual plants: plants germinating early in season are advantaged over late germinating ones due to intra- and interspecific competition (Symonides 1983;Coomes and Grubb 2003). However, this holds only when there is a temporally homogeneous environment and there are for example no drought or frost events that could damage early germinating plants more than those with germination delayed in the season, in which case dormancy becomes important (Silvertown 1999). Germination niche itself can offer several ways how to time and place the germination optimally. Reaction to diurnally fluctuating temperatures is interpreted as such a gap detection mechanism: in vegetation gaps temperature fluctuations are higher than in dense vegetation (Thompson *et al*. 1977;Grime *et al*. 1981;Thompson and Grime 1983). Annual and diurnal temperature fluctuations also decline with burial depth in the soil (Miess 1968). Therefore, enhanced germination to fluctuating temperatures permits a seed to detect in which depth it is. In greater depth, secondary dormancy is induced (Benvenuti *et al.* 2001). The smaller a seed the shallower the depth from which seedlings can emerge (Grundy *et al.* 2003). It is thus important especially for small seeds to detect in which depth they are and this is in congruence with smaller seeds being more dormant (Jurado and Flores 2005). Another mechanism that triggers germination is response to light: a light requirement blocks germination when a seed is buried as light penetrates only very little in the soil (Benvenuti 1995). Therefore this may be, together with primary and secondary dormancy a way to build up a soil seed bank (Grime *et al*. 1981;Baskin and Baskin 1989;Milberg *et al*. 2000). Again seeds react differently according to their size and smaller seeds are more dependent on light for germination than large seeds in cold temperate floras (Milberg *et al.* 2000). For climates

with dry and hot summers, like Mediterranean ecosystems, it has been proposed that there should even be higher germination in darkness because the soil humidity is higher below surface (Bell *et al.* 1995) and it is suggested that in dry environments larger seeds have advantages (Jurado and Westoby 1992). Studies on the temperature requirements for germination of Mediterranean annual plant species show that most species germinate at cold temperatures (Baskin and Baskin 1998). This can be explained by the higher rainfall in winter (see fig. I.8) and by lower evapo-transpiration and hence a higher fitness for species germinating in the cold.

DORMANCY

Initially, dormancy was used as a quasi synonym for no germination of resting seeds (Harper 1977). Now the widely accepted definition of dormancy states is that a seed is dormant when germination does not occur at optimal (water, light, temperature) conditions until a specific mechanism (chilling, scarification, after-ripening) breaks dormancy (Baskin and Baskin 1998). Baskin & Baskin (1998) also differentiate between primary dormancy, already present in mature seeds and secondary dormancy, acquired by non-dormant seeds often induced by environmental conditions (*e.g.* darkness or high temperatures). Many species show cycling dormancy: they are dormant in one season and non-dormant in another, optimising the chance of their offspring seed to establish successfully. Primary dormancy has been classified into several types which also can be combined (Baskin and Baskin 1998). Most important are (i) physical dormancy where an impermeable seed coat prevents imbibition (ii) morphological dormancy where an underdeveloped embryo needs time to fully develop before germination (iii) physiological dormancy where germination is prevented although seed coats are permeable and an embryo is well developed and generally a (warm or cold) stratification is needed to break dormancy. The 'enforced dormancy' type of Harper (1977), corresponding to not germinating seeds because

environmental conditions are not favourable is no longer considered as dormancy. Since the persuasive effort of Baskin & Baskin (1998) and Thompson *et al.* (2003) it has become clear that dormancy is only one stage of seeds in the soil and that types of primary dormancy alone are not sufficient to explain soil seed persistence.

SOIL SEED BANKS

Soil seed banks are the reservoir of viable seeds in a soil or at its surface. The discussion of population dynamics and models for coexistence points to the central role of soil seed banks for the understanding of population persistence, especially for annual plants. According to the time seeds stay viable in the soil, seed banks have been classified into transient (<1 year), short (> 1 and < 5 years) and long-term persistent (> 5 years) (Thompson *et al.* 1997). For temperate floras, Grime & Thompson (1979) used also a species' germination seasonality (separating spring and autumn) and the abundance of seeds for persistent seeds. Arable fields and dry grasslands are probably the most deeply studied vegetation types, including their seed banks (Poschlod and Jackel 1993;Dutoit and Alard 1995;Thompson *et al*. 1997). This has opposite reasons. Arable weeds are studied to predict and control weed emergence (for example Ball 1992) and only rarely for restoration (Dutoit *et al*. 2003). Soil seed banks of calcareous grassland species are studied for the potential to restore species rich communities with many rare species (van der Valk and Pederson 1989;Dutoit and Alard 1995;Hutchings and Booth 1996;Bakker *et al*. 1996b;Poschlod *et al*. 1998;von Blanckenhagen and Poschlod 2005;Bossuyt and Honnay 2008). For the plants of dry habitats, several works suggest higher mortality of seeds in moist environments than in their original dry habitat (Ellenberg 1996;Blaney and Kotanen 2001;Schafer and Kotanen 2003;Wagner and Mitschunas 2007). The importance of disturbances, bare soil and hence soil movement for seed bank formation and persistence of seed bank forming species has been shown empirically (Peco *et al*. 1998;Hopfensberger 2007).

The possibilities to obtain a high diversity and abundance of plant species contained in the soil seed banks has been shown very early by Darwin (1859). Nowadays, this potentiality to rapidly yield high plant diversity is a cornerstone in restoration of communities (van der Valk and Pederson 1989;Hutchings and Booth 1996;Bakker *et al*. 1996b;Schütz 2000;Dutoit *et al*. 2003;von Blanckenhagen and Poschlod 2005;Bossuyt and Honnay 2008). Seed mortality in soil under field conditions is the key factor that most heavily reduces important individual seed numbers to often only scarce seedlings (Silvertown 1982;Kalisz and McPeek 1993;Günter 1997). From early experiments we know that there can be considerable differences among species concerning soil seed mortality (Beal 1885;Duvel 1902;Telewski and Zeevart 2002), but for many especially rare species precise data are still scarce. A considerable amount of data on natural soil seed banks has accumulated giving insight on the existence, size and seasonal dynamics of soil seed banks (Thompson and Grime 1979;Poschlod and Jackel 1993;Ortega *et al*. 1997;Thompson *et al*. 1997). Soil seed banks conserve to a certain amount the diversity of a plant community, and therefore can serve as a means to restore it (van der Valk and Pederson 1989;Hutchings and Booth 1996;Bakker *et al*. 1996b;Schütz 2000;Dutoit *et al*. 2003;von Blanckenhagen and Poschlod 2005;Bossuyt and Honnay 2008). Evidently, this is not true for all species and the effectiveness depends thus much on the degree of soil seed persistence of the seeds for a given species and on the proportion of species forming a persistent seed bank. Number of persistent seeds necessarily decline with time, so restoration is also dependent on the time elapsed since the last seed input (Waldhart *et al*. 2001;Dutoit *et al*. 2003). Finally, the conditions of restoration, notably the season when the soil is disturbed to promote germination, can drastically decide on community composition (Lavorel *et al*. 1994;Ellenberg 1996). Another aspect of soil seed bank is its role for maintaining genetic diversity. Works on the genetic role of the soil seed bank show that the genetic diversity in soil seed banks is higher than in the above-ground population (McGraw 1993;Cabin *et al*. 1998). Additionally, there is a higher inter-population

genetic diversity in above-ground populations than in the soil seed bank (Cabin *et al.* 1998). Moreover, a comparative analysis of many species showed that species forming a persistent seed bank have a higher evolutionary rate than allied species without persistent soil seed bank as ageing seeds accumulate mutations (Whittle 2006).

MATING SYSTEM AND POLLEN:OVULE RATIO

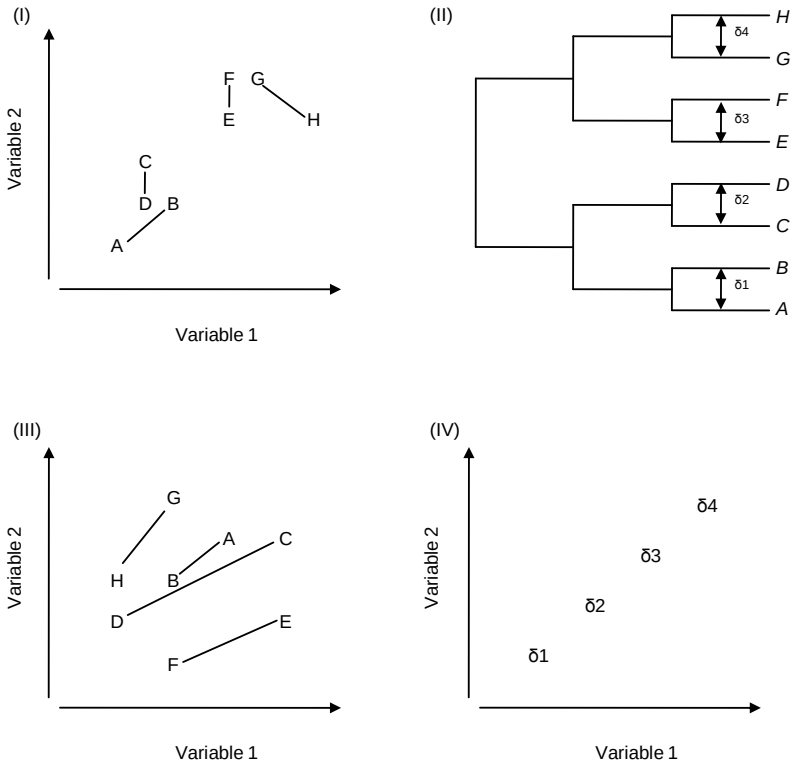
Mating system and pollination vectors are related to another set of plant traits that can trigger genetic diversity and in this way extinction threat of populations. (i) The mating system leads to levels of in- and outbreeding that result in low or high levels of heterozygosity respectively (Loveless and Hamrick 1984;Reed *et al*. 2002). These levels of heterozygosity in turn are related to high or low levels of local extinction. Mating systems with limited gene exchange lead also to a lower genetic diversity at the population scale, because indirectly they reduce effective population size (Silvertown and Charlesworth 2001) and therefore enhance genetic drift and accumulation of deleterious mutations. However, there are exceptions to the high extinction risk of limited gene exchange as illustrates the existence of apomictic and obligate autogamous plants. This decrease in potential to adapt increases the threat of local extinction (Ellstrand and Elam 1993). These factors can increase the genetic erosion in plants with already small populations or limited distribution, that have lowered gene flow compared to common plants (Falk and Holsinger 1991;Cole 2003). (ii) The pollination vector decides whether there is large scale gene exchange or very limited pollen transport (Hamrick *et al*. 1979;Loveless and Hamrick 1984). There are several traits such as spatial stigmate-anther separation, flower size and the pollen ovule ratio (P/O) that are related to different pollination vectors and degree of in- and outbreeding (Cruden 1977). Outcrossing species such as wind pollinated species with high genetic exchange, and a greater effective population size (Loveless and Hamrick 1984) have a high P/O ratio, whereas frequently inbreeding species, some of them obligate autogamous, have a low P/O

ratio (Cruden 1977). Inbreeding depression, that is the lower performance of selfed to outcrossed descendants, is the most important genetic cost of reduced genetic diversity (Charlesworth and Charlesworth 1987). Gene exchange, as a main determinant of local genetic diversity, is equally important for plant performance (Ellstrand 1992). Gene exchange and levels of genetic diversity have also consequences for the survival of entire local populations (Ellstrand 1992). This shows that beside simple mechanistic performance, genetic diversity and gene exchange can enhance plant performance at any life stage (Charlesworth and Charlesworth 1987).

COMPARATIVE BIOLOGY AND PHYLOGENETICALLY INDEPENDENT CONTRASTS

Comparative biology applied to ecological problems is an across species approach which aims to elucidate the differences in species morphological and life history traits and species ecological 'behaviour', that is, its niche, reaction to environmental factors, population ecological characteristics and so on. These parameters are then compared for a larger set of species. With this approach, species are used as independent replicates. However, the use of species as independent data points is controversial: some species are more related than others are and measured traits may have evolved only once for a large group of species. From an evolutionary point of view, these species are not independent realisations: closely related species often show similar characters and habitats as a consequence of common ancestry and therefore differences among species are not independent (Harvey and Pagel 1991). For these reasons several approaches take the phylogeny into account while comparing species (Felsenstein 1985;Harvey and Pagel 1991). Phylogenetically independent contrasts (PICs; Felsenstein 1985) offer the opportunity to recalculate data in order to retrace how often they appeared independently in the phylogeny, instead of analysing simply species as replicates.

However, there are also reasons to analyse comparative data without taking into account phylogenetic correction and to consider the observed variance to be correlated to ecological features (Westoby *et al.* 1995b). Species composition in different communities can be similar although they originated from different local species pools because species' traits match the environmental filters – in this vision, the outcome is strongly dependent on repeated environmental conditions in other words ecological processes and not evolutionary ones. In the present thesis, we chose the usage of both approaches giving the opportunity to evaluate how far observed patterns are correlated to phylogeny and to identify which are only revealed by the comparison of two closely related species. Phylogenetic explicit analyses depend on information of phylogenetic relationships among the species studied. We compiled a tree from recent works on phylogeny of the studied species and families, using APGII as a backbone (Angiosperm Phylogeny Group 2003). This tree was completely resolved down to species level for all of the 38 species studied here. Branch lengths can importantly change the outcome of analysis; this has been explored using three different trees. We visually examined the branch length of closely related species pairs and distantly related genera in our data set for three trees were (i) all branch lengths were set to 1; (ii) branch lengths according to Wikström *et al.* (2001) and (iii) branch lengths calculated according to Grafen (1989). Whereas (i) and (ii) gave no realistic branch lengths, whereas method (iii) retraced very well the many closely related species pairs and longer lengths for much more distant groups. For this reason, we decided to use Grafen's (1989) estimation of branch-length method and not the age estimations of Wikström *et al.* (2001). We then run linear regression through the origin as recommended by Garland *et al.* (1992) and presented phylogenies in the accompanying figures. We used the comparative method parallel to all analyses that were not phylogenetically explicit.



Box 1: Phylogeny and comparative analyses

When two variables are compared among species, the phylogenetic relationships can lead to contrasting interpretations of the correlation among the variables. In (I) both variables are correlated when each species (letters in fig. I) is taken as a replicate, but inspection of species pairs in the corresponding phylogeny (II) shows that the correlation is due to a single split in the phylogeny and that among species pairs (lines in fig. I) show no consistent relation between the two factors. In (III) both variables are uncorrelated, however all differences among closely related species pairs (II) go in the same direction and phylogenetically independent contrasts (δ1-δ4) for the two variables are hence strongly correlated (IV).

Study system and site

CEREAL WEEDS, HISTORY, EVOLUTION

Cereal weeds (German '*Segetalpflanzen*', French '*messicoles*') are arable weeds that are bound to cereal cultivation. Ecological reasons for the existence of a special set of plants in cereal fields are the disturbance characteristics such as timing of ploughing (Schneider *et al*. 1994;Roche *et al*. 2002) and reseeding of contaminated seed material known as 'speirochory' (Schneider *et al*. 1994;Jäger 2002). However, the many reasons for their regression highlight which factors explain their existence in cereal fields, very well documented in the extensive review of Schneider *et al.* (1994). There are also historical and biogeographic reasons for the existence of a particular cereal weed flora (Lososová *et al.* 2004). Cereal weeds were often supposed to originate in the same area as cereals themselves (Olivereau 1996) but caryologic and chorological work on rare arable species (Verlaque and Filosa 1997) showed that the simplistic opinion that arable weeds are foreign archaeophytes, regressing to their original distribution area does not hold and that those statements compromise conservation efforts. *Agrostemma githago* (Caryophyllaceae) appears in Central Europe very early in Neolithic sites (Willerding 1986). This species originated probably from Greece or Asia Minor because there a closely related species, *A. gracile,* exists in natural habitats such as rocky screes (Tutin *et al.* 1964). Although cereals are initially collected by hand and toxic seeds of *Agrostemma* would have surely been sorted out this species extended rapidly their range with the spread of agriculture (Willerding 1986). It is possible that it was even cultivated. A related species, *Vaccaria hispanica* (Caryophyllaceae) is still cultivated today as a complement to legumes in forage cultures. *Cnicus benedictus* (Asteraceae-Cardueae) is another example of a cultivated plant. From the different species of *Valerianella* (Caprifoliaceae) only *V. locusta* is cultivated today as '*mâche*' *i.e.* corn salad. *Camelina sativa* was cultivated as a source of oil seeds. These examples show that a part of this flora originated from cultivated species, in some cases from

eastern Mediterranean origin, which subsequently became spontaneous. Another part came probably from North Africa such as *Adonis, Ceratocephala* (both Ranunculaceae), *Hypecoum, Roemeria* (both Papaveraceae). There are written proves from several Latin authors (see box 2) that North Africa was exporting cereals to Europe in Roman times. That these species travelled together with cereals and have been re-sown somewhere is very likely in the light of cereal weed seeds present in today cereal samples from Europe or Northern Africa. Others have local, North-West Mediterranean origins such as *Legousia* (Campanulaceae), *Anagallis* or *Androsace (*both Primulaceae*)*. The same or close relatives of the species studied here still exist on rocky calcareous outcrops or gravels in the wild not far from actual cereal fields (Molinier 1981;Girerd 1991).

Even in the case of the relatively recent arrival of archaeophyte cereal weed species in agricultural landscapes of Europe, dating back at least to the Neolithic (Willerding 1986;Bonn and Poschlod 1998), the rapid local evolutionary processes registered throughout many organisms (Hairston *et al.* 2005) makes it evident that local adaptation and hence a form of specific local genetic diversity is present. The interesting discovery of Verlaque & Filosa (1997) who found a Provence-specific hexaploid caryotype of *Roemeria hybrida* is probably only the tip of the iceberg. A considerable part of original local and regional biodiversity exists in this group of plants and this cannot be preserved elsewhere or *ex situ*. The species analysed in the present work are necessarily a subset of a larger group of plants bound to cereal fields. We focus here on winter annuals with essentially the same cycle as the cultivated cereals. Cereal weeds are known to be rather xerophytes with a preference for soils with a high pebble content (Ellenberg *et al*. 1992;Ellenberg 1996).

VEGETATION TYPES AND FLORISTIC GRADIENTS IN ARABLE FIELDS

The vegetation of cereal fields has also been studied by phytosociologists for a long time; they have contributed interesting information on the realised niche, ecological species

groups and floristic vegetation types of annual plants in cereal fields according to soil parameters and agricultural practices (Braun-Blanquet *et al*. 1952;Ellenberg 1996). Earlier (Braun-Blanquet 1939), this approach identified two main gradients in floristic composition of cereal fields: soil reaction (pH) and time of ploughing. The time and kind of ploughing opposes the summer cultures grouped together in the 'Chenopodietea' to winter cereal fields 'Secalinetea' (Braun-Blanquet *et al*. 1952;Ellenberg 1996). Calcareous bedrock type results in high soil pH and dry soils, conditions that coincide with a set of species limited to the 'Caucalidion' alliance, opposing to acidic soils rarely used for cereal cultivation in Western Mediterranean with different species ('Scleranthion'). These works were also the first to highlight the functional differences between low temperature germinating annuals of winter cereal fields 'Secalinetea' and the high temperature germinating species of summer cultures 'Chenopodietea': the key factor that decides which vegetation will establish is not crop type but time of soil disturbance (Lauer 1953 and Salzmann 1939 cited in Ellenberg 1996). Additionally species germinating at high temperatures include rapidly growing species, often with C_4-photosynthetic pathways (Larcher 2001, p. 84), with high nutrient requirements, which become more and more numerous in the European flora. Whereas species germinating at low temperature grow slowly and are advantaged on oligotrophic soils with a little competitive environment (Ellenberg 1996).

TRADITIONAL MEDITERRANEAN CEREAL CULTIVATION AND FARM TYPES IN THE LUBERON AREA

The ecology of cereal weeds is closely bound to the agricultural practices of cereal cultivation (Schneider *et al.* 1994). In traditional systems in the Western Mediterranean, cereals are sown when the field has been prepared by ploughing in September and October just after the first autumn rains. Over winter, there is no treatment of the fields, in modern more intensive systems herbicides and chemical fertilizers are applied during autumn or spring. In some

cases, the field is rolled over to break primary shoots of cereals in order to produce several shoots per individual. In traditional systems, weeds, especially when they become apparent during flowering, were sometimes pulled out by hand and used as forage. Cereals are harvested between end of June and July, when they completed ripening and grains became tough. Cereals are stored dry in the storehouses. In autumn, cereal fields are used as pastures, Gerbaud *et al.* (2001) showed that cereal weeds constitute a high quality nutriment complement for sheep.

There are considerable differences among practices in farms in the Luberon area (see map below). Gasc (2005) identified three actual types of farms that cultivate cereals: cereal dominated farms, cereal culture with sheep flock and organic farmers with cereals.

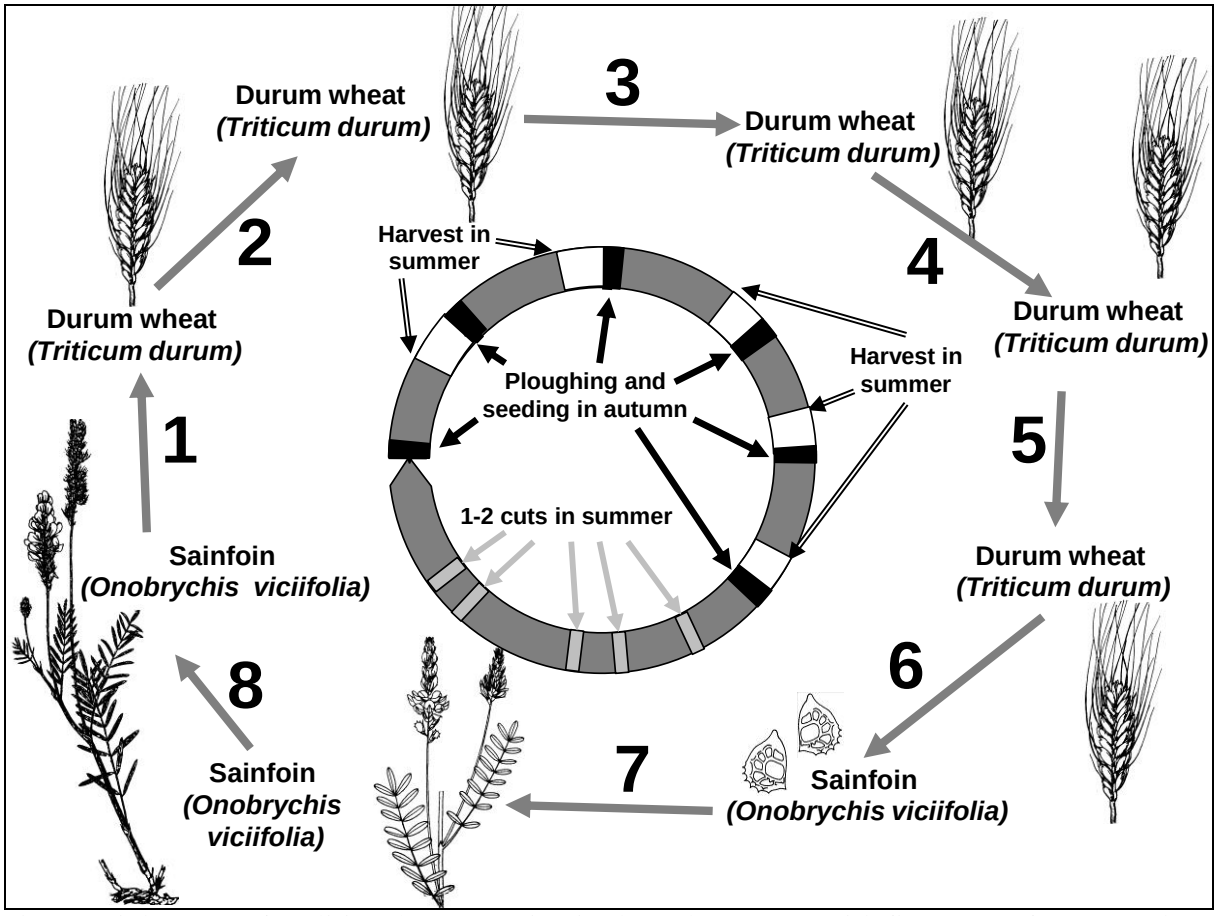


Fig. I.4: Eight years of traditional crop rotation in the Luberon area with five years of Durum wheat and three years of sainfoin as fodder intercrop with disturbance regime as inner circle, black: open bare soil between ploughing and crop germination, dark grey: standing crop, light grey: cut crop, white: wheat stubbles (drawings modified from Jávorka and Csapody 1979;Rothmaler 2000).

In cereal-dominated farms, herbicides are intensively used, seed material is not re-sown but bought every year and there is only a short fallow period or a rotation with another crop. In the remaining farms, herbicide use is an exception and rotation alternate one to five years of cereals with a period of fallow-pasture or legumes. In the Luberon area, the legume phase of the crop rotation is often three years of *Onobrychis viciifolia* (Fig. I.4). According to the farmers experience this reduces considerably the soil seed bank of weedy species. Farm produced seed material is frequently re-sown, in some cases, especially small farms with sheep flocks. Weed seeds are even not sorted out before reseeding (Gasc 2005). Jäger (2002) counted seeds in four replicates of 1 kg seeding material and could identify 43 different cereal weed species with a mean of over 12000 weed seeds per kg seeding material. We counted weed seeds from a cereal sample from traditional cereal agriculture in Algeria, where we identified only eight species and much less seeds per kg cereals (Saatkamp, unpublished data). This illustrates that 'speirochory' *i.e.* the transport and reseeding of weed seeds into cereal field is a general and important factor for dispersal of cereal weeds, which has probably an impact on weed population dynamics. According to Jäger's (2002) and our own sample from Algeria there is no strict selection of species with particular seed sizes, however a tendency to greater plant height. Both wheat samples contain small species *(Aegilops sp., Anagallis arvensis)* as well as small seeded species *(Papaver rhoeas, Legousia speculum-veneris, and Silene sp.)*. In the light of the very simple techniques of some small farms, the evolution of agricultural practices in the area from first appearance of agriculture in the Neolithic to today are the mechanisation of working steps, larger cultivated surfaces, deeper ploughing, watering and different cultivated crops together with probably a shorter field rotation. For the more intensive farms, synthetic herbicides and fertilizers, high performance crops and intensive field preparation are in sharp contrast with this traditional farming. In the study period (1983-2006), there was a shift to more winter wheat cultivation and abandonment of marginal fields and pastures (Gasc 2005).

ECOLOGICAL SERVICES OF CEREAL WEEDS

Cereal weeds are in a complex web of relations to other organisms in cereal fields, including pollinators, herbivores, predators and cereals themselves. Figure I.5 resumes some of the ecological services of cereal weeds for remaining wild life and human usage of agricultural landscapes (Gerbaud *et al*. 2001;Marshall *et al*. 2003;Gibson *et al*. 2006;Pinke *et al*. 2008). Plants developing after harvest and some of those found within cereals are a supply of complementary nutrients in farms where cereals are cultivated together or for sheep breeding (Gerbaud *et al.* 2001). Granivorous birds such as the hunted grey partridge (*Perdix perdix*) depend on seeds from wild plants in cereal fields (Marshall *et al.* 2003). This is certainly also the case for more heterophagous birds that can also feed on noxious insects such as the Ortolan bunting (*Emberiza hortulana*). The recent and general decline of pollinators (Biesmeijer *et al.* 2006) with its important economic consequences (Gallai *et al.* 2009) has various reasons and the quasi-absence of nectar sources for pollinators in landscapes dominated by intensive agriculture is one reason (Gibson *et al.* 2006). The decline of nectar sources has also consequences for the remaining flora (Biesmeijer *et al.* 2006) and especially on rare insect-pollinated taxa in cereal fields (Gibson *et al.* 2006). Presence of weeds, especially grasses, in cereal fields is also known to increase cereal aphid predating insects (Sotherton *et al.* 1989; van Emden 1990 cited in Eliott *et al.* 1998). Parasitoid wasps (*e.g. Aphidius rhopalosiphi*) are the most effective in controlling cereal aphids (*e.g. Sitobion avenae*) in cereal fields (Schmidt *et al.* 2003); these specialised parasitoid wasps develop larger and thus more effective populations for bio-control when monocot cereal weeds developed prior to cereals (van Emden 2002). These are just some examples, other beneficial effects of cereal weeds *via* other groups of predator insects are also favoured by cereal weeds.

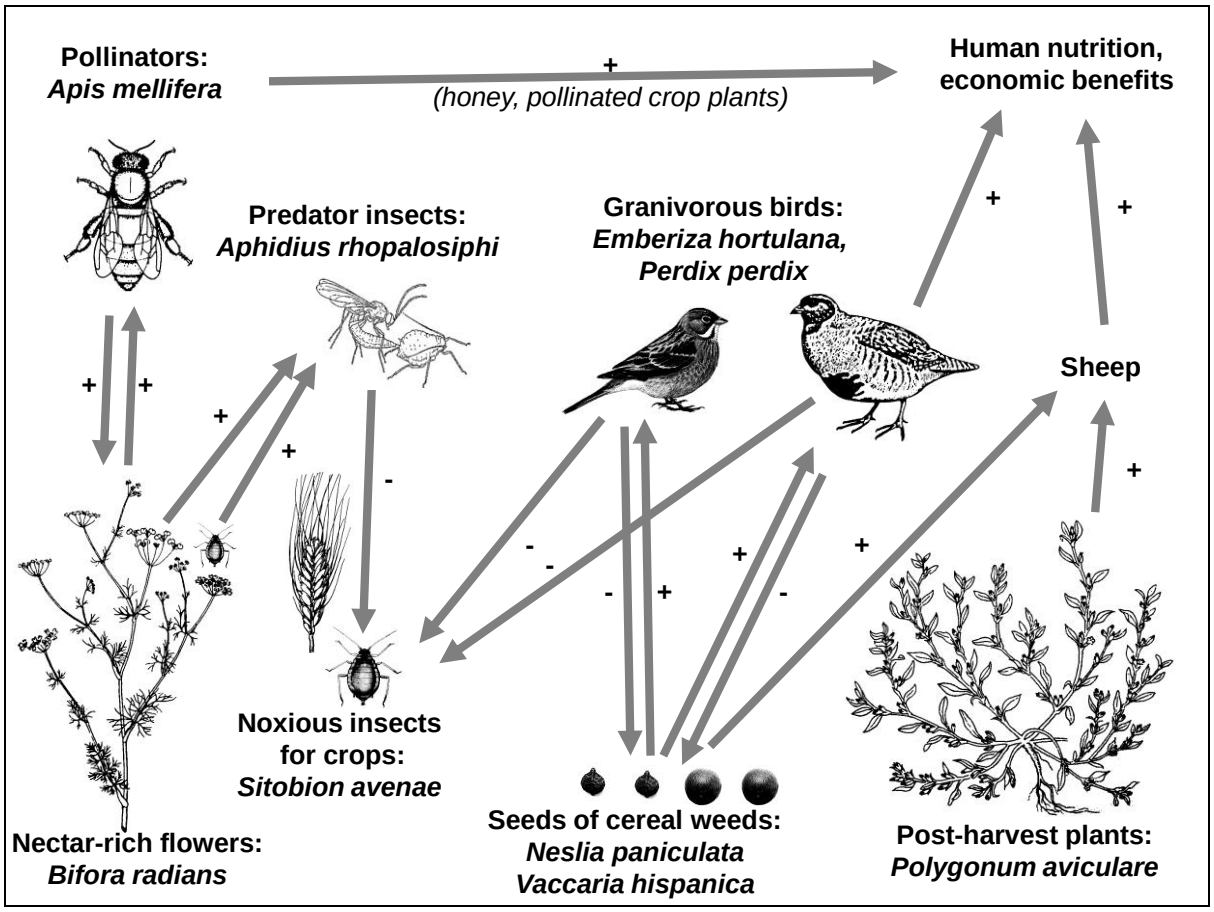


Fig. I.5: Examples of trophic relationships and ecosystem services of cereal weeds, for detailed discussion and bibliographic sources see text.

The relations of cereal weeds to these organisms show their economic importance but also the dependence of rare cereal weeds on other plants in these ecosystems *e.g.* for pollination (Gibson *et al.* 2006). It is evident that there can also be severe economic losses due to infestation of cereal fields by other than the crop plants and pertinent agronomic techniques evolved to reduce or even exterminate all other plants than crops. A small review in Ellenberg (1996) already showed that presence of other than crop plants up to 25% of cover is without major impact on crop yields. Smith *et al.* (1999) and Marshall (1989) showed that even the species richer field margins are not important sources of economically detrimental weeds in cereal fields. There is also sporadic evidence for a direct benefit of some cereal weeds on the crop yields, such as the positive interaction between *Scandix pecten-veneris* and wheat (Dutoit *et al.* 2001), probably due to allelopathic action of weed born substances on soil pathogens (Qasem and Foy 2001).

In the light of the evidence discussed, there is no longer an economic conflict between weed control for higher crop yields and tolerance of weeds for ecosystem services; a low degree of weed infestation should be tolerated to guarantee important ecosystem services of richer agricultural landscapes. In the meanwhile, research on how annual plant diversity maintains in changing agricultural landscape, can add helpful details for its conservation.

CAUSES OF MAINTENANCE OR REGRESSION

The maintenance of cereal weeds in cereal fields was related to traditional cultivation practices including *e.g.* seeding of not cleaned seed material (Schneider *et al*. 1994;Ellenberg 1996;Olivereau 1996;Jäger 2002). Their regression is related to change in agricultural practices (a detailed review in Schneider *et al*. 1994;Fried *et al*. 2009). Hence these species specialised to cereal fields are now the most heavily regressing plants all over Europe (Korneck and Sukopp 1988;Schneider *et al*. 1994;Andreasen *et al*. 1996;Sutcliffe and Kay 2000;Aboucaya *et al*. 2000;Robinson and Sutherland 2002;Pyšek *et al*. 2005;Baessler and Klotz 2006;Pinke *et al*. 2008).

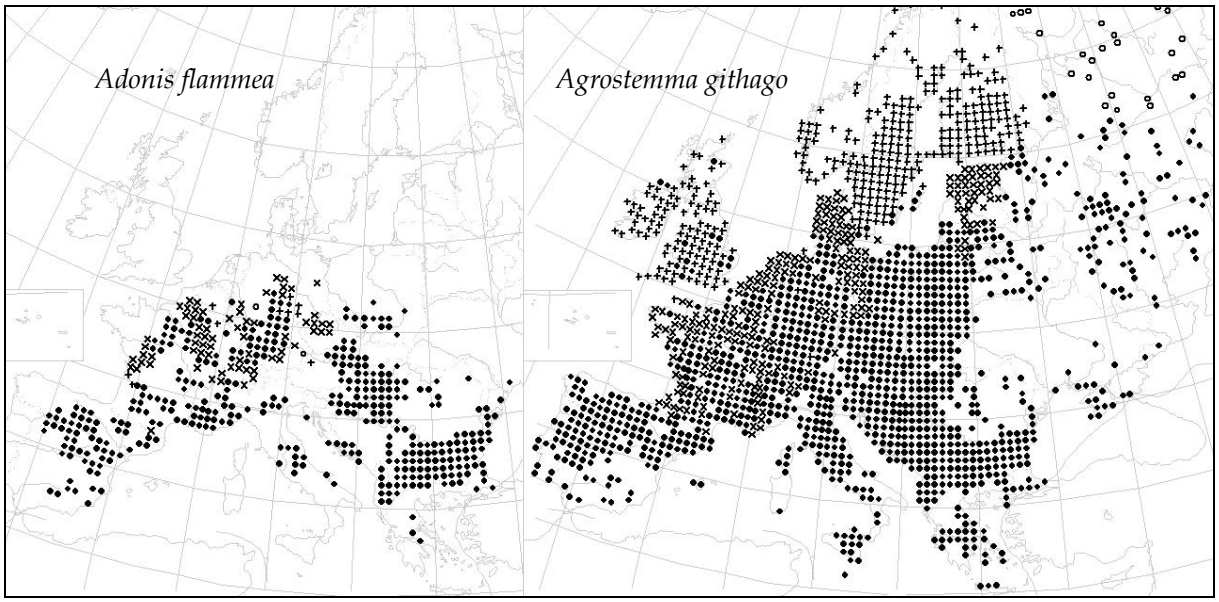


Fig. I.6: Regression of two cereal weeds, *Adonis flammea* and *Agrostemma githago* in Europe; plain dots are occurrences after 1930, crosses are extinct occurrences; note that for Central and Western Europe regression continued and *Agrostemma* is now near to extinct in Great Britain, France and Germany (source: Atlas Florae Europaeae).

Figure I.6 illustrates the early regression (post-1930) of two species in Europe, showing the important regression in industrialised agro-ecosystems of *e.g.* Northern France, Netherlands and Eastern Germany and the preservation in Southern and Eastern Europe. Several processes have been identified that trigger(ed) the regression of cereal weeds in present day landscapes and at the same time favoured other often more ruderal species. (i) The use of herbicides impacts both plant establishment and soil seed banks, favouring resistant lines (Ball 1992;Schneider *et al*. 1994;Fried *et al*. 2009). (ii) Change in characters, identity and density of crop plants yields a higher competition on weed species (Schneider *et al.* 1994), however Roche *et al.* (2002) clearly showed that higher cereal density favours cereal weeds with respect to other ruderal plants. (iii) The use of mineral fertilizers instead of organic manure triggered regression mediated by a changed nutrient status of soils but also less seed input (Schneider *et al.* 1994). (iv) Cleaning of seeding material before sowing leads to a lower seed input and enhanced isolation of populations among different fields (Schneider *et al*. 1994;Ellenberg 1996;Olivereau 1996;Jäger 2002) (v) change in soil preparation steps, ploughing depth and changed crop rotation systems. In general, arable weeds are known to form often long time persistent seed banks, but some cereal weeds like for example *Agrostemma githago* lack any dormancy or darkness inhibition of germination and do not form a persistent soil seed bank (Schneider *et al.* 1994; this work). In areas with traditional cereal agriculture, where these species still persist, it could be shown that cereal weed communities quickly lose the most interesting species after abandonment, and their regeneration from the soil seed bank is impossible (Jäger 2002;Dutoit *et al*. 2003). This can at least partly be explained by the striking differences in the longevity of soil seed banks, but also by the changes in dispersal processes at the landscape scale (Schneider *et al*. 1994;Ellenberg 1996;Bonn and Poschlod 1998;Jäger 2002;Dutoit *et al*. 2003). Because of its high diversity in rare cereal weeds and the traditional agriculture, the Luberon area is of high conservation interest on a European scale for these plants (Aboucaya *et al.* 2000).

WHY ANNUAL CEREAL WEEDS AS A STUDY SYSTEM?

We showed that annual cereal weeds could fulfil the requirements for the storage effect. Their coexistence may be mediated by differences in reaction to temporal variability and the storage function of the soil seed bank. Indeed, cereal fields with crop rotation and changing agricultural practices offer a highly temporally variable environment with variable recruitment success for annual plants of different competitive ability, seed size and germination requirements. The high temporal environmental variability should enhance extinction dynamics and population turnover, differences among species in these parameters may become apparent quickly. The short life span of annuals makes it also easier to observe the effects of temporal variability because the short life cycle reduces the number of factors that buffer against population turnover in perennials. Nonetheless, models on relevant factors for the dispersal in space and time from annual plants are also valid for perennials (Venable and Brown 1988). For these reasons, we decided to study an exemplary subset of rare and related common cereal weeds.

STUDY SITE

Localisation and topography of the study area

We gathered data on rare and common cereal weeds in an area of ca. 2500 km² around the Luberon ridge in South Eastern France (Fig. I.7). The Luberon mountain ridge is a representative of the vast surfaces of limestone mountain ridges in Southern France, North Eastern Spain and Northern Italy all with similar bedrock types, climate and agricultural landscapes.

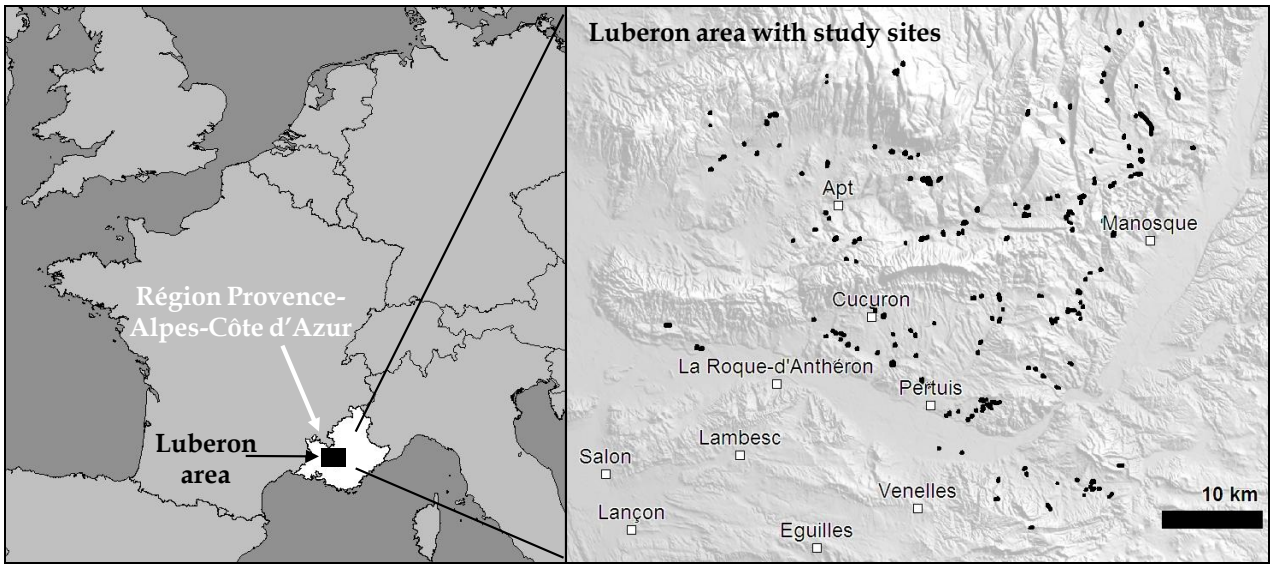


Fig. I.7: South Eastern France -*Région Provence-Alpes-Côte d'Azur*- in white, Luberon area marked with a black square and study sites with dots in the shaded relief map.

Climate of the study area and climate during the study period

This area is characterised by Mediterranean climate (Salon: mean rainfall$_{1971\text{-}2000}$: 623 mm, with maxima in April and October, data: Météofrance, 2009). There is one climatic gradient in the study area, *i.e.* a combined temperature and rainfall gradient due to the higher altitude of the Northern part of the study area. Figure 8 indicates details on the climate, for rainfall at *Roque d'Anthéron* in the western part of the study area, and for temperatures in *Manosque* in the eastern part. The rainfall pattern shows marked differences among years, notably a long dry period in summer 2007, a year when effective rainfalls did not occur until November. Drought triggers vegetation cover in Mediterranean ecosystems, a putative reason thus to influence the outcome of the dormancy patterns in the burial experiment, which took place at 12 km at *Cucuron*. The very dry winter and spring 2005 may have also had its effect on population abundance. The temperature patterns are uniform among years; summer 2006 was hotter and winter 2007/08 less cold. These temperature patterns had probably no marked effect on population dynamics or dormancy levels as only extreme cold winters leave considerable gaps in the vegetation in the study area.

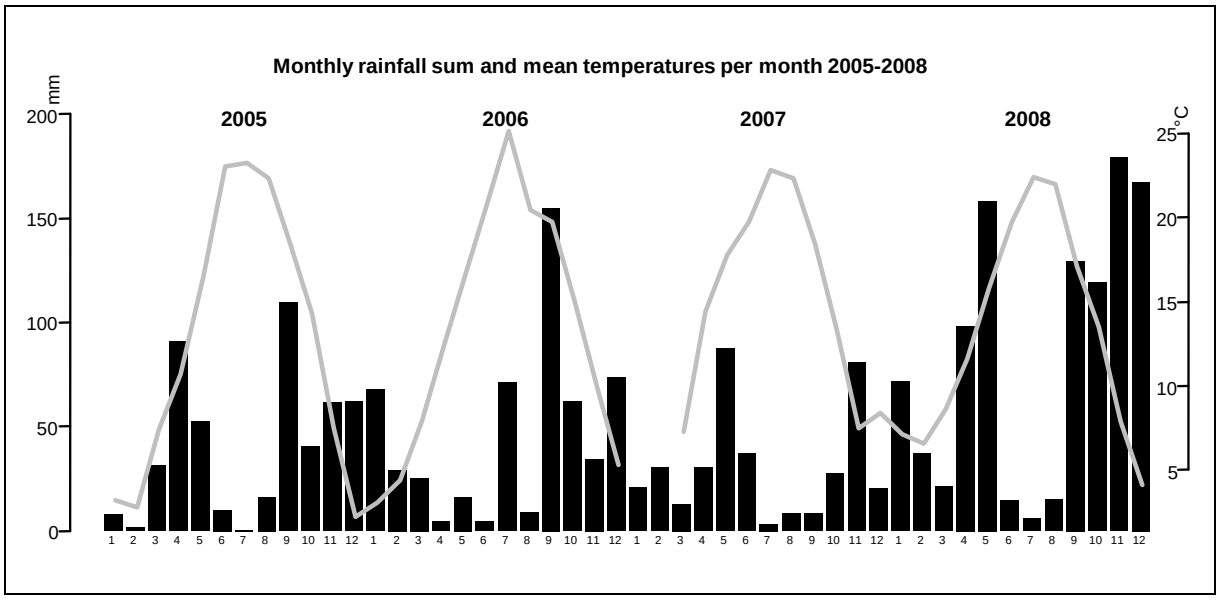


Fig. I.8: Monthly rainfall sum (black bars, scale at the left) and mean temperatures per month (grey line, scale at the right) for the study period, rainfall at La Roque d'Antheron, temperature at Manosque (data: www.infoclimat.fr).

