Seed Ecology VII - Book of Abstracts

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Preface

The seventh edition of the Seed Ecology Conference of the International Society for Seed Science takes place in Gijón/Xixón, Asturias (Spain) from September 6 to September 9, 2022.

Chapter 1

Keynotes

1.0.1 Macroecology of seed dispersal distance

KEYNOTE

Si-Chong Chen

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Seed dispersal is critically important in plant regeneration, but patterns in seed dispersal distance have been much less studied at a broad scale. We collated the most geographically and taxonomically broad database of seed dispersal distance. With this collation, we showed a latitudinal gradient in seed dispersal distance, with seeds dispersed on average more than an order of magnitude further at the equator than towards the poles. This pattern is partially explained by plant life-history traits that simultaneously associate with seed dispersal distance and latitude, including dispersal mode and plant height. Species that dispersed far are more likely to be those long-lived species with large, non-dormant seeds. This indicates that spatial dispersal and temporal dispersal are bet-hedging strategies. Since seed dispersal is a complex process, we also evaluate the relative importance of each phase in species having more than one dispersal phase. Dispersal distances of the two diplochorous phases were independent and comparable to that of single-phase dispersal. Therefore, the two phases of diplochory form a coordinated dispersal strategy. These findings allow a better understanding of plant reproductive strategies and a potential avenue to predict restoration potential, community assembly and species persistence under global changes.

1.0.2 Using seed ecology to inform biodiversity conservation

KEYNOTE

Mark Ooi

University of New South Wales, Australia

Many questions remain surrounding both the importance of less commonly measured seed traits and the response of well-studied traits to shifting conditions. Climate change is leading the shift in ecological processes, and fires are arguably one of the most spectacular examples of such shifts. Fire regimes are changing and recent megafires around the world are examples of the extremes that fire events can reach. Plant species are adapted to historic fire regimes, persisting within thresholds of fire frequency, seasonality, severity and extent. Developing an understanding of how shifting fire regime elements can impact persistence and recovery of plant species is therefore essential for conserving biodiversity. Seeds provide an incredible resource in this endeavor. They are key drivers of plant population dynamics and their study can allow us to predict the trajectories of functional groups of species. Seeds are also critical for applied restoration, where plant populations have been pushed past the limits for natural recovery. Drawing on the results from recent research, I will look at how different elements of the fire regime, including extreme fire severity and out-of-season burns, interact with plant and seed ecological processes to determine which species may persist and which will struggle. While the reduction of climate change impacts requires a global effort, a clear understanding of local processes can help to provide informed conservation management, while local restoration efforts can help to minimize negative impacts for those species most at risk.

1.0.3 Seeding the future: evolutionary perspective on seed-based restoration

KEYNOTE

Anna Bucharova

University of Marburg, Germany

Restoration of degraded habitats is an indispensable tool for combating the current biodiversity crisis. As degraded habitats often lack plant diaspores, successful terrestrial restoration commonly requires introduction of plant seeds from other sources. During the seed production and restoration seeding, plants repeatedly face novel environments, which provides an excellent opportunity to study rapid adaptation. Restoration seeds are collected in the wild and either directly used in restoration or propagated on seed farms to increase their amount. However, agricultural propagation imposes a specific selection pressure which shifts plant trait distributions towards larger size and synchronized flowering – in parallel with domestication syndrome in crops. Fortunately, these changes are rather small, affect only a fraction of cultivated species, and do not reduce plant adaptation to stress. The plants from farm-produced seeds even show signs of regional adaptation, suggesting that their natural integrity is largely maintained through the cultivation process. Restoration seeds are sown to degraded or destroyed habitats whose soils, water regime or management typically differ from the conditions at natural sites. We have shown that some species adapt to such new conditions; for example, plants from restored grasslands flower earlier and have stronger clonal propagation than conspecifics from natural grasslands that served as the seed source for the given restoration project. Such rapid adaptations may contribute to species persistence at restored sites and ultimately to restoration success.