Land use

The traditional land use in the study area is a highly diverse mixture of pastures, cereal fields, vineyards and specialised cultures such as cherry plantations in the *Vallée du Coulon* and lavender on the limestone highlands. However, there is a general shift from cereal fields to vineyard especially south of Luberon ridge (Müller 1991) and from cereal fields to lavender at higher altitudes in the North of the study area and more recently a shift from diversified to pure cereal farms (Gasc 2005). The dominating culture in the plots was winter wheat. Winter wheat is cultivated in a cycle which alternates two or three years of wheat with two or three years of fallow or legume (*Onobrychis viciaefolia* or *Medicago sativa*) for sheep fodder (Gasc 2005), see figure I.5. This relatively traditional agriculture compared to intensive wheat cultivation maintained a high diversity of rare cereal weeds elsewhere extinct in Europe (Filosa 1989;Filosa 1997).

Geology and soils of the study area

Geological units covering the study area comprise a nearly complete range between Middle Jurassic (Oxfordian limestone near *Mirabeau*) to recent Quaternary (Durance-alluvia near *Mérindol*). Cretaceous and Jurassic limestone characterize the mountain ridges of *Grand*

Luberon, *Petit Luberon* and the *Monts de Vaucluse* in the north of the study area. Most of the agricultural areas are on molasses and sands of the Miocene, sands of the Eocene and marls and smooth limestone of the Oligocene in the lowland parts and more rarely on Cretaceous limestone at the slopes of the mountain ridge, for a detailed map of geology in the study area see Moutier and Balme (1997). Soils that developed on these bedrock types have the common point of being very stony throughout and having always a very dry phase in summer when they completely dry out in the relevant layers for annual plants. In a limited set of parcels on marl bedrock, there can be water saturation in winter, but we had no parcel with the typical vegetation of winter-inundated fields, which is dominated by *Juncus bufonius* and *Lythrum hyssopifolium* outside the studied parcels.

Overall, gradients of water content, pH and stone content exist inside particular fields and among fields (Roche *et al.* 2002). We did not analyse the differences in soil properties in detail in this thesis because the focus of the thesis is on processes at the population and community level along time and not among different locations. This bears the risk of not capturing relevant changes in soil properties along time. We regret not to have the time to bring the precision needed for questions on changing soil properties; such a work would seriously be hampered by the lack of old data on soil properties in 1983. We therefore decided to consider changing properties indirectly through the use of indicator values, a very powerful tool to detect such changes *via* vegetation (Diekmann 2003).

'*Frumenta hieme in herba sunt, verno tempore fastigantur in stipulam quae sunt hiberni generis, at milium et panicum in culmum geniculatum et concavum, sesama vero in ferulaceum. omnium satorum fructus aut spicis continetur, ut tritici, hordei, muniturque vallo aristarum contra aves et parvas quadripedes, aut includitur siliquis, ut leguminum, aut vasculis, ut sesamae ac papaveris. milium et panicum tantum pro indiviso et parvis avibus expositum est; indefensum quippe membranis continetur*' (Pliny the Elder, *Naturalis Historia,* 18.52-53)
'Cereals are green in winter, in spring they climb into stalks which originated in winter; the millets however, have kneed or curved stems and sesame is quite a creeping herb. All sown crops are -or enclosed in spikes- like those of wheat and barley that are protected by a wall of awns -or they are enclosed in silics- like those of the legumes -or they are enclosed in small cups- like those of sesame and poppy. The millets on the other hand are exposed to the numerous small birds as they lie unprotected in skins.'

'*igitur quod nunc intra murum fere patres familiae correpserunt relictis falce et aratro et manus movere maluerunt in theatro ac circo, quam in segetibus ac vinetis, ac frumentum locamus qui nobis advehat, qui saturi fiamus ex Africa et Sardinia, et navibus*' (M.T. Varro, *Res Rustica,* 2.pr.3)
As therefore in these days practically all the heads of families have sneaked within the walls, abandoning the sickle and the plough, and would rather busy their hands in the theatre and in the circus than in the grain-fields and the vineyards, we hire a man to bring us from Africa and Sardinia the grain with which to fill our stomachs.

'*frumentique uim ingentem quod ex Africa P. Scipio miserat quaternis aeris populo cum summa fide et gratia diuiserunt*' (T. Livius, *Ab urbe condita,* 31.4.6-7)
They also distributed to the people with strict impartiality and to the general satisfaction a vast quantity of corn which Scipio had sent from Africa. It was sold at four ases the modius.

'*...legati terni in Africam ad Carthaginienses et in Numidiam ad frumentum rogandum, quod in Graeciam portaretur, missi, pro quo pretium solueret populus Romanus*' (T. Livius, *Ab urbe condita,* 36.3.1-2)
'(six commissioners) were sent to Africa to procure corn for Greece, the cost to be borne by Rome; three went to Carthage and three to Numidia.'

'*temperata apud transmarinas provincias frumenti subvectio, et ne censibus negotiatorum naves adscriberentur tributumque pro illis penderent constitutum. Reos ex provincia Africa, qui proconsulare imperium illic habuerant, Sulpicium Camerinum et Pompeium Silvanum absolvit Caesar*' (C. Tacitus, *Annales,* 13.51-52)
'In our transmarine provinces the conveyance of corn was rendered less costly, and it was decided that merchant ships should not be assessed with their owner's property, and that no tax should be paid on them. Two men under prosecution from Africa, in which province they had held proconsular authority, Sulpicius Camerinus and Pomponius Silvanus, were acquitted by the emperor'

Box 2: An early description of plant phenology, food webs and plant traits in cereal fields (Pliny) and four classical texts documenting the transport of cereals from Northern Africa to Europe in Roman times (Varro, Livius and Tacitus).

TRANSITION TO CHAPTER 1

Real world example of diversity at different spatial scales

Spontaneous populations and communities are the basis for understanding and the aim of prediction of ecology. The study of actual vegetation detects patterns of diversity, abundance and function, but also temporal changes and realised niches of species. In this approach, data are gathered on species composition and abundance of local communities complemented by data on abiotic properties, land use and its history.

Figure T1.1 illustrates how the γ-diversity at a larger spatial scale, exemplified by the species list of two plots, is subdivided into diversity contributed by α-diversity from two different plots. In figure T1.1, C marks species that are found in both plots, the species overlap. The species that are only found in one of two plots is the absolute β-diversity. This β-diversity can also be calculated among different γ-diversity on a higher spatial scale, leading to spatially nested components of β-diversity (Crist *et al.* 2003). Absolute β-diversity is measured on the same scale as α and γ-diversity, and is most easily obtained as the difference between γ-diversity and the species overlap: $\beta = \gamma - C$. Absolute β-diversity can be divided by γ-diversity yielding a relative β-diversity; this is useful when γ-diversity are very different and one is interested in the relative change not the total number of species added. These types of diversity measures have fundamental differences: α-diversity and β-diversity are not correlated and are not driven by similar factors; even relative and absolute β-diversity are quite different. Diversity at fine scales can have different determinants than diversity on coarser spatial scales: *e.g.*, factors that increase β-diversity at fine scales can enhance γ-diversity at a coarser scale. For this reason, an analysis at different spatial scales is important to understand factors determining plant diversity.

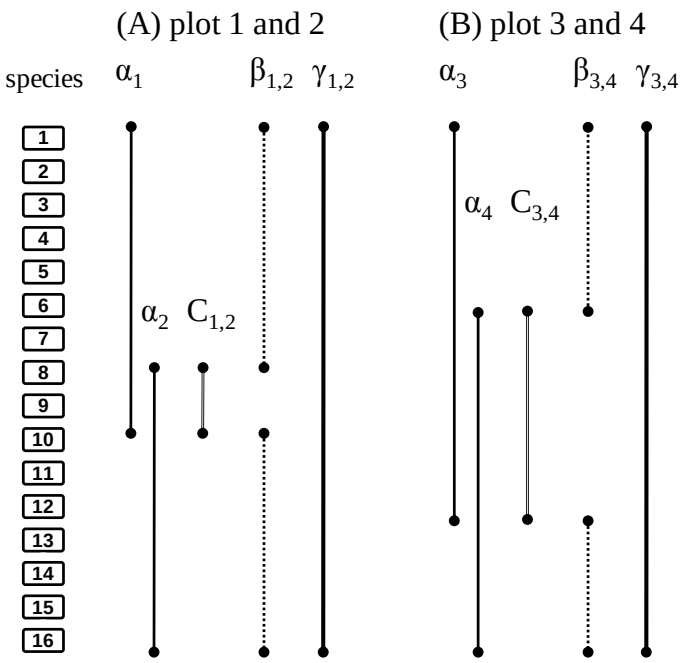


Fig. T1.1: Additive partitioning of plant diversity with α-diversity at the plot scale for two pairs of plots, 1 & 2 and 3 & 4, with different overlap and the resulting different β-diversity but similar γ-diversity.

Diversity in plant communities has many different aspects and can be analysed in a hierarchical framework, such as **additive diversity partitioning** (Crist *et al.* 2003). This approach aims at analysing biological diversity subdividing it according to different spatial scales. In chapter 1, we adopt this approach to analyse diversity patterns in an agricultural landscape dominated by vineyards before we tackle approaches that are more experimental or involving the use of larger data sets. In our case, an *a priori* stratification has been applied to study the factors landscape, habitat and agriculture. All three can influence species composition and species diversity. *A posteriori* supplementary data on the history of the studied fields was gathered and this added a historic dimension to the understanding of species presence.

CHAPTER 1

Plant diversity in agro-ecosystems influenced by vineyard structure, landscape class, land use intensity and past cereal cultivation

INTRODUCTION

Plant diversity in agro-ecosystems is in general decline in Europe (Andreasen *et al*. 1996;Sutcliffe and Kay 2000;Aboucaya *et al*. 2000;Jauzein 2001;Robinson and Sutherland 2002;Baessler and Klotz 2006). There is now growing concern about this loss of biodiversity, many specialised species from agro-ecosystems being on regional and national Red Data Books (Korneck and Sukopp 1988;Roux and Nicolas 2001). In Europe, some specialists, *e.g.* cereal weeds are among the most threatened plants on regional scales. In addition to that, there are also serious concerns due to the functional role of these plants in the agro-ecosystems and the services they provide for agriculture (Marshall *et al*. 2003;Pinke *et al*. 2008). There are indirect services of arable plants by supporting predator populations in fields (Sotherton 1984;Eliott *et al*. 1998;van Emden 2002;Schmidt *et al*. 2003) or on their edges (Thomas and Marshall 1999;Smith *et al*. 2008). Decline of arable plants in general also contribute to the decline of effective pollinator communities with important economic losses (Biesmeijer *et al*. 2006;Gallai *et al*. 2009). The decline of pollinators in turn trigger the decline of pollinator dependent plants including rare plants (Biesmeijer *et al*. 2006;Gibson *et al*. 2006). It is now clear that at moderate densities within fields and at high densities outside, arable plants have no important impact on the cultivated crop (Marshall 1989;Ellenberg 1996;Smith *et al*. 1999).

Which factors determine plant diversity at different scales in agricultural landscapes is therefore of prime interest for conservation of plant diversity and maintenance of ecological

services for agriculture. Reasons for the decline of arable plant diversity are the higher intensity agriculture, including herbicide use, higher competitiveness of crop plants due to different crop types, the use of synthetic fertilizers and the consolidation of arable land (Schneider *et al*. 1994;Robinson and Sutherland 2002). Moreover, plant diversity in agricultural systems is also determined by the landscape context, the number and size of different habitats and diversity of bedrocks and soils. Historical-geographical factors also determine plant diversity in arable systems (Lososová *et al.* 2004).

Species diversity can be measured at different scales –from the habitat to regions– and either as α-diversity, that is species richness, or as β-diversity, *i.e.* differences in species between stands at different spatial scales (Crist *et al.* 2003). The global diversity in a larger area or region summing up both is termed γ-diversity. This additive framework enables one to analyse the contribution of α and β-diversity at different spatial scales (Allan 1975;Lande 1996;Crist *et al*. 2003). This additive diversity partition, permits direct comparisons, whereas the classical Jaccard's and Sørensen's index analyse relative species-turnovers and do not measure β-diversity on the same scale as species richness.

For pollinators and predator populations, not only the diversity and abundance of arable plants in the fields themselves are important but also the diversity in crop edges or outside in adjacent habitats (Steffan-Dewenter *et al*. 2002;Gabriel and Tscharntke 2007). Therefore, β-diversity between different habitats has functional importance for biodiversity conservation and ecosystem services in present day landscapes. However β-diversity in arable systems with different intensities of agriculture has been analysed only recently, and this has only been done at the field centred scale, especially for cereal fields (Gabriel *et al*. 2006;Roschewitz *et al*. 2009). Much less is known on the β-diversity of structural elements which are part of the agro-ecosystem (Thomas and Marshall 1999;von Arx *et al*. 2002;Dutoit *et al*. 2007), especially in crop systems where these elements have a functional role, like in vineyards. Vineyard α-diversity has thoroughly been analysed by Maillet (1992), but little is known about diversity

at different spatial scales and the role of non cultivated habitats such as embankments or the areas for turning of machines (Dutoit *et al*. 2007). It can also be asked if in the transition zone between, cultivated field, field edges and adjacent communities there are new and characteristic species or not. This has been conceptualised *via* the distinction of ecotones and ecoclines, the former without the latter with specialised species in the transition between to different habitats according to van der Maarel (1990); similar definitions with terms inversed appeared earlier (Frochot 1987). In the terminology of additive diversity partitioning, these works compare in detail absolute to relative β-diversity in transition zones between two habitats and classify transitions according to the amount of absolute β-diversity. However, these works generally do not consider different spatial scales and they do not consider that β-diversity in these transition zones can be modified when landscape context or management change.

The main scope of this study is therefore to know (i) Which factors determine plant α-diversity in vineyard landscapes? (ii) How do these factors influence β-diversity among different habitat structures in vineyards, between vineyards and γ-diversity at the landscape scale? (iii) How recent historical factors such as cereal cultivation influence α-diversity and finally (iv) Which are plants of high conservation value and do they follow the same trends of α-diversity that the entire flora follows?

METHODS AND STUDY AREA

Study area

The study has been done in the agricultural landscape south of the Luberon ridge and north of the Durance river valley in Provence, South Eastern France. This area of about 300 km² is on molasses and sands of the Miocene, sands of the Eocene and marls and smooth limestone of the Oligocene (Moutier and Balme 1997). Climate is Mediterranean with mild winters and summer drought. Forest remnants in the study area are dominated by downy oak (*Quercus*

pubescens) on deeper soils and sclerophyllous evergreen Holm oak (*Quercus ilex*) on shallow skeletal soils especially on the south-facing slopes of the hills and the Luberon ridge itself. Initial successional stages after forest destruction include mattorals with kermes oak (*Quercus coccifera*), rosemary (*Rosmarinus officinalis*) and several *Cistus* species. Some of the species of initial successional stages and forests occur also in vineyards as a secondary habitat *e.g. Rubia peregrina* on the vine-rows and *Cistus* species on the embankments.

Stratification scheme and plant species survey

We used three main factors, landscape classes, agricultural intensity and habitat type to stratify plots, and resulting in a balanced sample for each subgroup. To do so, we first selected at random five areas for three different landscape classes according to a previous classification based on actual vegetation, geology and relief, which was run in a GIS on a 1km basis. The data sources for this classification include the geological map (Moutier and Balme 1997), a digital elevation model (DEM) with a 50 m resolution (IGN, Paris) and the CORINE land-cover map which is based on interpretation of satellite images (DRE-PACA 1999). This classification leads to three classes. The first class consists in a flat unit with slightly south facing slopes on calcareous molasses between Cucuron, Lourmarin and Ansouis (fig. 1.1). In these areas, vineyards are the dominating land use type lying mostly on Miocene molasses as geological unit and we refer to it as limestone landscape. Second, a landscape class with smooth north-facing slopes on Eocene and Miocene sands, discontinuous around Pertuis, La Tour d'Aigues and La Bastidonne with vineyards and cereal fields as co-dominant land use units, which we called sand landscape. Finally a third class with a complex relief in the northeast around the villages of Grambois, La Bastide des Jourdans and Mirabeau, has a more balanced land use mixture of forests, vineyards and cereal fields, predominantly on oligocene marls and limestones, we termed it marl landscape. We replicated these three landscape classes five times.

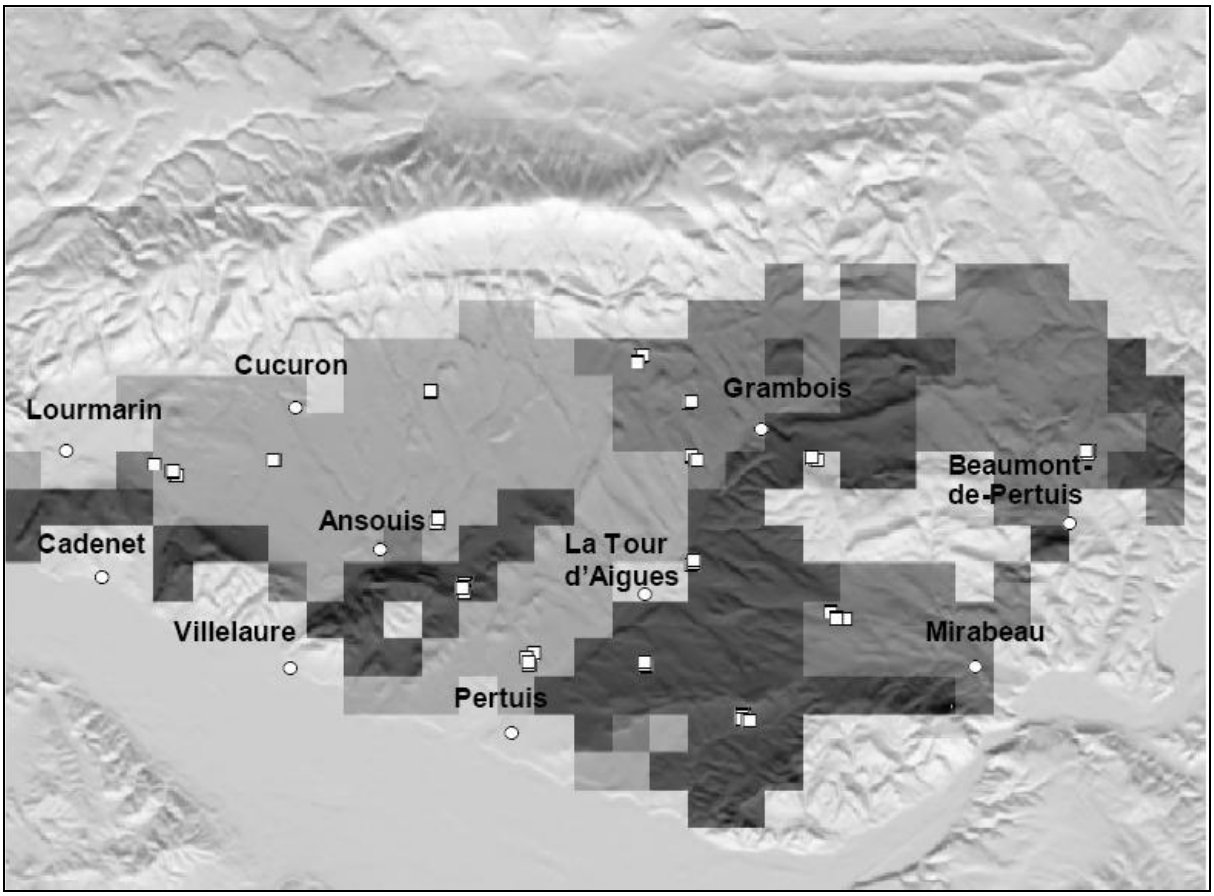


Fig. 1.1: Map of the study area with 1 km grid of the three landscape classes, major villages are marked by white rounds and plots by white squares; dark grey: 'sand landscape' class, middle grey 'marl landscape' class and light grey 'limestone landscape' class, scale is given by the 1 km grid.

Inside each of these fifteen zones, we selected three vineyards with extensive, intermediate and intensive agriculture. Intensity of agriculture was studied using three contrasted neighbouring vineyards that we identified in a previous survey of the entire study area based on indicators of herbicide use, ploughing frequency and economic value of vineyards (Saatkamp, unpublished map). We termed the classes 'H' for high intensity, 'I' for intermediate, and 'N' for extensive vineyards. For each of the resulting 45 individual vineyards, we studied three different habitat types. Therefore, we selected three very frequent habitats, which cover important surfaces in the study area. (i) Inside the vineyard 'P', where we placed a plot with a size of 10 x 20 m at a fixed 20 m distance from the border, with always 4 vine-rows and 3 inter-rows. (ii) the border habitat at the endpoints of the vine-rows where the machines use to turn from one to another vine-row, abbreviated as 'M'. This plot had also a size of 200 m² but the shape varied with the possibilities in the field. (iii) The

adjacent grassy embankment, which is not regularly ploughed or chemically weeded, again a plot with a size of 200 m², was always maintained, we termed it 'T'. Most of the embankment and border plots had 4 m x 50 m. Figure 1.2 resumes a vineyard with the three plot types.

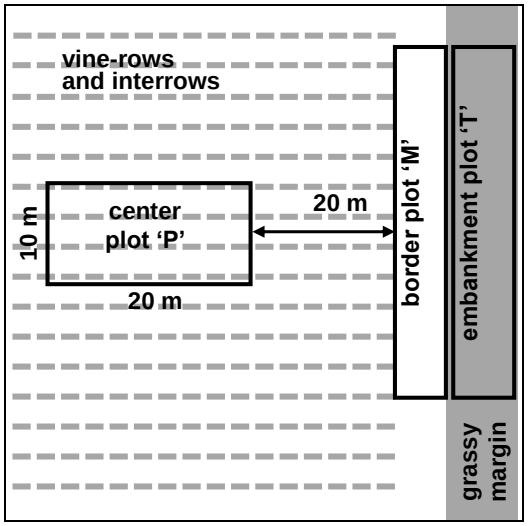


Fig. 1.2: The situation of a vineyard with its embankment (grey), vine-rows (black) and the size and position of the three habitat stratified plot types.

The resulting 3 x 5 x 3 x 3 =135 plots form the basic dataset used for all analyses. The size of each individual plot was set to 200m², because we aimed to find high species numbers, which we think to be more reliable for effects of plant diversity. After noting location (UTM coordinates) a species list of all vascular plants has been established. We visited plots between April and June 2004. We sampled all plants which we could not identify directly in the field and identified it later using Jauzein (1995), this is also the nomenclatural reference, complemented by Kerguelen (1998).

Documentation of former land-use type

The former cultivated crop for the study has been inferred from the French topographic maps at 1:25 000 (IGN, Paris) where several land cover types are noted. Vineyards, orchards and arable fields are differentiated. The most important crops cultivated in former times in this area are cereals (Müller 1991) and information from local farmers confirmed that all new vineyards were previously cultivated with cereals. We had however, no precise date of the

conversion but we have sure information that in 1986 the date of the revision of maps, all recent young vineyards were still cereal fields.

In our case, we calculated β_{TM} and β_{MP} at the individual vineyard scale, between embankment plots (T) and margin (M) of fields as the difference between sum of species of both, γ_{TM} and the species overlap between both C_{TM}. Similarly, we also calculated β_{MP} between margin plots (M) and inside of vineyard plots (P). We also calculated a β at the landscape scale between individual vineyards of different intensity that were grouped at one place as the difference between γ–diversity of two vineyards united and their species overlap C. We only calculated this β-diversity between extensive and intermediate vineyards, β_{NZ}, and between intermediate and intensively managed vineyards, β_{ZH}. Finally, we analysed α-diversity and γ–diversity at the plot, vineyard and landscape scale.

Tab. 1.1: Diversity levels, scales and independent factors analysed in this work.

Type	Variables	Measurement scale	Factors analysed
α-diversity	α	habitats	habitat type, intensity of agriculture, landscape class
β_1-diversity	β_{TM} and β_{MP}	vineyards	intensity of agriculture, landscape class
β_2-diversity	β_{NZ} and β_{ZH}	landscapes	landscape class
γ-diversity	γ_{NZ} and γ_{ZH}	landscapes	landscape class

Table 1.1 gives an overview on the diversity levels we analysed. In addition to the absolute β-diversity, we also measured the relative β-diversity using the percentage β-diversity on the corresponding γ-diversity, this resembles much classical community composition distances such as Sørensen's distance (Lande 1996;Legendre and Legendre 1998;Crist *et al*. 2003):

$$\% \beta = 100 \cdot \beta / \gamma$$

Data analysis

α-Diversity has been analysed as species richness per unit area according to three independent factors, habitat type, intensity of agriculture and landscape class. After testing the assumptions of normality (Shapiro-Wilk's test) and equality of variances (F-test) a

factorial ANOVA (Sokal and Rohlf 1995) has been conducted on the species number per plot as dependant variable and the three factors with three levels of stratification described above. In order to detect effects of cultural practices on vegetation differentiation between margins, borders and fields, or landscape class on differentiation between different intensities of agriculture, β-diversity has been analysed using ANOVA. For the analysis of Red List and cereal weed species, we used Kruskal-Wallis rank sum test to account for the non-normality of these data. Data handling, calculation of diversity measures and all statistical analyses have been done in the R environment (R Foundation for Statistical Computing 2008).

RESULTS

The ANOVA on the α-diversity showed that habitat types in vineyards explain most of species richness, followed by intensity of agriculture and landscape class (Tab. 1.2, fig 1.3). Furthermore, there is a significant interaction between habitat type and intensity of agriculture (Tab. 1.2, fig 1.4).

Tab. 1.2: Results of the analysis of variance on the species number per plot, factors were habitat type, landscape class and intensity of agriculture.

Factor	Degree of freedom	F	P	
Landscape class	2	5.33	0.0062	**
Intensity of agriculture	2	21.42	<0.0001	***
Habitat type	2	67.59	<0.0001	***
Landscape class x Intensity of agriculture	4	0.06	0.9914	
Landscape class x Habitat type	4	0.97	0.4232	
Intensity of agriculture x Habitat type	4	3.18	0.0162	*
Landscape class x Intensity of agriculture x Habitat type	8	0.77	0.6263	
Residuals	108			

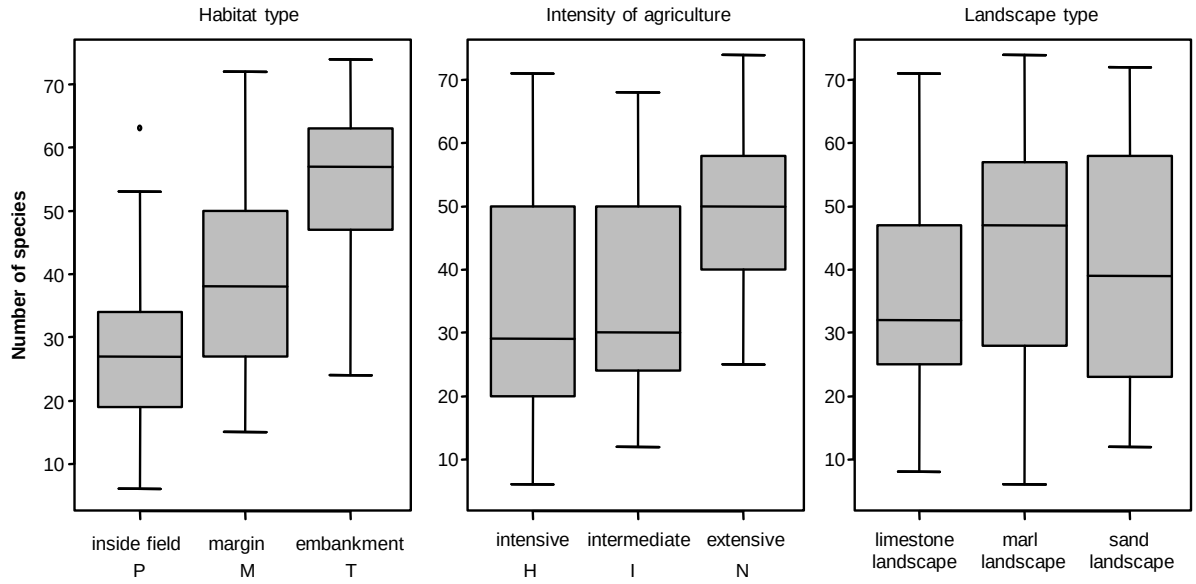


Fig. 1.3: Box plot of α-diversity (species richness on 200m²), each box represents 45 samples

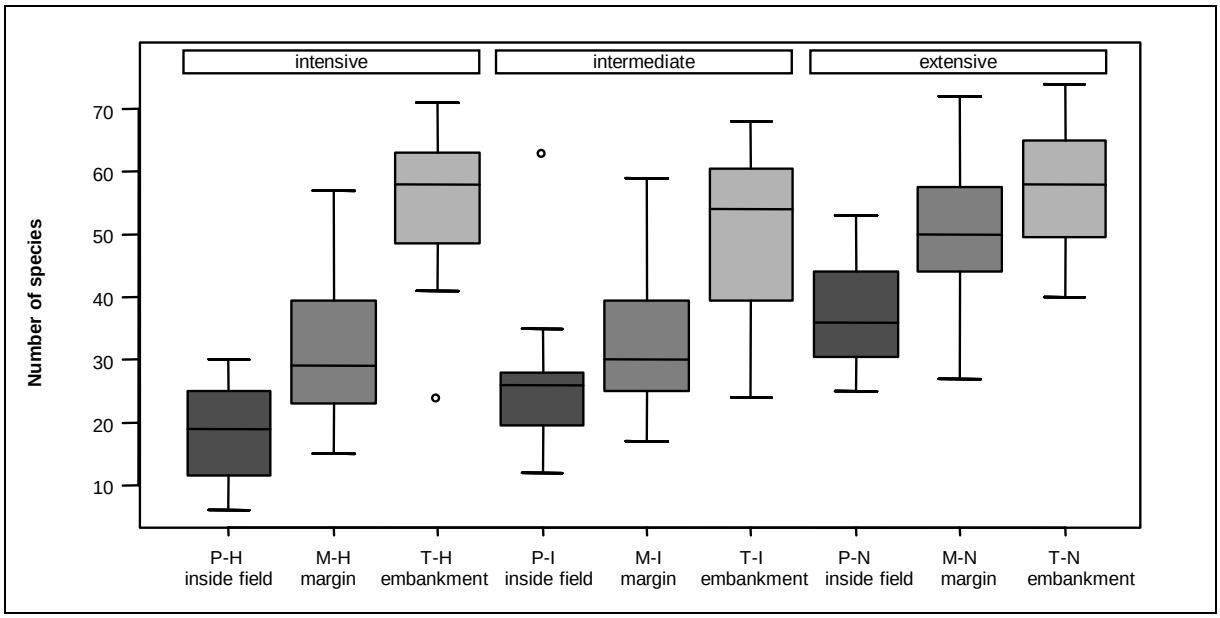


Fig. 1.4: Box plot of the interaction of habitat type and intensity of agriculture on the α-diversity, each box represents 15 samples, P-inside field, M-margin, T-embankment, H-intensive, I-intermediate, N-extensive agriculture.

The inspection of the interaction plot between habitat type and intensity of agriculture (Fig. 1.5) shows, that the differences in α-diversity between the three habitats become more marked with increasing intensity of agriculture.

Tab. 1.3: Synthesis of analyses of variance on the vineyard scale absolute and relative β_1-diversity; β_{TM}: β-diversity between margin and embankment plots, β_{MP}: β-diversity between margin and inside of vineyards.

Factor	Degree of freedom	*F*	*P*	
β_{MP} on intensity of agriculture	2	1.42	0.2529	
Residuals	42			
β_{MP} on landscape class	2	3.99	0.0257	*
Residuals	42			
β_{TM} on intensity of agriculture	2	1.75	0.1853	
Residuals	42			
β_{TM} on landscape	2	5.06	0.0107	*
Residuals	42			
$\%\beta_{MP}$ on intensity of agriculture	2	6.50	0.0034	**
Residuals	42			
$\%\beta_{MP}$ on landscape class	2	0.29	0.7436	
Residuals	42			
$\%\beta_{TM}$ on intensity of agriculture	2	2.88	0.0668	.
Residuals	42			
$\%\beta_{TM}$ on landscape	2	1.19	0.3128	
Residuals	42			

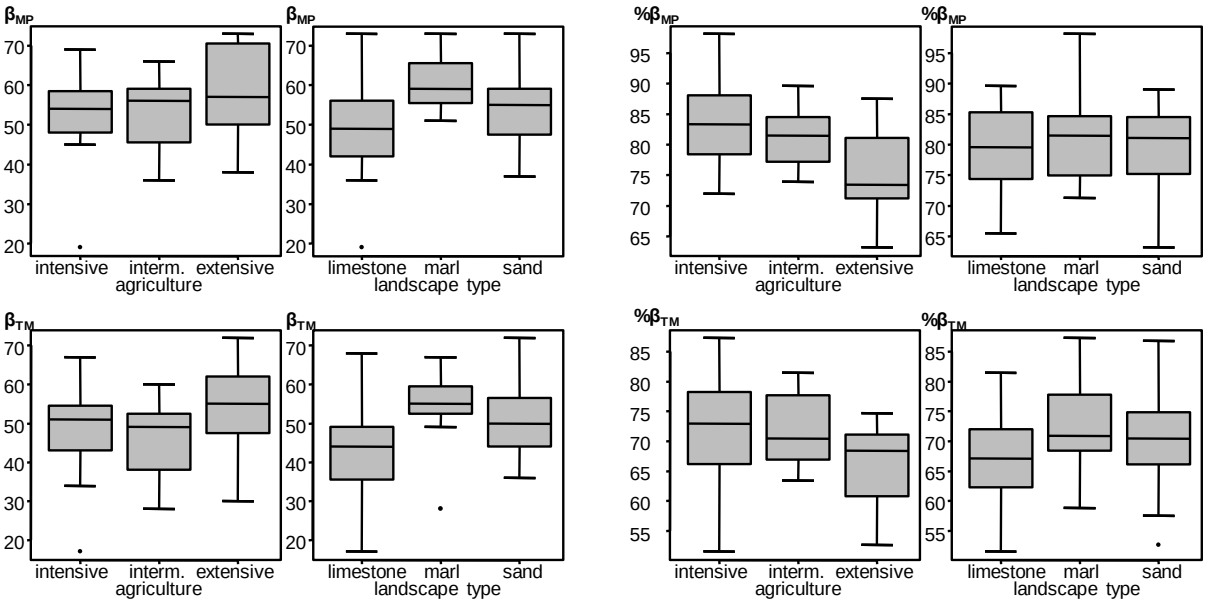


Fig. 1.5: Box plot of absolute (left) and relative (right) β-diversity, each box represents 15 samples; β_{TM}: β-diversity between margin and inside of vineyards (above), β_{MP}: β-diversity between margin and embankment plots (below).

The analysis of β-diversity at the vineyard scale (Tab. 1.3, fig. 1.5) reveals that landscape class had a significant effect on absolute β-diversity for both habitat contrasts, *i.e.* margin to inside field plots (β_{MP}) and margin to embankment plots (β_{TM}). Intensity of agriculture had no effect on absolute β-diversity. However, inspecting the analysis of relative β-diversity shows that

there is a striking effect of agriculture on the contrast between margin plots and plots inside the field, indeed, there is a higher relative β-diversity when agriculture is intensive compared to extensive.

Tab. 1.4: Synthesis of analyses of variance on the landscape scale of absolute and relative β_2-diversity and γ-diversity; β_{NI}: β-diversity between extensive and intermediate vineyards, β_{IH}: β-diversity between intermediate and intensive vineyards.

Factor	Degree of freedom	*F*	*P*
γ on landscape class	2	1.26	0.3167
Residuals	12		
β_{NI} on landscape class	2	1.18	0.3379
Residuals	12		
β_{IH} on landscape class	2	0.16	0.8535
Residuals	12		
$\%\beta_{NI}$ on landscape	2	1.54	0.2524
Residuals	12		
$\%\beta_{IH}$ on landscape class	2	0.63	0.5474
Residuals	12		

The analysis of β-diversity and γ-diversity at the landscape scale (Tab. 1.4) showed no significant effect for landscape class, neither for absolute nor relative β-diversity and this for both contrasts, *i.e.* extensive to intermediate (β_{NI}) or intermediate to intensive vineyards plots (β_{IH}).

The scan for species of high conservation interest in recent Red Data Books and Floras relevant for the study area showed that the vast majority of rare and threatened taxa belong to the group of cereal weeds according to previous floristic works (Braun-Blanquet *et al*. 1952;Guende and Olivier 1997) (Tab. 1.5, bold and italic species). We therefore looked for all typical cereal weeds in our data set according to these floristic works to obtain a complete list for this ecological group (Tab. 1.5, species names in italics).

Tab. 1.5: List and status of typical cereal weeds found among 359 species of this study; species of high conservation value are marked in bold; indented: all other species of high conservation value that are not cereal weeds. Status: (1) Roux & Nicolas (2001): 2, threatened; 3, rare; 5, quite rare but not threatened; 6, neither rare nor threatened; (2) Filosa & Verlaque (1997); (3) Jauzein (1995): AC – quite common; AR – quite rare; R – rare; TR – very rare; * special conservation efforts would be beneficial; (4) Montégut (1997).

Species	Vaucluse (1)	Western Provence (2)	France (3)	France (4)	Frequency
Adonis annua	**5**	**threatened**	**R***	**decreasing**	**4**
Adonis flammea	**5**	**threatened common**	**R***	**rare**	**2**
Allium rotundum			**R***		**1**
Anthemis altissima					1
Anthemis arvensis					22
Bunias erucago					12
Caucalis platycarpos		**less threatened**	**AR**	**decreasing**	**3**
Ceratocephalus falcatus	**5**	**threatened common**	**R**	**very rare**	**2**
Cnicus benedictus	**5**	**threatened**	**AR**	**rare**	**3**
Euphorbia falcata					5
Fumaria parviflora			**AC**	**decreasing**	**3**
Galium tricornutum		**less threatened**	**AR**	**decreasing**	**4**
Gladiolus italicus					14
Hypecoum pendulum	**2**	**rare & threatened**	**TR***		**1**
Iberis pinnata		**threatened**	**AR**		**1**
Legousia hybrida		**threatened**	**AR**		**3**
Lithospermum arvense					10
Medicago coronata	**5**		**TR**		**1**
Orlaya intermedia	**3**	**threatened common**	**R**		**2**
Papaver argemone		**threatened common**	**AC**	**decreasing**	**4**
Papaver dubium					14
Papaver hybridum		**threatened common**	**AR**	**decreasing**	**1**
Papaver rhoeas					52
Polycnemum majus					1
Ranunculus arvensis					7
Roemeria hybrida	**3**		**TR***	**rare**	**2**
Salsola kali	**5**		**TR**		**3**
Scandix pecten-veneris					1
Sclerochloa dura			**TR***		**3**
Valerianella coronata					4
Velezia rigida	**3**		**TR***	**decreasing**	**1**
Vicia narbonensis	**3**				**1**
Vicia pannonica					21
Vicia peregrina			**AR**	**decreasing**	**15**
Viola arvensis					1

The analysis of the α-diversity of cereal weeds in vineyards using the stratification of plots and the data on historical land use (Fig. 1.6) shows a higher number of cereal weed species on the embankment and margins of the vineyards than inside. More cereal weed species have been found in extensively managed vineyards than in intensive ones. There is a

conspicuous effect of the former cereal cultivation on cereal weed diversity. These relations were significant in a Kruskal-Wallis rank sum tests (habitat types, p = 0.0063; intensity of agriculture, p = 0.0003; landscape class, p = 0.0067; former cultivation type, p = 0.0006).

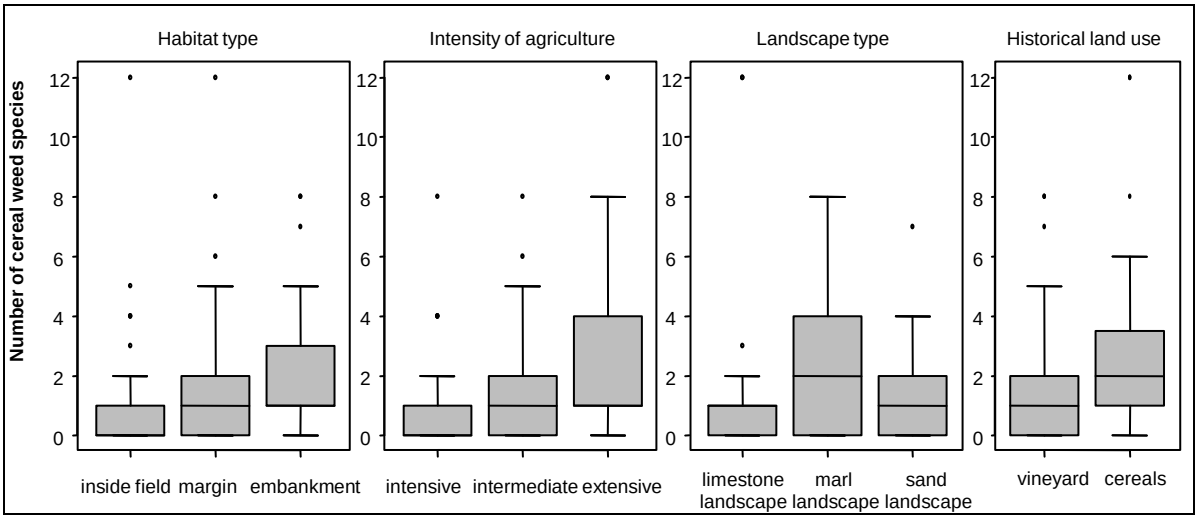


Fig. 1.6: Number of cereal weed species in the studied plots according to habitat type, intensities of agriculture, landscape class and the former cultivation type; N = 45 for each box, except for vineyards, N= 111 and cereals N= 24.

DISCUSSION

First, the analysis of different levels of α-diversity showed that, in this order, habitat types, intensity of agriculture, landscape class and historical factors determine plot scale α-diversity of vascular plants in vineyard landscapes. The high importance of vineyard margins and embankments for plant diversity is in line with the higher arable plant diversity previously found for arable field edges (Marshall 1989;Wilson and Aebischer 1995;Dutoit *et al*. 1999;Gabriel *et al*. 2006;Roschewitz *et al*. 2009) and can be explained by less fertilizer and herbicide application (Schneider *et al*. 1994;Robinson and Sutherland 2002). Therefore, this trend can be different in very extensive and long-term herbicide free systems (Dutoit *et al*. 1999). This can also be the result of influences from surrounding habitats such as dispersal from species rich adjacent habitats such as the 'mass effect' (Shmida and Wilson 1985;Kunin 1998).

Second, we observed a decline in α-diversity with increasing intensity of agriculture. This is an important result from a conservation point of view, because for intensive vineyards in non-ploughing systems even higher diversity has been predicted previously (Maillet 1992). However, practices in vineyards changed since that work was published and have become more diverse. A similar decrease as the one here is documented for α-diversity (and γ-diversity in small scale studies) between organic and conventional fields (Hald 1999;Hyvönen *et al*. 2003;Gabriel *et al*. 2006;Roschewitz *et al*. 2009). The α-diversity in this study corresponds to γ-diversity in certain other studies due to the coarse scale we used here. This decline has similar reasons (*i.e.* less or no herbicide applications) as the decline from field edges to centre in the extensive vineyards studied here -an important reason for species decline in agro-ecosystems (Schneider *et al*. 1994;Robinson and Sutherland 2002).

There are also differences at the landscape scale. The 'marl landscape' with the highest α-diversity is richer in non-arable land and has a higher and more diverse land use, where vineyards are little represented. The low diversity 'limestone landscape' is completely dominated by vineyards with a very low part of other habitats. The higher diversity in a more diverse landscape can be explained by easy dispersal from a huge local species pool resulting from many different habitats. Maillet (1992) already showed that potentially a large part of the regional flora can grow in vineyards, hence recruitment limitation may not be the major determinant. There is considerable evidence now of the importance of species pools for local species diversity (Zobel 1997;Pärtel 2002;Zobel *et al*. 2006) and the importance of dispersal limitation for local diversity (Ehrlen and Eriksson 2000;Coulson *et al*. 2001;Poschlod and Biewer 2005;Zobel *et al*. 2006).

Rare and threatened taxa, future targets for conservation efforts could be revealed by a scan of our species list using Floras, Red Data Books and floristic works (Girerd 1991;Jauzein 1995;Verlaque and Filosa 1997;Montégut 1997;Roux and Nicolas 2001). This showed that most species of high conservation value are known to be cereal field specialists, an

astonishing result for vineyards. The analysis of their diversity in the same schemes as the entire flora showed that they are influenced by the same factors as α-diversity of the entire flora, a finding that is not supported by other works in agro-ecosystems (Roschewitz *et al.* 2009). The analysis of recent historical factors such as cereal cultivation, complemented the interpretation of this species group of conservation interest: the highest number of cereal weeds are found on vineyards with recent cereal cultivation and show the persistence of these species after land use changes.

The absolute β-diversity among different habitats in vineyards was influenced by landscape class and intensity of agriculture. The landscape class modified the absolute β-diversity between the different habitats of field margin and centre and field margin and embankment; in marl landscape the floristic differences among these habitats are greater than in *e.g.* limestone landscapes. There is no effect of landscape class on relative β-diversity; this indicates that it is due to supplementary species added in one habitat, creating difference by the higher number of specialist species rather than by sharp differences in composition without additional species in one of the compared habitats. The importance of dispersal limitation and species pool concept (Shmida and Wilson 1985;Zobel 1997;Kunin 1998;Pärtel 2002;Zobel *et al*. 2006) explains how richer structured landscapes can provide more species to fit into different habitats than monotonous landscapes, as also reported elsewhere (Roschewitz *et al.* 2009). This enhances the diversity of the vineyard in general, so the part of β-diversity on γ-diversity, that is, the relative β-diversity, may not be higher; this is indeed what we could show here. The change of absolute β-diversity according to the characteristics of the surrounding landscapes questions the concepts of transition zones between adjacent communities *via* the distinction of ecotones and ecoclines. According to the landscape type, the presence of specialised species in the transition between to different habitats changed here making the definitions according to van der Maarel (1990) or Frochot (1987) dependent on characteristics outside the system and not on characteristics of the transition system itself.

It would be a challenge to follow up more tightly the consistency of these definitions in landscapes with contrasting diversity.

The relative β-diversity was modified by intensity of agriculture, with a higher relative difference in intensively managed vineyards than in extensive vineyards. This is the opposite situation to the effect of landscape class on β-diversity: in intensive vineyards, the floristic differences between centre, margin and embankment of vineyards are sharp, whereas in extensive vineyards species of the embankment and margin are also present inside the vineyard. Similar findings are known from arable fields (Marshall 1989;Wilson and Aebischer 1995;Gabriel *et al*. 2006). The analysis of α-diversity discussed above is complementary for the understanding in this context: intensive fields have much less species than extensive. This means that only very few specialised species can maintain populations in the centre of intensive vineyards and therefore this vegetation is quite different from the surroundings. The low diversity of these fields does not increase the total number of species, hence we did not observe a difference in absolute β-diversity, similar to studies from arable fields (Gabriel *et al*. 2006;Roschewitz *et al*. 2009). In the light of this findings, it is not astonishing that the different landscape classes had no effect on higher level β-diversity contrasting vineyards of different intensity of agriculture, to few species withstand the intensive treatments and intensive agriculture fails to add relevant plant diversity at higher scales. Therefore, extensive agriculture can be advised in order to enhance diversity at larger scales (Gabriel *et al*. 2006).

CONCLUSION

Considering ecosystem services which depend on a rich flora (Sotherton 1984;Eliott *et al*. 1998;van Emden 2002;Schmidt *et al*. 2003;Gibson *et al*. 2006), it is important to notice that the enhanced relative β-diversity in intensive systems does not mean more species and hence has no effect. Extensive systems offer the necessary increased diversity and have also effects

beyond the study system itself *e.g.* by providing pollinators (Gabriel and Tscharntke 2007). It is also interesting to consider surroundings, it can indeed be argued that the higher diversity in richly structured landscapes enhances ecosystem services, directly by higher levels in the field (Sotherton 1984;Eliott *et al*. 1998;van Emden 2002;Schmidt *et al*. 2003) or by higher species richness in surrounding habitats (Thomas and Marshall 1999;Smith *et al*. 2008). We could show that α-diversity and absolute β-diversity are higher in some landscape classes. It is therefore likely that there are differences in levels of services according to landscape classes. Gibson *et al*. (2006) showed that these services can have an impact on some rare species *via* their pollination mode. Therefore, no doubt remains that from the conservation point of view the less intense the agriculture is the more diverse the flora is and the better and longer rare and threatened species are maintained.

TRANSITION CHAPTER 1 TO 2

From community diversity to the meaning of soil seed bank longevity

The chapter 1 we evaluated the importance of different factors for the outcome of diversity in plant communities in agro-ecosystems. Different factors constrain diversity, some of them are anthropogenic like land use – resulting in different habitat structures or in fields of different intensities -others are of physical geographical origin or related to spatial structure such as coarse or fine grained habitats in different landscape types. Finally, history of communities such as past land-use can explain important parts of diversity, such as for example the presence of threatened and rare species. These target species are at the same time influenced by actual land use which is in itself directionally changing –from traditional to industrialised agriculture- but bears also yearly climatic and several year crop cycles which in contrast create temporally predictable changes.

Annual plants are those plants that cope best with this set of interfering disturbances that can be temporally predictable or not. A key aspect why annuals persist in these communities is their persistence in the form of seeds. Therefore, soil seed bank persistence attracted the interest of naturalists (Darwin 1859) and agronomists (Beal 1885) and later also conservation biologists (van der Valk and Pederson 1989;Bakker *et al*. 1996b;Willems and Bik 1998;von Blanckenhagen and Poschlod 2005;Bossuyt and Honnay 2008) leading to different methods and measures of soil seed bank persistence. The different methods differ largely in meaning and quality of data produced (Thompson *et al*. 1997). Before key aspects of soil seed bank ecology, such as soil seed bank persistence can be studied, it has to be checked which methods are sufficiently accurate and unbiased and what should be sampled and defined as soil seed bank.

In chapter 2, we study the accuracy and meaning of the most largely used method, which uses count and identification of seedlings emerging from soil samples and one widespread index, the longevity index (L.I.). This chapter contrasts experimental seed decay from conditions very close to the target community to data from literature coming from seedling emergence from soil samples. Then we follow up what meaning the seedling emergence method has. We check for relations between seed persistence gathered by this method and seed production for a large set of species. Then we discuss this in the light of the seed size seed number trade-off and its importance for above ground populations.

CHAPTER 2

The seed bank longevity index revisited - limited reliability evident from a burial experiment and database analyses

(Annals of Botany 104: 715–724, 2009)

INTRODUCTION

Soil seed banks are a key to understanding the dynamics of plant populations, species and ecosystems (Silvertown 1982;Kalisz 1991;Kalisz and McPeek 1992;Günter 1997;Cabin *et al*. 1998;Bekker *et al*. 1998b). Notably, seed persistence in soil has been shown to be an important correlate of population persistence (Stöcklin and Fischer 1999;Rees and Mills 2002). The importance of high seed survival in soil seed banks to ensure persistence of local populations has also been demonstrated in theoretical models (Pake and Venable 1995;Pake and Venable 1996). Species coexistence in communities is enhanced by the 'storage effect' of seeds (Chesson and Warner 1981;Warner and Chesson 1985;Levine and Rees 2004;Facelli *et al*. 2005). Thus, seed bank attributes such as seed persistence or survival account for a considerable part of diversity of plant communities *via* coexistence and may be one of the traits corresponding to α-niche differentiation (Silvertown *et al*. 1999;Silvertown *et al*. 2006). Additionally, it has been shown that soil seed banks are important for community composition in open and highly disturbed habitats (Thompson *et al*. 1997;Hopfensberger 2007) and on a smaller scale for bare soil communities in particular habitats (Peco *et al*. 1998;Wellstein *et al*. 2007). This explains the substantial practical use of soil seed banks for restoration of these communities (van der Valk and Pederson 1989;Bakker *et al*. 1996b). The correct identification of transient, short- and long-term persistent species and levels of seed survival is therefore crucial for the feasibility and success of restoration efforts for plant communities (Poschlod 1993;Hutchings and Booth 1996;Willems and Bik 1998;Dutoit *et al*.

2003;von Blanckenhagen and Poschlod 2005) and populations (Adams *et al*. 2005), and for understanding the maintenance of rare species in man-made ecosystems. Evidently, the same is true for more basic questions on vegetation and population dynamics as well as on species coexistence.

There are various methods to study soil seed bank persistence of seeds, which can be classified into: (i) direct age determination by C^{14}-dating (McGraw *et al*. 1991;Moriuchi *et al*. 2000) (McGraw *et al.,* 1991; Moriuchi *et al.,* 2000); (ii) burial experiments of seeds and subsequent testing of germinability or viability (Telewski and Zeevart 2002); (iii) determination of the depth distribution of germinable seeds in the soil (Bekker *et al.* 1998a); (iv) determination of soil seed banks along successional seres (Poschlod *et al*. 1998;Wäldchen *et al*. 2005); and (v) comparative analysis of seasonal dynamics of seed rain and seed bank (Thompson and Grime 1979;Poschlod and Jackel 1993). However, the methods are not equivalent with respect to quality of results. Whereas methods (i) and (ii) accurately identify soil seed bank survival, methods (iii) to (v) produce results, which are not accurate for several reasons. First, they may be affected by seed input – only species, which are frequent and/or have a high seed production will be found. Second, the results of using depth distribution will depend on the importance of soil movement and disturbances. Finally, methods (iii) to (v) are based on the so-called seedling emergence method, where soil samples are exposed to 'favourable' conditions for germination in order to identify and count seedlings. Since ungerminated but viable seeds are not quantified, levels of dormancy can influence the results of these methods. For data from the indirect methods (iii to v) based on seedling emergence we use the term 'seed bank persistence' and for direct measures coming from burial experiments (ii), we use the term 'soil seed survival'.

Methods that determine seed survival (i and ii) are expensive and time consuming, therefore Thompson *et al.* (1998) proposed the calculation of a 'longevity index' (*LI*) which summarises seed bank persistence and soil seed survival data from different studies (methods ii to v) for

a species and is measured on a continuous scale. *LI* is the proportion of the number of records in a database that report species as having a persistent seed bank relative to all records, including those classifying the species' seed bank as transient. *LI* is now widely used in fundamental ecological studies when a single continuous value is needed to describe the soil seed bank type for a given species, *e.g.* to study ecological correlates of seed bank persistence at species (Thompson *et al*. 1998;Hodkinson *et al*. 1998) and community levels (Thompson *et al.* 1998) or even searching trade-offs to other traits (Ozinga *et al.* 2007).

For several local floras, the use of seedling emergence data to determine soil seed bank persistence has revealed that persistent seeds tend to be smaller, more compact, dormant and dependent on light for germination, while transient seeds are larger, often elongated or bear appendages. (Thompson and Grime 1979;Thompson *et al*. 1993;Bekker *et al*. 1998a;Moles *et al*. 2000;Cerabolini *et al*. 2003;Peco *et al*. 2003;Funes *et al*. 2007). In contrast no seed size - seed longevity relation was demonstrated for the Australian flora by Leishman and Westoby (Leishman and Westoby 1998), who used dormancy patterns to estimate soil seed bank persistence.

The seedling emergence method to study seed bank persistence, can, even in intensive studies, fail to pick up species with short dispersal distance, short seed shedding period or short germination season and with primary dormancy (Thompson and Grime, 1979). Indeed, Bakker *et al.* (1996) and Thompson *et al.* (1997) have already pointed out that rare species can be absent in seed bank studies even if the species is present in the above ground vegetation and although its seed bank may be persistent. These aspects raise the question whether seed bank persistence measured by seed counts from soil seed samples is reliable, and thus correlated to, independent measures of seed longevity, such as soil seed survival in burial experiments.

It is widely acknowledged that seed size is related to seed production by a fundamental trade-off (Shipley and Dion 1992;Jakobsson and Eriksson 2000;Turnbull *et al*. 2000). High

seed production enhances dispersal efficiency (Tackenberg *et al*. 2003;Poschlod *et al*. 2005;Bruun and Poschlod 2006) and it has also been suggested to increase seed bank persistence (Thompson 2000). Surprisingly, this has never been tested directly and it has not been asked if the different measures of seed bank persistence all relate to levels of seed production. This is especially interesting because the trade-off supposes that many small seeds are equally efficient for reproduction as few large seeds. The latter compensate for their lower number at other life stages, beginning with the seedling (Leishman *et al*. 2000b;Moles *et al*. 2004). In order to understand population dynamics and community diversity it is important to distinguish between number and survival of seeds, and to know whether seed bank persistence estimates can be influenced by seed number. Seed production influences seed rain (Jackel and Poschlod 1994), therefore we can hypothesise that it also influences seed bank persistence estimates which are based on seedling emergence from soil samples but not so for soil seed survival.

The understanding of soil seed bank persistence is based primarily on works from arable fields since they contribute a large part of available data (Thompson *et al.* 1997). The difficulty of detecting rare species in seed bank studies using soil samples (Bakker *et al*. 1996a;Thompson *et al*. 1997) means that the work of conservation biologists is hampered by the lack of reliable information on the longevity of seeds for the rarest arable weed species (Schneider *et al*. 1994;Wäldchen *et al*. 2005). Thus, rare arable weeds are ideal candidates to study the importance of seed counts in soil samples for the estimation of seed bank persistence together with data on seed survival from burial experiments. For these reasons and because annuals depend on long-term persistent soil seed banks for their persistence, we explicitly studied a mixed set of rare and more common annual arable weeds in an experimental study (Appendix 1) and more generally in a wide set of habitats and species.

Our questions were studied using two different approaches. First, we used an 'experimental approach' (i) to gather reliable data on survival of seed in the soil for a quantified seed

population. This experiment was complemented by an analysis of seed production and seed bank persistence from literature for the same species to (ii) answer the question on the reliability of seed bank persistence estimated by seedling emergence from soil samples in the light of experimental soil seed survival and to (iii) explore whether experimental soil seed survival is related to seed production.

In a second 'data base approach', we studied further the questions (ii) and (iii) in a more general way using databases on a wider set of species. This allowed us (iv) to analyse whether published soil seed survival data from burial experiments show a relation to literature data on seed production and (v) to determine whether the longevity index based on published burial experiments and seed production from literature are related.

MATERIALS AND METHODS

(1) Experimental approach

Study system

Annual arable weeds were chosen as the study system because of the well-known interspecific differences in seed bank persistence and their short life cycle, making them heavily dependent on mortality in the seed bank. A burial experiment was carried out at Cucuron (43°46'5''N, 5°21'2''E, South Eastern France). The surrounding agricultural landscape in the Luberon area was chosen as our study region because, here, traditional agriculture has maintained a high diversity of rare arable weeds that are extinct elsewhere in Europe. This region is characterised by Mediterranean climate (autumn rain and summer drought).

Seed material was collected in the study region between June and September 2005. For each species, ripe seeds were taken from at least ten individuals of a single large population and mixed. Seed material was stored under dry conditions in paper bags until October 2005, when we started the burial experiment and the initial viability test. We cannot exclude a loss

of viability or a loss of dormancy due to after-ripening because seeds were not studied directly after harvesting (Baskin and Baskin 1998). However, this is what normally happens under Mediterranean climate, where seeds after-ripen in dry summer and germinate in autumn after the first rains or after ploughing (Baskin and Baskin 1998). Every seed sample was randomly taken from a single well-mixed seed lot.

Experimental design of the burial experiment

A burial experiment was set up using 38 annual arable weed species (Appendix 1), for which seed samples were buried for at maximum 2.5 years. Viability was tested every 6 months to capture the two main germination periods, in autumn and spring. The burial experiment was done in young fallow land with no disturbance during the time of the experiment. The seed samples were divided into 30 sub-samples with 25 to 50 seeds for most species (see Appendix 1). For each species, five samples were assigned at random to each of five retrieval dates (t1-t5), and five samples were kept for the initial test (t0). The experiment was set up as a randomised block design with each block containing groups of samples for each of five time intervals (t1-t5), placed at random in the block (Fig. 2.1).

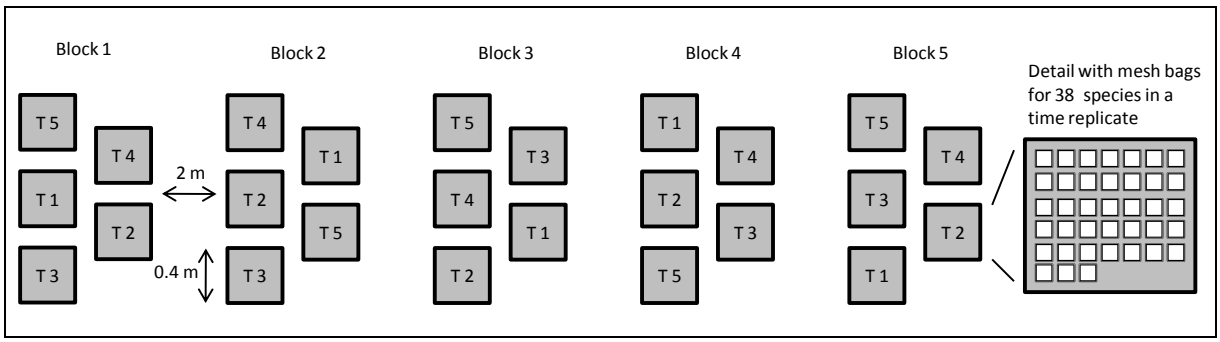


Fig. 2.1. Experimental layout: position of blocks, time step replicates (T1-T5) and mesh bags for each species inside blocks.

Each of these time step groups contained 38 nylon mesh bags, one for each of the 38 species. Samples were buried at 10 cm depth. At each retrieval date, one group of 38 mesh bags per block was removed and studied in the laboratory. Seeds were retrieved twice a year: in spring (t1, t3, t5) and in autumn (t2, t4); the initial test (t0) was done in autumn 2005. In the

burial experiment, 39 400 seeds were buried, and an additional 7 880 seeds were tested in the initial test. In all tests, 9 802 seeds germinated in the three germination test phases (see below), 16 574 ungerminated seeds were tested after the germination tests for viability using a tetrazolium test and 20 897 seeds presumably died during burial. The burial experiment was started in October 2005 and the last tetrazolium tests were finished in September 2008.

Testing experimental seed survival in the burial experiment

Germinability was tested using a sequence of germination conditions standardised for all seed retrieval dates. After seeds were exhumed, the empty seeds were counted. These were apparent by their shape or colour or being soft when pressing them with a needle (Ter Heerdt *et al.,* 1996). Firm seeds were then incubated at 22°C for 14h in light (fluorescent tubes, ±10 000 lux) and at 14°C for 10h in darkness in a growth chamber on moist filter paper. After 28 days, seeds were cold stratificated for 6 weeks at 4°C in darkness. Seed samples were then again subjected to 22°/14°C (14h/10h) for 28 days. Positions of Petri dishes were randomised in the growth chamber. Seeds were counted as they germinated and discarded when the tip of radicle emerged.

Seeds that did not germinate were tested for viability with tetrazolium chloride (International Seed Testing Association 1996). Seeds of *Consolida regalis, Legousia hybrida* and *Legousia speculum-veneris* stained well without previous bisection. Seeds of *Papaver rhoeas, P. argemone, P. hybridum* and *Roemeria hybrida* did not stain in the tetrazolium test. However, the embryos were firm and white, and thus the seeds were classified as viable. In some cases (*e.g. Adonis annua;* morphological dormancy in Ranunculaceae), a very small, underdeveloped embryo made the use of tetrazolium impossible in the first stages of the experiment. Thus, we used the highest number of viable seeds (germinable + dormant) detected from a later seed retrieval date as the initial number of living seeds.

Compiling seed bank persistence estimates from literature: the longevity index

The longevity index for the species in the burial experiment was calculated using literature data. Thus, we compiled a database using the entries for our species in Thompson *et al.* (1997), and results of a survey of the recent literature. Records on seed bank persistence were classified into one of the following soil seed bank types Thompson *et al.* (1997):

1 **Transient** species persist for less than one year

2 **Short-term persistent** seeds to persist living for more than one but less than five years after dispersal; and

3 **Long-term persistent** seeds persist viable in the soil for at least five years.

The longevity index (*LI*) was calculated for each species (Thompson *et al.*, 1998):

$$(1) \qquad LI = \frac{R_{sp} + R_{lp}}{R_t + R_{sp} + R_{lp}}$$

Where *LI* is the proportion of the number of records (*R*) classifying a species as short (sp) and long term persistent (lp) to the sum of all records, including the number of transient records (R_t) for a given species. Initially we used all types of data. We then wanted to test if data from seedling emergence from soil samples changed the reliability of *LI*, so we used only this data. Due to limited data in the literature on our initial 38 species, we only had *LI* values for 26 species using all data and for 21 species using only data from seed bank persistence estimates by seedling emergence from soil samples.

Seed production

Seed production was determined for the 38 species, *i.e.* mean individual seed production of 10 individuals in the field. Some species had multi-seeded fruits (*e.g. Papaver sp. pl.*), others had many fruits per infructescence (*e. g.* Apiaceae), therefore we counted the number of fruits or infructescences per individual for these species. Then the number of seeds per fruit or infructescence was counted in two fruits or infructescences. Seed production per

individual was calculated as the mean number of seeds per fruit or infructescence multiplied by the number of fruits or infructescences counted per individual.

(2) Database approach

Data on seed bank studies

A second approach compared seed bank persistence with seed production and completed our (necessarily) limited data set on arable weeds. Here, we explored a larger database on seed bank studies (*i.e.* Thompson *et al.,* 1997), together with another published database on seed production in the field (Šera and Šery 2004). We extracted all species for which there were data on both seed bank persistence and on seed production. The database of Thompson and co-workers (1997) includes a large number of seed bank studies using seedling emergence from soil seed samples and a relatively small number of burial experiments. Each record included information on the seed bank type for the species (transient, short or long-term persistent) according to the key in Thompson *et al.* (1997). We subdivided the data into those from seed burial experiments and data from seedling emergence studies. For the latter, only species with at least five entries were used (Thompson *et al.,* 1998). For burial experiments, all species were used because seed bank type is more reliable with this method. *LI* was calculated for all species in both subsets as explained above.

Data on reproductive capacity

Šera and Šery (2004) measured reproductive capacity by counting seeds per surface of sampled vegetation and using cover percentages of a species to calculate the potential seed production of a species at 100% cover; they provide data for 492 species. For 227 of these species there were seed bank data using the seedling emergence method and 174 species with data from burial experiments in the database of Thompson *et al.* (1997). Five of these species were also used in our own burial experiment.

Statistical analysis

The data were analysed using linear regression to test relationships between continuous parameters. All analyses were run in R statistical environment (R Foundation for Statistical Computing 2008).

RESULTS

(1) Experimental approach

Mortality of buried seeds at the end of the experiment ranged from very high, reaching 100 % in some cases, as exemplified by *Agrostemma githago, Asperula arvensis* and *Nigella arvensis* (Fig. 2.2, Appendix 1) to very low (down to 3.5 %) for species such as *Androsace maxima, Bupleurum rotundifolium, Adonis annua* and *Carthamus lanatus* (Fig. 2.2, Appendix 1). Other species had intermediate mortalities. There were marked differences in the proportion of surviving seeds and shape of the mortality curve between species. In some cases, final mortalities were similar but mortality curves were different, compare *e.g. Nigella nigellastrum* and *N. damascena* in figure 2.2.

There was no relation between soil seed mortality in the burial experiment and the longevity index of the same species (R^2= 0.02, $F_{1,25}$=0.58, p = 0.45; fig. 2.3). When the analysis was restricted to *LI* calculated from seedling emergence from soil samples data only, we still found no relation to experimental soil seed survival (R^2= 0.02, $F_{1,20}$=0.50, p = 0.49). Clearly, seed mortality under field conditions is not related to seed bank persistence determined using the seedling emergence method in soil seed samples. Furthermore, there was no significant relation between individual seed production and experimental seed survival after 2.5 years (R^2= 0.01, $F_{1,36}$=0.46, p = 0.50). This indicates the independence of the two parameters.

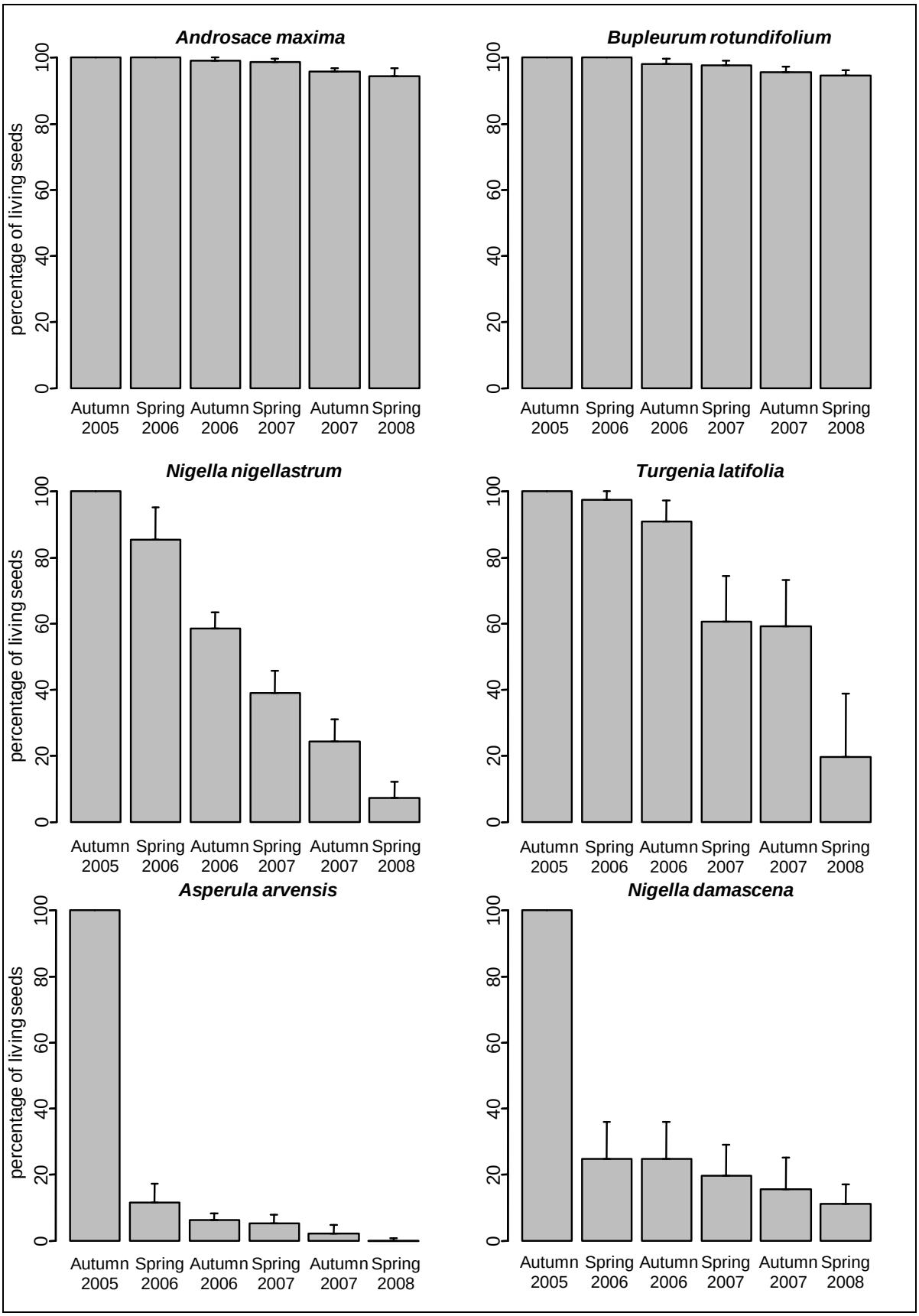


Fig. 2.2: Percentage survival for five retrieval dates for six representative species. Initial viability in autumn 2005 is presented as 100% to give a scale among species; the survival percentages are relative to this initial viability. Bars are standard errors.

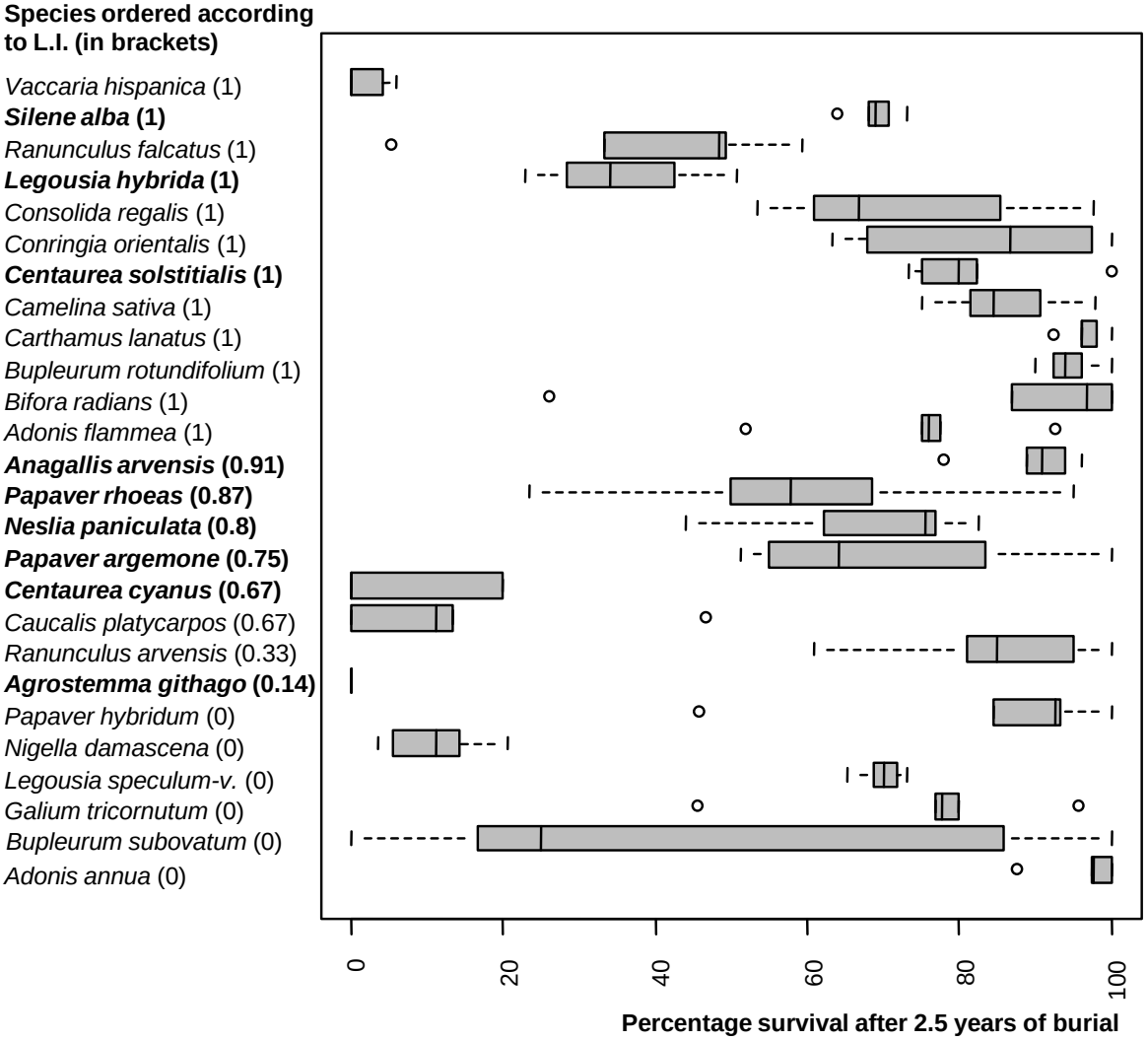


Fig. 2.3. Box plots of percentage survival of seeds for 26 species after 2.5 years of burial (five replicates per species), boxes and central bars represent interquartile range and median, dashed lines represent range of sample, dots are outliers. Species are ordered according to their longevity index (LI). Species in bold are those for which at least five records were used for calculation of LI.

(2) Database approach

The relation between reproductive capacity (seed production) and *LI* using counts of emerging seedlings in soil samples was significant and positive (R^2= 0.10, $F_{1,225}$=25.23, p < 0.001; T=5.02, p < 0.001; fig. 2.4), indicating that soil seed bank persistence determined in this manner can be related to the number of seeds produced per surface unit. However, the parallel analysis of soil seed bank persistence using only burial experiments yielded no

significant relationship (R^2< 0.01, $F_{1,172}$=0.12, p = 0.73), indicating that maximum longevity in burial experiments is not related to the number of seed produced per surface unit. The joint analysis of the two subsets is not shown because the results were completely dominated by the data from seedling emergence studies since they are the majority in the studied data sets.

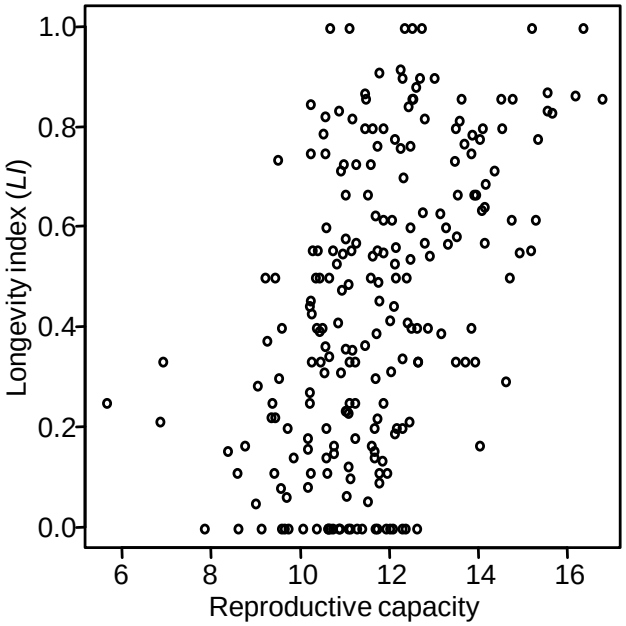


Fig. 2.4. Relation of a species' reproductive capacity (logarithm of seeds produced per m², Šera and Šery, 2004) and its longevity index (*LI*, Thompson *et al.*, 1997) based on ≥5 studies per species using seedling emergence from soil seed bank samples; *LI* is high when many studies classify the species as persistent, and low when there are many transient records, details in the text (R^2= 0·10, $F_{1,225}$=25·23, P < 0·001).

DISCUSSION

(1) Experimental approach

This study shows that seed survival measured from a burial experiment is not correlated to the commonly used seed bank persistence estimates from literature when it is estimated from seedling emergence. The absence of relation leads us to the following questions: How these seed bank persistence estimates have been generalised as a measure for seed longevity? What can explain the differences between our experimental 'soil seed survival' and seed bank persistence from other studies? What affects seed survival in the soil?

Bekker *et al.* (1998a) tested the general validity of seed bank persistence estimates based on the depth distribution of viable seeds; they detected seeds with the seedling emergence method. In order to show that their 'depth derived' method reflects soil seed longevity, they used a database without 'depth derived' data. However, their database still contained many entries using the seedling emergence method mixed with entries using seed burial experiments. The mixture Bekker *et al.* (1998a) used in their validation database makes it difficult to know whether the seedling emergence method is related to experimental soil seed survival, and therefore it is also not clear if data from 'depth derived' methods are related to experimental soil seed bank survival. There is, to our knowledge, no other analysis that tested the generality of seed bank persistence estimated on seedling emergence from soil samples in the light of experimental seed survival in soil.

The differences between our 'soil seed survival' and 'seed bank persistence' estimates based on seedling emergence from soil samples can be interpreted by methodological differences. Classically, the seedling emergence method uses 10 plots, each with 10 soil samples of 4 cm diameter yielding a total sampled surface of just 0.125 m^2 to represent a community (Hutchings 1986;Bakker *et al*. 1996a;Bekker *et al*. 2005). Thompson and Grime (1979) argued that species with low seed production are difficult to detect in the soil seed bank even if seeds are long-lived in the soil. Consequently, there is a strong risk of an erroneous classification since species present in the vegetation but absent in the seed bank are classified as transient. Especially rare species or species with low seed production are absent from samples, although they have long-lived seeds in the soil.

In addition to this, environmental factors acting on soil seed mortality can also explain the differences between our experimental data and literature data. For example, studies on fungi indicate that there are differences in soil seed mortality within species related to soil properties (Blaney and Kotanen 2001;Schafer and Kotanen 2003;Chee-Sanford *et al*. 2006;Wagner and Mitschunas 2007) but consider also Leishman *et al.* (2000a). Dry habitat

species have higher seed mortality under moist than under dry conditions due to pathogenic fungi attack (Blaney and Kotanen 2001;Schafer and Kotanen 2003). Thus, soil seed survival varies greatly from one site to another for a given species and differences among sites may contribute to the differences between our experimental data and the data from the literature. Moreover, the conditions in our mesh-bags may not reflect conditions in natural seed banks; this point was addressed by Van Mourik *et al.* (2005). This might imply that we overestimated seed depletion, but overall we found rather high survival rates and in addition, we did not have particularly wet conditions compared to the fields from which the seeds originated. Furthermore marked differences in seed decay among species appeared in our burial experiment as exemplified by figure 2.2. This suggests that our experimental seed survival is realistic and that the seed bank persistence estimates from the literature may reflect another aspect than only seed survival. According to suggestions of Thompson (*e.g.* Thompson and Grime, 1979; Thompson 2000), seed production is a possible candidate to influence it. However, absence of a significant relationship between seed production and experimental seed survival in our work suggests that both are independent. We can only draw limited conclusions with our experimental data because they represent only a single habitat and a limited number of species.

(2) Database approach

Use of two larger databases on soil seed bank studies and on reproductive capacity from the literature, including many species from many different habitats, explored whether reproductive capacity is related to seed bank persistence based on seedling emergence (Fig. 2.4). The regression showed that seed production influences seed bank persistence estimates. It is not surprising that this relation to reproductive capacity disappears when soil seed survival from burial experiments is used, a finding confirmed by our experimental data. No study so far has explored the relation between seed bank persistence and reproductive capacity. This leads to the conclusion that the seed bank persistence estimates used until now

do not only represent seed longevity but they mix both seed production and soil seed survival. This has to be considered for all studies using the seedling emergence method without an estimate of the total initial seed population. Furthermore, this also concerns studies that directly count seeds in soil samples (*e.g.* Moriuchi *et al.* 2000). Here we add empirical data showing that seed production is an important factor for the formation of a persistent seed bank (Parker *et al*. 1989;Simpson 1989;Thompson 2000). Bruun and Poschlod (2006) showed that seed production is a relevant component of dispersal through space, and therefore, seed production may also be related to dispersal through time (also see Poschlod *et al.* 2005). Our data suggest that seed production and seed mortality are two independent processes, since there is no relation between experimental seed survival and seed production. We think both contribute to soil seed bank formation. In contrast to seed production, seed mass and shape have been frequently used to explain soil seed bank formation (Bekker *et al.* 1998a). This should be reconsidered in the light of our findings - which emphasise the role of seed number- and *a fortiori* in the light of the seed size-seed number trade-off (Shipley and Dion 1992;Jakobsson and Eriksson 2000;Turnbull *et al*. 2000).

The correlation between seed production and persistence reported here suggests that size and detectability of the soil seed bank of smaller seeds are probably in the same trade-off with seed size than seed number. This offers a new and parsimonious explanation for the seed size-seed bank persistence relation (Thompson and Grime 1979;Thompson *et al*. 1993;Bekker *et al*. 1998a;Moles *et al*. 2000;Cerabolini *et al*. 2003;Peco *et al*. 2003;Funes *et al*. 2007). Using the seedling emergence method, seed longevity estimates for smaller seeds (*i.e.* more numerous!) are higher without a higher soil seed survival, because mechanisms that compensate larger seeds for their lower number act *after* germination, at the seedling stage (McGinley *et al*. 1987;Louda 1989;Jakobsson and Eriksson 2000;Leishman *et al*. 2000b;Coomes and Grubb 2003;Moles *et al*. 2004;Pizo *et al*. 2006;Bladé and Vallejo 2008). This has the consequence that species with a higher seed bank persistence estimate do not yield higher

numbers of established plants (Hillier *et al.* 1990). Seed bank persistence estimates based on seedling emergence methods are therefore potentially meaningless to explain population persistence or community diversity.

CONCLUSION

Our results question the use of seed bank persistence estimates based on seedling emergence in the current literature (Thompson *et al*. 1998;Bekker *et al*. 1998b;Ozinga *et al*. 2007). The strong relation between seed production and seed bank persistence estimates based on seedling emergence presented here should encourage us to carefully re-evaluate this literature. Moreover, we think that a clear distinction between seed quantity related parameters and seed age related ones could significantly increase our understanding of mechanisms generating soil seed banks and give new insights to what role seed banks play in vegetation and population dynamics.

Finally, there is a need to describe the two fundamental characteristics of soil seed banks that are longevity and abundance in future studies. For longevity, differences in survival of seeds between species become already apparent after 1.5 years (Fig. 2.2). A longer burial period (> 2.5 years) would confound transient and short-term persistent species because –at least in our data- both have similar final mortalities (Fig. 2.2) and additionally would greatly limit available data. Soil seed viability determined after only one year of seed burial does not discriminate between transient and persistent species. We therefore propose that two parameters should be used: (i) classes of soil seed abundance, and (ii) mean percentage survival of seeds after 1.5 years of burial. These two parameters are independent in the datasets studied here and represent two main factors for the formation of soil seed banks.

TRANSITION CHAPTER 2 TO 3

From soil seed persistence measures to functional ecology of soil seed banks

The persistence of seeds in the soil is a very important factor for the persistence of local populations and the reestablishment of plant communities after disturbances (van der Valk and Pederson 1989;Kalisz 1991;Kalisz and McPeek 1992;Cabin *et al*. 1998;Bekker *et al*. 1998b;Stöcklin and Fischer 1999;Bossuyt and Honnay 2008). Chapter 2 pointed out that the widely used longevity index is connected to seed production. L.I. may thus bias our perception of seed decay in the soil suggesting an overly high persistence of seeds of plants with numerous seeds compared to the low experimental longevity of few large seeds. This clearly can hamper our knowledge on seed persistence of seeds in the soil and there is a risk that current knowledge on seed decay is biased by seed number. Notably, the functional aspects of how seeds persist in the soil and which seed internal factors can limit persistence must be re-evaluated. However, even without this bias, the functional ecology of soil seed banks has many gaps of knowledge.

In the chapter 3, we complement therefore the experimental data from chapter 2 with a series of germination experiments on the same set of species. We also make use of other seed traits such as seed number and size. In this way, we aim to answer how seed persistence and one of its main limits -fatal germination at the wrong time or in the wrong depth- can be triggered by physiological and morphological adaptations of the seed. This gives also insights which ecological conditions may be the reason for the observed phenomena.

CHAPTER 3

Functional ecology of seed persistence in the soil – insights from germination experiments and seed traits with cereal weeds

INTRODUCTION

Seeds are the fascinating stage in a plant life cycle that permits dispersal in space and time and originate the regeneration of individuals and populations. Seed persistence, that is seed dispersal through time, is one of the key aspects for maintaining population persistence and also for plant diversity in some communities (Chesson and Warner 1981;Kalisz and McPeek 1993;Menges 2000). The germination ecology of seeds and, moreover, the formation of a persistent soil seed bank has two fundamental evolutionary reasons: (I) delayed germination or bet hedging of offspring under maternal control to avoid competition and maximise fitness of the mother plants (Ellner 1986;Silvertown 1999;Venable 2007) and (II) avoid germination under lethal or unfavourable conditions to maximise offspring fitness, which results in dormancy and complex reactions to temperature, light, water and other factors to detect favourable conditions (Baskin and Baskin 1989;Baskin and Baskin 1998;Silvertown 1999). Bet hedging in the form of seed banks and varying germination percentages is an important pattern in population dynamics of annual plants (Kalisz and McPeek 1993;Venable 2007). Here, germination is the necessary end of a successful dispersal phase and the link to plant establishment, triggered by conditional germination and dormancy. However, germination can also be a source of seed mortality as 'fatal germination' at the wrong time or the wrong position in the soil (Benvenuti *et al*. 2001;Fenner and Thompson 2005;Davis and Renner 2007). Indeed, seed plants developed a wide variety of adaptations to

avoid fatal germination especially in seasonal climates, including unexpected abilities to detect levels of many environmental factors and dormancy (Baskin and Baskin 1998;Benvenuti 2003;Jurado and Flores 2005). Beyond simple detection of environmental factors, dormancy prevents immediate germination even if temperature, light and water are at optimum and it makes a specific dormancy breaking mechanism necessary to enable germination (Baskin and Baskin 1998). Whereas primary dormancy can be short and lost before a seed enters the soil –with the exception of physical dormancy-, the capacity to develop secondary dormancy may be a reliable factor for seed bank formation (Baskin and Baskin 1989). Until now, processes leading to fatal germination have received much less attention (Fenner and Thompson 2005), some hints however indicate that depth-mediated fatal germination is often avoided by the means of secondary dormancy (Benvenuti *et al*. 2001;Davis and Renner 2007). The complex changes in the dormancy state over time make it necessary to study germination characteristics of species after burial, together with their seed decay in the soil (Baskin and Baskin 1989;Milberg and Andersson 1998), to test the influence on soil seed mortality of dormancy, light requirement for germination, reaction to diurnally fluctuating temperatures (DFT) and seed traits.

Physiological dormancy and cycles of secondary dormancy have been emphasised to be the main adaptations that permit seed bank formation (Baskin and Baskin 1989). Cycles of secondary dormancy illustrate how species can time their germination under seasonal climates (Milberg and Andersson 1997;Baskin and Baskin 1998;Mennan 2003). A shift from the simple analysis of primary dormant *versus* non-dormant species to a more comprehensive analysis integrating at the same time primary and secondary dormancy can significantly enhance our understanding of interspecific differences in soil seed longevity.