1.0.4 Seeds and climate change: what can we learn from alpine and arctic biomes?

KEYNOTE

Andrea Mondoni

University of Pavia, Italy

Plant regeneration from seeds will undoubtedly be affected by climate warming because it is a highly sensitive and climatically driven stage of plant development. Furthermore, the genetic diversity, phenotypic plasticity and dispersal ability of seeds might be crucial for species persistence and/or migration. The alpine and arctic life zones, with their cold-adapted species and some of highest rates of warming, provide excellent opportunities to assess climate-related impacts on plants. Here, we synthesize current knowledge on seed traits and functions that largely determine the ability of plants to disperse, persist, germinate, and establish. Using this information, we identify the main effects of climate change on regeneration by seeds for species typical of alpine and arctic environments and suggest future research on the topic.

Chapter 2

Macroecology and evolution of seeds

2.0.1 Ecological correlates and evolutionary history of post-dispersal embryo growth and embryo growth rate in angiosperms

ORAL

Vandelook, F.[1], Saatkamp, A.[2], Pritchard, H.W.[3], Rosbakh, S.[4], Mattana, E.[3], Fernández-Pascual, E.[5], Dickie, J.[3], Carta, A.[6]

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Several angiosperm lineages disperse seeds with small embryos and copious endosperm or perisperm. Nutrient reserves are transferred to the embryo either before germination, during post-dispersal embryo growth; or following germination, with the embryo acting as haustorium. Until now post-dispersal embryo growth has been viewed mainly as a dormancy mechanism, termed 'morphological dormancy'. To re-assess the ecological role and evolutionary history of post-dispersal embryo growth and embryo growth rate, we performed a literature search for studies documenting embryo growth and analyzed relations with other plant traits, climate and habitat variables. We compiled a list of over 150 studies presenting data on embryo growth for over 250 angiosperm species. Wherever possible, for each species we collected data on the extent of embryo growth, embryo growth rate, seed mass and germination timing. In addition, for all species we collected data on adult plant and seed functional traits (e.g., seed mass) and the climate and habitat conditions the species grow in. When we related the total growth of the embryo prior to germination to the time required to complete embryo growth only a weak positive linear relationship was observed, suggesting a poor relationship between embryo growth and germination timing. Post-dispersal embryo growth predominantly occurs in species growing in moist and shaded habitats and has been studied mostly in temperate climates. Plausible explanations for the variation in post-dispersal embryo growth are not limited to germination timing and include predation escape, efficient nutrient supply, seedling growth and avoidance

of parental conflicts.

2.0.2 Macroevolutionary correlation of seed traits in flowering plants: current advances and future prospects

ORAL

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Seeds show important variation as plant regenerative units among species, but their macroevolutionary co-variations with other plant characteristics are still poorly understood. Since the evolution of single seed traits cannot be understood without considering their relation with other traits, assessing and understanding co-variations is crucial. For example, whilst a positive association of seed mass with genome size (GS) has already been documented, a broad-scale quantification of their evolutionary correlation and adaptive selection has only recently conducted. GS is highly variable across angiosperms, but there is strong evidence that it is correlated with both cell division and tissue growth rate. This correlation has important implication for plant functions, including seed germination regulating-processes. Recent evidences also highlight the importance to consider not only seed size, but also internal seed morphological traits, like embryo and endosperm sizes. Using exemplar case studies we explored the co-variations of seed mass, embryo and genome sizes over a dataset containing several hundred angiosperm species. We first estimated whether these traits are phylogenetically clumped; then using multivariate modelling we evaluated what evolutionary pressures may drive the observed patterns. Specifically, we highlighted the need to test for an asymmetry in the correlated evolution acting on seed traits and genome sizes due to life form and macroclimate. We believe that the availability of high quality data and modern analytical tools provide a new macroevolutionary framework for a deeper understanding of seed traits integration and the functional roles of reproductive traits.