Light diminishes rapidly below soil surface (Benvenuti 1995;Cussans *et al*. 1996). Therefore, it has been proposed that species can form persistent seed banks if they require light for germination (Grime *et al*. 1981;Baskin and Baskin 1989 and literature cited therein;Milberg *et*

al. 2000). A species needing light for germination does not germinate when dispersal takes place under conditions unfavourable for germination (*e.g.* drought, high temperatures) and becomes buried under soil or litter before conditions become favourable. By this means, it can accumulate in the soil seed bank and germination takes place after disturbance when species are brought to the soil surface or litter is removed. In parallel with secondary dormancy, a light requirement can also be acquired by seeds germinating without light initially (Baskin and Baskin 1989). The light requirement for germination has been related to seed size, showing that small seeds are more heavily dependent on light for germination (Grime *et al*. 1981;Milberg *et al*. 2000). It can also be hypothesised that larger seeds should germinate better in darkness (from deeper depth) as predation is high for large seeded species at the soil surface (Abramsky 1983;Hulme 1998;Moles and Drake 1999) and water and temperature conditions are better in deeper soil layers in arid climates (Bell *et al.* 1995).

Diurnally fluctuating temperatures (DFT) are more extreme in large gaps than in small gaps or under closed vegetation (Bullock 2000). Relative better germination under DFT permits the detection of disturbances from below the soil surface; a mechanism described as 'gap detection' (Thompson *et al*. 1977;Grime *et al*. 1981;Thompson and Grime 1983). Diurnally fluctuating temperatures also offer a mechanism to detect end of flooding (Schütz 2000). In greater soil depth, *DFT* are smaller than at the soil surface (Miess 1968). Thus, *DFT* may also permit to detect the position of the seed in the soil profile and hence to be a predictor of soil seed survival. To our knowledge, the relation of *DFT* to soil seed survival has not yet been tested. This is astonishing as *DFT* are well known to prevent fatal germination under unfavourable conditions (Bullock 2000).

Benvenuti (2007) showed that in no till systems, and for fallow lands seeds are buried naturally at different depth according to their sizes, and this may lead to a higher degree of dormancy and soil seed longevity of small respective to large seeds because small seeds can only emerge from relatively shallow depths (Bond *et al*. 1999;Grundy *et al*. 2003). However,

there is also evidence against a strong effect of predation on the size of persistent seeds, explaining the absence of relation between seed predation and seed size in some cases (Kollman *et al*. 1998;Moles and Drake 1999). Large seeds resist better to partial predation (Leishman *et al.* 2000b) and have adaptive strategies to avoid predation (Louda 1989;Lokesha *et al*. 1992). Both counteract a strong seed size-seed longevity relation. The very small negative or insignificant effect of seed size on dormancy offers no support to the view that small seed size enhances seed persistence (Garwood 1989;Rees 1996;Leishman and Westoby 1998;Jurado and Flores 2005). Finally, seed size is related to seed production by a fundamental trade-off (Jakobsson and Eriksson 2000;Leishman *et al*. 2000b;Benvenuti *et al*. 2001). It can therefore be argued that any relation to seed size can also be a relation that appeared in relation with the higher number of seeds. There is thus a need to show that soil seed survival is related to seed size, dormancy, light requirement and diurnally fluctuating temperatures independently from seed number.

Seed traits are phylogenetically conserved (Shipley and Dion 1992). Therefore, it can be expected that at least some of these relations have a component correlated to phylogeny. In this case phylogenetically explicit analysis can elucidate to which degree traits are conserved and relations are correlated to phylogeny (Harvey and Pagel 1991). Nonetheless, caution has to be paid not to assign overly much variation to phylogeny that can equally well be explained by ecology (Westoby *et al*. 1995a;Westoby *et al*. 1995b). The survival of seeds in the soil seed bank is often studied on single or two species simultaneously (e.g. Leishman *et al*. 2000a;Mennan 2003) although comparative studies of a larger part of a flora have turned out to be particularly informative (Grime *et al*. 1981;Noronha *et al*. 1997;Milberg *et al*. 2000;Thompson *et al*. 2003). Previous evidence, came from among species comparisons using compiled data on soil seed persistence without simultaneous comparative studies on the soil seed survival (Thompson *et al.* 2003). This can be circumvented by burial experiments that give sound soil seed mortality data (Baskin and Baskin 2006).

In annuals the constraints on soil seed persistence are higher than in perennials but in the same direction (Venable and Brown 1993a). Furthermore, in annuals, adaptations can more easily be explained because for annuals, there is no competition with the mother plant. This makes annuals an interesting model to test hypotheses on seed traits and soil seed persistence.

Using a seed burial experiment and an experiment testing different germination ecological characteristics, we study the relations between seed traits, germination characteristics and soil seed survival. We tried to answer the questions: (a) Is light inhibition an important factor for soil seed persistence? (b) Is a gap-detection mechanism risky for seed persistence? (c) Is the level of dormancy of buried seeds related to their persistence? (d) Is final soil seed survival explained by a factor acting equally over time or is this factor more important at a particular period after burial? (e) Which relations are phylogenetically correlated and to what degree?

MATERIALS AND METHODS

Study site and species

The study region is the agricultural landscape around the Luberon ridge in South Eastern France. This region is characterised by Mediterranean climate, with rainfall peaks in October and April followed by summer drought, and moderate frost occurs during winter (mean rainfall 1971-2000: 623 mm/ 60 d; Salon). The beginning of vegetation period and cereal cultivation is in October, resulting in autumn as the main germination season of herbaceous species (Espigares and Peco 1993;1995;Baskin and Baskin 1998). Rarely, species also germinate in winter and spring, especially in relation with disturbances (Lavorel *et al.* 1994). Traditional agriculture in this area maintained a high diversity of rare arable weeds elsewhere extinct in Europe (Filosa 1997;Aboucaya *et al*. 2000).

The selection of species focussed on 35 cereal weeds (Tab. 3.2), some regressing in the study region (Filosa 1985;1989). Seed material was collected in the study region between June and September 2005 for the burial experiment and between June and September 2006 for the study on germination characteristics. Ripe seeds were taken from at least ten individuals of one single large population and mixed before usage. Seed material was stored dry in paper bags at conditions similar to those in field until burial in October 2005 (or until begin of germination studies in October 2006). Every seed sample was randomly taken from a single well-mixed seed lot.

Experimental design of the burial experiment

To test seed viability under field conditions, we set up a burial experiment using 35 of the 38 annual arable weed species. For each species, we buried 25 seed samples, enabling us to retrieve five replicated samples for five different dates. These five retrieval times are noted t1 to t5. We retrieved and tested seeds for viability every 6 months for 2.5 years. The burial experiment was done near Cucuron (43°46'5''N, 5°21'2''E). Seed retrieval took place twice a year: in spring (t1, t3, t5) and in autumn (t2, t4), an initial viability test (t0) was run in autumn 2005. We chose these time steps to capture the two main germination periods in autumn and spring. The burial experiment was conducted in a fallow land with no disturbance at the time of the experiment. Collected seed lots were sub-divided in 30 sub-samples with a fixed number of 10, 25 or 50 seeds per sample. For each species, 25 samples were assigned at random to one of five burial dates (t1-t5) and one of five experimental blocks; five samples were kept for the initial test (t0). We set up a randomised block design with five blocks. Each block contained groups of samples for each of five time steps (t1-t5), placed at random inside the block (Fig. 3.1). Each of these time step groups contained one mesh bag for each of 35 species. Seed samples were put in 4 x 4 cm nylon mesh bags and buried at 10 cm depth. At each retrieval date, all mesh bags of one time step group per block

were removed and studied in laboratory. The burial experiment was started in 2005 and the last tetrazolium tests were finished in September 2008.

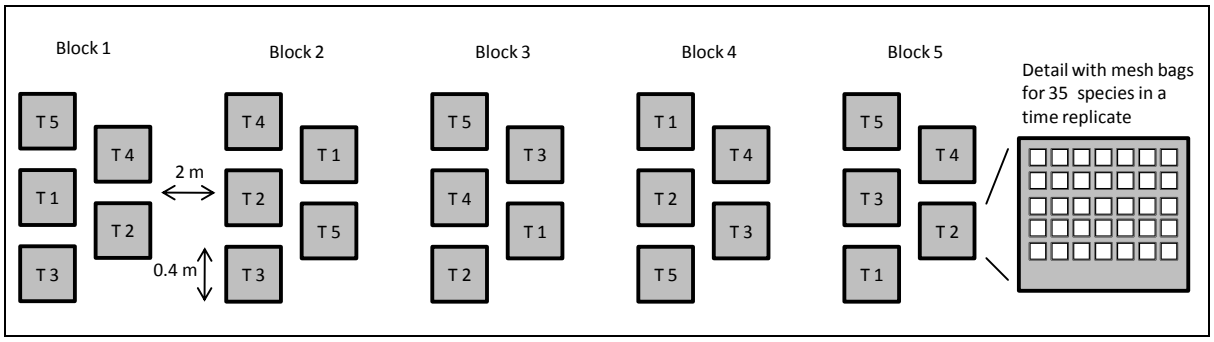


Fig. 3.1. Experimental layout: position of blocks, time step replicates (T1-T5) inside blocks and mesh bags for each of 35 species.

We used a sequence of germination tests under standardised conditions to test germinability at each seed retrieval dates. After sample retrieval, we counted the empty seeds. Firm seeds were then exposed to experimental conditions of 22°C at 14h of light and 14°C at 10h of darkness in a growing chamber on filter paper in regularly watered Petri dishes. After 28 days, seeds were subjected to cold stratification for 6 weeks at 4°C in darkness. Then seed samples were subjected to the initial temperatures for 28 days. Position of Petri dishes was randomised in the growth chamber. We counted seeds as germinated and removed them when the tip of radicle emerged. Remaining seeds have been tested by means of tetrazolium test (TZ) which we applied according to the ISTA rules (International Seed Testing Association 1996), including surface sterilisation with a NaOCl solution. Seeds of *Consolida regalis, Legousia hybrida* and *Legousia speculum-veneris* turned out to stain well without previous bisection so we tested them without bisection. In some problematic cases, the tetrazolium test was not meaningful: seeds tested as viable increased with time of experiment. This concerned species with morphological dormancy such as *Adonis annua,* where embryos are very small initially and therefore difficult to detect. They grew after burial. In this case, we used the maximum number of viable seeds in a subsequent test from the same block as initial number of viable seeds. Seeds of *Papaver rhoeas, P. argemone, P.*

hybridum and *Roemeria hybrida* did not stain in the initial test. Their seeds have been examined after removal of teguments and seeds with stiff white embryos were classified as viable.

At the end, we calculated soil seed survival as the proportion of living seeds at the retrieval data on the number of viable seeds in the previous test, yielding five independent sets of soil seed survival data t0-t1, t1-t2, t2-t3, t3-t4 and t4-t5. Additionally, we calculated the soil seed survival at the end of the experiment (t5) as proportion of viable seeds in the initial test (t0-t5).

Seed testing conditions in the germination ecological experiments

We then set up a series of experiments to study the effects of temperature, diurnally fluctuating temperatures (DFT) and light on germination. We did not stratify seeds in cold prior to these experiments because the Mediterranean species studied here germinate directly in autumn after a dry summer period (Espigares and Peco 1995;Bell *et al*. 1995;Baskin and Baskin 1998;Mennan 2003). Every temperature and light condition was studied using eight seed replicates for each of 35 species. According to the annual temperature range, we chose four different temperature conditions with alternating (high and low) temperatures and one constant temperature of 12°C, all received 14h of light (day) and 10h of darkness (night) in a growing chamber. The temperature conditions were (day/night °C): 10/2, 16/8, 22/14 and 28/20. Seeds were placed on filter paper in Petri-dishes, controlled and watered regularly. As soon as radicles emerged, we counted seeds as germinated and removed them. These temperature regimes correspond to three contrasting situations in the vegetation period and conditions in early summer/autumn.

Parallel to two temperature conditions, *i.e.* the 16°C / 8°C daily fluctuating and 12°C constant conditions, we conducted a darkness experiment. The darkness experiment started with watering of all prepared samples in complete darkness, without green safety light. Petri-dishes were then closed with a stretch of Parafilm and all samples placed together in

specially prepared lightproof boxes. All preparation steps were conducted in complete darkness and not in safety green light because the latter can induce germination in some species (Baskin and Baskin 1998). After 10 days, we controlled water content in complete darkness. Germinations were counted for the first time after four weeks using indirect weak green light. Within each experimental unit, all samples were placed at random in the growing chamber or the darkness box.

Relative light germination, index for germination in diurnally fluctuating temperatures and degree of dormancy

In order to present information from the burial and the germination ecological experiment we calculated a number of indices. We classified species according to their relative light germination (*RLG*) modified from Milberg *et al.*, (2000) extending the scale below zero, negative values accounting for better germination in darkness:

$$(1) \qquad RLG = \frac{G_{light} - G_{darkness}}{G_{light} + G_{darkness}} \times 100$$

We calculated *RLG* as the ratio of the number of seeds germinating in light (G_{light}) minus the number of seeds germinating in darkness ($G_{darkness}$) on all seeds germinating in the pair of the experiment, ($G_{light} + G_{darkness}$). We used data from the germination ecological experiment under diurnally fluctuating temperatures of 16°C for 14h and 8°C for 10h. When *RLG* is +100%, seeds germinated only in light; at 0% light and darkness germination were equally important. When *RLG* is -100% seeds germinated in darkness and never in light.

Similarly, we calculated an index for the relative germination in diurnally fluctuating or constant temperatures, *RFG*, being positive when germination percentages are higher under diurnally fluctuating than at constant temperatures and negative when germination was higher under constant temperatures relative to diurnally fluctuating (in darkness):

$$(2) \qquad RFG = \frac{G_{fluctuating} - G_{constant}}{G_{fluctuating} + G_{constant}} \times 100$$

Here, we used the difference between the number of germinated seeds in darkness at diurnally fluctuating temperatures of 16°C (14h) and 8°C (10h), $G_{fluctuating}$, and number of germinated seeds under constant 12°C $G_{constant}$, relative to the sum of seeds germinated in this two experimental conditions ($G_{fluctuating}$ + $G_{constant}$) in the germination ecological experiment. We did not use values measured in light because we think that the most realistic situation of diurnally fluctuating or constant temperatures is when seeds are buried (in other words in darkness), whereas when seeds are in light (that is, on the soil surface) temperatures are always fluctuating daily.

We also ordered species according to their degree of dormancy (*DD*), we therefore used the data from the seed burial experiment. In this experiment, we tested all seeds for each seed retrieval date (t1- t5) and the initial test (t0) under three subsequent conditions (see above) in a growth chamber and in a final tetrazolium test. We calculated the degree of dormancy (*DD*) for each species as following:

$$(3) \qquad DD = \text{mean}_{t0\text{-}t5} \left(\frac{G_{viable\ in\ TZ}}{G_{germinating} + G_{viable\ in\ TZ}} \right) \times 100$$

We calculated the degree of dormancy as the mean ratio of seed numbers over all retrieval dates and the initial test. The ratio is the proportion of ungerminated seeds in the three testing phases detected in the tetrazolium test ($G_{viable\ in\ TZ}$) respective to all viable seeds, that is, the sum of germinated ($G_{germinating}$) and ungerminated viable seeds. This value takes 100% when no seed germinated under the experimental conditions and 0 % when all living seeds germinated in the testing phase.

Seed traits

We determined seed production for all species sticking to the methodological suggestions in Poschlod *et al.* (2000) and Kleyer *et al.* (2008). Seed production was determined as mean individual seed production of 10 individuals in the field. For species with capsules or many seeds per infructescence, we counted the number of capsules or infructescences and sampled

two of them per individual to count in the laboratory. Seed production per individual was then calculated as mean seed number per fruit (infructescence) multiplied by the number of fruits (infructescences) counted per individual.

Statistical analysis and phylogenetically independent contrasts (PICs)

The burial experiment was analysed using a factor by factor non-parametric Kruskal-Wallis Test because data were non-normal and residuals not uniform and because often a high number of zero values (Sokal and Rohlf 1995). Relations among the numerous continuous parameters were analysed using linear regression. We arcsine-transformed *DD* and log-transformed seed sizes to meet the normality assumptions of linear regression. For comparisons of means, we used T-tests for normal data and Mann-Whitney (U) test for non-normal data. We applied a Wilcoxon-rank sum test and a subsequent correction for multiple comparisons (Holm 1979) to test at which particular moment of the burial experiment the difference in soil seed mortality was significant between two groups of high or low *DD*, high or low *RLG* and high or low *RFG*.

We used phylogenetically independent contrasts (PICs; Felsenstein 1985) to study correlation of parameters with phylogeny and to complement analyses with species as replicates. We used the phylogenetically explicit method parallel to all other comparative analyses. We compiled a phylogeny for our species from recent works on phylogeny of the studied species and families, using APGII as a backbone (Angiosperm Phylogeny Group 2003). We preferred Grafen's (1989) method of branch-length estimation and to age estimations of Wikström *et al.* (2001) because in our data set with many closely related species pairs, Wikström-ages gave no realistic branch lengths. We calculated PICs for seed size, seed number, *RLG*, *RFG*, *DD* and soil seed survival percentages. We log-transformed seed number and size before calculating PICs. We then run linear regression through the origin as recommended by Garland *et al.* (1992). All analysis where run in R statistical environment (R Foundation for Statistical Computing 2008).

RESULTS

(1) Seed Burial Experiment

The seed burial experiment yielded two types of data: proportions of seeds that died in the soil that we analysed as soil seed mortality and fractions of living seeds germinating in the three test phases or remaining ungerminated and then detected in the TZ test (Fig. 3.2).

Tab. 3.1. Soil seed mortality analysed as dependent variable with block, time of burial and species as independent factors, using Kruskal-Wallis' test for each factor separately

Factor	df	χ^2	p-value
block	4	1.815	0.7697
time of burial	4	144.2385	<0.0001
species	34	440.4036	<0.0001

This analysis showed that there was no significant 'block' effect on soil seed mortality overall. Moreover, there were highly significant effects of 'time of burial' and highly significant differences among species in the soil seed mortality.

Secondly, the seed burial experiment yielded a large data set on the germinability of seeds after different times of burial in the soil and the reaction of seeds to four-week stratification at 4°C. Figure 3.2 illustrates the dormancy patterns of the different species in the burial experiment.

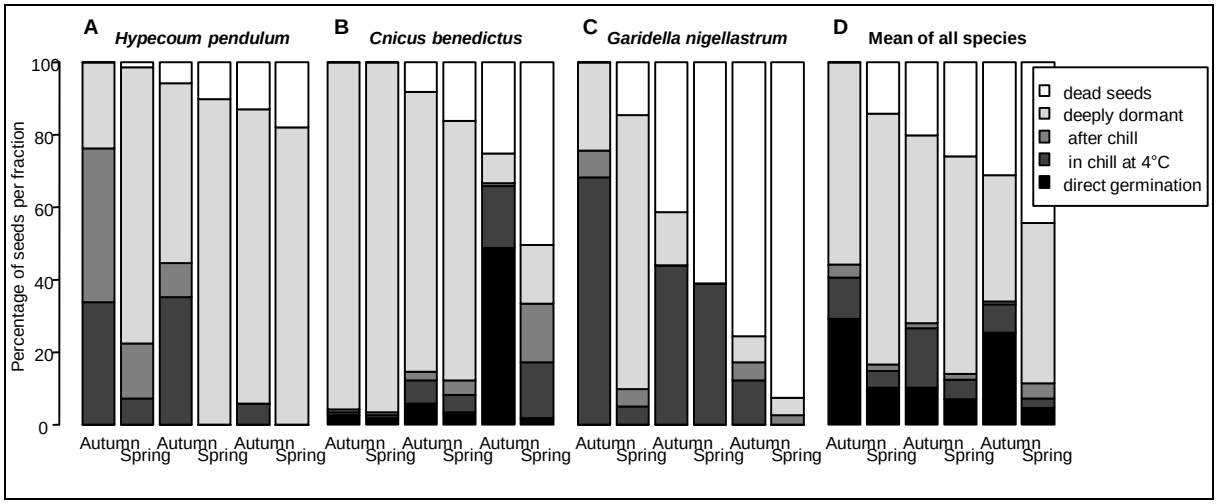


Fig. 3.2. Dormancy cycles in three contrasting species (A-C) and mean dormancy cycles of 35 species (D); black: seeds germinating directly after retrieval in 22°/14°C, dark grey: germination in chill phase (4°C), medium grey: germinated seeds after chilling in 4°C in 22°/14°C, light grey: non-germinated but viable seeds (TZ test) and white: dead seeds.

Figure 3.2D shows the mean proportions of dormant, germinating and dead seeds of all species per time step, with a marked cycling of dormancy in the experiment and the importance of autumn respective to spring germination. Figure 3.2D shows that species are generally non-dormant in autumn, the main germination season, and are dormant in spring. There is a marked cycling dormancy for many species. However, the proportions of seeds germinating in the different seed testing phases versus the viable seeds detected only in the tetrazolium test varied greatly among species.

Dormancy

To order species on a continuous scale of dormancy, we calculated a degree of dormancy (*DD,* see methods) which is tabulated for each species in table 3.2.

Tab. 3.2. Degree of dormancy (*DD*) of species and their four letter codes used in the plots and phylogenetic trees; in bold, species which germination patterns are illustrated in figure 3.2 A-C.

Species	Family	Code	*DD* %
Adonis annua	Ranunculaceae	Adan	100,0
Adonis flammea	Ranunculaceae	Adfl	94,9
Agrostemma githago	Caryophyllaceae	Aggi	0,0
Anagallis arvensis	Primulaceae	Anar	56,9
Androsace maxima	Primulaceae	Anma	94,0
Asperula arvensis	Rubiaceae	Asar	58,7
Bifora radians	Apiaceae	Bira	94,4
Bifora testiculata	Apiaceae	Bite	92,7
Bupleurum rotundifolium	Apiaceae	Buro	40,3
Bupleurum subovatum	Apiaceae	Busu	82,3
Cartahmus lanatus	Asteraceae	Cala	85,4
Caucalis platycarpos	Apiaceae	Capl	99,7
Camelina microcarpa	Brassicaceae	Casa	59,2
Centaurea cyanus	Asteraceae	Cecy	29,0
Centaurea cyanus	Asteraceae	Ceso	13,7
Cnicus benedictus	**Asteraceae**	**Cnbe**	**73,2**
Conringia orientalis	Brassicaceae	Coor	77,3
Consolida regalis	Ranunculaceae	Core	62,5
Galeopsis angustifolia	Lamiaceae	Gala	88,3
Garidella nigellastrum	**Ranunculaceae**	**Gani**	**40,3**
Galium tricornutum	Rubiaceae	Gatr	61,6
Hypecoum pendulum	**Papaveraceae**	**Hype**	**73,0**
Legousia hybrida	Campanulaceae	Lehy	32,6
Legousia speculum-veneris	Campanulaceae	Lesv	62,2
Neslia paniculata	Brassicaceae	Nepa	97,0
Nigella damascena	Ranunculaceae	Nida	51,7
Papaver argemone	Papaveraceae	Paar	92,5
Papaver hybridum	Papaveraceae	Pahy	75,2
Papaver rhoeas	Papaveraceae	Parh	68,2
Ranunculus arvensis	Ranunculaceae	Raar	53,7
Ranunculus falcatus	Ranunculaceae	Rafa	62,8
Roemeria hybrida	Papaveraceae	Rohy	83,9
Silene latifolia	Caryophyllaceae	Sila	13,4
Turgenia latifolia	Apiaceae	Tula	59,7
Vaccaria hispanica	Caryophyllaceae	Vahi	8,7

Soil seed mortality and dormancy

We then analysed the relation of a species' degree of dormancy (*DD*) together with its soil seed mortality in two ways. First, we divided species into two subsets tailored by the median: deeply dormant species (*DD* > 62.2 percentage, N = 18) and little or non-dormant species (*DD* < 62.2 percentage, N = 17). We did five separate plots, corresponding to five burial times in the soil, *i.e.* from first burial to first retrieval date (t0-t1), from first to second retrieval data (t1-t2) and so on, see figure 3.3. Second, we compared the mean soil seed

mortality for each of these burial time steps among deeply dormant and little or non-dormant species. Species with deep dormancy had lower soil seed mortality in all phases and the paired test was highly significant, (see fig. 3.3 for details). We also tested whether the differences declined with time: but there was no significant effect in a linear regression (inlay in fig. 3.3).

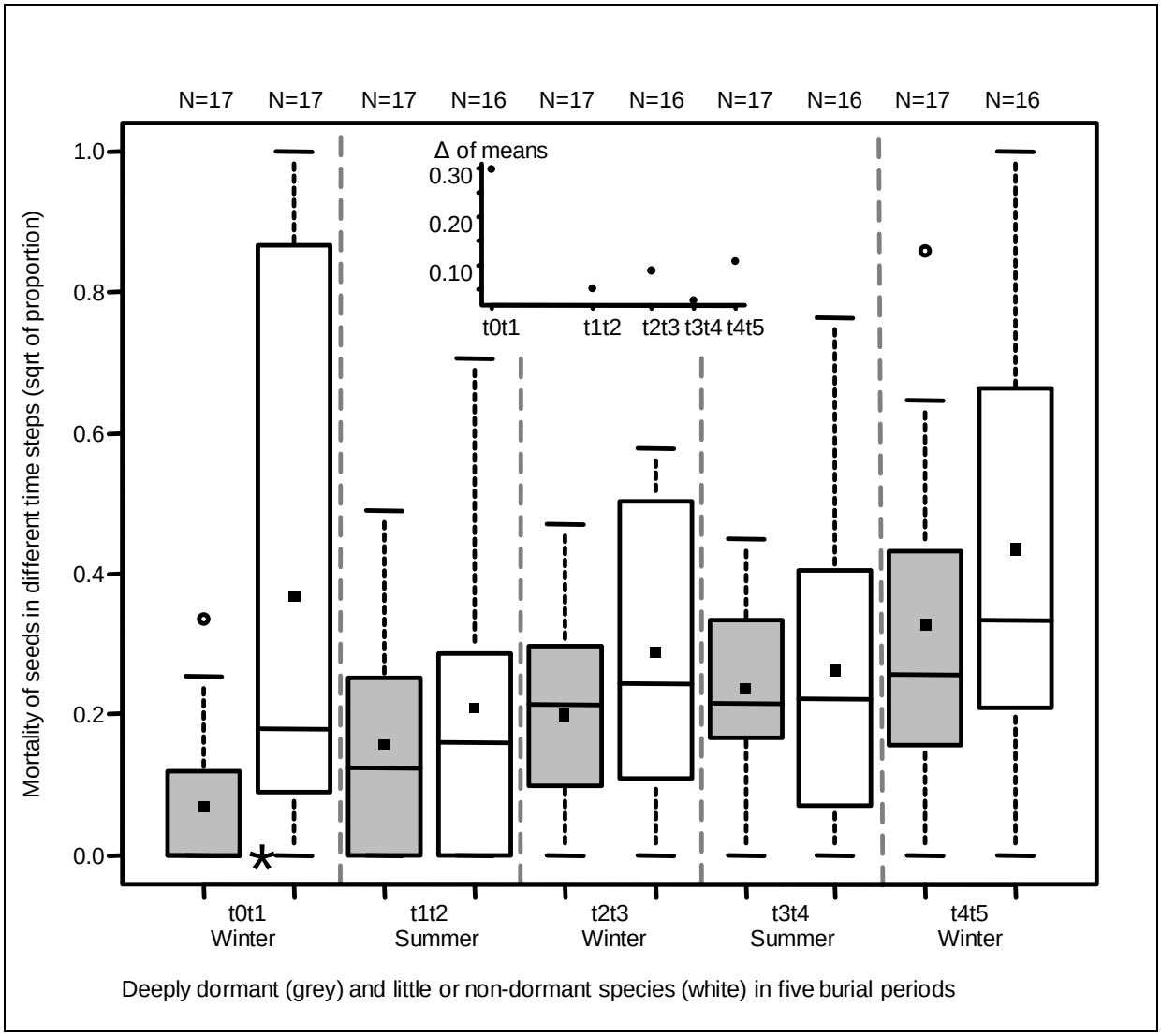


Fig. 3.3. Box plots of the soil seed mortality of deeply dormant (grey) and little or non-dormant species (white) in five burial periods of six months each; for dormancy definition see text; the only significant difference in a particular burial phase is marked with an asterisk (U-test, $p < 0.05$, after correction); note that mortality is square root transformed and that squares design mean values, inlay: differences in mean soil seed mortality between the two degrees of dormancy along time.

The mean soil seed mortality of deeply dormant species is significantly lower (one-sided T = -2.38, p = 0.0376). The pairwise comparisons of soil seed mortality between species with high

or low degree of dormancy showed a significant effect after the first six months of burial (asterisk in fig. 3.3).

In a second approach we analysed the effect of degree of dormancy on the final soil seed mortality (t0-t5) using linear regression of the mean soil seed mortality at the end of the burial experiment for a species on the degree of dormancy of the same species (Fig. 3.4A). This showed the marked effect of dormancy on soil seed mortality.

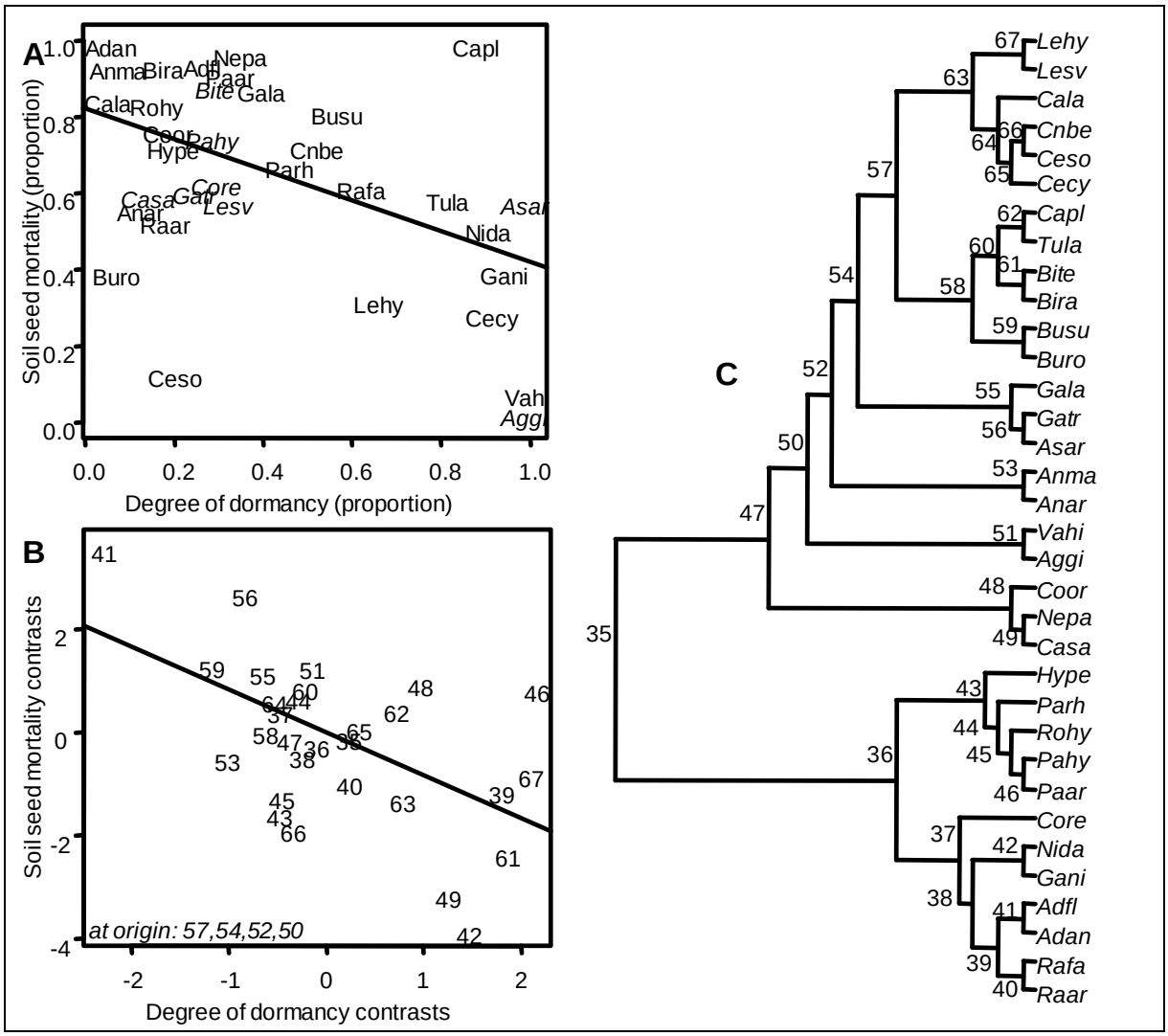


Fig. 3.4. Soil seed mortality after 2.5 years of burial decreases significantly with the degree of dormancy in simple regression (A, R^2 = 0.2344, $F_{1,32}$ = 9.796, p = 0.0037) and using contrasts of mortality and degree of dormancy (B, R^2 = 0.3135, $F_{1,32}$ = 14.61, p = 0.0006), numbers in the tree (C) correspond to PICs used in the analysis. Whenever we moved numbers or species codes for legibility, we put them in italic; codes for species names in A and C are in table 3.2.

Finally, we wanted to know whether phylogenetic constraints had an effect on the outcome of this relation. Therefore, we calculated phylogenetically independent contrasts (Felsenstein

1985) for soil seed mortality t0-t5 and for degree of dormancy. The regression through the origin showed a marked effect of degree of dormancy contrasts on soil seed mortality contrasts (Fig. 3.4B). Neither degree of dormancy nor soil seed mortality showed important contrasts in basal splits of the phylogeny; the grouping of the contrasts of the higher nodes at the origin indicates that most of the differences appear at low phylogenetic level (Fig. 3.4B,C).

We also tried to analyse cycling of dormancy in a similar manner but there were no clear pattern (data not presented).

Dormancy and seed traits

There was no relation between seed size and dormancy ($R^2 < 0.01$, $F_{1,32} = 0.2123$, $p = 0.648$, *DD* arcsine-transformed, seed size log-transformed). Nevertheless, there was a significant relation of seed number per plant and dormancy ($R^2 = 0.1356$, $F_{1,32} = 5.022$, $p = 0.0321$). There was no significant relation between seed number contrasts and dormancy ($R^2 = 0.037$, $F_{1,32} = 1.244$, $p = 0.2731$) or seed size contrasts and dormancy ($R^2 = 0.024$, $F_{1,32} = 0.803$, $p = 0.377$).

Seed mass, seed number and soil seed mortality

There were no differences among species in their soil seed mortality that were correlated to their seed mass in a regression of soil seed mortality at the end of the experiment (t0-t5) with seed mass as the explanatory variable. Figure 3.5A illustrates that there was no significant relation in this regression ($p = 0.1280$, $F_{1,33} = 2.437$, $R^2 = 0.0688$, seed size log-transformed). We then tested whether these differences were at least apparent in opposing closely related groups of species and therefore we used a regression of phylogenetically independent contrasts of soil seed mortality and seed mass. This showed that seed mass contrasts had a highly significant effect on soil seed mortality contrasts (see fig. 3.5B, $F_{1,33} = 11.7$, $R^2 = 0.2617$, $p = 0.0017$).

We then wanted to know whether the differences in seed size were more marked at particularly phases of the burial experiments (not illustrated). We subdivided the species in

large (N = 17) and small seeds (N = 16) and compared the soil seed mortality of these two subsets of species in the five burial periods. Regarding only the means of these two groups, big seeds have a higher seed mortality in four out of five burial time steps (paired, one-sided T-test, T = 2.3084, p = 0.0411). Testing each individual burial phase yielded no significant differences after correction for multiple comparisons ($p > 0.05$, Kruskal-Wallis).

There was no relation between soil seed mortality and seed number ($R^2 < 0.01$, $F_{1,32} = 0.0718$, p = 0.7904) even if phylogenetically independent contrasts were applied ($R^2 < 0.01$, $F_{1,32} = 0.2739$, p = 0.6043).

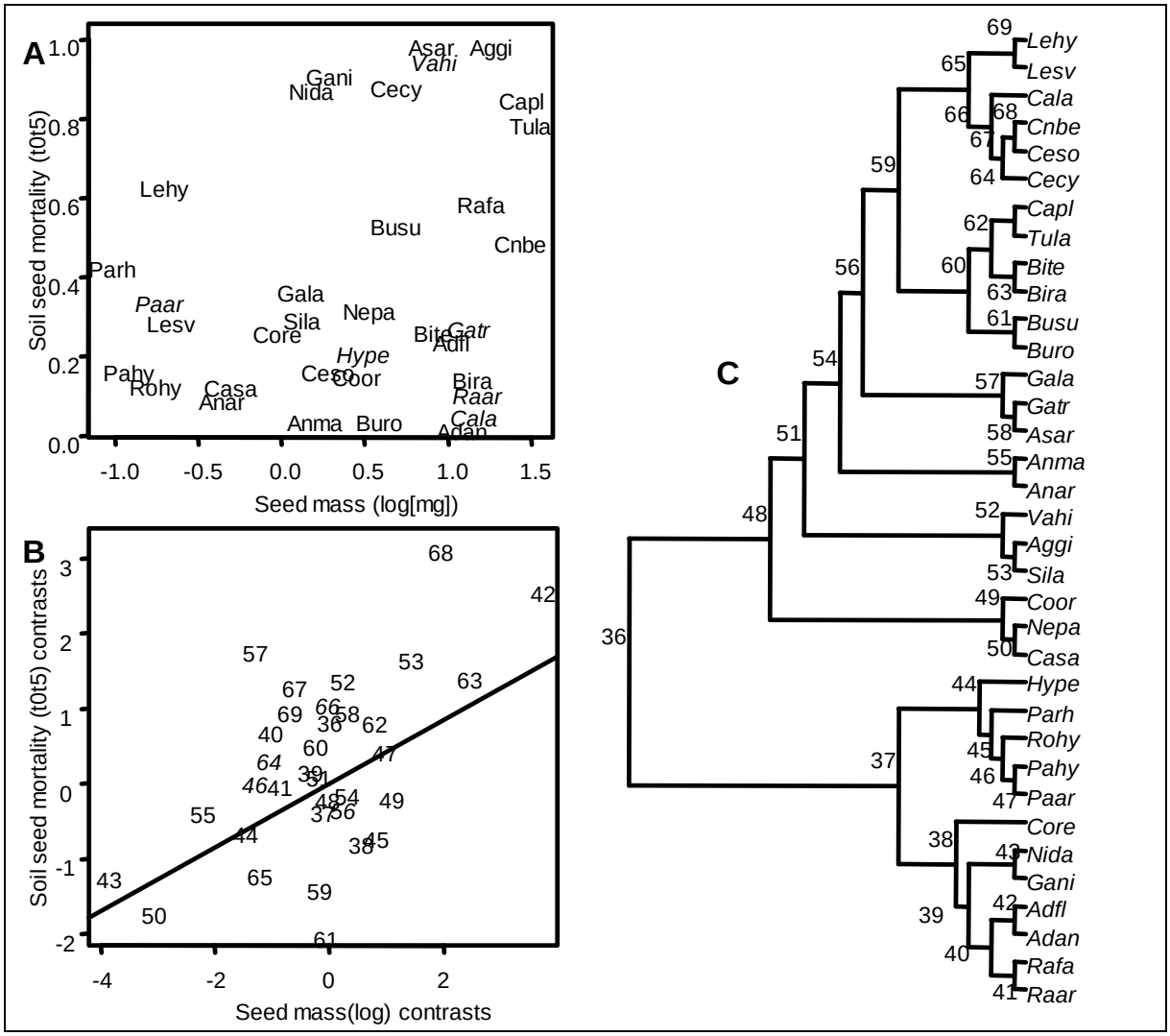


Fig. 3.5 Soil seed mortality after 2.5 years of burial and seed mass are not significantly related in simple regression (A), but contrasts of mortality and seed mass are (B, $R^2 = 0.2617$, $F_{1,33} = 11.7$, p = 0.0017), numbers in the tree (C) correspond to PICs used in the analysis. Whenever we moved numbers and species codes for legibility, we put them in italic.

(2) Germination ecological experiments

In the burial experiment, we showed that most species germinated best directly without stratification or after warm summer periods in autumn under Mediterranean climate (Fig. 3.2D). This was the reason why we tested seeds directly after harvest and a short dry storage period without stratification in the germination ecological experiments, because this reflects best the conditions in the field. We tested the germination of eight samples of 25 to 50 seeds per species under controlled conditions of light and temperature parallely in five growth chambers. Most species showed a maximum of germination at low and diurnally fluctuating temperatures (16/8°C) temperatures, with the only marked exception of *Conringia orientalis,* which germinated best under high fluctuating temperatures of 28°/20°C and much less at 16°/8°C. Some species, such as *Ranunculus falcatus* germinated a little better in the lowest temperature conditions of 10°/8°C and this was consistent with the frequent germination of this species in the chilling phase of the germination tests in the burial experiment. Because of the marked maximum of nearly all species at 16°/8°C, we conducted the experiments on light/darkness and fluctuating/constant temperatures at this temperature level, this experimental subset was a 2 x 2 factorial design (see fig. 3.6A-C and tab. 3.3).

Germination under diurnally fluctuating temperatures

In darkness, the majority of species germinated better under diurnally fluctuating temperatures (N = 3; tab. 3.3). We often found no meaningful differences between constant and diurnally fluctuating temperatures in light (see for example fig. 3.6). For that reason, we did not represent the differences between fluctuating and constant temperatures in light and why we did not used these values later in our analysis.

Tab. 3.3. Relative germination under diurnally fluctuating temperatures in darkness (*RFG*) and relative light germination (*RLG*, under fluctuating temperatures) for 26 species, ordered according to *RFG*; we excluded nine species with no darkness germination in bold species illustrated in figure 3.6 (see below).

Species	*RFG%*	*RLG%*
Conringia orientalis	100	33,3
Papaver argemone	**65**	**60**
Ranunculus arvensis	64	62,5
Centaurea solstitialis	43	95
Bifora radians	35	-31,8
Legousia speculum-veneris	33	75, 6
Roemeria hybrida	33	92,7
Centaurea cyanus	25	3,2
Consolida regalis	25	52,4
Androsace maxima	**24**	**-48,3**
Nigella damascena	16	-81,6
Hypecoum pendulum	13	-73,3
Bupleurum rotundifolium	11	3,1
Silene latifolia	1	-1,3
Agrostemma githago	0	-3,1
Vaccaria hispanica	-4	-39,7
Papaver rhoeas	-7	49,4
Neslia paniculata	-8	-89,5
Garidella nigellastrum	-18	0
Bifora testiculata	-33	-100
Caucalis platycarpos	-43	0
Asperula arvensis	**-59**	**-100**
Legousia hybrida	-60	98,8
Ranunculus falcatus	-75	93,5
Anagallis arvensis	-100	100
Turgenia latifolia	-100	100

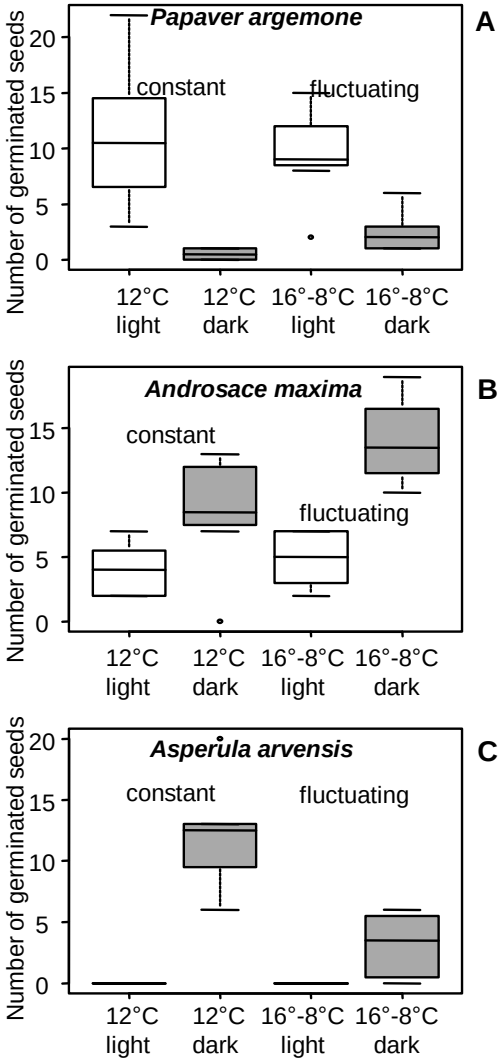


Fig. 3.6: Germination of *Papaver argemone* (A), *Androsace maxima* (B) and *Asperula arvensis* (C) in diurnally fluctuating and constant temperatures in darkness (grey) and in light (white); note that *Asperula* (C) does not germinate in light.

Seed traits and germination ecological parameters

Light and darkness germination are related to seed size and number

Figure 3.7A illustrates the relation between light requirement and seed size. In the darkness experiments, some species did not germinate at all and others only to a very little amount. Therefore, we weighted the regression by the number of seeds germinated, because we think that differences involving high numbers of seeds yield more reliable data than with numbers. Large seeds germinate more easily in darkness than in light, and vice versa (R^2 =

0.14, $F_{1,27}$ = 4.49, p = 0.043) . This relation is similar under constant temperatures (R^2 = 0.15, $F_{1,27}$ = 4.64, p = 0.04).

The weighted regression showed no significant relation when seed number is used as an explanatory variable: figure 3.7B shows species germination as a function of seed number under diurnally fluctuating (R^2 = 0.05, $F_{1,27}$ = 1.42, p = 0.24), and constant temperatures (R^2 = 0.03, $F_{1,27}$ = 0.85, p = 0.36).

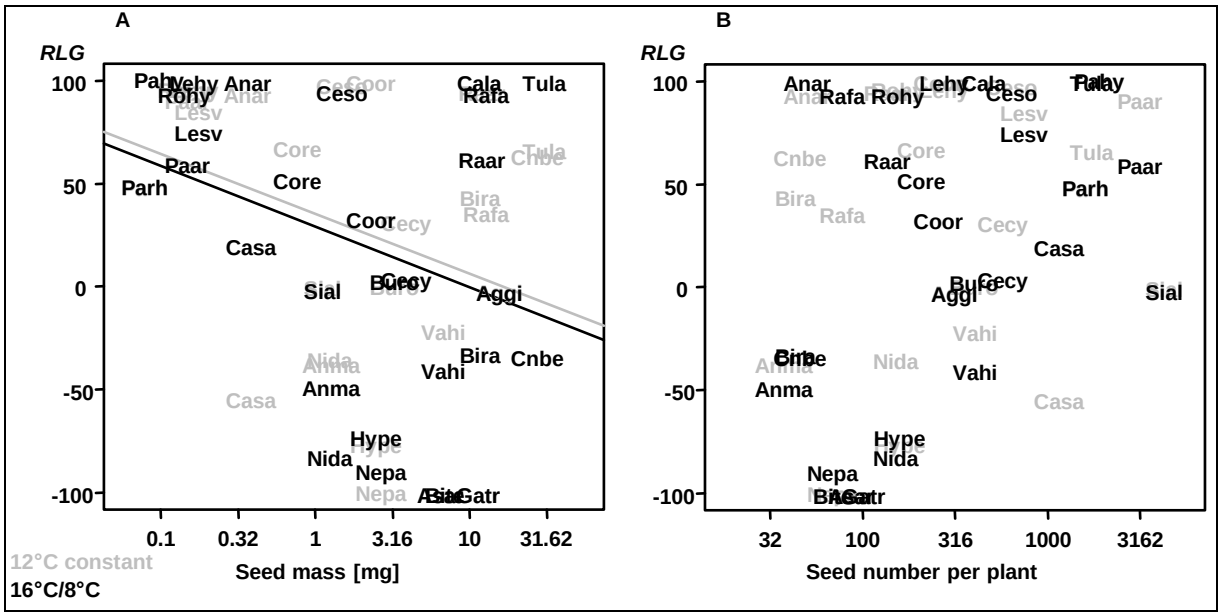


Fig. 3.7. Germination in light (*RLG* > 0%) and darkness (*RLG* < 0%) for species with different seed size (A) and number (B) under diurnally fluctuating (black) and constant (grey) temperatures, lines show the significant relationships in weighted regression (black: R^2 = 0.14, $F_{1,27}$=4.49, p = 0.043; grey: R^2 = 0.15, $F_{1,27}$=4.64, p = 0.040); note the back-transformed logarithmic scale for seed mass and seed number.

Diurnally fluctuating temperatures are not related to seed size or number

We then used weighted linear regression to test if seed size or number were related to the degree to which species reacted on diurnally fluctuating temperatures. Because some species germinated little or not at all in this experiment, we used the number of all seeds germinated in this experiment as weights in the regression. In darkness there was no significant relation between seed mass and relative reaction to diurnally fluctuating temperatures (R^2 = 0.06, p = 0.21, $F_{1,25}$ = 1.609) . This relation is similar in light (R^2 = 0.04, p = 0.35, $F_{1,25}$ = 0.90).

Seed number showed no significant effect on relative reaction to diurnally fluctuating temperatures in the weighted regression neither in light (R^2 = 0.02, $F_{1,24}$ = 0.56, p = 0.46), nor in darkness (R^2 = 0.02, $F_{1,27}$ = 0.02, p = 0.88).

Combined results of (1) soil seed mortality and (2) germination ecological parameters

Light and darkness germination and soil seed mortality

Moreover, we wanted to know whether there were relations between germination ecological characteristics and soil seed mortality. We therefore used the data from the germination ecological experiment to explore differences in soil seed mortality among species.

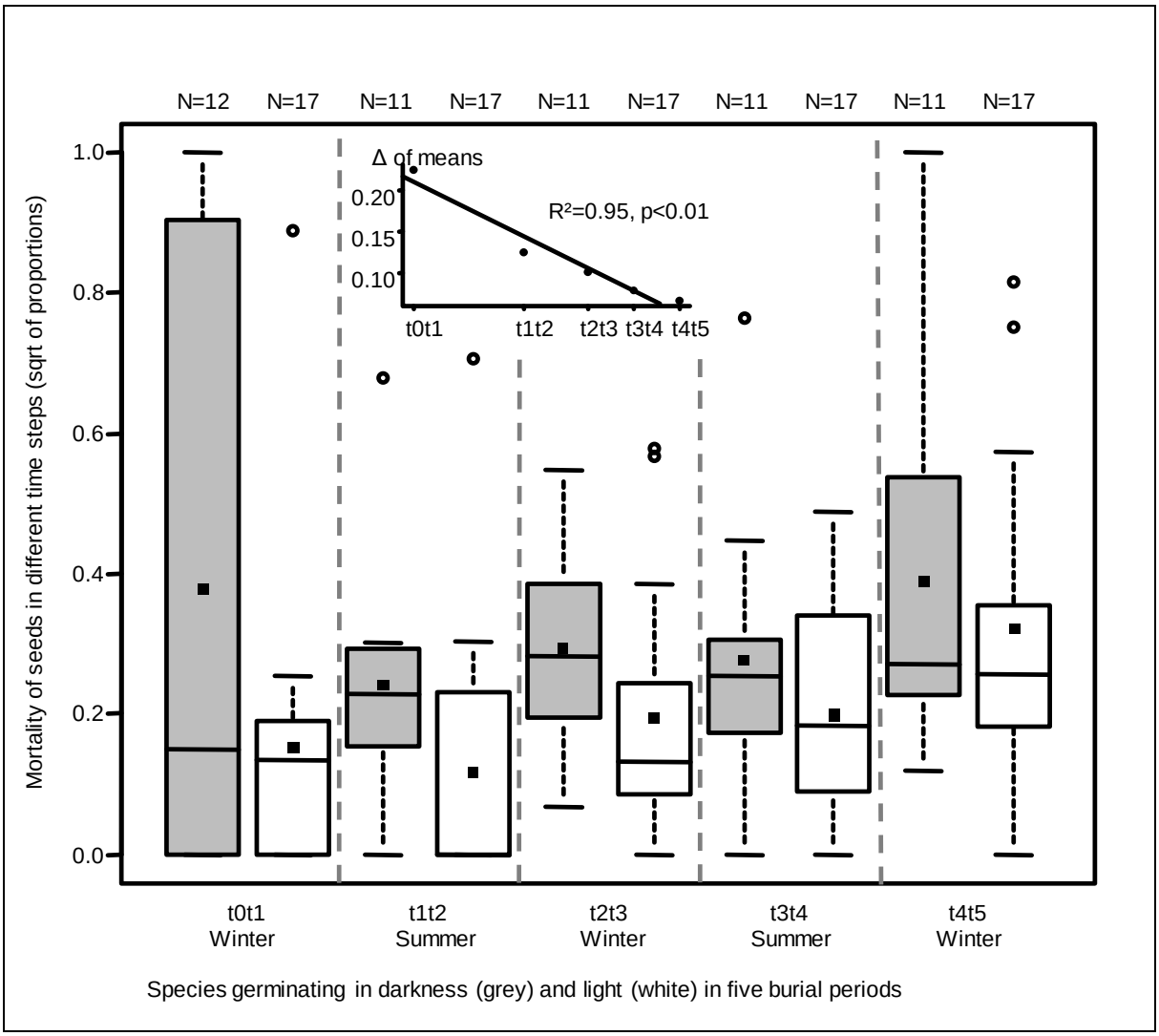


Fig. 3.8. Box plots of the soil seed mortality of species germinating in darkness (grey) and light (white) in five burial periods of 6 months each; inlay: differences in soil seed mortality between light and dark germinating species decline significantly with time; note that mortality is square root transformed and squares designate mean values.

We subdivided the set of species tested in the germination ecological experiment in species that germinated better in darkness than in light (N = 12) and species that germinated better in light than in darkness (N = 17), for this we used the sign of the *RLG* reported above. The mean mortality of darkness germinating species at five burial dates is significantly higher than mean soil seed mortality for species with light requirement for germination (one-sided T-test, $T = 4.21$, $p = 0.0068$). We then tested whether there were significant differences in the soil seed mortality of the associated species in the five burial periods of six months each (fig. 3.8). There was a highly significant overall difference in the means of darkness germination species between darkness germinating species and species with a light requirement for germination (see fig. 3.8). The differences between the two groups declined in a significant way with time (inlay in fig. 3.8). However, comparing darkness germinators to light germinators in each single burial period showed no significant difference after correction of the *p*-values for multiple comparisons (fig. 3.8).

Reaction to diurnally fluctuating temperatures and soil seed mortality

Finally, we tested whether a species' reaction to diurnally fluctuating temperatures had an effect on soil seed mortality. We therefore subdivided the species set in two groups: one with species germinating much better under diurnally fluctuating temperatures (*RFG* ≥ 0%, N = 15) and species that germinated better under constant temperatures (*RFG* < 0 percentage, N = 11). Using the means of the diurnally fluctuating temperature reactors versus the constant temperature germinators there was no overall difference (one-sided T-test, $T = -1.7$, $p = 0.9176$, see fig. 3.9). We then tested whether there were differences in particular burial periods by comparing the two groups in each time step and applying a correction for multiple comparisons. Figure 3.9 and table 3.4 show that there are significant differences in winter. Species that germinate better under diurnally fluctuating temperature have significantly lower soil seed mortality in the second and third winter of burial.

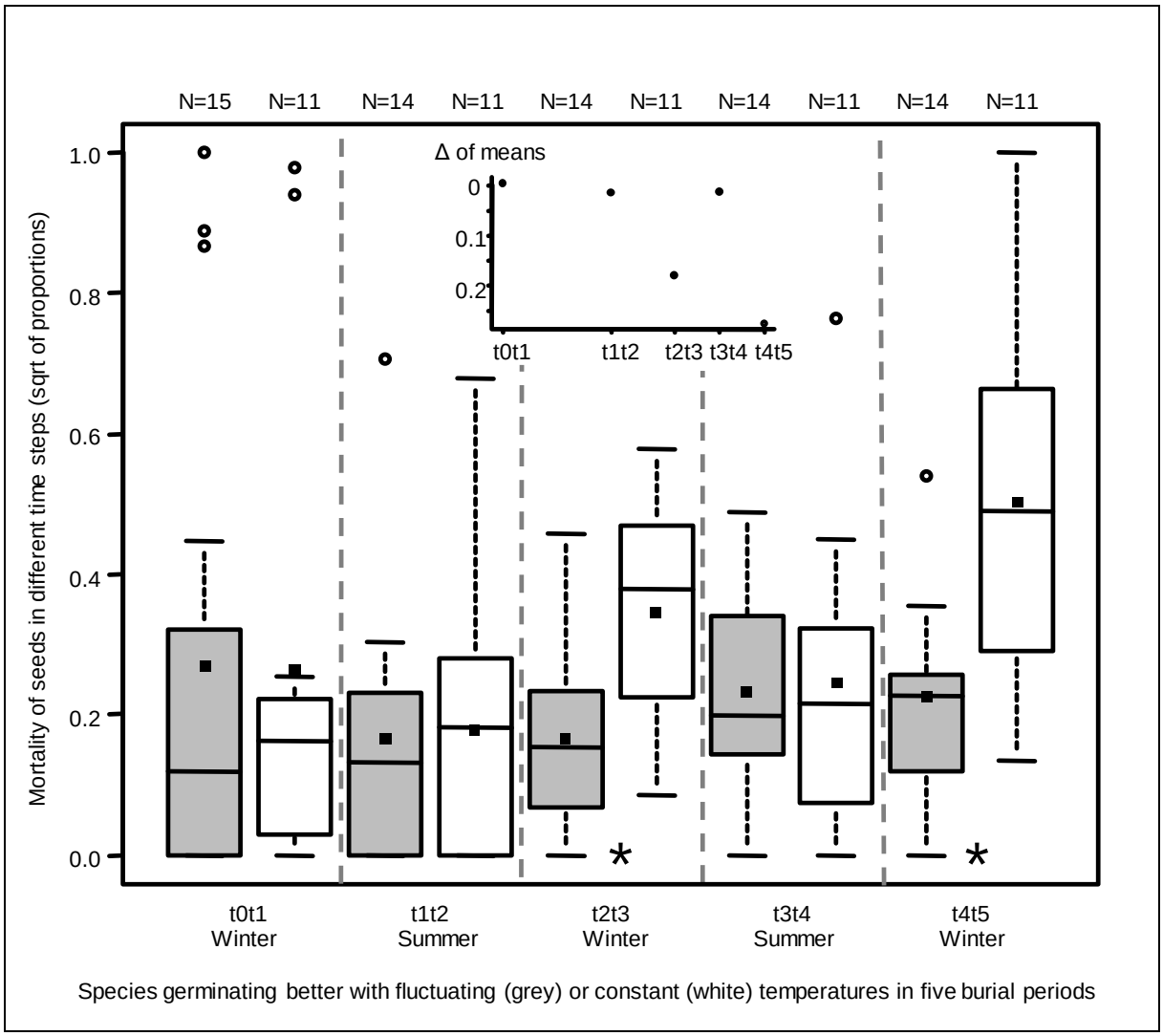


Fig. 3.9. Box plots of soil seed mortality of species germinating better under diurnally fluctuating temperatures (grey) than under constant (white) in five burial periods of 6 months each, the significant differences in 2nd and 3rd winter are marked with * (U-test, $p < 0.05$, after correction for multiple comparisons; tab. 3.4 for details); note that mortality is square root transformed and that squares design mean values, inlay: differences in soil seed mortality between species germinating better under diurnally fluctuating and constant temperatures along time.

At the end, we tested if there was an effect of *RFG* on the final soil seed mortality, using linear regression, which indicated that there was a marginal significant relationship (R^2 = 0.08, $F_{1,33}$ = 2.9, p = 0.0978).

Tab. 3.4. Comparisons of soil seed mortality between species germinating better under diurnally fluctuating or constant temperatures

Time step	W	p-value	corrected p (Holm)
t0t1	78.5	0.8538	1
t1t2	79.5	0.9114	1
t2t3	30	0.01089	0.04356
t3t4	76.5	1	1
t4t5	26	0.004194	0.02097

We then wanted to know whether there were marked differences among closely related species, using phylogenetically independent contrasts. The regression of soil seed mortality contrasts on *RFG* contrasts showed that there was no such effect (R^2 = 0.0308, $F_{1,33}$ = 1.05, p = 0.313, regression forced through origin).

DISCUSSION

Dormancy and soil seed survival - dormancy and seed traits

Dormancy had an important effect on soil seed survival during and at the end of our experiment (Fig. 3.4 and 3.5). This indicates that species with a higher degree of secondary dormancy germinate less easily while buried and in this way are less depleted from the soil seed bank. These findings confirm that dormancy is a very relevant factor for soil seed survival (Baskin and Baskin 1998;Thompson *et al*. 2003;Baskin and Baskin 2006). The use of an experimental data set on soil seed survival rather than data on seed persistence estimated from the literature and the integration of secondary dormancy may explain why this pattern is much clearer here than in previous works (Thompson *et al.* 2003). In deed, we studied soil seed depletion and degree of dormancy in the same experiment, both on a quantitative scale, whereas Thompson *et al.* (2003) studied the qualitative relation between dormancy and soil seed persistence on a qualitative basis. The fact that dormancy is so closely related to soil seed survival is clear from an evolutionary point of view: in our experiment dormancy illustrates the degree to which seeds germinate easily when conditions are optimal (our standardised testing conditions). This degree of delayed germination is a typical bet hedging trait that is evolutionarily triggered by the risk in reproductive success for each species (Venable 2007). Our data confirm that dormancy is the main proximate way how soil seed persistence is controlled in the long run in buried populations of seeds. This points also to dormancy as a primary quantitative predictor of soil seed persistence with a sound

evolutionary basis, explaining general across species trends and holding true after removal of phylogenetically correlated variation (Fig. 3.5C).

Dormancy is especially important just after seed burial (Fig. 3.3), because here seeds of some species, such as *Agrostemma githago* and *Asperula arvensis* decrease rapidly to a low level. Apparently, these species maximise their fitness by immediate germination at the earliest possible germination period that is consistent with the missing light requirement (tab. 3.3 and discussion below) for these species. Light requirement is therefore a second possibility to achieve seed survival in burial (Baskin and Baskin 1989;Baskin and Baskin 1998). That neither seed size nor seed number have a relation to dormancy even when phylogenetically contrasts are used is astonishing in this context. One can think that larger seeds reduce their risk by a higher survival to partial predation (Leishman *et al.* 2000b) and by meaning out spatial heterogeneity (Fenner and Thompson 2005). Larger seeds should therefore have lower levels of dormancy. If phylogeny is not accounted for, there is a significant higher degree of dormancy in species with high seed production. This may be in relation to the fact that more numerous seeds have always a higher risk of competition due to crowding. Delaying germination through dormancy is a way to escape this density dependent effect.

The inspection of soil seed mortality at different times after burial (Fig. 3.3) shows the tendency that soil seed mortality of little dormant species is higher in winter than in summer (Fig. 3.3), a similar finding to the one reported for diurnally fluctuating temperatures (Fig. 3.9) were it is much clearer and discussed in more detail.

Light requirement, darkness germination, soil seed survival and traits

Our results show clearly that larger seeds are less dependent on light for germination. This is in congruence with previous findings (Milberg *et al.* 2000) and the observations that larger seeds can emerge from deeper soil layers (Bond *et al*. 1999;Grundy *et al*. 2003) together with the fact that light penetrates only extremely little into soil (Benvenuti 1995). We could add to these findings that larger seeds not only depend less on light, but also that, in our

experiments (Fig. 3.7A), they show an even higher germination in darkness than small seeds. Bell *et al.* (1995) found that some species germinated better in darkness than in light under the Mediterranean climate of Western Australia. We interpret our findings in a similar way to Bell *et al.* (1995), that is, species that can detect favourable moments to germinate, but which germinate in soil layers were moisture and light conditions are less extreme than at the surface may have an advantage over light dependent germination under Mediterranean climate. It has to be noted here, that we conducted our experiments in complete darkness and that there were not even short light stimuli in our experiment, so our data do not apply to *e.g.* shaded environments and more importantly, there is no bias by light stimuli that are not intended. We discuss later that fluctuating temperatures in darkness are an important feature to understand germination in darkness.

Species with a light requirement showed a lower soil seed survival in our data set than species capable of germination in darkness (Fig. 3.8). This is in congruence with the view that a light requirement can be sufficient to form persistent seed banks even if there is no dormancy, and *vice versa* darkness germinators do not form seedbanks (Baskin and Baskin 1989;Baskin and Baskin 2006). The differences in soil seed mortality between light- and darkness germinators in different times after burial (inlay in fig. 3.8) shows that the importance of a light requirement decreases with time of burial, this can be so for several reasons: some dark germinating species disappear completely after short times of burial, as it is the case with *Agrostemma githago*. This however cannot explain why the effect lasts so long and does not completely break down after the first burial phase (Fig. 3.8). Another explanation is that, once seeds are buried, different mechanisms such as secondary dormancy or reaction to diurnally fluctuating temperatures, become more important to control germination than a light requirement. In our data set, *Agrostemma githago, Asperula arvensis* and *Vaccaria hispanica* the most rapidly declining species show no light requirement for germination. On the other hand, there are some species with better germination in

darkness (that are light inhibited!), *e.g. Neslia paniculata* or *Bifora testiculata* that have high soil seed survival; in these cases the degree of dormancy is (not astonishingly) very high showing the complementary strategies to control germination below ground.

'Gap detection', diurnally fluctuating temperatures and soil seed survival – diurnally fluctuating temperatures and seed traits

The inspection of soil seed mortality at different times after burial (Fig. 3.9) shows that soil seed mortality of species germinating better without diurnally fluctuating temperatures (*DFT*) is higher in winter than in summer (asterisks in fig. 3.9). This is consistent with the dormancy cycles for nearly all species, which are markedly dormant in spring and less dormant in autumn (Fig. 3.2). An effect in relation with how we quantified dormancy: we did not use a particular germination season for the calculation of the *DD* but averaged over all available seasons, making our measure relatively independent from degree of cycling dormancy. While species without gap detection mechanism germinate easily in winter, when temperatures are relatively constant and buffered by high soil water content and so are depleted from the soil seed population by fatal germination, species with gap detection mechanism do not (Fig. 3.9). With increasing depth diurnally temperature fluctuations are lower (Miess 1968). Our data therefore significantly extend the classical view of the 'gap detection' role of diurnally fluctuating temperatures (Bullock 2000), because *DFT* can also prevent germination in situations when seeds are buried too deep to emerge to the soil surface (Fig. 3.9). Diurnally fluctuating temperatures are therefore a more general feature to trigger germination, as this suggests also the reaction of *e.g.* mud flat species to the end of flooding periods (Schütz 2000). In the context as *DFT* as a way to detect burial depth, it would be probable that seed size has a negative effect on the strength of the reaction to *DFT*. However, in our results there was no relation between seed size or number and germination in diurnally fluctuating temperatures. This may indicate on the one hand, that the gap detection mechanism is equally often evolved in large and small seeded species. On the other

hand, we may not have measured mortality at the right depth or *DFT* on the right amplitude of fluctuations to detect such a relationship. In order to definitely answer the question whether depth of burial and *DFT* are related to seed size, more detailed data are needed, which include different, especially shallower burial depths and different temperature fluctuation amplitudes (especially smaller amplitudes).

Soil seed survival and traits

We also tested if seed number and seed size were related to soil seed mortality measured in the burial experiment. There was no strong relationship to seed number. Seed size had an effect when all burial periods are used but no significant effect on the final soil seed mortality. In the analysis of phylogenetically independent contrasts, however, there was a strong negative effect of seed size on soil seed survival, indicating that larger seeds have a lower soil seed survival when closely related species are compared (Fig. 3.5B). This is the first test that explicitly uses data from a burial experiment with a defined seed input and quantitative measures of soil seed survival that shows this relationship. Therefore, this adds significant data to sustain the seed size-seed persistence relationship reported from a series of works (Thompson *et al*. 1993;Bekker *et al*. 1998a;Moles *et al*. 2000;Cerabolini *et al*. 2003;Peco *et al*. 2003). However, it is astonishing that there was no clear effect on final soil seed mortality when PICs were not used. A possible bias in our experiment may be that we used fixed numbers of seeds for all species and a unique size of mesh bags for burial without substrate, in this design small seeds are more distant in average than large seeds and propagation of fungi can be enhanced in more densely packed seeds in mesh bags (Van Mourik *et al.* 2005). This bias would lead to higher soil seed mortality in larger seeds due to fungi attack, one of the most important factors acting in soil seed decay (Schafer and Kotanen 2003;Davis and Renner 2007).

CONCLUSION

This work shows that different germination ecological parameters can be used to explain soil seed mortality; notably diurnally fluctuating temperatures and secondary dormancy have both to be considered when soil seed survival is analysed from the seed's perspective. The previous positions emphasised primary dormancy and light requirement to predict soil seed survival (Baskin and Baskin 1989;Milberg *et al*. 2000;Thompson *et al*. 2003). We also found similar relations for light requirement, but we could extend the understanding by integrating explicitly species with pronounced darkness germination. Darkness and light germination are related to seed size in our data set as in the work of *e.g.* Milberg *et al.* (2000). This is additional support for the view that there is a relationship between seed size and seed longevity based on selective forces that trigger an earlier depletion of larger seeds in relation with darkness germination. That may be the explanation why in our data set we found a seed size-seed survival relation but in the absence of a strong global relation between seed size and seed survival at the end of our experiment this point has still to be studied.

We also could elucidate that different strategies exist to control germination below ground that can be complementary in their importance for soil seed mortality as in the case of light requirement and dormancy. This is important if one wants to predict soil seed mortality from simple germination and seed traits. Both, information on the dormancy state and the need of a light requirement are needed to predict whether a species is rapidly declining, that is forming a transient soil seed bank. This observation has also an importance for conservation efforts: here it becomes clear which parameters identify high soil seed depletion and thus more vulnerable species to changes of habitat quality.

TRANSITION CHAPTER 3 TO 4

From functional ecology of soil seed banks to population persistence

In chapter 3, we studied how seeds trigger their emergence from the soil seed bank and this gives insights into how persistence in the soil seed bank is regulated by the timing of germination. The germination experiments showed that level of dormancy, reaction to diurnally fluctuating temperatures and reaction to light all showed a direct relation to soil seed mortality. This re-emphasises the high constraints on the timing of seedling emergence, corresponding to the low temperatures in Mediterranean type ecosystems because the cold season is the only season with enough and predictable moisture. Equally, after disturbance the first germinating seeds have advantages by pre-emption of space and resources as long as moisture conditions are sufficient. The high dependence of annual species on moisture in the top soil layers put an important evolutionary constraint – genotypes detecting better this conditions will have a greater reproductive success. This shows the constrain of the optimal timing of germination *via* dormancy, detection of light and diurnally fluctuating temperatures for seed persistence in the soil.

Soil seed bank persistence was related to population persistence by different authors (Kalisz and McPeek 1992;Kalisz and McPeek 1993;Pake and Venable 1996;Stöcklin and Fischer 1999;Venable 2007). This has been done by comparing persistence of populations between many species of different soil seed bank types, which however have been classified using seedling emergence method (Stöcklin and Fischer 1999). Another approach which points in this direction comes from studying population dynamics of a single or rather limited set of species (Kalisz and McPeek 1992;Kalisz and McPeek 1993;Venable 2007) or from modelling (Venable and Brown 1993a;Pake and Venable 1996). There is hence scarce evidence about this

point and comparing a larger set of species with precise data on soil seed bank persistence, related germination traits and complementary performance traits can be a profitable way to analyse population dynamics in annuals.

In chapter 4, we study long and short-term population dynamics of annuals in relation to these traits. There are two aspects of population dynamics: turnover, *i.e.* the importance of colonisation and extinction events respective to stable populations and, second, extinction rate, *i.e.* the extinction of populations in a given observation frame. Most extinction dynamics are driven by change of environment. We explicitly place our study in the rapidly changing environment of arable fields. We study the importance of traits from functional ecology of soil seed banks. However, there can be many other processes than differences in soil seed bank parameters that influence population dynamics. Because it is difficult to study all influences at the same time, we focussed on soil seed mortality as an identified source of variation among species. In a first step, we did not study other important processes such as dispersal in space, predation and competition. The disturbance intensity and frequency in arable fields change with agricultural techniques, which can also modify soil factors such as nutrient status and soil acidity. We analysed the importance of the change in abiotic factors for extinction dynamics using indicator values.

CHAPTER 4

Is there an effect of soil seed mortality and seed production on local population dynamics in annual plants? – the case of rare cereal weeds

INTRODUCTION

Annual plants are an important part of plant diversity in habitats with frequent and unpredictable disturbances, however mechanisms that maintain high diversity are still not well understood.

First, on the community level, the 'storage effect' offers an explanation for coexistence in habitats where species differ in response to disturbances and levels of competition change (Chesson and Warner 1981;Warner and Chesson 1985;Levine and Rees 2004;Facelli *et al*. 2005). Storage effect suggests that a life stage that buffers population growth and decline, *e.g.* a persistent soil seed bank, enhances the coexistence of species. Several studies show the applicability of the storage effect (Bonis *et al*. 1995;Cáceres 1997;Facelli *et al*. 2005). In the same context, plant diversity effects mediated by enhanced local population persistence have been studied to determine which species are more under risk due to their specific trait configuration (Fischer and Stöcklin 1997;e.g. Stöcklin and Fischer 1999;Ozinga *et al*. 2007). Like storage effect, they all point on the importance of longevity of seeds in the soil. On the one hand, the formation of a soil seed bank has been related to gap availability or bare soil cover in vegetation (Peco *et al*. 1998;Hopfensberger 2007). Arable fields are on the top of this disturbance and bare soil cover scale. On the other hand, soil moisture varies from year to year, between and among habitats. Soil moisture was identified as enhancing fungal activity and thus increasing soil seed mortality for dry habitat species (Blaney and Kotanen 2001;Schafer and Kotanen 2003). However, flooding events can create favourable

environments for seed bankers (Stromberg *et al.* 2008) and anoxia in water logged soil enhances seed longevity in aquatic plants (Baskin and Baskin 1998). Directional changes in soil humidity can act probably directly on soil seed mortality and in this way influence extinction of populations for dry habitat species. In a similar way, burial depth and physical soil factors influence soil seed bank dynamics and mainly determine the fractions of germinating and dormant seeds (Benvenuti *et al*. 2001;Benvenuti 2003), triggering in this way the soil seed mortality of seeds. To compare the rapidity of seed mortality in soil seed banks among different species, several approaches can be used. The most widespread method is the use of seedling emergence from soil samples and to classify the species according to their depth distribution, presence/absence in the surrounding vegetation, seasonality or position in successional seres (Thompson *et al.* 1997). A different approach measures directly soil seed mortality in burial experiments (Telewski and Zeevart 2002). In analysis of large databases, both approaches are sometimes mixed (Bekker *et al.* 1998a). We have shown elsewhere, that seed production influences the classification of seed bank types when it is determined by seedling emergence and quantified as 'longevity index' (Saatkamp *et al*. 2009). Seed mortality from burial experiments and seed production are not correlated, showing that soil seed mortality and seed production are two independent factors for soil seed bank formation (see also Jakobsson *et al*. 2006;Saatkamp *et al*. 2009). Species with high seed mass compensate the higher seed production of small seeded species by higher survival in the seedling and other life stages. Hence, many small seeds and few large seeds are equally effective for regeneration (Leishman *et al*. 2000b;Moles *et al*. 2004). For these reasons, we think it is important to separate clearly between soil seed mortality and seed production (and correlated measures) when studying the effects of soil seed bank parameters on local population persistence and dynamics.

Second, on the population level, Poschlod *et al.* (2000) showed that there are many different traits or processes contributing to population persistence and they highlighted soil seed

persistence. This is especially true for annuals, where there is no other resting stage than the seed and there is no clonal growth (Menges 2000;Venable 2007). Many works sustain the idea that soil seed mortality is an important parameter for population size fluctuation in annuals (Silvertown 1982;Kalisz and McPeek 1993;Schneider *et al*. 1994;Schmid and Matthies 1994;Günter 1997;Stöcklin and Fischer 1999;Menges 2000;Adams *et al*. 2005). However most of these works evaluated the relation on a species level (Silvertown 1982;Kalisz and McPeek 1993;Adams *et al*. 2005) or used measures of seed persistence confounding soil seed mortality and seed production (Schneider *et al*. 1994;Stöcklin and Fischer 1999). High species numbers make study of several population parameters difficult, so studies including several species are scarce (Harrison and Ray 2002) whereas comparative population dynamics including a larger set of species are expected to give new insights (Menges 2000). Furthermore, regeneration is hampered and plant populations decline when environment changes. Not only in such a regression context, small populations go extinct easier than large ones (Matthies *et al.* 2004). We think similarly that species with high soil seed mortality should go extinct more easily than species with low soil seed mortality irrespective of seed production or seed size because of the seed size –seed number trade-off. Detailed works on the latter show that there is a seed quantity being equally effective for regeneration *i.e.* corresponding to some large or to many small seeds (Leishman *et al*. 2000b;Moles *et al*. 2004). The buffering effect soil seed banks against extinction depends thus less on the number of seeds but on their soil seed mortality. In this respect, it is of high importance not to use seed persistence estimates based on seedling emergence methods that are biased by seed production.

In cereal fields, population extinctions are mostly due to changing agricultural practices such as herbicides, fertilizers and high densities of crop plants (Schneider *et al*. 1994;Fried *et al*. 2009). Therefore, annual plants in cereal fields give a good opportunity to test effects of species life history traits and habitat requirements on population dynamics and notably the respective roles of seed production and proportional soil seed mortality. Because habitat

requirements such as moisture or nutrient status can also directly influence soil seed mortality or seed production it should be illustrated if and which directional habitat change exists in the studied habitat.

In this study, we used data on population fluctuations for 30 cereal weed species together with experimental soil seed mortality and seed production data to answer (i) whether the initial size of a population has an impact on population extinction and (ii) how the habitat requirement of a species is related to population turnover and the extinction/colonisation ratio. Subsequently, we wanted to know how population turnover and extinction/colonisation rates are influenced by (iii) soil seed mortality and by (iv) individual seed production.

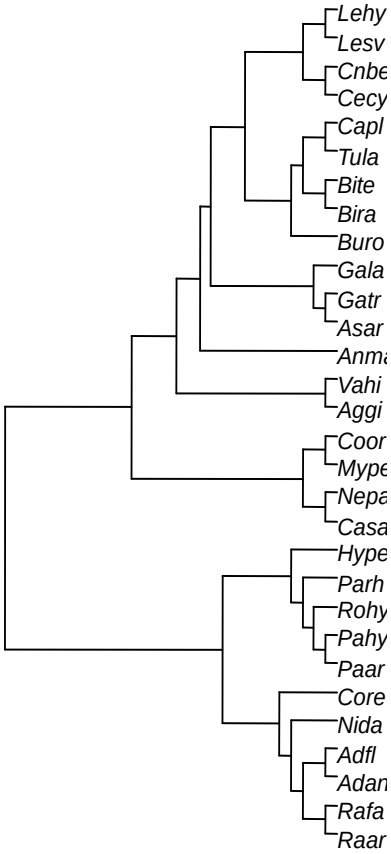
MATERIALS AND METHODS

Study site and species

We gathered data on rare and common cereal weeds in an area of ca. 2500 km² around the Luberon ridge in South Eastern France (see fig. I.7). This area is characterised by Mediterranean climate (mean $rainfall_{1971\text{-}2000}$: 623 mm, maxima in April and October). Traditional agriculture in this area maintained a high diversity of rare cereal weeds elsewhere extinct in Europe (Filosa 1989;Filosa 1997). We surveyed 30 species of rare cereal weeds (Filosa 1989) which were still present in 2005 (relative to their presence in 1983-1985) and for which we were able to study more than two populations (see tab. 4.1).

Tab. 4.1. Species studied, their four-letter code, the number of populations (1983-2006) studied per species and phylogenetic relationships.

Species	Family	Code	Populations
Adonis annua	Ranunculaceae	Adan	30
Adonis flammea	Ranunculaceae	Adfl	45
Agrostemma githago	Caryophyllaceae	Aggi	11
Androsace maxima	Primulaceae	Anma	12
Asperula arvensis	Rubiaceae	Asar	6
Bifora radians	Apiaceae	Bira	57
Bifora testiculata	Apiaceae	Bite	10
Bupleurum rotundifolium	Apiaceae	Buro	11
Caucalis platycarpos	Apiaceae	Capl	41
Camelina microcarpa	Brassicaceae	Casa	17
Centaurea cyanus	Asteraceae	Cecy	23
Cnicus benedictus	Asteraceae	Cnbe	28
Conringia orientalis	Brassicaceae	Coor	20
Consolida regalis	Ranunculaceae	Core	25
Galeopsis angustifolia	Lamiaceae	Gala	16
Galium tricornutum	Rubiaceae	Gatr	52
Hypecoum pendulum	Papaveraceae	Hype	5
Legousia hybrida	Campanulaceae	Lehy	29
Legousia speculum-veneris	Campanulaceae	Lesv	40
Myagrum perfoliatum	Brassicaceae	Mype	8
Neslia paniculata	Brassicaceae	Nepa	49
Nigella damascena	Ranunculaceae	Nida	21
Papaver argemone	Papaveraceae	Paar	42
Papaver hybridum	Papaveraceae	Pahy	32
Papaver rhoeas	Papaveraceae	Parh	83
Ranunculus arvensis	Ranunculaceae	Raar	64
Ranunculus falcatus	Ranunculaceae	Rafa	21
Roemeria hybrida	Papaveraceae	Rohy	17
Turgenia latifolia	Apiaceae	Tula	35
Vaccaria hispanica	Caryophyllaceae	Vahi	13



Seed production

Seed production was determined as mean individual seed production of 10 individuals in the field. Seed production was not calculated for given surface unit (Šera and Šery 2004). For species with multi-seeded fruits or infructescences, we counted the number of fruits or infructescences and sampled two of them per individual to count number of seeds per fruit/infructescence. Seed production per individual was then calculated as mean seed number per fruit/infructescences multiplied by the number of fruits/infructescences counted per individual (Kleyer *et al*. 2008).

Soil seed mortality

We studied soil seed mortality using a burial experiment presented in more detail in Saatkamp *et al.* (submitted). For each of the 30 species studied, we collected seed material between June and September 2005 in the field. Ripe seeds from at least ten individuals were mixed and stored dry in paper bags until burial in October 2005. After seed burial, we retrieved buried seed every six months from October 2005 to April 2008. In an initial test, we determined the germinable fraction of the seed lots used. Each retrieved seed sample was tested for viability using first a germination test in a growth chamber and then a tetrazolium test on all ungerminated seeds (International Seed Testing Association 1996). This burial experiment yielded mortality percentages for five time steps (t_1 to t_5). We used soil seed mortality from the beginning of the experiment (t_0) until 6 months (t_0t_1); until 18 months (t_0t_3) until the end of the experiment (32 months, t_0t_5) and one intermediate period in the middle of the burial experiment, t_2t_3, to test for effects on population viability. Here we refer to this as 'soil seed mortality'.

Population fluctuations

We used a census data set on rare cereal weeds dating from 1983 and 1985 to gather population sizes and localisations (Filosa 1989). This data set contained 100 cereal fields with 863 populations of 30 rare annual plants. Data from 1983 and 1985 were pooled, we refer to them as 1983. The locations were dispersed over an area of 2500 km². The populations were documented by a single visit per year at the flowering to fruiting time of the species before wheat harvest. We included 20% of cereal fields without any population of the studied species in 1983. The remaining fields had often populations of several different species. In 2005 and in 2006 we revisited all fields and conducted the census at the same level of precision as in 1983. Hereafter, we use long time step for the population dynamics between 1983 and 2005 and 'short time step' for the population dynamics between 2005 and 2006. We counted all flowering or fruiting plants of the 30 cereal weeds in the same fields. When the

total number of individuals exceeded 50, we estimated the number of individuals. For 338 populations, size has been documented in sufficient detail in the 1983 data set; this enabled us to study its relation to extinction. In the remaining analyses, we only used presence/absence data. Evidently, no detection of a population in one single year can be viewed as above-ground absence but still presence in the soil seed bank, we therefore use the term 'new populations' for those populations that have been found in 2006 not in 2005. However, for the long time step, it is more realistic to assume that new populations are effectively colonisations and that disappearances are definitive extinctions. For each species, we counted population extinctions (P_{ext}), populations found at both dates (P_{per}) and new populations (P_{new}) on places not inhabited in 1983-1985. We analysed separately the data for the two observation time steps 1983-2005 and 2005-2006. We calculated two different ratios to investigate the relationships between population fluctuations and seed traits, (1) a measure of population turnover, *i.e.* relative change of populations, R_{cp} (modified after Morrison 1997;Morrison 1998) and (2) a measure of regression/progression, *i.e.* $R_{ext/new}$ the ratio of extinction to colonisation (Crooks *et al.* 2001).

$$(1) \qquad R_{\mathrm{cp}} = \frac{P_{\mathrm{ext}} + P_{\mathrm{new}}}{P_{\mathrm{ext}} + P_{\mathrm{new}} + P_{\mathrm{per}}}$$

$$(2) \qquad R_{\mathrm{ext/new}} = \frac{P_{\mathrm{ext}}}{P_{\mathrm{new}}}$$

R_{cp} varies between one (only new or extinct populations) and zero, $R_{\mathrm{ext/new}}$ is positive. We omitted species without new populations because this gave undefined values of $R_{\mathrm{ext/new}}$.

Species habitat requirement

To evaluate whether population dynamics were driven by some environmental factors that enhance soil seed mortality we used indicator values of a species to detect potential directional changes (Ellenberg *et al.* 1992). Indicator values are a very sensible way to assess changes in habitat quality (Diekmann 2003). Ellenberg *et al.* (1992) provided position of a

species in gradients of light, temperature, continentality of climate, moisture, soil reaction and soil fertility. We complemented species missing in (Ellenberg *et al.* 1992) using the ecological information from three local floras (Molinier 1981;Girerd 1991;Jauzein 1995).

Statistical analysis

The relation between a species' position on the gradient and the number of extinct (P_{ext}) or new (P_{new}) populations was analysed using Spearman's rank correlation coefficient. We run a logistic regression for the effect of population size in 1983 and persistence until 2005 (Crawley 2000;Harrison and Ray 2002). To give weight to the numerous populations studied here, we used generalised linear models (GLM) using a binomial response variable, to investigate ratio data such as relative change of populations, R_{cp} and extinction/colonisation ratio $R_{ext/new}$ (Crawley 2000). For R_{cp}, this led us to use P_{ext} +P_{new} and P_{ext} +P_{new} +P_{per} as binomial denominator. Similarly, we analysed $R_{ext/new}$ with a binomial response variable contrasting extinct populations (P_{ext}) to colonised (P_{new}) populations. The independent variables studied were mortality percentage data and individual seed production. In all cases, we square-root transformed mortality data to remove distortion of percentage data and we used logarithm of seed production to account for left-skewness (Sokal and Rohlf 1995). The use of several data for soil seed mortality leads to a higher false discovery rate, so we corrected p-values for multiple comparisons (Holm 1979). Generally, we reported the results of the tests using the intermediate burial period, t_2t_3 in all analysis using seed mortality. If significant, we also reported the tests for the other burial periods (t_0t_1; t_0t_3 and t_0t_5). All analysis where run in R statistical environment (R Foundation for Statistical Computing 2008).

Phylogenetically explicit analyses

The use of species as independent data points is controversial in ecological literature and often for comparative analyses between species using some phylogenetic correction (Felsenstein 1985;Harvey and Pagel 1991). Closely related species often show similar

characters and habitats as a consequence of common ancestry and therefore differences among species are not always independent from a statistical point of view (Harvey and Pagel 1991). Phylogenetically independent contrasts (PICs; Felsenstein 1985) offer the opportunity to recalculate data in order to retrace how often they appeared independently in the phylogeny, instead of analysing simply species as replicates. We compiled a tree from recent works on phylogeny of the studied species and families, using APGII as a backbone (Angiosperm Phylogeny Group 2003). We used Grafen's (1989) estimation of branch-length method and not age estimations of Wikström *et al.* (2001) because in our data set with many closely related species pairs, this gave no realistic age estimations. We calculated PICs for $R_{ext/new}$ of the two observation time steps, seed production and soil seed mortality. We log10 transformed seed production and square root transformed $R_{ext/new}$ before calculating PICs. We then run linear regression through the origin as recommended by Garland *et al.* (1992). We used the comparative method parallel to all analyses that were not phylogenetically explicit. There are also reasons to analyse comparative data without taking into account phylogenetic correction and to consider the variance correlated to ecological features (Westoby *et al.* 1995b). We chose the usage of both which gives insight in how far the relations are correlated to phylogeny or to ecology.

RESULTS

Effect of initial population size on extinction

A logistic regression analysis showed that populations noted small in 1983 went extinct more easily compared with large ones ($Z_{337} = 3.46$, $p < 0.001$, Fig 4.1).

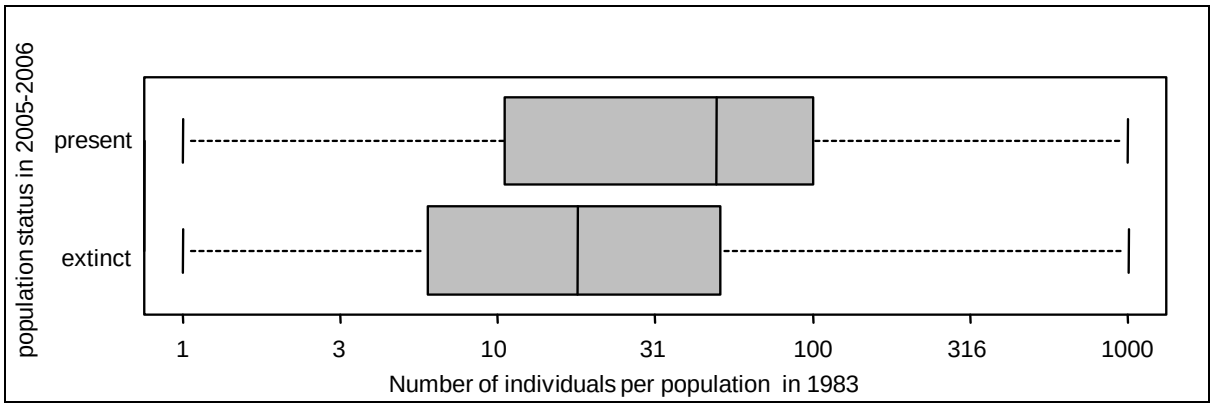


Fig. 4.1. Box and whisker plots showing the relationship between the size of a plant population in 1983 and its probability of survival until 2005/2006, population size is on a logarithmic scale.