2.0.3 The global seed germination spectrum of alpine plants

ORAL

Fernández-Pascual, E.[1], Carta, A.[2], Mondoni, A.[3], Cavieres, L.[4], Rosbakh, S.[5], Venn, S.[6], Satyanti, A.[7], Guja, L.[8,9], Briceño, V.F.[10], Vandelook, F.[11], Mattana, E.[12], Saatkamp, A.[13], Bu, H.[14], Sommerville, K.[15], Poschlod, P.[5], Liu, K.[14], Nicotra, A.[10], Jiménez-Alfaro, B.[1]

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We present a quantitative synthesis of the seed germination spectrum of a coherent global biome: temperate alpine habitats above the treeline. We created a collaborative database of 9,799 primary records from laboratory germination experiments, contributed by 12 research groups specialized on the topic. The database included data from four continents and 661 species. To analyse the database, we used Bayesian meta-analysis of primary data, estimating the influence of six environmental cues (scarification, stratification, GA3, average temperatures, alternating temperatures and light) on germination proportion, mean germination time and germination synchrony; accounting for possible effects of seed morphology (mass, embryo:seed ratio), phylogeny and between-study variation. In general, alpine plants (1) have physiological seed dormancy and thus need cold stratification to release dormancy, (2) germinate better at warm temperatures (20-25 ºC) and (3) show positive germination responses to light and alternating temperatures. Specialist species of the alpine belt have a more pronounced warm-cued germination and a stronger response to cold stratification than gener-

alist species that occur both in the alpine belt and below the treeline. Germination responses to the environment are constrained by seed mass, embryo size and phylogeny; with smaller and more endospermic seeds being more responsive to warmth, light and alternating temperatures. Globally, overwintering and warm-cued germination are key drivers of germination timing in alpine habitats. The interaction between germination physiology and seed morphological traits further reflects pressures to avoid frost or drought stress. Our synthesis indicates the convergence, at the global level, of the seed germination spectrum of alpine species.

2.0.4 No-dormancy in *Argyreia populifolia* seeds, an example of dormancy reversal in genus *Argyreia*

ORAL

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Phylogeny of seed dormancy of Convolvulaceae showed that Physical dormancy (PY) have evolved under seasonal environments, while PY has revert to no-dormancy under aseasonal environments. Thus, we hypothesized that as the endemic wet zone *Argyreia* species in Sri Lanka evolved under aseasonal environment, produce non-dormant seeds. Seed germination, storage behavior, dormancy and anatomy of *Argyreia populifolia* an endemic wet zone species in Sri Lanka were compared with those of *Argyreia nervosa* a widely distributed species in the Indian subcontinent. Intact seeds of *A. populifolia* germinated 92%, revealing that they are non-dormant. In contrast, manual scarification increased the germination percentage of *A. nervosa* (95%), compared to the non-scarified seeds (10%). Mass increment of manually scarified seeds of *A. nervosa* after 24hrs is significantly higher than that of the non-scarified seeds ($p < 0.05$) confirming PY. Dye tracking confirms the water permeability and impermeability of seed coats of *A. populifolia* and *A. nervosa*, respectively. Within 10min, stain was observed inside the seeds of *A. populifolia* while the dye did not diffuse in to seeds of *A. nervosa* even after 72hrs. Drying and germination experiments showed that seeds of both species have orthodox storage behavior. Palisade layer was observed in the seed coat of *A. nervosa* with a light line, while the palisade layer is restricted to the hilum area in *A. populifolia* seeds. According to the phylogeny of seed dormancy in Convolvulaceae, tropical recalcitrant, non-dormant seeds producing species given rise to orthodox, PY seed producing species when inhabiting dry dominated seasonal climates. PY seems to be reserved to non-dormancy when wet zone endemic species like *A. populifolia* has evolved from more widely distributed species like *A. nervosa* which may have evolved under dry-dominated tropical cli-

mate. Our observation provides an example of reversing PY to non-dormancy in another clade in Convolvulaceae.