Population turnover, extinction and habitat requirements

We analysed the importance of the position of species in gradients of humidity and productivity for R_{cp} and $R_{ext/new}$ as shown in figures 4.2 and 4.3 respectively. The relation between R_{cp} 2005-2006 and species habitat requirements was significant for light but not for temperature, moisture and productivity values (Fig 4.2, light: ρ = -0.49, $p < 0.01$, temperature: ρ = -0.32, p = 0.10, moisture: ρ = 0.19, p = 0.33; fertility: ρ = 0.16, p = 0.38). Only temperature values were significantly correlated with R_{cp} 1983-2005 (ρ = -0.37, $p < 0.05$).

There was no significant relationship for a species' habitat requirements and $R_{ext/new}$, on neither the short nor the long time steps for any of the studied habitat requirements. Figure 4.3 shows the trend of higher moisture value species to have a lower colonisation/extinction ratio for the short time step (ρ = -0.30, p = 0.13). *Papaver rhoeas* and *Papaver hybridum* were two species with high moisture values appearing heavily in 2006 compared to 2005. Whenever we included the 2006 data to calculate $R_{ext/new}$ or R_{cp}, this trend between 2005 and 2006 override the trends between 1983 and 2005. In all subsequent analysis, we only used the 2005 data to calculate R_{cp} and $R_{ext/new}$ for the long time step.

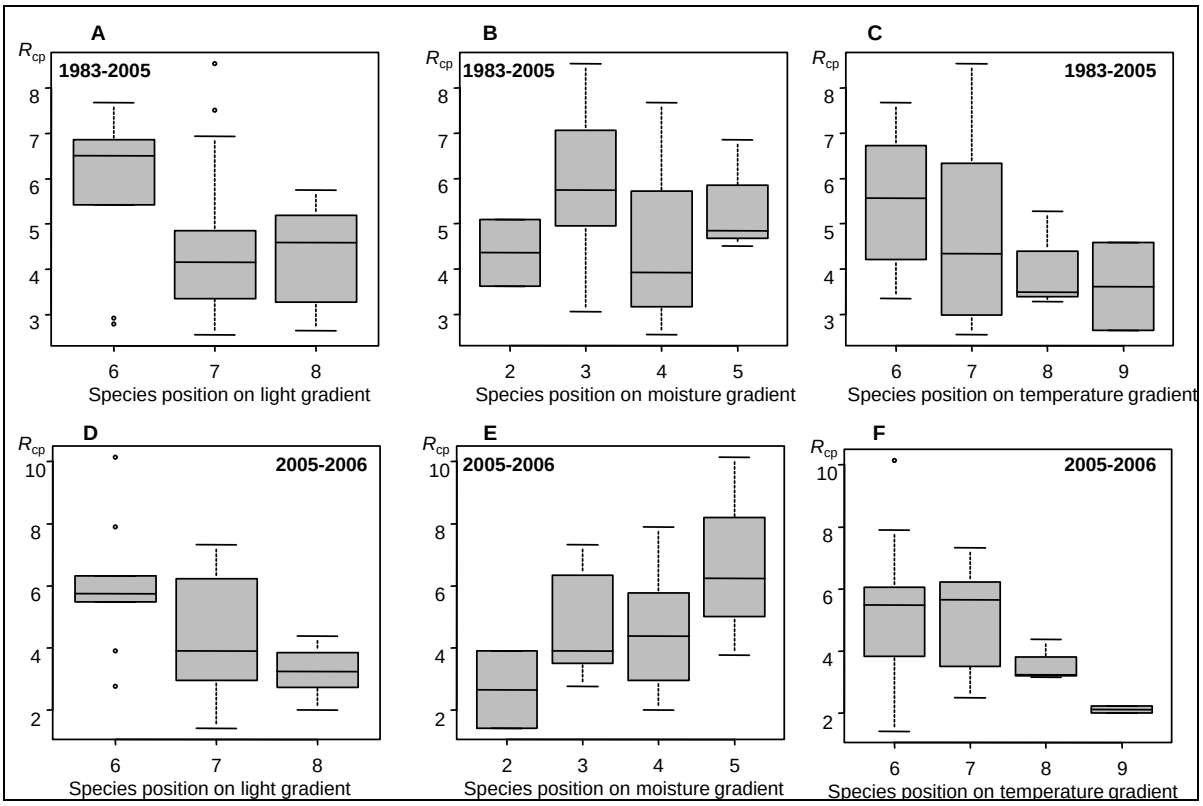


Fig. 4.2. Box and whisker plots of relative change of populations R_{cp} for 2005-2006 and 1983-2006, the thirty species are presented by one box plot per ecological group ordered along gradients (abscissa) of light (6-8), moisture (2-5) and temperature (6-9).

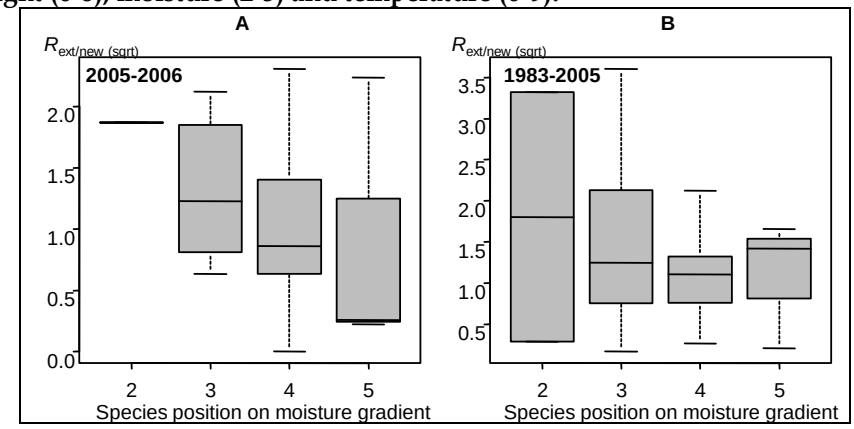


Fig. 4.3. Box and whisker plots of the extinction/colonisation ratio $R_{ext/col}$ for 2005-2006 and 1983-2006 according to a species' moisture requirement.

Effect of seed production, soil seed mortality on Rcp

For the short time step, relative change increased significantly with seed production (GLM, $T_{29} = 2.62$, $p = 0.014$, seed production log-transformed). We found no significant relationship (GLM, $T_{29} = 1.38$, $p = 0.1778$, seed production log-transformed) for the long time step.

Soil seed mortality had no significant effect on R_{cp} for the short time step (GLM, T_{28} = 0.21, p = 0.839, soil seed mortality square root transformed), similarly for the long time step (GLM, T_{28} = 1.43, p = 0.1638, soil seed mortality square root transformed).

Effect of seed production on $R_{ext/new}$

We found a significant negative relation between $R_{ext/new}$ for the short time step and individual seed production (GLM, T_{28} = -3.37, p = 0.0023, fig. 4.4). The regression of this relationship shown in figure 4.3 has an R^2 of 0.13 ($F_{1,25}$ =4.622, p = 0.0629, $R_{ext/col}$ square root transformed, seed production log-transformed, zero values omitted).

We found no significant relation between $R_{ext/new}$ and seed production for the longer time step 1983 to 2005, neither in GLM (T_{29} = 1.135, p = 0.266, seed production log-transformed) nor in linear regression (R^2 = 0.0954, $F_{1,27}$ = 2.85, p = 0.1031, $R_{ext/col}$ square-root transformed, seed production log-transformed, fig. 4.4).

Effect of soil seed mortality on $R_{ext/new}$

The relation between soil seed mortality and $R_{ext/new}$ for the long time step 1983 to 2005 was significant in the binomial GLM (1983 to 2005: T_{28} = 2.133, p = 0.0421, seed mortality square root transformed). This finding was confirmed by linear regression (R^2 = 0.23, $F_{1,26}$ =8.38, p = 0.0096, $R_{ext/col}$ and seed production square root transformed, fig. 4.4). In this analysis, we tested four different periods in the burial experiment: t_0t_1, t_0t_3, t_0t_5 and t_2t_3, all showing a similar trend (Tab. 4.2).

Tab. 4.2. Effect of soil seed mortality at different time steps in the burial experiment on long term (1983-2005) extinction/colonisation ratio $R_{ext/new}$

Burial period	coefficient	R^2	$F_{1,26}$	p	p-value after Holm's correction
t_0t_1	0.84	0.10	3.07	0.091	0.182
t_0t_3	0.99	0.14	4.29	0.048	0.144
t_0t_5	0.84	0.10	2.89	0.1	0.182
t_2t_3	4.53	0.23	7.81	0.009	0.038

We found no significant relation between $R_{ext/new}$ and soil seed mortality for the short time step neither in GLM (T_{29} =1.021, p =0.3160, seed mortality square root transformed) nor in

linear regression (R^2 < 0.000, $F_{1,26}$ = 0.0003, p = 0.9865, $R_{ext/col}$ and seed mortality square root transformed). The analysis of the other time steps in the burial experiment revealed similar relations (data not shown).

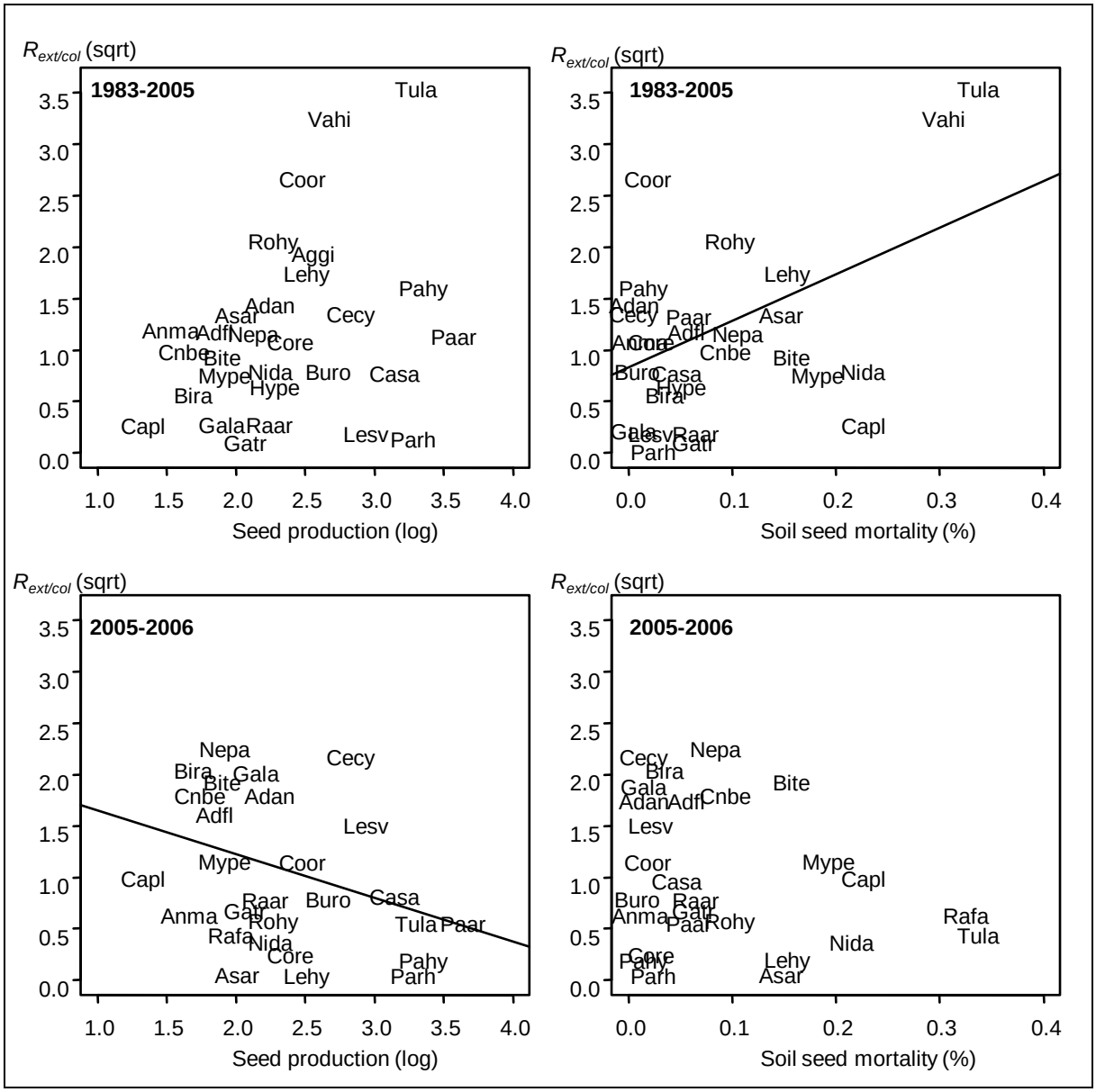


Fig. 4.4. Effect of seed production and soil seed mortality on extinction/colonisation ratio, lines indicate relations significant in binomial regression (p < 0.05). Whenever we moved species codes for better legibility we put them in italics, codes for species are in table 4.1.

PICs and the effect of soil seed mortality and seed production on $R_{ext/new}$

We redid all regressions reported above using their corresponding phylogenetically independent contrasts. Soil seed mortality contrasts showed a significant relation to extinction/colonisation ratio $R_{ext/col}$ 1983-2006 (R^2 = 0.164, $F_{1,26}$ =5.106, p = 0.0324). This is not

the case for the short time step $R_{ext/col}$ 2005-2006 (R^2 = 0.02, $F_{1,25}$ = 0.59, p =0.448). The high contrasts for soil seed mortality appeared inside the family and generic levels in the phylogeny with the exception of the contrast (N° 53, Fig 4.5) between Asteraceae and Campanulaceae.

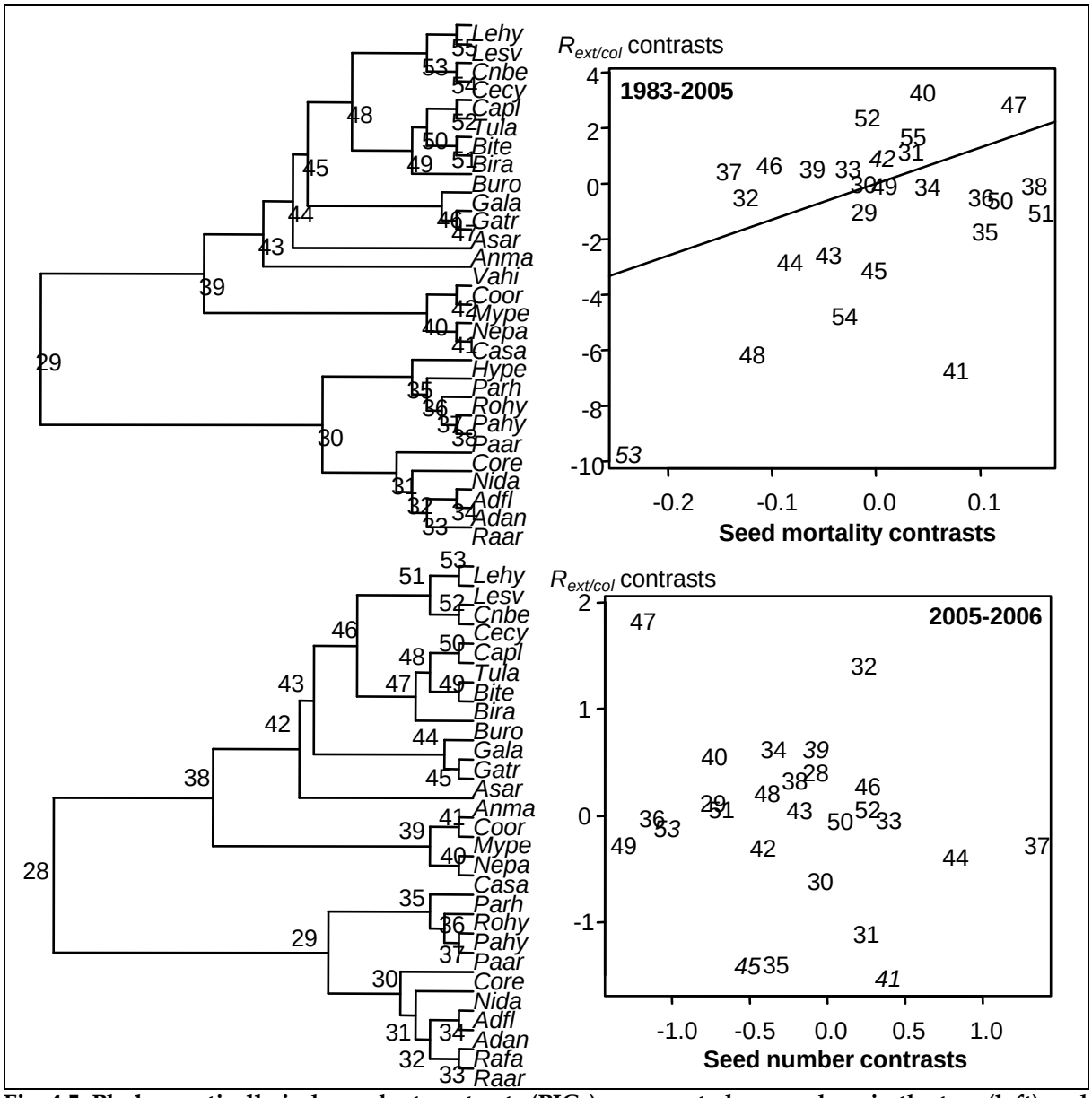


Fig. 4.5. Phylogenetically independent contrasts (PICs) represented as numbers in the tree (left) and in the relation of seed production and of soil seed mortality on extinction/colonisation ratio ($R_{ext/col}$, right); note that we present here only the regressions that were significant in figure 4.4 and therefore different time steps; the line indicates a significant relation (p < 0.05), species codes in table 4.1. Whenever we moved numbers for better legibility, we put them in italics (right).

The analysis of seed number contrasts and $R_{ext/col}$ 2005-2006 showed no significant relation (R^2 = 0.06, $F_{1,25}$ =1.597, p = 0.218) although the analysis without PICs does. Seed number and

contrast had no effect on the $R_{ext/col}$ 1983-2006 long-term extinction/colonisation rate contrasts (R^2 = 0.08, $F_{1,27}$ =2.45, p = 0.129). Interestingly, the most important extinction/colonisation ratio contrasts for the long time step, 1983-2006, appear at relatively deep nodes in the phylogeny. The corresponding ratio contrasts for the short time step, 2005-2006 come from more derived nodes.

DISCUSSION

Population size related extinction and habitat requirements

Our results show that we met the conditions necessary for testing the relationships between population fluctuations and soil seed mortality and seed production. This includes notably that smaller populations going extinct more easily than larger ones. This is significant supplementary evidence for the higher extinction risk of small populations, for which there is still little empirical evidence (but see e.g. Fischer and Stöcklin 1997;Matthies *et al*. 2004). Extinction of the smaller fraction of populations implies that there are processes compromising reproduction. Reproduction of wild and cultivated plants is impeded by fragmentation of habitats, lack of pollinators or seed dispersal (Poschlod and Biewer 2005;Biesmeijer *et al*. 2006;Cousins and Eriksson 2008). These circumstances lead to a decline on the long run of the cereal weeds studied here and this is in congruence with other recent findings on cereal weeds in other regions of France (Aboucaya 2000, Fried 2009) and elsewhere in Europe (Schneider *et al.,* 1994, Andreasen *et al.,* 1996, Sutcliffe and Kay 2000, Robinson and Sutherland 2002, Pyšek *et al.,* 2005, Baessler and Klotz 2006, Pinke *et al.* 2008). Moreover, the regression of these species is a phenomenon observed since the industrialisation of agriculture after World War II (Aymonin 1962, Schneider *et al.,* 1994, Robinson and Sutherland 2002). This shows that our study system is a case of regression of formerly more common species and that the observed processes are general processes for declining populations.

A second aspect consists in a lack of a clear long term trend in change of the abiotic environment as highlighted by the analysis including indicator values (Ellenberg 1996). There are year-to-year fluctuations in indicator values for moisture and, most probably triggered by this, nutrients (Ellenberg 1996). Additionally, an analysis within ecological groups (indicator values) showed that the main outcome is robust to environmental changes (analysis not presented). They are likely to be connected to climatic fluctuations rather than to changes in agricultural practices, because the studied fields extend over 2500 km² and cover a very large range of different types of farms; a synchronous directional change from one year to another in their agricultural practices can therefore be excluded.

Soil seed mortality and population turnover

Species with higher soil seed mortality in the burial experiment suffer a more rapid extinction of populations from 1983 to 2005. Lower soil seed bank persistence has been supposed on several occasions to be related to population extinction threat (Fischer and Stöcklin 1997;Stöcklin and Fischer 1999;Menges 2000;Poschlod *et al*. 2000). Studies comparing several species respective to their soil seed mortality and population fluctuations are scarce. Stöcklin & Fischer (1999) for example found lower local extinction rates of species with high seed persistence using fragmented grassland remnants. This work included perennial species and was conducted in calcareous grasslands. Additionally, the classification of seed persistence in their work was based on seed presence in soil samples, giving implicitly some weight to seed production beside soil seed mortality as a factor potentially contributing to classify a species' seed bank as persistent (Saatkamp *et al.*, submitted).

The higher extinction rate of species with high soil seed mortality is also in congruence with the idea that longer soil seed longevity enhances species coexistence, the 'storage effect' (Chesson & Warner 1981). In our case, this applies to the coexistence of a species-rich weed community with cereals, a highly competitive part of the environment. Cereals also fulfil another condition of the storage effect hypothesis that is different reaction of coexisting

species to temporal changes (disturbances). When cereals are sown they germinate instantly, and cereal seeds have very high soil seed mortality. There are different reactions of cereal weeds, which can have low (or high) soil seed mortality, delayed (or immediate) germination and which react to a wide range of different germination cues, some of them related to recent changes (*e.g.* Baskin and Baskin 1998, see also Otte 1994).

Seed production and population turnover: the importance of trade-offs

Another important result in this study is clarified that it is seed mortality and not seed production that importantly influences population turnover in the long run. This is astonishing at the first sight, because the size of the storage compartment, *i.e.* the number of produced seeds, should also count for the effectiveness of the storage effect (Chesson and Warner 1981, Facelli *et al.,* 2005). To understand this, let us turn back to the reflections of Moles *et al.* (2004) on the seed size-seed number trade-off: Moles *et al.* (2004) showed, why many small seeds are not more advantageous as few large ones in a comparative study including many species. They re-emphasized that large seeds compensate for the smaller number in generating a higher survival of seedlings (Leishman *et al.* 2000, Jakobsson & Eriksson 2000), and suggest that there is additional compensation in life stage other than seedlings. In the light of this, it becomes a both plausible and parsimonious explanation to say that seed number *per se* is not so important for the size and effectiveness of the storage compartment of the soil seed bank. Here, it is proportional soil seed mortality that – irrespective of the number or size – will affect a proportion of the storage compartment. We have to re-emphasise that soil seed mortality should be measured on a quantified seed population in this context and not with methods that could give estimates correlated to seed production (Saatkamp *et al.*, submitted).

Phylogenetically independent contrasts

The analysis of PICs supports the previous views of a relation between population dynamics and soil seed mortality on the long run but not for seed production at the short term. The

analysis of PICs added to this that the studied soil seed mortality is not a phylogenetically conserved trait. Perhaps the rapid modification of this trait is necessary to react to episodic changes in disturbance frequencies which otherwise would be fatal. Seed production is a conserved trait to a degree that it makes it impossible to show with our data that it triggers extinction/colonisation ratio in the short term independently from phylogeny. This is nothing new in front of other findings that report seed size as phylogenetically conserved and the trade-off between both (Shipley and Dion 1992). More interestingly, the extinction/colonisation ratio for the short time step was less phylogenetically conserved than the ratio for the long time step. This points to different processes and different traits (themselves more or less conservative) implicated in the population turnover over the short and the long term.

CONCLUSION

Our findings imply that there are differential extinction risks among species according to their soil seed mortality and that soil seed mortality as studied here is not just a workaround to describe incomplete population surveys. Finally, Venable and Brown (1993, p 47.) already showed using models that 'perenniality per se does necessarily change the E[volutionnary] S[table] S[trategy] dispersibility for escaping crowding or sib interactions' (our brackets). Soil seed mortality has therefore a general effect for local persistence of all seed plants. Consequently they should be more generally be used for extinction risk evaluation. The above findings underline and precise the importance of soil seed mortality as a parameter for extinction risk assessment on a species level (Poschlod *et al.* 2000, Menges 2000). In population viability analyses, there are still many difficulties bound to the functional roles of life stage of the seed banks (Poschlod *et al.* 2000, Menges 2000). For these reasons, soil seed mortality should be considered more in detail also in population viability analyses for plants (Menges 1990;Schmid and Matthies 1994;Menges 2000;Harrison and Ray 2002;Brigham and

Schwartz 2003). Finally, we suggest giving more attention to soil seed mortality measured independently from the seed size-number trade-off, to understand the underlying mechanisms that enhance or reduce diversity not only in annual plant communities.

TRANSITION CHAPTER 4 TO 5

From population dynamics to rarity and abundance

In chapter 4, we evaluated the relationships among a set of traits of two aspects of population dynamics at two temporal scales. In this approach, we detected an effect of soil seed mortality on extinction rate over the long term and seed production over the short term, with only the first being also related when using PICs. This shows that species differing in their traits also differ in rapidity of population dynamics according to the value by this trait. In our case, species with high soil seed mortality also have high extinction rates.

Evidently, high extinction rates have also consequences for regional frequency of populations: species with high extinction rates can be less frequent because many populations went extinct before the observation date. Rarity at a regional population frequency scale can thus be related to factors that historically decreased the frequency of a species. However, geographical distribution is just the first of three main axes of rarity defined by Rabinowitz (1981); local abundance is the second and niche width is the third axis. Beside historical factors acting on populations, there can be actual environmental parameters that limit species, therefore niche width of a species, the third axis of rarity, can rather be a reason than a dimension of rarity (Gaston 1997). The second axis, local abundance is also constrained by both environmental limiting factors and traits. The relation of traits with abundance, such as body size, is very well documented for animals (White *et al.* 2007). Much less is known about plant traits and plant abundance, seed size has been evoked as a putative trait negatively correlated to local abundance, but there is still little evidence on such a relationship and recent attempts rather refute a general relationship (Murray *et al.* 2002). However, there have been propositions that mechanistic models involving traits can predict abundance at the local scale (Shipley *et al.* 2006). A functional trait approach can give additional insight what triggers plant rarity at local scales. However, there are still many

gaps in the understanding of how traits contribute to rarity and local abundance (Murray *et al*. 2005).

Additionally, many works focus on very rare and endemic species, so here we focus on the rare-common contrast but not strictly endemic species, which permits us to have a second focus on annuals that are little represented among endemic species and hence in previous work on rarity in plants.

In the chapter 5, we study hence differences between regionally rare and common species and between locally abundant and scarce species. This corresponds to two axes of rarity. In this study, we ask if life-history traits such as soil seed survival, dormancy and germination characteristics are related to these differences. Further on, we also study the pollen:ovule ratio, a measure that is related to the rate of gene exchange *via* breeding system (Cruden 1977;Charlesworth and Charlesworth 1987;Reed *et al*. 2002), and putatively can increase survival in small populations by increasing connectiveness and effective population size (Laporte and Charlesworth 2002;Charlesworth 2009).

CHAPTER 5

Comparison of traits between rare and common cereal weeds and implications for conservation

INTRODUCTION

The contrast of rare to common plants is not only one of the main motivations for nature conservation but also an important source for giving sound advice in this area. Since the key-stone work of Rabinowitz (1981) it is therefore a research focus in comparative plant ecology (Hegde and Ellstrand 1999;Lavergne 2003;Farnsworth and Ogurcak 2008;Römermann *et al*. 2008). Rarity is a complex term covering at least three independent aspects: narrow niche width, small distribution range and low local abundance (Stebbins 1942;Rabinowitz 1981;Rabinowitz *et al*. 1986;Hubbell and Foster 1986;Bawa and Ashton 1991). All of range size, niche width and local abundance potentially contribute to higher extinction threats in plants and local extinction risk (Harrison and Ray 2002;Reed *et al*. 2002;Matthies *et al*. 2004). However, even if effects and importance are general, possible ecological correlates of these three aspects of 'rarity' are not.

Distribution range, for instance, is obviously a result of very diverse historical and actual ecological factors and in this light, it seems meaningless to search for a general causal pattern for its explanation (Fiedler 1986;Hegde and Ellstrand 1999;Gitzendanner and Soltis 2000). Indeed the reasons are potentially as numerous as plant's adaptations and histories, even if in restricted regions with a common set of physical and historical factors the biological solutions in many rare versus common taxa can be of insightful parallelism (Hubbell and Foster 1986;Bawa and Ashton 1991;Médail and Verlaque 1997;Lavergne 2003). Distribution range in itself covers total range size (Gaston 1991), fragmentation (Morgan 1998) and regional frequency of populations (Hodgson 1986;Eriksson *et al*. 1995;McCollin *et al*. 2000).

Range size has been correlated to genetic diversity at the population level among species (Hamrick and Godt 1997) implying that larger distribution range with a higher number of different populations may be reflected in higher local genetic diversity.

Niche width has been supposed to be rather a cause than a type of rarity (Gaston 1997). This is intuitive given the many 'rare' plants on specialised habitats such as coasts or scarce geological units. This is even more true for the regression from commonness to extreme rarity of specialised species, *e.g.* rare weeds from flax (*Linum usitatissimum*) fields (Schneider *et al.* 1994) or inversely the actual ubiquity of formerly exceptional subtropical weeds in corn or rice fields in northern temperate floras (Jauzein 1995). In our eyes, studying ecological factors that define a plant's niche is a powerful way to understand causes of distribution and abundance. Therefore, comparing rare and common, abundant or scarce species in a narrowly defined environment is a good opportunity to study plant's adaptations and relevant factors in this habitat. This discussion already shows that not all three aspects of rarity have necessarily a relation to a plant's biological traits and general relations between rarity and species' traits are therefore questioned (Fiedler 1986;Gitzendanner and Soltis 2000). Moreover, it is possible that reasons for rarity are more complex to elucidate than consequences, since they involve historical factors of the environment. Biological consequences of rarity or low local abundance are probably more easy to identify, allowing a plant to survive in small ranges and population sizes (Gaston and Kunin 1997). This has already been confirmed by Lavergne (2003). There is however strong evidence of a general relationship between rarity in form of local abundance and genetic diversity at the population level (Leimu *et al.* 2006). There are also relations of local abundance to body size according to allometric relations (White *et al.* 2007).

For both range size and local abundance, things are complicated by historical factors, leaving us with actual patterns because of past processes and mechanisms how plants escaped complete extinction. However, being aware of this dimension enables us to better

understand how plant regression and extinction works and to explain more consistently actual patterns. Rarity in form of narrow distribution range is one necessary passage in naissance of many plant species except in some cases of allopatric speciation, where two common species can generate from one common. It therefore gives insight how plant diversity is limited or can spread. The patterns observed, especially when not consistent at different times should also be interpreted in the light of environmental changes to explain them.

Most works on rarity in plants take a global view on all plants of one or several floras (Rabinowitz *et al*. 1986;Hodgson 1986;Hegde and Ellstrand 1999) or focus on endemic and widespread relatives in a given region (Menges 1991;Lavergne 2003;Farnsworth and Ogurcak 2008). Only a very few works include annual species (Lavergne 2003) and –to our knowledge- no work focussed explicitly on annual species in the comparison of rare to common plants. From a global point of view, this can be deplored for two reasons: (i) the rapid turnover at both population and range size levels make annuals a more severe test for hypotheses on rarity and (ii) annuals are model systems where functional relationships of population dynamics are more easily modelled and understood than for perennials. Moreover, annuals are underrepresented among narrow ranged (endemic) species (Médail and Verlaque 1997), probably because a short life cycle prevents relict endemism and hampers range restriction. In order to test if relations of plant traits to rarity and abundance hold also for annual plants, we wanted to test relevant quantitative traits for 37 annual plants including 14 species pairs of rare and common species.

We therefore wanted to know, if: (i) the pollen:ovule ratio is related to local population size and regional frequency, (ii) seed size has a relation to regional frequency and local population size, (iii) degree of dormancy and soil seed mortality have a relation to the local population size and finally if (iv) plant size, specific leaf area (SLA) and leaf dry matter content (LDMC) can related to local population size or regional frequency.

MATERIALS AND METHODS

Study site and population data

We gathered data on 37 rare and common cereal weeds species in an area of ca. 2500 km² around the Luberon ridge in South Eastern France. This area is characterised by Mediterranean climate (mean rainfall$_{1971\text{-}2000}$: 623 mm, maxima in April and October). Traditional agriculture in this area maintained a high diversity of rare cereal weeds elsewhere extinct in Europe (Filosa 1989;Filosa 1997). We used a survey from 1983 (Filosa 1985;Filosa 1989) for which we re-evaluated population size for the same species, giving us population sizes on two different dates separated by 22 years. We revisited all populations in 2005 and 2006, so we had also data on a short time step. The initial data set included mostly rare species, in our later survey, we complemented it with closely related common species; see appendix 1 for a detailed list of species. In our survey, we counted population size when there were less than 50 individuals, above, we visually estimated total population size using density of population and total area covered by the population. This results in three data sets of population sizes in 1983, 2005 and 2006 with 24 species in the old survey and 37 species in the recent survey. We used log-transformed data on the mean population size in 1983 and 2006 for these species and classified species into rare and common according to regional frequency of population, *i.e.* the number of populations documented in our survey.

Traits

Specific leaf area (SLA) and leaf dry matter content (LDMC)

Leaf material was collected in the field from living individuals at the start of flowering (April to June) using only green, intact and undamaged leaves. For each species, five individual plants (A, B, C, D, and E) were chosen at random. For each individual, two leaves were collected, one more basal and one more apical leaf. Position of leaves was noted on a small

piece of paper placed together with each individual leaf (*e.g.* A1 – basal leaf, A2 – cauline leaf of individual A). We did not remove petioles. Leaves were transported in sealed plastic bags with a small amount of additional water to prevent desiccation. Leaves were stored in sealed plastic bags in a fridge at 4°C, and processed within 24h after collection. Prior to measurements, soil remnants were removed, wet leaves were rubbed dry with cotton tissue and we re-hydrated leaves using deionised water according to the recommendations in Garnier *et al.* (2001). This ensured that we measured leaf fresh mass and leaf area only on living and fully turgid leaves. Leaves were then placed individually in tagged paper bags and dried at 50°C under circulating air until weight constancy. Weight was measured using a fine balance (± 0.001 mg). We used scanned images along with reference surfaces of known size to detect leaf area using the lafore software package (Lehsten 2005). Leaf size was expressed in mm^2. Specific leaf area was calculated for each single leaf as the ratio of fresh leave area on leaf dry mass and expressed in $mm^2 \cdot mg^{-1}$. Leaf dry matter content was calculated as the dry leaf mass on fresh leaf mass and noted in $mg \cdot g^{-1}$.

Pollen Ovule ratio

In spring and summer 2006, we collected three stamens per flower for each of five randomly chosen individuals per species. We only used closed but near to flowering buds. We stored the three stamens for each bud dry together in Eppendorf tubes. At sampling, we also kept the remaining flower and stored it in 70 % alcohol plastic tubes for counting total number of stamens and ovules. Once all species sampled, we dissolved stamen tissue using 550 µl of sulphuric acid per tube for 24-48h. After that, we crushed remaining tissues with a small glass pestle, added 1650 µl of water with 2% triton tenside, and mixed it with the pestle. We then centrifuged for 5 minutes, decanted the superfluous liquid and added 1000 µl of alcohol (95 %), and mixed again. We centrifuged again for 5 min, removed liquid, and evaporated to obtain dry pollen samples. We then added 40 µl of a 30 % sucrose/20 % glycerol solution

('counting solution') and sonicated. We counted pollen grains under microscope using a 1 µl hematocytometer and ovules under a binocular lens. The number of pollen grains for one stamen was then calculated as follows: number of pollen grains in 1 µl x 40 µl of counting solution, divided by three. We then calculated pollen:ovule ratio (P/O) as the number of pollen grains for one stamen x number of stamens per flower, divided by the total number of ovules. When needed, P/O ratios were log10-transformed to meet the normality assumption.

Seed mass, seed number and plant size

We measured seed weight for three samples of 10 seeds for each species; seeds were collected from at least ten individual plants. Seed production was determined for all 37 species, *i.e.* mean individual seed production of 10 individuals in the field. Some species had multi-seeded fruits (*e.g. Papaver sp. pl.*), other had many fruits per infructescence (*e. g.* Apiaceae), we counted the number of fruits or infructescences per individual for these species. Than we sampled two fruits or infructescences per individual and counted number of seeds per fruit or infructescence. Seed production per individual was then calculated as mean number of seeds per fruit or infructescence multiplied by the number of fruits or infructescences counted per individual. Plant size was measured as height in mm in the field for 25 random individuals from one population per species at time of fruit set.

Degree of dormancy and soil seed survival

We conducted a burial experiment and subsequent germination and tetrazolium test for the 38 species. This experiment is described in detail in Saatkamp *et al.* (2009). This experiment was a randomised block design with species grouped into time steps and the latter into blocks. At six months intervals for 2 ½ years we retrieved seed samples for each species from burial and exposed them to standardised germination conditions; ungerminated seeds at the end of these germination tests were tested for viability using tetrazolium (International Seed Testing Association 1996). For each species, we determined soil seed survival as the

proportion of living seeds after 1 ½ years of burial out of the number of living seeds in the seed lot before burial, we chose 1 ½ years because differences among species were most marked at this date. Degree of dormancy was determined as the proportion of ungerminated but living seeds (*i.e.* number of seeds tested using tetrazolium) out of the number of all living seeds retrieved at a date; we averaged this value for the five retrieval dates together with the initial test; this gave quantitative values of the proportion of germinating seeds, which we preferred over a qualitative classification into dormancy types.

Data analysis

Phylogenetically independent contrasts

We took phylogeny into account in our analysis using phylogenetically independent contrasts (PICs) in the sense of Felsenstein (1985). These are differences in character (phenotypic) values between phylogenetic sister groups. They are calculated starting from species values down to deeper nodes of the phylogenetic tree. PICs were computed using the algorithm pic() in the ape software-package (Paradis *et al.* 2006) for R (R Foundation for Statistical Computing 2008). In the case of seed number, population size and pollen/ovule ratio that were left-skewed, data were log-transformed prior to PIC calculation. We calculated internal node averages and divergences incorporating branch lengths according to Felsenstein (1985). These difference data have an arbitrary sign and an inherent mean of zero, therefore regression analysis of independent contrasts was forced through the origin (Garland *et al.* 1992).

Construction of phylogeny

We constructed a hypothesis on phylogenetic relationships including all species using published trees from the literature. A 'supertree' hypothesis was constructed using APGII (Angiosperm Phylogeny Group 2003) and Phylomatic (Webb and Donoghue 2005) with branch length estimates taken from Wikström *et al.* (2001) using the bladj-algorithm (Webb *et*

al. 2006). Polytomies among basal angiosperms in this tree where completely resolved using APGII (Angiosperm Phylogeny Group 2003), Soltis *et al.* (2000) and Jansen *et al.* (2006a;2006b). For derived taxa, we resolved polytomies using recent molecular phylogenies from recent works (see below). In the case of species pairs belonging to the same genus, a sister group relationship was inferred. Genus names and phylogenetic relationships are somewhat contradictory where genera turned out to be paraphyletic in recent phylogenetic works as in the case of *Centaurea* (*Cnicus benedictus* inside *Centaurea*) and *Papaver* (*Roemeria hybrida* forms a clade with *Papaver argemone* and *P. hybridum; P. rhoeas* is sister to this clade). We detailed the phylogenetic relationships according to recent molecular works for Apiaceae (Downie *et al*. 2000a;Downie *et al*. 2000b), Asteraceae (Garcia-Jacas *et al*. 2000;Susanna *et al*. 2006), Caryophyllaceae (Fior *et al.* 2006), Brassicaceae (Al Shebhaz *et al*. 2006;Beilstein *et al*. 2006;Warwick *et al*. 2006), Papaveraceae (Hoot *et al*. 1997;Soltis *et al*. 2005;Carolan *et al*. 2006) and Ranunculaceae (Jensen *et al*. 1995;Paun *et al*. 2005). We compared three different ways to obtain branch lengths for this phylogeny: all branch length set to one; second, branch length according to Wikström *et al.* (2001) adjusted using the phylocom/bladj algorithm (Webb *et al.* 2006) and third group-size estimated branch lengths using the algorithm proposed by Grafen (1989). We chose the method according to Grafen (1989), because it was the only that showed relatively short and meaningful branch length for the many species pairs relative to deeper branches in the phylogeny. Missing data complicate analyses of PICs, we removed missing taxa using the drop.tip()-algorithm in the ape software package (Paradis *et al.* 2006).

Statistical analysis

We chose species in this comparative work based on their preference for the target community of winter cereal fields (Secalinion, Braun-Blanquet 1939) and the definition of Jauzein (1997) and Guende & Olivier (1997). We included whenever possible two close sister species within a genus or a family and this across the whole system of recent angiosperms.

The choice of species used in the two cases when the species pairs are not the closest possible relative in our data-set was made to maximize differences in regional frequency of populations. The data were analysed using linear regression of population sizes at two dates on continuous trait values using species as replicates. In parallel, we did linear regression through the origin for the same parameters using phylogenetically independent contrasts (see above). We also compared the species pairs of rare and common species according to the regional frequency of populations using a paired Wilcoxon-test. All analyses where run in R statistical environment (R Development Core Team 2008).

RESULTS

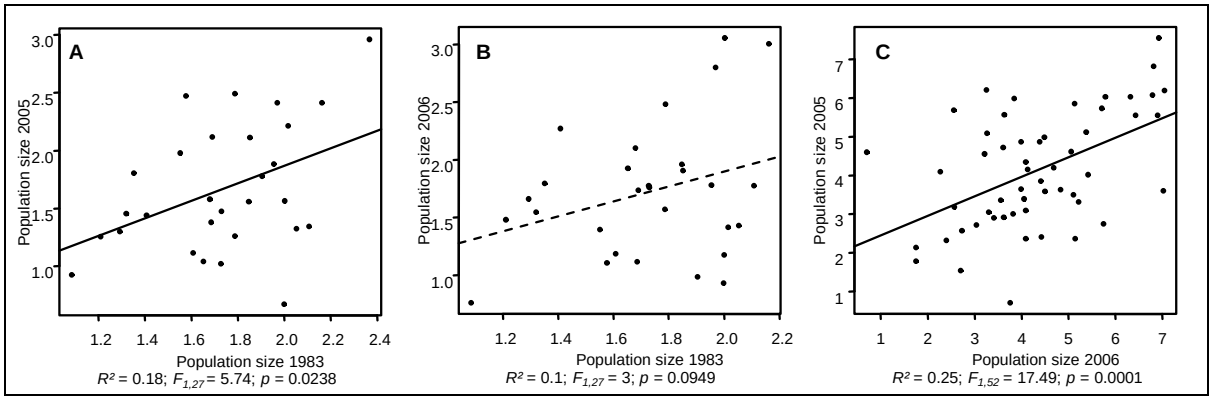


Fig. 5.1. Relation between population sizes at different dates, a regression line is drawn when coefficient was significant (straight line $p < 0.05$; broken line $p < 0.1$).

First, we found a clear relation of mean population sizes among different dates, with tight relations among subsequent years and less close relations for the 22 year time step (Fig. 5.1). The regression shows a positive overall linear relationship between population sizes in 1983 and population sizes in 2005 and 2006. However, some points below the regression line indicate clearly smaller population sizes in 2005/2006 than in 1983.

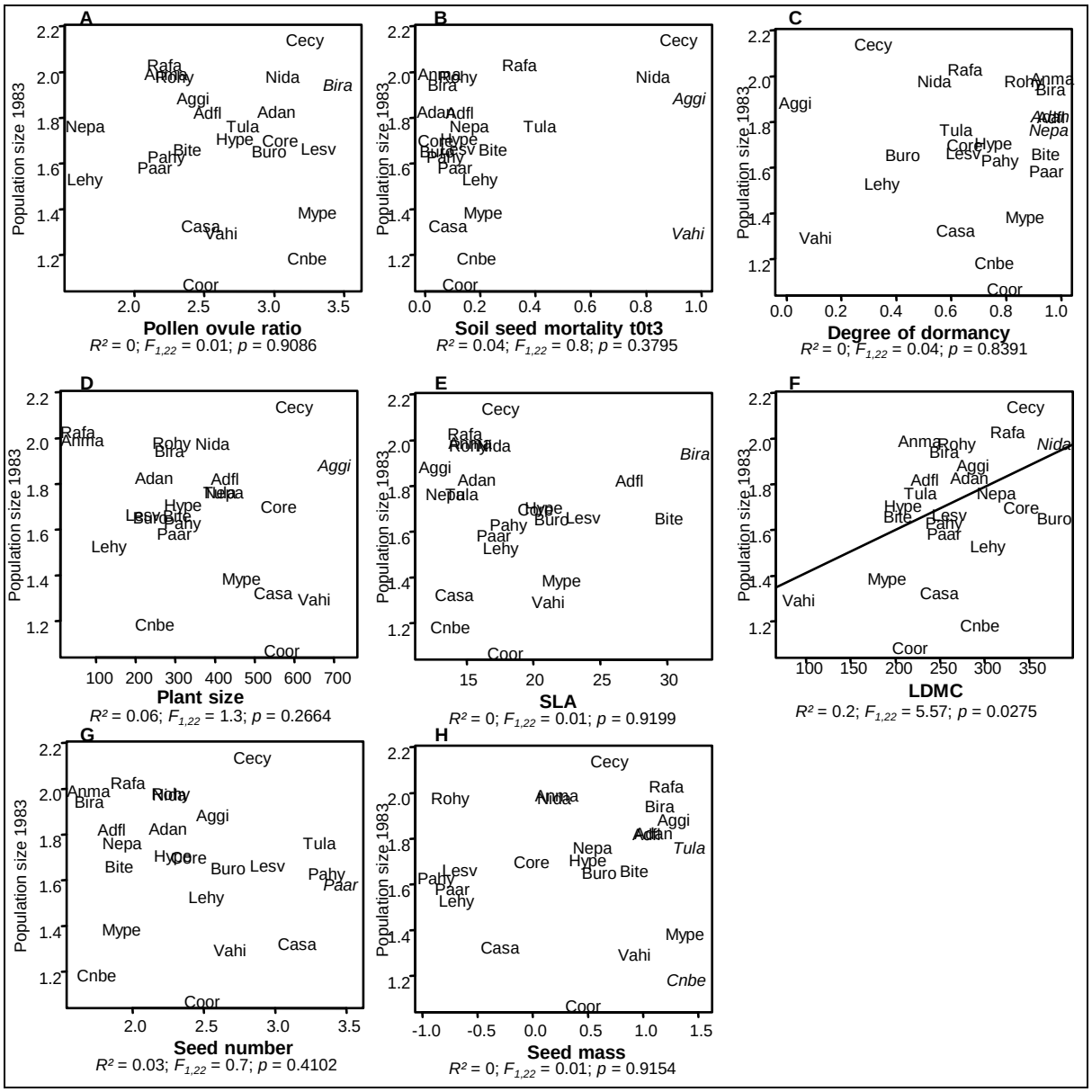


Fig. 5.2. Correlates for population size in 1983 for 24 species, the regression line indicates a significant ($p < 0.05$) relationship. Species codes are in table 4.1.

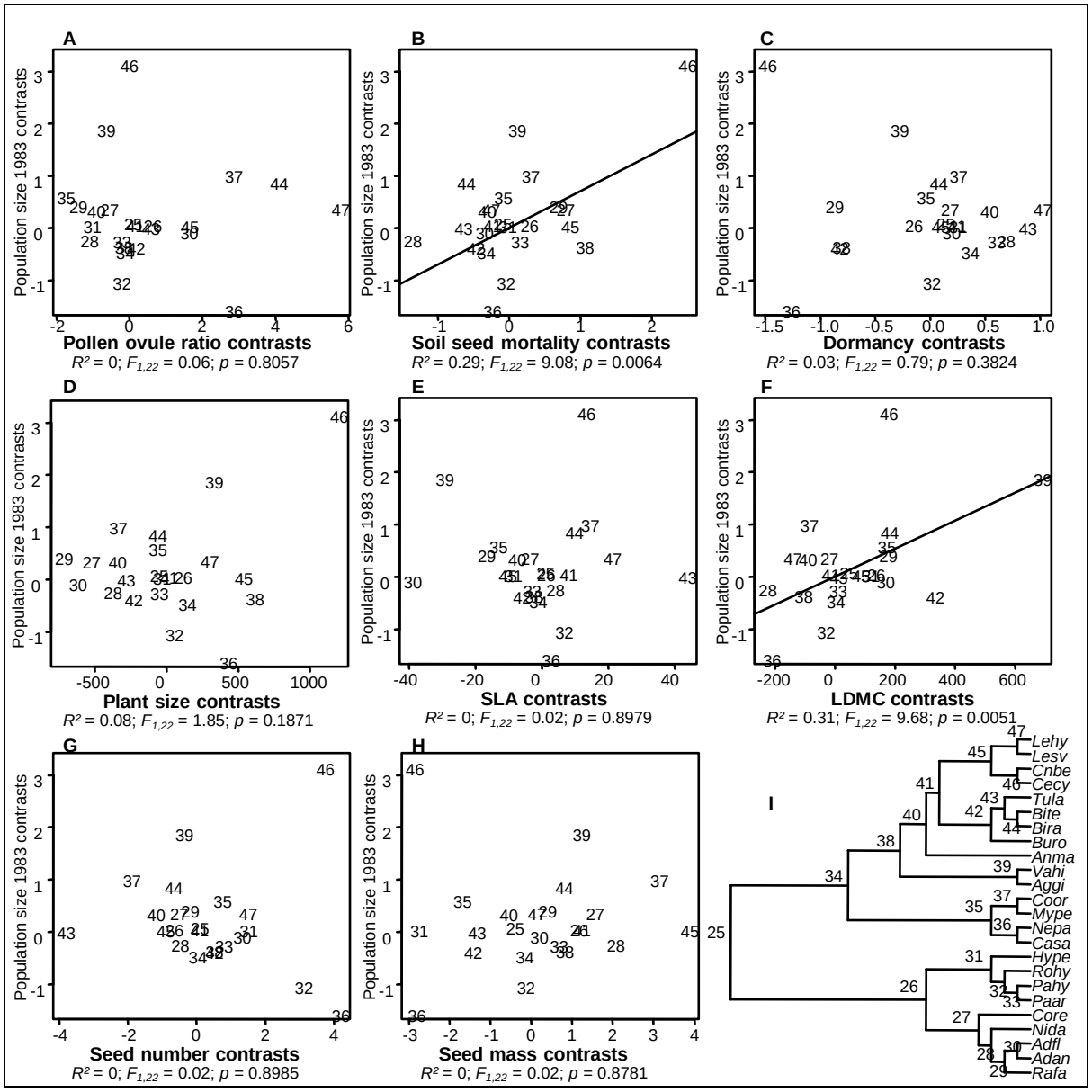


Fig. 5.3. Population size contrasts (in 1983) and trait contrasts (numbers in plots and tree). The regression lines indicate significant ($p < 0.05$) relationships. (I): phylogeny used for calculation of PICs. Species codes are in table 4.1.

Second, we found a number of significant relations between species traits, trait PICs and population size in 1983 as well as in 2006 (Figs. 5.2, 5.3, 5.4 & 5.5). However, it was not the same traits that showed a significant relation to population size at the different dates. In 1983, using no phylogenetic correction, leaf dry matter content (LDMC) was positively correlated to population size (fig. 5.2). When we considered phylogeny, again LDMC contrasts were related to population sizes (fig. 5.3). Additionally, soil seed mortality

contrasts were positively related to population size contrasts, in other words, when comparing related species or taxa, the one with high soil seed mortality has often larger population sizes in 1983 (fig. 5.3).

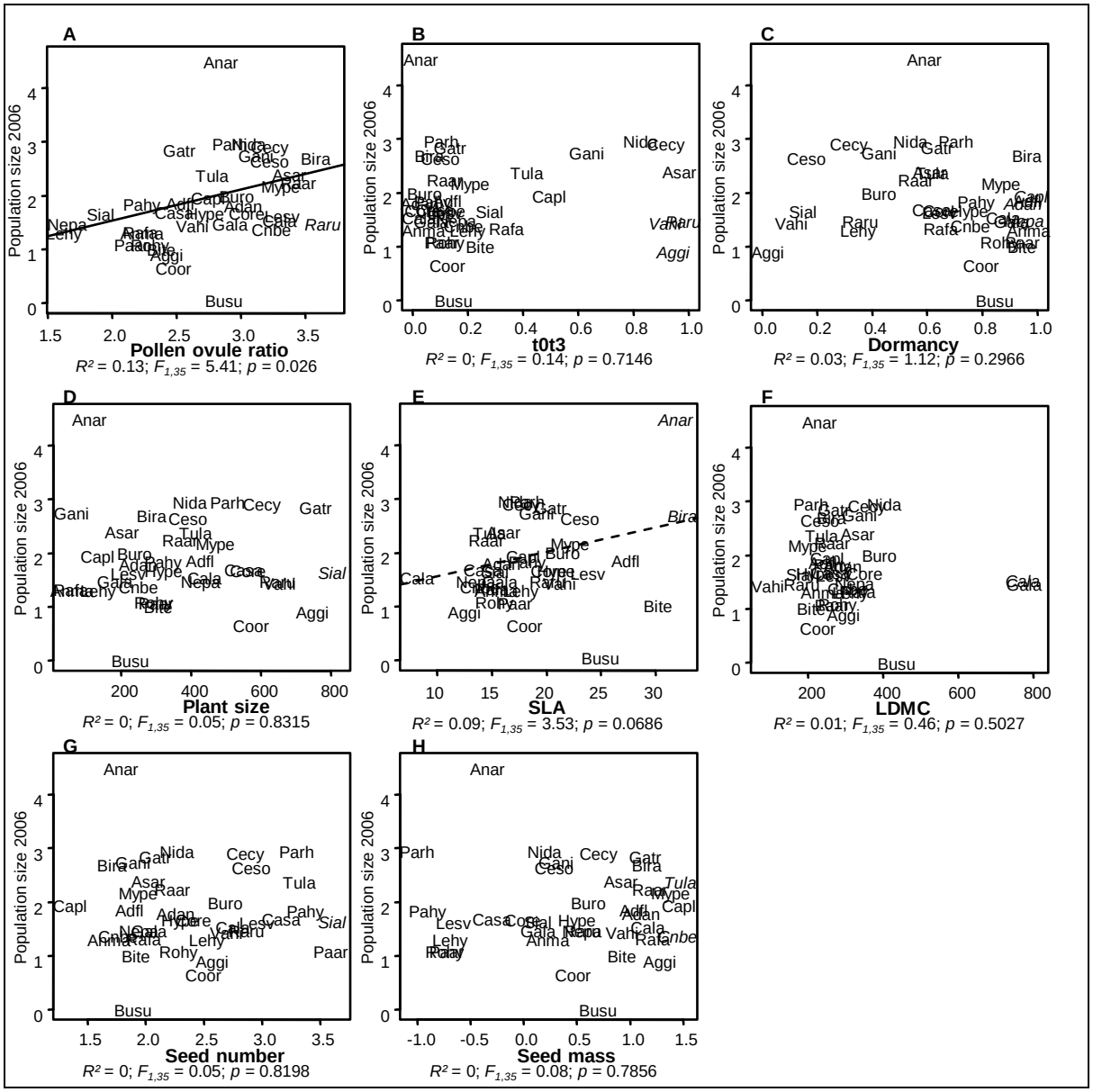


Fig. 5.4. Correlates for population size in 2006 for 37 species. The straight regression line indicates a significant (p < 0.05) relationship, the broken line a weakly significant (p < 0.1) relationship. Species codes are in table 4.1.

For the population sizes in 2006 using no correction, we found a significant positive effect of Pollen/Ovule (P/O) ratio on population size. Specific leaf area showed a marginally significant effect, with high SLA-species having larger population sizes (fig. 5.4).

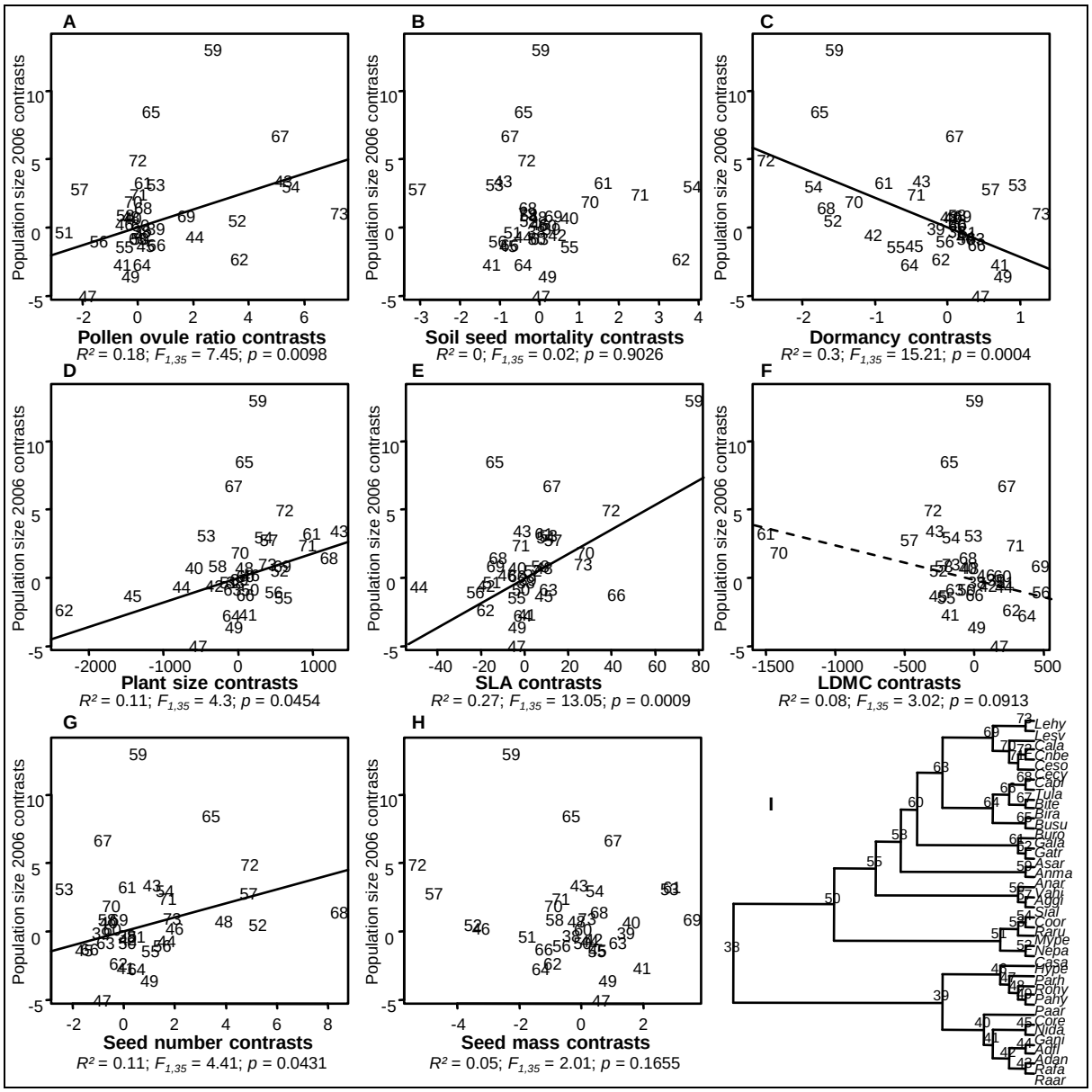


Fig. 5.5. Population size contrasts (in 2006) and trait contrasts (numbers in plots and tree). The straight regression lines indicate significant relationships ($p < 0.05$), the broken line weakly significant relationships ($p < 0.1$). (I) The phylogeny used for calculation of PICs. Species codes are in table 4.1.

This picture changed when phylogeny was taken into account (fig. 5.5). Again, P/O ratio contrasts were significantly related to population size contrasts, with an even slightly stronger relationship, indicating that among closely related taxa, the one with the higher P/O ratio has the higher population size (fig. 5.5). SLA contrasts showed a stronger effect on population size contrasts than without phylogenetic correction (fig 5.4 and 5.5). In addition to this, degree of dormancy contrasts were negatively correlated to population size contrasts;

this indicates that among two taxa, the one with low germinating fractions has very generally the smaller population size in 2006 (fig. 5.5). Using PICs, plant size and seed number were both positively related to population size (fig. 5.5). Finally leaf dry matter content showed a marginally negative effect on population size contrasts for 2006 (fig. 5.5), the inverse of the relation for 2005 (fig. 5.3).

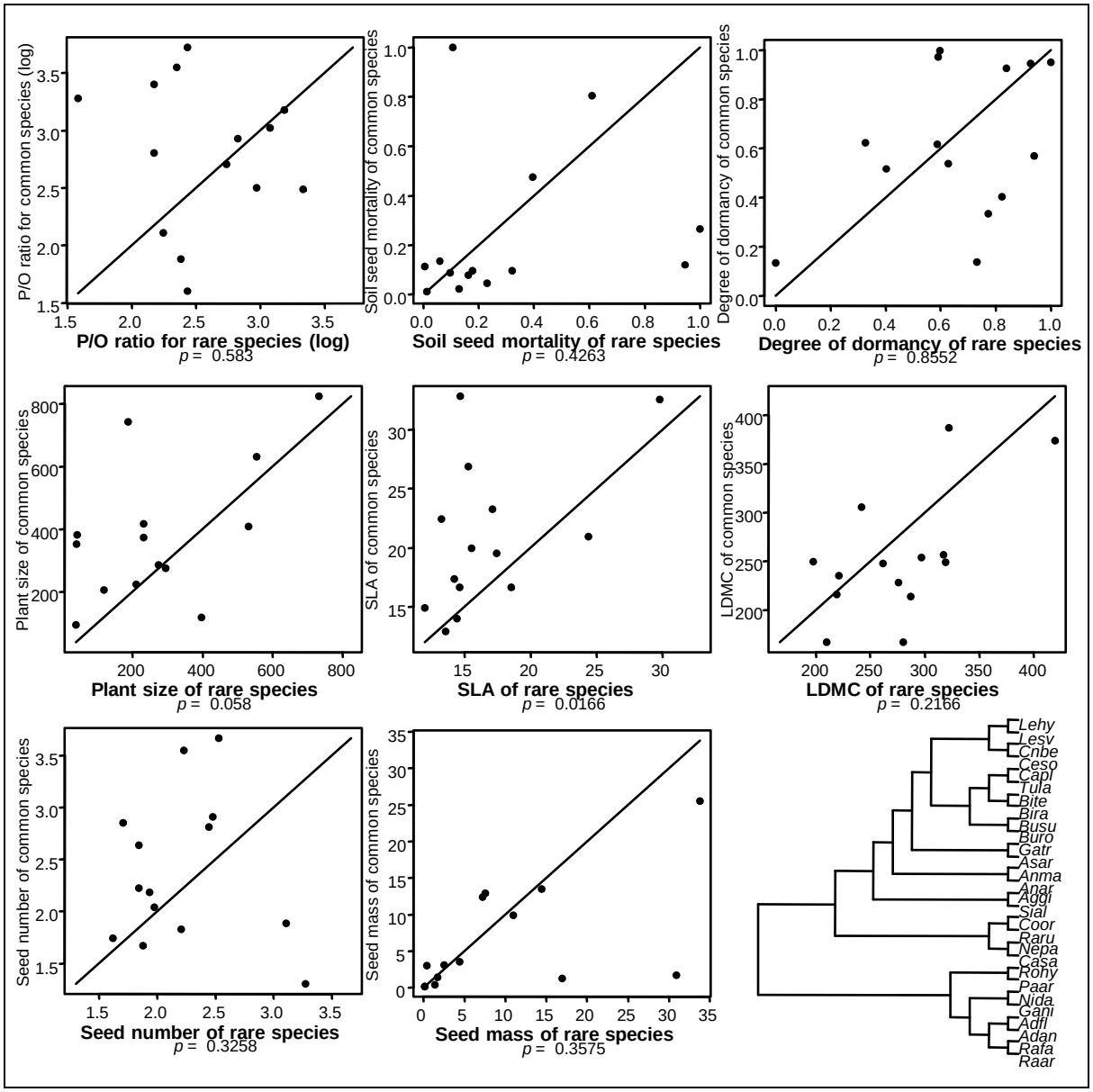


Fig. 5.6. Comparison of regionally rare and common species according to their traits: each dot represents a species pair. Dots are placed according to the values of the rare species on the x-axis and according to the common species on the y-axis, dots on the line indicate no difference between trait values of rare and common species. The phylogeny indicates the species pairs used here. Species codes are in table 4.1. P-values are values from a paired Wilcoxon-test.

For the second measure of rarity, the frequency of populations per species in our study area shows that rare and common annual species differ in their SLA and marginally in their size (fig. 5.6). Common species have a higher SLA than their rarer relatives and common species are also larger in size (fig. 5.6).

DISCUSSION

The first feature of our work shows, that there are no consistent relationships among years between resource capture or resource conservation traits, such as SLA and LDMC, and population size. Astonishingly, there were no relationships at all between seed size or number and population size or regional abundance. Also for the pollen ovule ratio, there were inconsistencies among years. The interpretation of these results can therefore not been disconnected from directional changes in the environment of the cereal fields. Previous works on breeding system and P/O ratio suggested that this trait may be related to the ability of a species to withstand changing environmental conditions (Lavergne 2003). Pollen:ovule ratio is related to a plant's breeding system, with low P/O for inbreeders and high P/O for outbreeders (Cruden 1976;Cruden 1977). Furthermore, inbreeders are known to have lower genetic diversity and levels of heterozygosity than outbreeders (Hamrick and Godt 1997). Therefore, it is possible that species with low pollen:ovule ratio are disadvantaged due to less exchanges of genetic information. In a severely changing environment, this can lead to larger population sizes for high P/O species and smaller sizes for low P/O species, because outbreeders adapt more quickly than inbreeders do. Additionally, low P/O species can eventually persist in smaller populations without being pollen limited and hence continue to form seeds, even if the environment is suboptimal whereas high P/O species disappear completely or show higher fluctuations, due to the pollen triggered seed set (Leimu *et al.* 2006). There is however, a problem with this interpretation as it fails to explain why there are still species with low P/O and why this is

not already the case in the earlier survey of 1983. It is possible that these species had a high gene exchange among populations *via* seed dispersal in former times, in relation with their higher seed production due to self-compatibility, and that nowadays there are small relictual populations persisting for inbreeding species. These populations bear however, a very high extinction risk because they are relatively small and show high levels of inbreeding.

Second, both dates together indicate that smaller populations show a higher degree of dormancy and longer soil seed viability than larger populations, in other words an important long-lived soil seed bank. It can be argued that for species with long-lived seed bank, each year only parts of the seeds germinate and thus total population size is underestimated by surveys. Long-lived seed banks or low germination fractions are life history traits that buffer against failure in reproduction in annual plants by bet hedging (Venable 2007). A possible interpretation of the data would be that a long-lived soil seed bank enables species to persist even in small populations because effective population size from a genetic (Silvertown and Charlesworth 2001) and life history (Kalisz 1991;Kalisz and McPeek 1992) point of view is higher in seed bankers than in species with short lived soil seed bank. However, it is now clear that there are considerable genetic (McGraw 1993;Cabin *et al*. 1998;Hock *et al*. 2008) and functional (Kalisz 1991;Kalisz and McPeek 1992) differences between above ground and below ground populations.

Third, the results of plant size and specific leaf area (SLA) are consistent between the population sizes in 2006 and regional frequency of populations, in both cases, common species or species with higher number of population at a regional level are taller and have a higher SLA. SLA is linked to rapid growth and nutrient uptake can potentially be relevant (Weiher *et al*. 1999;Garnier *et al*. 2001). This indicates that species with rapid growth, fast resource acquisition and big final size have advantages for both, building up larger populations, disperse, and establish better at a regional level. It is interesting to note here that this is not the case in the 1983 data set, which -in contrast to 2006- showed a higher leaf

dry matter content (LDMC) for species with larger populations and no relation to plant size or SLA. This data can be interpreted as a shift from resource conserving plants being advantaged in the environment of the 1980ies, and this due to dryer climate, less competitive environment and less fertilizer, all three factors changed over time for the studied ecosystem in the study area (Gasc 2005;Fried *et al*. 2009). The differences at the regional scale can probably be interpreted in a similar manner even if they act at different temporal and geographical scales. Here, we studied indeed a set of species that were notoriously common at the beginning of the 20th century and that is now becoming increasingly rare. Herbicide use, changed ploughing techniques and the use of fertilizer are implicated in these changes. This makes the surrounding vegetation and especially the standing crop more competitive and favours plants with rapid nutrient uptake (Bischoff and Mahn 2000;Fried *et al*. 2009).

In the light of the important changes over time among annual plants, it has to be emphasised that most of the traits that can be related to local population size or regional frequency of populations are not general predictors or correlates of population size, but merely indicators to what limits the studied set of species in the actual landscape. In the light of the data advanced here, the survival of seeds in the soil seed bank and degree of dormancy may be excepted: both buffer against the fatality of exceptional years and small population sizes and may by this promote persistence in small populations.

The relations we document here have an importance to actual conservation of rare annual plants in the North West Mediterranean. If rare annuals have to be preserved, the nutrient status of the growing places, in our case cereal fields, have to be kept low at least somewhere, in order not to disadvantage small plants with slow nutrient uptake. We can exclude a decline in pollinators and hence pollen mediated reproductive failure as a major source for the loss of species and populations (Biesmeijer *et al.* 2006). Because, at the time scale studied for our study region, outbreeding species, with a high P/O ratio show actually the larger population sizes. It is rather inbreeding species that face a major actual extinction

risk. This is in contradiction to claims of a general pollinator mediated plant regression in other parts of Europe (Biesmeijer *et al.* 2006). Here we also add significant data to support the idea that changing nutrient status of the environment –eutrophication– is also a cause for plant rarity in Mediterranean Europe, for which this general trend in remaining Europe was often excluded.

Beyond these geographically more restricted conclusions of conservation interest, our findings suggest that for a vast majority of functional traits relations to 'rarity' are not general but bound to a temporal context. Soil seed bank is a promising research area to explain some of the contrasts in population sizes among species.

GENERAL DISCUSSION, CONCLUSIONS AND PERSPECTIVES

In the general discussion, we resume and evaluate the main insights from each chapter and relate them to the theoretical context given in the general introduction. Later we try to point on the limits of our research. In the general conclusions, we discuss some more basic questions appearing in the thesis. Finally, we give perspectives which questions can complete our approach and how future research can successfully answer them.

General discussion

PLANT DIVERSITY IN AGRO-ECOSYSTEMS: THE MAIN INFLUENCE OF DISTURBANCES AND THE ROLE OF SPATIAL HETEROGENEITY FOR DIVERSITY MAINTENANCE

The analysis of diversity in an agro-ecosystem in the first chapter showed that habitat types, intensity of agriculture and historical factors all had an important influence on plant diversity in agro-ecosystems. Because habitat types depend only on differences in land-use and are only little different in their soil conditions the largest part of biodiversity in these agricultural landscapes is determined by human disturbances. This result has been shown by a number of works on herbicide use and plant diversity (Schneider *et al*. 1994;Robinson and Sutherland 2002), organic farming and plant diversity (Hald 1999;Hyvönen *et al*. 2003;Gabriel *et al*. 2006;Roschewitz *et al*. 2009) and agricultural practices in vineyards (Maillet 1992). There are however new insights coming from chapter 1. For instance, the different habitat types have only rarely been taken into account for a landscape scale analysis of plant diversity (von Arx *et al.* 2002) and, to our knowledge, this has not yet been done for vineyards. The differences in α-diversity among habitats showed that field margins and embankments play a major role for maintaining plant diversity in vineyards and that this is especially true for plants of high conservation interest. Similar findings come from works on intensive arable fields (Marshall 1989;Wilson and Aebischer 1995;Gabriel *et al*. 2006;Roschewitz *et al*. 2009)

and this view lead to programs which specially aim on the maintaining of plant and animal diversity in field margins by reducing herbicide use and crop density (Thomas and Marshall 1999;Moonen and Marshall 2001;Smith *et al*. 2008). For vineyards, our data indicate however, that embankments, *i.e.* the surfaces that are never ploughed, bear by far the most important remnants of plant diversity. There are several reasons why previous works do not point on non-ploughed surfaces for maintaining of plant diversity in agricultural landscapes. (i) These embankments do not necessarily exist in intensive arable land, where they are much smaller and often bear woody vegetation like hedges in Central and North Western Europe. (ii) Mediterranean type climate implies severe summer drought with nearly complete drying of above ground biomass. Drought as a cycling disturbance enhances especially diversity of annual plants and can give similar niches as in arable land itself. This is not the case for moist climate field embankments. Evidently, diversity in field embankments in vineyards or arable land depends also on their management.

The higher diversity on embankments are interpreted to influence adjacent habitats such as field margins to have a higher diversity due to dispersal and establishment of sink populations a concept termed 'mass effect' (Shmida and Wilson 1985;Kunin 1998). However, embankments not only increase diversity in adjacent habitats by the establishment of a high number of sink populations. The higher diversity of embankments so close to the other habitats can also increase the possible species pool, which provides more species to fit into the niches available in field margins and centres -a diversifying effect termed 'species pool concept' (Zobel 1997;Pärtel 2002;Zobel *et al*. 2006). The higher number of different species in more diverse landscapes that are dispersed into a particular habitat can also explain the higher absolute β-diversity in landscapes with many different habitats. Both aspects -the gradient from embankment to field centre and the increased diversity in diversified landscape- show that plant diversity including target species for conservation cannot be explained independently from the spatial surroundings. Surroundings can play a crucial role

by constantly dispersing seeds into the studied habitats, and its opposite, dispersal limitation, is known to be one of the most severe limits to plant diversity (Ehrlen and Eriksson 2000;Coulson *et al*. 2001;Poschlod and Biewer 2005;Zobel *et al*. 2006).

In the introduction we presented the principles of 'storage effect' which explains the coexistence of dominant and subordinate species *via* temporal heterogeneity in habitat quality, correlated competition and the buffering effect of persistent soil seed banks. In the light of the high importance of dispersal from species rich surroundings, we can now better understand how diversity maintains. In addition to the seed bank, surrounding habitats that have not the same disturbance regimes than the target community can provide seeds to fill up vacant niches when conditions become suboptimal in the target community. For arable fields the work of Dutoit *et al.* (2003) showed indeed that after long non-crop rotation in arable field the return of the typical plant community is hampered. However, this is a transitional condition in the light of the high number of arable plant seeds in seeding material from traditional systems (Jäger 2002). This gives insights into how plant diversity can best be maintained in agro-ecosystems: not to long crop rotations and increasing of dispersal mechanisms, like *e.g.* the use of not cleaned seeding material. Not surprisingly, the most threatened arable weeds are those that do not have a persistent soil seed banks and that relay on seed dispersal by uncleaned seed material to maintain in agricultural landscapes (Schneider *et al.* 1994).

METHODS AND ESTIMATES OF SOIL SEED BANK PERSISTENCE REVISITED – WHICH SEED BANK ESTIMATE CAN PREDICT LOCAL PLANT DIVERSITY AND ABUNDANCE?

In the second chapter, we use seed survival measured from a burial experiment and show that it is not correlated to the commonly used seed bank persistence estimates from literature when these are estimated from seedling emergence. This let us ask what quality of data the methods for the study of soil seed banks give and how soil seed bank persistence estimates

have been validated in the past. We had a closer look on the work of Bekker *et al.* (1998a), who tested the general validity of seed bank persistence estimates based on the depth distribution of viable seeds. We realised that the data mixture that Bekker *et al.* (1998a) used in their validation database makes it difficult to know whether the seedling emergence method is related to experimental soil seed survival. Our analyses do not support a close general relationship of the both. We then asked what could influence the seedling emergence method to yield different data than burial experiments. One putative candidate to bias seedling emergence is seed production, which is already known to be strongly related to dispersal in space (Tackenberg *et al*. 2003;Poschlod and Biewer 2005;Poschlod *et al*. 2005). In a subsequent analysis of data from a literature survey, we therefore tested whether seed production was correlated to soil seed bank estimates. Using the data of Šera and Šery (2004) and Thompson *et al.* (1997), we could demonstrate that there was a clear and significant relationship indicating that higher seed production is related to higher soil seed bank persistence estimates. Seed production is caught in a fundamental trade-off with seed size (Shipley and Dion 1992;Jakobsson and Eriksson 2000;Turnbull *et al*. 2000). The processes that compensate larger seeds for their smaller number, such as seedling mortality, act after germination (McGinley *et al*. 1987;Louda 1989;Jakobsson and Eriksson 2000;Leishman *et al*. 2000b;Coomes and Grubb 2003;Moles *et al*. 2004;Pizo *et al*. 2006;Bladé and Vallejo 2008). We therefore think that the classical soil seed bank persistence estimates are not useful when one wants to predict diversity or population persistence. This finding is confirmed by field observations showing that the emergent seedling composition in gaps is quite different from what finally establishes in the gaps (Hillier *et al.* 1990). This results also question the existence of a positive relation between seed size and seed longevity which have been shown using soil seed bank estimates coming from the seedling emergence method (Thompson *et al*. 1993;Bekker *et al*. 1998a;Moles *et al*. 2000;Cerabolini *et al*. 2003;Peco *et al*. 2003). In short, high seed number enhances the probability that one viable seed will survive until formation of a

gap or sampling by an ecologist, but this will not mean that the resulting seedling will grow to an adult plant under natural conditions. The probability to reach adult age for one seedling is much higher in large seeded species. There are different numbers of seeds in the soil seed sample according to differences in abundance and seed production, which itself is connected to seed size by a trade-off. However, both -many small and few large seeds- will result in equal numbers of adults or equal chance to establish in the gap. This gives insights how to scale abundance in the seed bank in order to give sound predictions about diversity and abundance in the resulting vegetation. Seed size may here be a potential scaling factor: multiplied by the seed number it yields the relative investment of plants for the given sample. This should be explored in future works.

Probably, seed size may be near to neutral for the 'storage effect'. From an evolutionary point of view, one can imagine many other processes that trigger soil seed persistence. According to the storage effect, this can also include the adult plant niche, because this may decide whether immediate or delayed germination yields the highest fitness. Evidently, the timing of germination can change as a function of environmental conditions. These germination cues may be one path to a deepened understanding what decides on the longevity of seeds in the soil - a point that we explored in chapter 3.

FUNCTION OF SEED PERSISTENCE IN THE SOIL: HOW GERMINATION AND SEED TRAITS OPTIMISE A PLANT'S RESOURCE USE IN DISTURBANCE DRIVEN ECOSYSTEMS

Dormancy had an important positive effect on soil seed survival in our experiment. This finding confirms that dormancy is an important adaptation to achieve soil seed survival in the soil (Baskin and Baskin 1998;Thompson *et al*. 2003;Baskin and Baskin 2006). Inhibition of germination after burial can also be regulated by a light requirement for germination; a light requirement that is known to be negatively related to seed size (Milberg *et al.* 2000). The light requirement for germination could be confirmed for small seeds and it could be

complemented by a darkness requirement for large seeds. The latter is a reasonable expectation because larger seeds can emerge from deeper soil layers (Bond *et al*. 1999;Grundy *et al*. 2003) and because light penetrates only extremely little into soil (Benvenuti 1995). Under Mediterranean climate, it has been shown by Bell *et al.* (1995) that some species germinated better in darkness than in light. The argumentation behind this is that species germinate in soil layers were moisture and light conditions are moderated compared to the soil surface. Species germinating in darkness may therefore have an advantage over light dependent germination under Mediterranean climate. We also confirm with experimental data that a light requirement enhances soil seed persistence with a clear decline of its importance from the moment seeds entered into the soil. This confirms experimentally what has been argued earlier on smaller experimental basis (Baskin and Baskin 1998;Thompson *et al*. 2003;Baskin and Baskin 2006). This factor may sustain a negative seed size-seed longevity relationship independently from the seed size-seed number trade-off.

The long survival of seeds in the soil may be connected to mechanisms that detect favourable conditions for establishment and that trigger germination below the surface. We demonstrated that 'gap detection', *i.e.* reaction to diurnally fluctuating temperatures (*DFT*) is one mechanism to enhance soil seed persistence after one and a half year of burial and -in our Mediterranean example- especially for the winter burial periods. To our knowledge, a relationship between soil seed survival and *DFT* has not been reported previously. Diurnally temperature fluctuations (*DFT*) are lower with increasing soil depth (Miess 1968). Therefore, an effect on soil seed persistence can also be an adaptation to detect depth of burial and to avoid fatal germination in deeper soil layers. This extends the classical view of the 'gap detection' mechanism of *DFT*, which is restricted to the detection of above ground gaps in the vegetation. The reaction to *DFT* had a seasonal component: soil seed mortality is higher in winter than in summer for species that germinate better without *DFT*. If *DFT* is a way to detect burial depth, one could suggest a relation to seed size, which could have a negative

effect on the strength of the reaction to *DFT*. In our data set, we could not show such a relation, which indicates, that the gap detection mechanism evolved equally often evolved in large than in small seeded species. However, data is scarce and this point would better be addressed with a burial experiment especially designed to detect the importance of burial depth, DFT and seed size.

We also confirmed in an analysis of phylogenetically independent contrasts, that seed size was negatively related to soil seed survival. This is the first test showing this relationship only with experimental data. This is thus important independent evidence to sustain the seed size-seed persistence relationship (Thompson *et al*. 1993;Bekker *et al*. 1998a;Moles *et al*. 2000;Cerabolini *et al*. 2003;Peco *et al*. 2003). However, we want to highlight that there was no strong relation of soil seed mortality without the use of PICs. One interpretation would be that the possibility of detection is enhanced using close relatives. This ignores however, that seed sizes even in this experiment vary over several orders of magnitude. However, we could not find any threshold value, *i.e.* no value beyond which all species have persistent or transient seeds. An alternative explanation would be to admit a bias in the burial experiment that is related to seed size: *e.g.*, we used fixed numbers of seeds for all species and a unique size of mesh bags for burial without substrate. In such a design, small seeds are more distant on average than large seeds and propagation of fungi can be enhanced in the more densely packed mesh bags for large seeds (Van Mourik *et al.* 2005). This leads to higher soil seed mortality in larger seeds due to fungi promoting seed decay in the soil (Schafer and Kotanen 2003;Davis and Renner 2007). Finally, this illustrates how difficult it is to escape the seed number –seed size trade-off even under experimental conditions of a seed burial experiment and that the relation of seed size to soil seed mortality remains unresolved. Future works should explore different seed sizes and buried seed densities to account for density dependent effects and to give a definitive test of the relation.

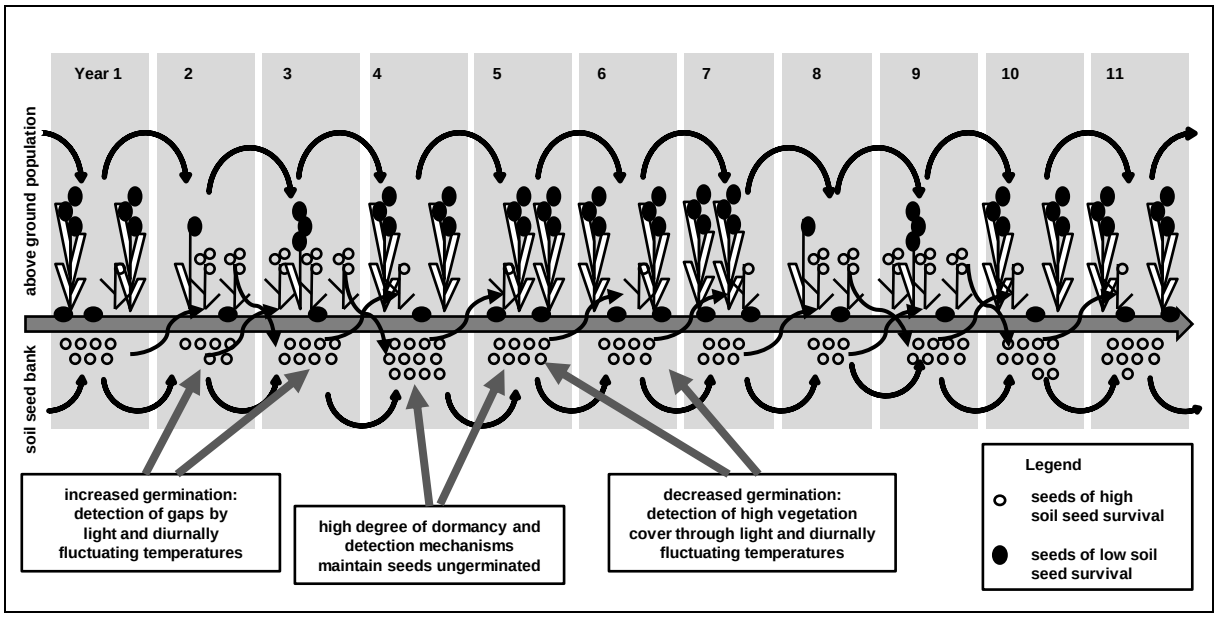


Fig. C.1: Traits and germination ecological characteristics important for soil seed persistence.

Figure C.1 summarises the different ways in which soil seed persistence can be controlled by adaptations in germination ecology. DFT and a light requirement permit the detection of disturbances and gaps and hence trigger germination (fig. C.1). On the other hand, dormancy, DFT and light requirement also permit seeds to stay ungerminated while conditions for establishment are unfavourable (fig. C.1). The complexity of these adaptations shows how crucial timing of germination is for the individual fitness. According to storage effect, these adaptations can avoid competition as a limiting factor for individual fitness. Seed size has been supposed to be correlated to competitive ability in seedlings: larger seeds can manage to establish in denser vegetation, with a litter cover, in later successional states or in more shaded habitats (Hodkinson *et al.* 1998). The advantage of adaptations that detect the best moment to germinate becomes clear in the light of storage effect. They permit to detect favourable periods (fig. C.1) and limit the decrease of the soil seed population in unfavourable years (fig. C.1). Moreover, it can be argued that adaptations that detect favourable conditions for germination and establishment enhance the storage effect. This would mean that these traits promote coexistence and diversity in plant communities where

annual plants are common, favourable years are unpredictable and disturbances are frequent.

TRAITS AND LOCAL POPULATION DYNAMICS IN ANNUAL PLANTS: CAN POPULATION TURNOVER AND EXTINCTION DYNAMICS BE PREDICTED?

In chapter 4, we show that a part of the studied species is regressing and notably that the smaller the size of their population the higher the extinction risk during the observation period 1983-2005. However, there was no clear trend in light conditions, moisture content, soil pH and soil nutriment status during the study period. We concluded that the reasons for regression must be elsewhere than in changes of habitat quality in these factors. Indeed, the major reason for regression is herbicide use, and its increase is possible during the study period. Furthermore, change in timing of disturbances such as a shift towards more summer crops can influence the studied species. This would lead to a flora richer in summer germinating species ('Chenopodietea', see introduction), but we could not observe this in the field. Finally, the disruption of dispersal processes can be an important source for the disappearance of local populations (Poschlod *et al*. 1998;Poschlod and Biewer 2005;Poschlod *et al*. 2005;Ozinga *et al*. 2008).

We then analysed functional plant traits and their relation to population dynamics and found that only soil seed mortality showed a significant relationship to rate of local population extinction. This is in congruence with previous suggestions and findings (Stöcklin and Fischer 1999;Menges 2000;Poschlod *et al*. 2000). Why do plants with rapid soil seed decay suffer from more rapid local extinction? One answer may come from the storage effect: when there is no buffer, competition with a temporally dominant other vegetation will make disappear subordinate species. There is no doubt that this is the case in the study area because Dutoit *et al.* (2003) could show that many species disappear after only 10 years of fallow land. Several years of fallow land are no exception in traditional cereal cultivation

(Ellenberg 1996;Dutoit *et al*. 2003;Gasc 2005). Evidently, one has to answer the question how these species maintained so long time without soil seed bank in the studied system. We think that alternative dispersal processes, nowadays disrupted, such as transport with seeding material and sheep dispersed cereal weeds in former times. This is confirmed by the high number of non cereal seeds transported with cereal seeding material (Schneider *et al*. 1994;Jäger 2002) and the comparatively low number of species that are dispersed by sheep dispersal for the same set of species in the same study area (Jäger 2002).

It is astonishing in this context that other traits did not have a major influence. According to the storage effect, species with a high competitive ability should more easily be able to withstand unfavourable years without seed bank. We were not able to show that larger plants or plants with a high SLA have populations that persist better, so we think that most is explained by adaptations on the seed and germination level.

TRAITS AND THEIR RELATION TO RARITY AND ABUNDANCE

Today's plant diversity, abundance and distribution cannot be understood without knowledge on the history of study area and the plants themselves. What is true for evolution of floras on continental scales involving geological times is also true for the diversity of individual fields and the cultivation practices only some years ago as we could illustrate this in chapter 1 for the cereal weeds that were marker species for former cereal cultivation in vineyards.

In chapter 5, we study the relationships of plant traits to two different axes of rarity: local abundance and regional frequency of populations (Rabinowitz 1981;Rabinowitz *et al*. 1986). We study essentially the same traits as in chapter 4, but we aim now to relate them to rarity and abundance at a given date without considering the temporal changes. A striking result of this analysis is, that there are only very few traits that are consistent among different years.

One of the traits that were consistently related through time was the smaller population size across different dates for species with high degree of dormancy and high soil seed viability. This is an important result from fundamental point of view, because long-lived seed banks or low germination fractions are life history traits that buffer against failure in reproduction in annual plants by bet hedging (Venable 2007). This is also in line with the predictions of storage effect, meaning that a considerable part of plant diversity can be maintained in unpredictable environments by species that form a persistent seed bank. Their small population sizes also have only little effect on neighbouring plants *via* competition because of their low abundance. For conservation efforts, this means that a diversified disturbance regime with unpredictable disturbances can enhance local plant diversity in ecosystems with a characteristic dominance of annual or short-lived plants.

A second complementary explanation for the smaller population sizes of species with long lived seeds in the soil is the increase in effective population size both by higher gene pool (Silvertown and Charlesworth 2001) and buffering life history stages (Kalisz 1991;Kalisz and McPeek 1992). In other words, the soil seed bank extends the above ground population and provides thus supplementary genetic diversity and this enhances local population performance. It is therefore interesting to pursue the researches on the genetics comparing above and below ground populations, an aspect which is still little explored (McGraw 1993;Cabin *et al*. 1998;Hock *et al*. 2008).

Our study provides the first account for a relation between soil seed bank persistence and local population size using comparative species data. It would now be interesting to explore this relationship notably for annual plants in other regions, such as rare and common steppe and desert annuals or for other growth forms such as perennial herbs or woody species, which could elucidate which functional role the soil seed bank plays for their population structure.

Astonishingly, seed size or number had no effect on rarity or abundance and this is in contrast to the body size-abundance relationship (White *et al.* 2007) and the seed number-seed size trade-off (Shipley and Dion 1992;Jakobsson and Eriksson 2000;Turnbull *et al*. 2000). Both point to more individuals for smaller than for large seeds. The absence of a relation can have two reasons. First, we did not study population sizes on fixed surfaces so density dependent effects leading to fewer individuals for large plants could not be elucidated. Second, the processes that compensate by disadvantaging small seeds for their higher number are very effective and lead really to equal numbers of adult individuals (Leishman *et al*. 2000b;Moles *et al*. 2004). This confirms the difficulties to demonstrate body size-abundance allometries for plants (White *et al.* 2007).

There were conspicuous differences among years for the resource capture and resource conservation traits, such as SLA and LDMC (Garnier *et al*. 1997;Kazakou *et al*. 2006). These differences indicated larger populations for high SLA species in 2006 and larger populations for high LDMC species for 1983-85, in other words opposite resource use strategies were performing in these two dates. Again, this result is only clear in front of a background data of changing environment. Our interpretation is hampered by the lack of such a data set. We had no data on nutrient status in 1983-85 and it was not possible to gather data for 2006. The analysis of Ellenberg indicator values along time in chapter 4 provides a clear signal for higher water and nutrient status in 2006 compared to 2005 and 1983-85 and this is in line with the findings of other works. (Gasc 2005;Fried *et al*. 2009). Therefore, the change in these two parameters will be a plausible explanation for the observed relationship between leaf traits and abundance. This temporally invertible relation between leaf traits and abundance illustrates why they are not generally relied to abundance: the environmental conditions determine completely the sense of the relationship. On the other hand, they are good predictors of reaction to changing environmental conditions. Indeed, in the global change of climate with altered precipitations, LDMC and SLA are putative traits that can predict future

regression and extinction directly bound to climatic factors (Shipley *et al*. 2006;Pakeman *et al*. 2008).

However, caution has to be paid in systems with changing influences of agriculture, because changes in cultural practices can also act on species composition according to their leaf traits: the crop type can change and favour more competitive plants with rapid nutrient uptake (Bischoff and Mahn 2000;Fried *et al*. 2009) and consequently high SLA.

POLLEN:OVULE RATIO AND POPULATION DYNAMICS

In chapter 4, we did not present an analysis of pollen:ovule ratio and its influence on rate of local population extinction. We excluded this P/O from chapter 4 because this trait differs from other traits in being related to the evolutionary flexibility of plants and populations and not to the mechanistic performance of single individuals. We think that this is a fundamental difference. Pollen:ovule ratio relates to gene exchange *via* pollen and breeding system (Cruden 1976;Cruden 1977;Hamrick *et al*. 1979;Loveless and Hamrick 1984).

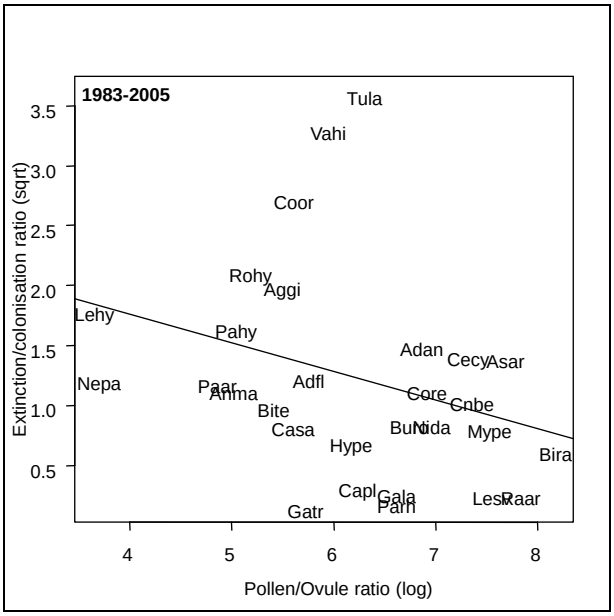


Fig. C.2: Extinction:colonisation rate and pollen:ovule ratio for the studied species (GLM, quasibinomial, $T_{1,29}$ = -2.66, p = 0.0126); for species codes and details of analysis see chapter 4, methods section, for pollen:ovule ratio methods see chapter 5.

Higher levels of gene exchange relate to the evolutionary flexibility of plants. This enhances performance indirectly and more directly, by higher levels of cross-fertilization, more

connected population structure and can have effects in this way on population dynamics. The opposite, low levels of gene exchange *via* pollen can lead to inbreeding depression, *i.e.* the reduced performance of selfed to outcrossed descendants. Figure C.2 shows that species with high pollen:ovule ratio have lower local extinction rates than species with low pollen:ovule ratio. What is the relation of P/O to extinction? It has been shown that P/O is closely correlated to the breeding system and the pollen vector of plants (Cruden 1977). The breeding system influences levels of genetic diversity in plants and among plants and it has also consequences on the genetic diversity within populations and genetic structure within and among populations, because it influences the extent of gene exchange among plants (Loveless and Hamrick 1984;Reed *et al*. 2002). Pollen dispersal vectors also have an influence on the importance and the distance of gene exchange (Loveless and Hamrick 1984;Reed *et al*. 2002). Higher gene exchange and a larger effective population size can be related to a high P/O (Silvertown and Charlesworth 2001), with anemophileous species having very high gene exchange and very large populations (extending to large geographic areas) and obligate autogamous species or apomictic species with no or very limited gene exchange. For the latter, the population definition based on gene exchange is probably not applicable. In the light of this, it can be argued that species with a low P/O go more easily extinct because of limited gene exchange and decreased genetic diversity making them more vulnerable to environmental and biotic changes. An alternative way to exchange genetic information *via* pollen among populations is the dispersal of seeds. For our study system, important seed dispersal processes break down recently (Gasc 2005). It becomes therefore plausible that previously widespread plants, which rely more on seed dispersal for gene exchange than on pollen, become more now easily extinct. Why gene exchange by pollen plays such a crucial role? Several observations on herbicide resistance in *Amaranthus* and *Chenopodium* illustrate that species with an important long-distance pollen dispersal in these two anemophileous genera have advantages in the severely changing environments with very effective

herbicides (Bettini *et al*. 1987;Darmency and Gasquez 1990;Culpepper *et al*. 2006). In these two species, herbicide resistances spread rapidly. They are indeed the only species that manage to grow in the most industrialised arable fields of Europe and North America due to the spread of genetically transmitted herbicide resistance to a trazine for *Chenopodium* in the 1980s and for glyphosate in *Amaranthus* since 2000.

This shows the high importance of dispersal *via* pollen or seeds for the maintenance of local populations. Our findings together with Biesmeijer (2006), point on a general shift of the flora due to the breakdown of dispersal processes. Here, we only observe tendencies in the local extinction of some species. We think that this may be only the beginning of a process that may lead in future to complete disappearance of entire floristic groups with *e.g.* seed dispersal relying on disappearing agricultural practices or pollen dispersal by disappearing pollinators. A special attention should therefore be paid to maintain dispersal processes and dispersal vectors, when the conservation of 'nature', 'biodiversity' or a complete set of 'ecosystem services' is the aim.

DISPERSAL OF SEEDS AND POPULATION PERSISTENCE

Seed dispersal in space has not yet been considered in detail in this thesis. However, we mentioned it in the chapters 1, 2, 4, 5 and the introduction, where we discussed that seed dispersal in space has an impact on diversity and population maintenance. Our results suggest, especially for species with limited pollen transport and short-lived seeds in the soil, that the disruption of spatial dispersal of seeds may be responsible for the strong recent decline of these species. The lack of seed dispersal processes in cereal fields has been shown to be the reason for the regional extinction of rare cereal weeds (Schneider *et al*. 1994). Schneider *et al*. (1994) showed that the cleaning of seeding material is responsible for the disappearance of species that do not form persistent soil seed banks such as *Agrostemma githago* or *Asperula arvensis*. They also discuss several aspects of new dispersal mechanisms

by harvesting machines that can favour other species, especially grasses with small seeds. The replacement of organic by mineral fertilizer constitutes another reason for the decrease in seed dispersal processes at the farm or landscape scale, because dung is very rich in viable diaspores (Bonn and Poschlod 1998). Finally, the work of Jäger (2002) showed that some species with seeds bearing appendages, such as *Caucalis platycarpos*, can also be transported exozoochorously by sheep. The decline of itinerant sheep flocks in the study region can therefore accentuate the decline of these species. However, according to Jäger (2002) this concerns only very few species. Jäger (2002) also showed the very high importance of uncleaned seeding material that can transport several 10 000s of seeds for each hectare of seeded cereals and he also showed that this concerns species with a high variability in plant heights and seed sizes. Complementing this work, Gasc (2005) studied in detail the use of uncleaned seeding material in the Luberon area. He could show that uncleaned seeding material is most likely to occur in small farms with cereals being most often cultivated as a fodder crop for sheep, a situation where herbicides are rarely used and conditions are thus optimal for the maintenance of a rich cereal weed flora. Other dispersal vectors such as ants and small mammals have been considered by Gerbaud and Dutoit (2002) but they seem to play only a minor role compared to other ecosystems. In chapter 1, we showed that seed dispersal processes are important to explain the diversity of arable field species at a vineyard and landscape scale and we suggest according to Dutoit *et al*. (2003) that they are crucial for the recolonisation after longer crop abandonment. Moreover, in chapter 4, we suggested also that the disruption of dispersal processes is probably an important source for the disappearance of local populations in line with previous findings (Poschlod *et al*. 1998;Poschlod and Biewer 2005;Poschlod *et al*. 2005;Ozinga *et al*. 2008). In conclusion, seed dispersal through uncleaned seed material is crucial for the maintenance in the field for the most endangered cereal weeds with limited soil seed survival.

INTERACTIONS BETWEEN CEREALS AND ANNUAL CEREAL WEEDS

We did not include in this thesis an experiment that we conducted on the competition between cereal weeds and wheat. In this experiment, we tried to quantify the impact of wheat competition on cereal weed reproduction and the impact of different cereal weeds on wheat in a greenhouse and common garden experiment. A small subset of the experiment tried to test the importance of changed edaphic conditions, in our case stony soils on the outcome of competition (see fig. C.3). The experiment was hampered by the uneven germination of the different species tested and the low number of species we finally were able to grow. However, there were striking results showing notably that positive interactions exist at intermediate wheat densities that enhance wheat yield in presence but not in absence of cereal weeds. Similar findings have already been reported by Dutoit *et al*. (2001) for one species. One putative explanation for these results is the release of allelopathic substances repressing wheat parasites in the soil (Qasem and Foy 2001), but this should be tested in an experimental approach in more detail. Dutoit *et al*. (2001) show however that rare cereal weeds can be have a high competitive effect on wheat, especially for the species with higher plant height.

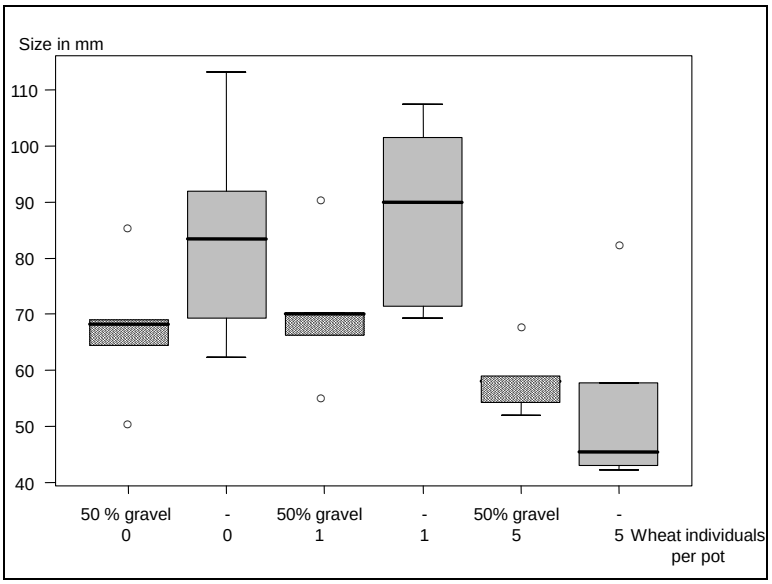


Fig. C.3: Size of *Agrostemma githago* in mm under competition between Durum wheat (*Triticum durum*) in normal soil conditions (grey boxes) and stony (dotted boxes) soil with 50 Vol % stones in a watered common garden experiment, each box plot represent 5 replicates.

Figure C.3 shows one important competitive effect of wheat on cereal weeds: there is a proper decrease in the performance of cereal weeds under strong densities. In the same way, this experience also showed that the decrease due to competition with the cultivated crop is only important in the stone free soil, i.e. under good soil nutrient and moisture conditions. High densities of the cultivated crop have, however, only limited effect on the performance of cereal weeds when the amount of fine soil is reduced by the presence of many stones in the soil. This moderates the findings of Roche *et al.* (2002) who showed that rare cereal weeds perform relatively better than ubiquitous weeds under high wheat density. First, they reported 'relatively' that is the absolute abundance of rare cereal weeds was smaller under high density than under low and it is only the part of rare cereal weeds on all weeds that increased. Second, they worked on a large field data set giving a mean view, this corresponds probably better to the 'no stone' condition in our experience, which diminished by half the performance of *Agrostemma githago* compared to the best condition. In conclusion, the competition of high-density cultivated crop can severely decrease rare cereal weed performance, a point of view that is shared by Schneider *et al.* (1994). In unproductive environments, such as very stony soil, this is not an issue; here the low performance decreases only by less than 20%. Therefore, the competitive effect may be important only on small spatial scales, on the best parts of a particular field, whereas the stony parts of the field, and this is a widespread situation in our study area, are not concerned.

General conclusions

DISPERSAL TRAITS AND A BASIC CONSIDERATION OF DIFFERENT PLANT TRAITS

The discussion of both the influence of pollen:ovule ratio and the survival of seeds in the soil showed that these traits had no direct function in the performance of plants. They modified the evolutionary flexibility of plants by enhancing gene exchange *via* pollen or *via* seeds

among populations but also among individuals of the same population (Jain 1976). In this way, they increase the effective population size and the diversity in terms of individual genetic diversity or genetic diversity at a population level (Silvertown and Charlesworth 2001). The importance of genetic diversity for population survival is evident. However, the examples of herbicide resistance showed that even singular genetic information could have an enormous impact on population growth and size. When comparing performance traits such as SLA, plant height or even seed size and number to dispersal traits on their generality to predict extinction or population sizes, dispersal traits have a much larger explanatory power because they consider evolutionary flexibility and not only mechanistic reaction.

This is a fundamental difference between performance and dispersal traits and emphasises the importance of evolutionary sound interpretations in ecology. Without taking into account the possible effects of evolution, any prediction in biology will remain very limited. Evolution constantly changes biotic conditions and environmental changes constantly the abiotic conditions. In both cases, there is no return to exactly the same conditions, which makes ecology necessarily a historical discipline. This is confirmed by the fact that evolution acts on sufficiently small temporal scales, as illustrated by the examples on herbicide resistance and 'rapid evolution' (Hairston *et al.* 2005).

Our data highlight dispersal traits as a reliable indicator for ecological parameters such as population persistence and local abundance. This underlines the importance of creation, spread and function of biological information, which does not follow mathematical rules, but is rather characterised by uniqueness. Many biological innovations are unpredictable; functional trait and phylogenetically explicit analyses try to sieve out the predictable or repeated part of evolutionary history (Harvey *et al*. 1995;Westoby *et al*. 1995a;Westoby *et al*. 1995b). However, these works systematically disregard the uniqueness of biological innovations as insignificant and only focus on general rules. At the long sight, these

approaches risk to withdraw from ecology its independence towards other, notably physical, sciences where information cannot change the behaviour of the system (Mayr 2004).

OBSERVATION INFLUENCES RESULTS: THE CASE OF SEED BURIAL AND GERMINATION

When analysing the burial experiment in chapter 3 and in the general discussion above, we suggested that large seeds become more crowded in the mesh bags we used. The higher available biomass and lower distance between each seed can enhance propagation of fungi and in this way lead to a lower soil seed survival of these seeds compared to smaller seeds or to situation outside the experiment. This is one example where the experiment itself modifies the relation between seed size and seed survival and is a possible experimental bias we did not expect. Similarly, we studied germination in complete darkness, that is, we prepared Petri dishes with seeds and filter paper in them in light and started the darkness experiment by watering them in complete darkness and closing the Petri dishes with Parafilm in order to minimize water losses. These dishes have only been controlled once, after four week of exposition to the different temperature regimes tested. Even the small amount of safety green light which we while counting the seedlings later is known to enhance germination for some species (Baskin and Baskin 1998), which would overly alter the outcome in a comparative work among species. In this case, we had to make a choice between observing these seedlings at this moment (altering the experimental conditions) or not observing, lacking probably important data.

These two examples show that experimental data have always limited value for the prediction in real world situations and that in some cases it is even impossible to gather data without changing important conditions of the system under study. The observation of spontaneous systems in the field can thus still yield data with a different quality than field or laboratory experiments and can still add significant complementary evidence on the function of ecological systems, *e.g.* soil seed banks and seedling emergence.

SCALING UP FROM SOIL SEED PERSISTENCE TO POPULATION PERSISTENCE AND DIVERSITY

In chapters 2 to 5, we could illustrate the importance of soil seed persistence for population persistence. The burial experiment and the analyses of germination tests in chapter 3 showed, that this factor acts on a smaller number of individuals than the entire population, on a faster time scale than population extinction and on a smaller spatial scale than population persistence. However, this factor had a clear influence to processes at the next higher, population dynamic scale. That this factor is not very tightly related is evident as population persistence involves any individual at one place, also standing vegetation and migrants and it is related by dispersal two other populations and can be influenced by them and therefore may act on longer time scales than seed persistence. On the next higher scale, population persistence is linked to community diversity by persistence of species *in situ* but also by dispersal to locally adjacent communities. This shows that diversity at local community and regional scales cannot be disconnected from processes at the single stage and population scale. There is a shift of importance of different factors according to scale. Some traits such as dormancy or soil seed survival, which are important on a local scale, are less important on the regional scale.

STORAGE EFFECT EXPLAINS SOIL SEED BANK ECOLOGY IN AGRO-ECOSYSTEMS

Storage effect explains how coexistence of a subordinate species is possible in the presence of a superior competitor. The model predicts that a life stage that buffers against reproductive failure, such as soil seed bank can promote coexistence when there are unpredictable environmental changes with correlated changes of competitive intensity. Is this model is applicable in arable land? In arable land under cereal cultivation, there is competition of cereals that diminishes reproductive success of cereal weed species. However, cereal weeds perform relatively better in dense cereals as standing crop than in other cultures or mixtures

with non cereal weeds (Dutoit *et al*. 1999;Roche *et al*. 2002), but this is relative abundance, the absolute abundance follows the opposite trend.

One of the main competitive adversaries of cereal weeds in arable land, are other plants in the crop rotation, in our case legumes that are planted as intercrop every 3-5 years. In this time, most cereal weeds do not or only little reproduce and disappear sometimes completely from the above ground vegetation.

Additionally, the high interannual rainfall variability leads consequently to low predictability of favourable years (see introduction and fig. C.4).

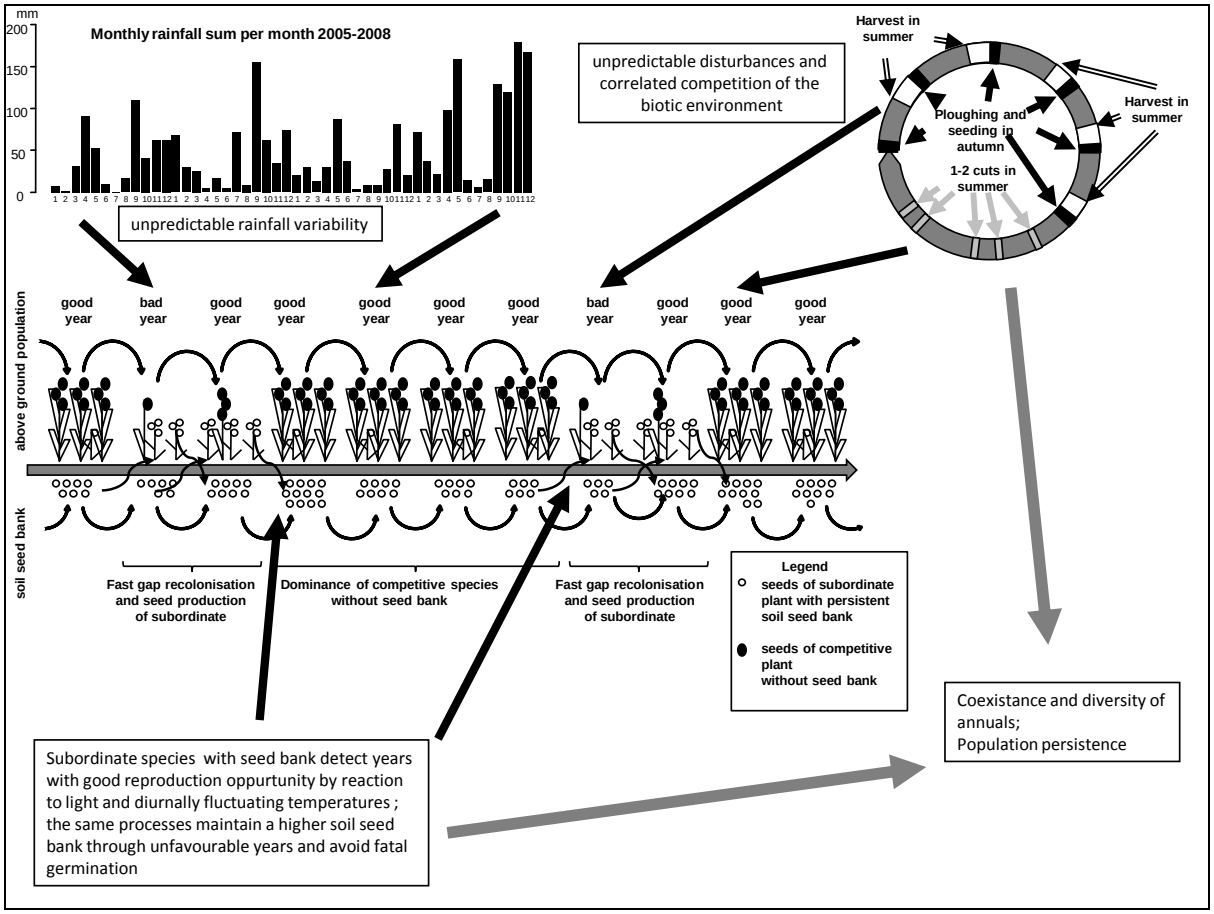


Fig. C.4: Storage effect in cereal fields and adaptations of cereal weeds to overcome unfavourable years in the crop rotation.

This is correlated to year-to-year fluctuations in cereal yield and one can imagine that the competitive strength of cereals towards cereals weeds goes in the same direction. Competition has been shown to be less important in constrained, especially dry environments (Michalet *et al*. 2006) and we showed that there are (i) many positive

interactions between cereal weeds and wheat and (ii) limited interactions in stony soils, with a much better performance of the cereal weed in stony compared to normal conditions when density is high (Fig. C.3). Therefore, one may think that dry years represent years with a better reproduction of cereal weeds because competition is less heavy, a hypothesis worth to be tested under field conditions. In addition, it is well known that the fields with the richest cereal weed flora are on very dry, stony fields at the margin of economic equitability.The importance of unpredictable disturbances for community diversity together with the intense human land use and climatic instability may also be an explanation why there are so many annual plants in the European Mediterranean climate zone. In European Mediterranean climate zone, annual plants are much more important in number of species compared to the other Mediterranean zones where rainfall is higher, more predictable and intense human disturbances are of much younger date (Hobbs and Huenneke 1992).

CONSERVATION ISSUES

There was a clear recent decline of species with short-lived soil seed bank in the last two decades in the study area. Visibly, species with short-lived seeds in the soil are more threatened than species with a persistent seed bank. This complements the findings of Dutoit *et al*. (2003) who showed that most species cannot recover from the seed bank after long abandonment and thus restoration in this way is impossible. We therefore suggest for conservation of these species to pay particular attention not to abandon fields longer than 2 years or to cultivate other crops than winter cereals for more than 2 years. Evidently, for the shortest-lived species, this is still too long and alternative conservation strategies, such as uncleaned seeding material discussed below, should be used.

The correlation of pollen:ovule ratio with extinction rate at the long time scale (fig. C.2) showed the importance of exchange of genetic diversity and pollen dispersal processes for the maintenance of populations. Equally, the data on the local abundance presented in

chapter 5 shows that species with small populations have low P/O and probably low gene exchange *via* pollen. This permits to identify low P/O species as particularly threatened with extinction in the studied context.

The paragraph on dispersal of seeds in space showed that for an identified set of species, those with low soil seed survival and limited pollen exchange, the reseeding with uncleaned seed material is essential for their conservation in the field. This is crucial for species such as *Agrostemma githago, Vaccaria hispanica* and *Asperula arvensis* (Schneider *et al*. 1994) for all of which we could show very low seed survival in the soil in chapter 2. However, the use of uncleaned seeding material should also generally favour the maintenance of rare cereal weeds, because even if there are surviving seeds in the soil survival is very variable among species, with the most interesting species declining fast and only a very limited set of species can re-establish after abandonment from the soil seed bank under field conditions (Dutoit *et al*. 2003). The high diversity and abundance of rare cereal weeds in uncleaned seeding material found by Jäger (2002) is a promising path to restore high diversity and populations of rare species.

The discussion of the storage effect and the adaptations of species to detect favourable years illustrated in chapter 3 show that inter-annual variability plays a role to promote the diversity of annual cereal weeds. This inter-annual variability of favourable years for cereal weeds is enhanced in Mediterranean system by the occurrence of dry years. Low moisture conditions of dry years, similarly to the competition experiment with stony soil, can promote coexistence and hence reproduction the subordinate species (Cáceres 1997;Facelli *et al*. 2005;Sears and Chesson 2007). We therefore suggest the following scheme for cereal fields: in good years, cereal growth is optimal and rare cereal weed reproduction hampered, in dry years, cereals are less performing and cereal weeds can reproduce. Nevertheless, this effect can also be triggered by years with suboptimal agricultural practices such as low density seeding, bad cereal seed material, accidents in the soil preparation etc. This re-emphasises

that the very standardised and homogenised disturbances in industrialised agriculture are not compatible with the maintenance of a rich weed flora, a finding that is confirmed by the observation that in some well-organised organic cereal fields there are no rare cereal weeds.

Finally, there are details in the soil preparation for cereal fields that can increase the occurrences of rare cereal weeds such as autumn ploughing and seeding (Schneider *et al*. 1994;Roche *et al*. 2002). We could confirm in chapter 3 that most of the studied species germinate directly, without stratification at low temperatures a situation that occurs in autumn after the first rainfalls at cold temperatures and is typical for the germination conditions of Mediterranean annuals and rare cereal weeds (Baskin and Baskin 1998;Baskin and Baskin 2006). For the conservation of a typical cereal weed flora ploughing and seeding in the cold part of the year should therefore be followed. However, there are exceptions to this, one of the rarest cereal weeds in our study area, *Conringia orientalis*, only germinated under high temperatures. A set of cereal weeds, such as *Ranunculus falcatus, Consolida regalis* and *Bupleurum rotundifolium* germinate best at very cold temperatures (< 10°C) and after retrieval from burial germinate not immediately but in the chilling phase at 4°C. The latter pattern is conspicuous in the Mediterranean rare cereal weeds *Hypecoum pendulum, Garidella nigellastrum* and *Papaver hybridum*. We have no precise information of when these species germinate in the field, and it would be particularly interesting to know if they germinate only late after ploughing in autumn or if ploughing at very low temperatures is mandatory.

In conclusion, precise management suggestions can be given on the grounds of the data presented in this thesis and previous work on rare cereal fields. The discussion on ploughing dates and cold germination of rare cereal weeds, the dispersal processes and soil seed survival showed also that there are big differences among species. On the grounds of the presented data and data from literature, we should synthesise the data in order to classify species in highly and low vulnerable and different management groups.

Perspectives

Seed banks are known to form a considerable genetic reservoir for local populations. In fact, from a population genetic point of view, seed banks of species with persistent seeds are more diverse and less geographically differentiated than above ground populations (Cabin *et al*. 1998;Hock *et al*. 2008). It is also clear that seed banks have an important role for population genetics, as they enhance the stability of local populations with respect to environmental hazards (Venable 1989;Venable 2007), but also when competing with other plants for regeneration niches (Chesson and Warner 1981;Warner and Chesson 1985;Facelli *et al*. 2005). The role of this reservoir of seeds has been only little studied from a functional and genetic point of view. In future work it would be interesting to answer questions such as: (i) What is the germination niche of the seed bank population compared to a one year seed generation? – We expect that the regeneration niche is much larger with respect to general gradients important to germination. Reasons are the higher number of genotypes in the seed bank; the higher number of different seed generations with a particular dormancy state for each one; a general effect of after-ripening and stratification in the soil leading to a broader germination niche. (ii) What is the genetic differentiation of seeds along gradients of germination conditions (temperature, light, fluctuations) compared to one single year of seed generations? – We expect that there is a genetic differentiation of seeds germinating under different germination conditions, and that there is a higher among germination conditions than within experimental germination conditions. We expect also that for a set of regional populations genetic differentiation is high when comparing different population inside the same germination conditions.

A second aspect, is the re-interpretation of diversity of annual plants in the light of predictability of climate. This has not yet been explored in more detail, there is still only little evidence how annual plant diversity is distributed around the globe, and relatively simple

biogeographic analyses can enhance the understanding of the relations between annual plant diversity and climatic predictability.

Third, all the long of the chapters 2 to 5, we refer to storage effect as a model to better understand adaptations at the seed bank and germination level (see also fig C.4). In the chapter 3 on functional ecology, we demonstrated adaptations in germination and seed traits that optimise establishment in temporally variable habitats. Additionally, in chapter 2 on the measure of soil seed bank persistence, we point out that the seed size–seed number trade-off has a major influence on how we perceive soil seed bank composition and abundance and that our previous perception is not necessarily useful for prediction of successful establishment. These two points can have effects on the strength of the storage effect. A good detection of opportunities for reproduction enhances the longevity of soil seed bank and can enhance the coexistence of species. How can we measure the importance of these detection mechanisms for coexistence? In experimental communities, do species without detection mechanisms coexist for shorter time than species with?

A second issue is the spatiality of storage effect. This aspect has recently been addressed and the model has been extended (Sears and Chesson 2007). It would now be interesting to test hypothesis to evaluate the spatial aspects on more natural systems.

The importance of different adaptations for coexistence and diversity exemplified by light requirement for germination, gap detection mechanism and dormancy has also consequences for conservation: species that bear these adaptations and ecosystems that are characterised by many of such species diversity can best be maintained by unpredictable conditions and disturbances and not by exclusion of disturbances. For practical conservation, there are hence questions such as: Can disturbances that destroy above ground populations enhance long-term population persistence? Are there differences in genetic diversity between populations in high compared to low disturbance frequency or high and low predictability of favourable years? Obviously, frequent disturbances and unpredictability of

favourable years are only important for diversity of a part of flora and cannot enhance diversity *e.g.* for long-lived perennial or woody plants. However, these plants also contribute to local plant diversity. In a first step, it would therefore be interesting to know how disturbances vary spatially and how diversity at different scales of long-lived compared to short lived plants can be related to spatially heterogeneous disturbances.

From a conservation point of view, several aspects need to be checked in order to give consistent suggestions for management. A first aspect is the germination in the field and the timing of ploughing. A detailed field work should figure out at which moment seedlings emerge after ploughing and if there is a direct relation between temperature at ploughing date and germinating species. It should be cleared if there can be delayed germination of species that need cold stratification or very cold temperatures for germination. Second, the importance of environmental conditions, such as dry habitats, dry years, heterogeneous or low cereal density and stony soils are suggested on several occasions to be of high conservation interest. Future studies should estimate if these conditions are sufficient. This is an opportunity to run an experimental field work that quantifies the importance of habitat conditions on rare cereal weed reproduction. Such a detailed experimental work on temporally changing habitat conditions could also more formally test the theoretical expectations of the storage effect and enhance very generally our understanding how rare annual plant diversity can persist.

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APPENDIX

RESUME FRANÇAIS

Dynamique des populations et traits fonctionnels des plantes annuelles - une étude comparative sur la persistance des adventices rares et communes dans les agro-écosystèmes

L'industrialisation continue de l'agriculture est responsable d'importants changements de composition et d'un appauvrissement des cortèges de plantes vasculaires dans les agro-écosystèmes Européens. Cependant, les fragments d'espaces agricoles encore exploités par une agriculture plus traditionnelle conservent de nombreuses espèces végétales annuelles des cultures de céréales –les messicoles– notamment dans le Sud Est de la France. Leur conservation *in situ* est difficile en raison du manque d'informations sur les caractéristiques biologiques qui déterminent leur rareté et la régression de leurs populations. Des données sur la longévité de leurs semences – un facteur primordial pour la dynamique des populations de plantes annuelles- peut aider à hiérarchiser les efforts de conservation. L'objectif principal de cette thèse est donc d'étudier la persistance des populations à long terme, l'abondance et la rareté des messicoles en relation avec leurs traits d'histoire de vie, leurs traits physiologiques et traits de graines afin d'identifier les espèces les plus menacées. Nous prenons comme modèle les espèces annuelles en raison de la simplicité et de la rapidité de leur cycle de vie et les champs cultivés comme modèle pour leurs changements d'usages rapides et intenses dans le temps et l'espace.

Premièrement, nous analysons les diversités α et β à différentes échelles dans un paysage viticole du Luberon en utilisant le concept du partage additif de diversité. Nous avons identifié le type d'habitat, l'intensité des pratiques, le type de paysage et le passé cultural comme patrons de la diversité végétale et du maintien des espèces à fort enjeu de conservation. Comme la majorité des espèces cibles sont habituellement liées aux champs de céréales, nous avons limité par la suite cette étude aux messicoles.

Deuxièmement, en raison de l'importance de la longévité des semences dans le sol pour expliquer la dynamique des populations, nous avons conduit une expérience d'enfouissement des graines pour étudier la survie et les degrés de dormance des espèces. D'importantes différences avec les données auparavant connues sur ces espèces nous ont motivé de réévaluer les méthodes actuelles de l'étude des banques de graines dans le sol. La méthode de l'émergence des plantules et l'indice de longévité qui en dérive se révélaient en effet corrélé à la production de graines et non pas à la survie des graines dans le sol.

Troisièmement, nous analysons à nouveau la survie des graines dans le sol grâce aux données sur la germination. La survie des graines se révèle déterminée par une germination dépendante de lumière ou de températures fluctuantes au cours de la journée ainsi que des niveaux de dormance et de taille des graines. Ces mécanismes expliquent comment les graines peuvent se maintenir viables dans le sol.

La régression entre 1983 - 2005/2006 et la dynamique de populations ont été corrélées à la survie des graines dans le sol, au niveau de dormance, à la dépendance de lumière et de températures fluctuantes pour la germination, à la surface spécifique et la teneur en masse sèche des feuilles ainsi qu'au ratio pollen/ovule (P/O). Ces analyses ont été complétées par l'utilisation de contrastes phylogénétiquement indépendants. Il en découle que la survie des graines est un facteur majeur pour l'extinction sur une période de vingt années et que cela ne peut pas être expliqué par des changements édaphiques directionnels, car ils ne sont pas détectables pour le niveau trophique, l'acidité du sol ou l'humidité.

Finalement, nous analysons deux aspects de la rareté des espèces végétales –l'abondance locale et la fréquence régionale des populations- et leur relations avec les traits biologiques et d'histoire de vie. Nous mettons en évidence que peu de relations sont identiques à différentes dates à l'exception de la survie des semences dans le sol. Cependant, les analyses de la régression et de la rareté soulignent toutes les deux le rôle important du P/O –traceur des échanges génétiques *via* le pollen- pour la rareté et la régression des espèces messicoles.

En conclusion, les espèces à forte mortalité séminale dans le sol et à P/O faible sont les plus menacées de disparition locale et devront être ciblées prioritairement pour la conservation. L'écologie de la germination et de la survie de graines dans le sol indiquent que la variabilité temporelle mésologique et des années défavorables peuvent augmenter la diversité des messicoles au travers de l'effet de stockage. Néanmoins, des différences fortes existent entre espèces. Pour les espèces les plus menacées un maintien de l'utilisation de semences fermières non triées semble nécessaire pour leur maintien à long terme dans les agro-écosystèmes.

Mots clés: banque de semences du sol – messicole – dispersion en espace – agriculture – mauvaises herbes des champs de céréales – germination – détection de niches – fluctuations de température journalières – diversité – méditerranéen.

DEUTSCHE ZUSAMMENFASSUNG

Populationsdynamik und funktionelle Merkmale von einjährigen Pflanzen-
eine vergleichende Studie zur Populationspersistenz von seltenen und häufigen Segetalpflanzen in Agroökosystemen

Die Industrialisierung der Landwirtschaft führt zu kontinuierlichen Veränderungen in der floristischen Diversität und Zusammensetzung Europäischer Agroökosysteme. Im Südosten Frankreichs erhalten die Reste traditioneller Landwirtschaft viele der sehr selten gewordenen Segetalarten, obwohl auch hier ein starker Rückgang zu beobachten ist. Für den Schutz dieser Arten mangelt es an detailliertem Wissen über die biologischen Gründe für die großen Unterschiede in Populationspersistenz, Abundanz und Seltenheit dieser Arten. Daten zur Langlebigkeit der Diasporen im Boden –ein wichtiger Faktor für die Populationsdynamik einjähriger Pflanzen- kann helfen Schutzbemühungen sinnvoll zu orientieren. Gegenstand dieser Dissertation ist es daher die Bezüge von langzeitlicher Populationsdynamik, Abundanz und Seltenheit zu Lebenszyklus-, Samen- und physiologischen Charakterzügen dieser Arten zu untersuchen, auch um Unterschiede in der Gefährdung der Arten herauszustellen. Wir untersuchten explizit einjährige Pflanzen wegen ihres Einfachheit und schnellen Reaktion auf Veränderungen und Agroökosysteme wegen deren starken und schnellen Veränderungen.

Zuerst untersuchten wir α- und β-Diversität auf verschiedenen räumlichen Skalen in einer Weinbaulandschaft und konnten Habitattypen, Landbauintensität, Landschaftstyp und frühere Bewirtschaftung als wichtige Einflussfaktoren für pflanzliche Diversität und das Vorkommen von Zielarten herausstellen. Da nahezu alle für den Naturschutz bedeutsamen Pflanzen der Segetalflora angehören, beschlossen wir im Weiteren nur seltene und nah verwandte häufige Segetalarten zu betrachten.

Die Bodensamenbank ist eine wichtige Phase im Lebenszyklus einjähriger Pflanzen. Daher untersuchten wir gezielt die Samenmortalität und Dormanz an 38 Arten in einem vergleichenden Vergrabungsexperiment. Die auffälligen Unterschiede zu anderen Studien ermutigte uns bisherige Methoden neu zu evaluieren. Dies zeigte, dass das Schätzwerte der Langlebigkeit in der Bodensamenbank die auf der Sämlingsauflaufmethode und dem davon abgeleiteten Langelbigkeitsindex beruhen mit der Samenproduktion aber nicht mit der Samenmortalität im Boden zu korrelieren sind.

Die Samenmortalität im Boden wurde danach zusammen mit Daten zur Keimungsökologie untersucht. Lichtkeimung, Dormanz, Reaktion auf täglich fluktuierende Temperaturen und Samengröße hatten alle einen Einfluss auf die Samensterblichleit im Boden. Diese Faktoren erklären wie langlebige Samen Keimung im Boden steuern können.

In einem weiteren Ansatz verglichen wir Daten zur Samenmortalität, Dormanz, Samengröße, und -produktion, spezifischer Blattfläche, Blatttrockenmassengehalt und dem Pollen/Ovulen-Verhältnis mit lokalem Populationserlöschen und Populationsturnover zwischen 1983 und 2005/2006. Dazu nutzten wir auch phylogenetisch unabhängige Kontraste. Samenmortalität im Boden stellte sich als einer der wichtigen Faktoren für lokales Aussterben auf lange Sicht heraus, dies konnte nicht mit gerichteten Veränderungen in edaphischen Faktoren erklärt werden.

Zuletzt untersuchten wir zwei Aspekte der Seltenheit, lokale Abundanz und regionale Frequenz von Populationen und deren Bezüge zu funktionellen und Lebenszyklusmerkmalen. Nur wenige Bezüge waren unabhängig vom Beobachtungszeitpunkt, darunter Samenmortalität im Boden, ein Faktor möglicherweise durch effektive Populationsgröße wirkt. Beides, der Rückgang und sowie die Seltenheit von Populationen zeigten enge Korrelation zum P/O-Verhältnis, der als ein Indikator des Genaustausches über Pollen gelten kann.

Zusammenfassend kann gesagt werden, dass Arten mit hoher Bodensamenmortalität und niedrigem P/O-Verhältnis besonders gefährdet sind und bei Naturschutzmaßnahmen besonders berücksichtigt werden sollten. Keimungsökologie und Bodensamenbankexperiment zeigten zudem, dass zeitliche Variabilität und „schlechte" Jahre zur Diversität von Segetalpflanzen durch die Wirkung des „storage effects" beitragen können. Es gab allerdings große zwischenartliche Unterschiede. Um viele der stark zurückgehenden Arten dauerhaft im Freiland erhalten zu können ist die Nutzung von ungesäubertem Saatgut unumgänglich.

Schlüsselwörter: Bodensamenbank – Segetalpflanzen – zeitliche Samenausbreitung – Landwirtschaft – Getreideunkräuter – Keimung – gap detection – im Tagesrythmus schwankende Temperaturen – Vielfalt – Mediterran.

ABSTRACT

The continuing industrialisation of agriculture leads to important changes in composition and decrease of plant diversity in European agro-ecosystems. Remnants of traditional agriculture conserved many but declining rare cereal weeds in South Eastern France. Conservation efforts for them are hampered by the little evidence on which differences in their biology are related to population persistence, abundance and rarity among species. Data on longevity of seeds in the soil, an important factor for annual plant population dynamics, can help to prioritise conservation efforts. The main aim of the present thesis was therefore to study the relations between long-term population persistence, abundance and rarity together with life history, physiologic and seed traits in order to identify species most at risk. We studied annual plants -simple and rapidly reacting- in arable fields -an environment with rapid and drastic changes.

First, we analysed plant α- and β-diversity in vineyards at different spatial scales, using additive diversity partitioning. We identified habitat types, intensity of agriculture, landscape type and land use history as main determinants for plant diversity and maintenance of species of conservation interest. Nearly all target species are known to be cereal weeds; we therefore restricted the study on rare and common relatives of annual cereal weeds for the remainder of the study.

Soil seed banks are known to be an important life stage in annual plants for population dynamics. Therefore, we did a comparative seed burial experiment with 38 species to study soil seed survival and levels of dormancy. The striking differences with previous data motivated us to re-evaluated current methods. This showed that the soil seed bank persistence estimates from seedling emergence method and derived seed longevity index (L.I.) are correlated to seed production but not to soil seed mortality.

Third, we re-analysed soil seed survival with data from germination experiments. Light requirement, degree of dormancy, reaction to diurnally fluctuating temperatures and seed size were related to survival of seeds in the soil giving insights into how long-lived species can stay ungerminated while buried.

Fourth, we compared data on soil seed survival, degree of dormancy, seed size and number, specific leaf area, leaf dry matter content and pollen:ovule ratio (P/O) to data on local population extinction and turnover from 1983 to 2005 and 2006, in an approach using phylogenetically independent contrasts (PICs). This revealed that soil seed survival was a major correlate of extinction on the long time step and that this could not be explained by directional changes in edaphic factors.

Finally, we studied two axes of rarity, local population size and regional frequency of populations and their relation to biological and life history traits. This yielded few results consistent among different dates. An exception was soil seed survival; probably in relation with increased effective population size. Both analyses - on rarity and on regression- emphasised also the role of P/O –a monitor for gene exchange via pollen- for rarity and regression of annual cereal weeds.

In conclusion, species with high soil seed mortality and low P/O are most at risk of local extinction and should be considered in conservation efforts. The germination ecology together with the soil seed survival indicated that temporal variability and unfavourable years can trigger annual cereal weed diversity through storage effect. However, there are striking differences between species. For the most regressing species seed dispersal via uncleaned seed material is necessary to conserve them at the long run in rapidly changing agro-ecosystems.

Keywords: soil seed bank – segetal – dispersal in time – agriculture – cereal weeds – germination – gap detection – diurnally fluctuating temperatures – diversity –Mediterranean.