2.0.5 Two life-forms - two patterns of seed morphology in Orchidaceae

ORAL

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Dust-seeds are one of the most common characters in the family Orchidaceae. In general, the seeds have a thin coat of dead cells and a pluricellular embryo surrounded by an amount of free air space. Morphological studies of seeds have been mainly developed in terrestrial taxa of the Northern hemisphere, focusing on qualitative and quantitative characters. Qualitative traits were referred to seed shape, morphology of the testa cells and their anticlinal and periclinal walls, ornamentation in the walls and the presence of testa extensions and verrucosities. Due to seed traits are considered more conservative than others, they have been used in taxonomic, phylogenetic and phytogeographic studies. The vast majority of orchids are terrestrial or epiphytic. Terrestrial species can be found throughout the distribution range of the family, but epiphytic orchids are mostly restricted to tropical and subtropical areas. Few studies have related the seed morphology with the habit. To address whether terrestrial and epiphytic orchids consistently differ in seed morphological characters, we analyse 14 traits in a comparative framework. We report a detailed morphological survey with the aim to identify the most diagnostic characters of seed morphology related to the habit. This survey includes a large representation of genera from the tribes Neottieae (exclusively represented by terrestrial orchids) and Vandeae (mostly represented by epiphytic orchids). Co-varying with the orchid habit, the seed coat showed differences in the orientation of the cells along the longitudinal axis, the width of periclinal walls, and the presence of waxes or testa extensions. Two main patterns have been observed according to the two main life-forms in Orchidaceae.

2.0.6 Orchid seed morphometrics as a predictor of germination behaviour and some ecological implications

ORAL

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Although, several members of Orchidaceae can be germinated easily on sterile nutrient media, there are numerous species where propagation has been completely unsuccessful. On the other hand, seed morphology of orchids has been previously associated with their dispersal ability but relations to germinability have not been generally addressed. We have gathered seed morphometry and germination data for 203 orchids species world-wide from the available international literature, complemented with unpublished results of our lab. On the basis of: 1) final germination percentage and 2) pre-treatment duration, two major groups of germination behavior (defiant and compliant) are identified. Seed morphometric and germination data along with corresponding data of several ecologically important parameters (habitat shadiness, mycoheterotrophy level, growth form, climatic zone, subfamily) are correlated and statistically analyzed. A strong relationship between the ratio of embryo length to testa length (E:T) and germination behavior is detected. E:T values tend to be lower for species with defiant germination, which generally abound in shaded habitats in contrast to compliant germinating species, which prefer open habitats. Based on the mycoheterotrophy continuum, we suggest that the relative reduction of the embryo size (as a fraction of the entire seed length) seems to be linked with several lineages adapted to shaded habitats and their increased dependence on fungal symbiosis.

Chapter 3

Seed dispersal and plant-animal interactions

3.0.1 Directed endozoochory: a hitchhiker's guide for clonal ericaceous plants to successful sexual reproduction

ORAL

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Many plant populations are both seed and microsite limited. Microsites are often spatially unpredictable and if they are also short-lived, they are called Recruitment Windows of Opportunity (RWO). Sexual reproduction then depends on the arrival of enough seeds at the right time. When endozoochorous dispersal vector behaviour helps seeds reach such suitable microsites, this is known as directed dispersal and can be distinctly advantageous. *Vaccinium* species are clonal, berry-producing ericaceous species for which both seed-supply and microsite limitation have been demonstrated, despite the fact that they produce copious amounts of berries. Seedlings must have suitable abiotic conditions for growth but also avoid competition from the standing vegetation. We hypothesized that directed endozoochorous seed dispersal by birds towards decomposing stumps facilitates seedling recruitment in *Vaccinium* species which utilize RWOs for their sexual reproduction. We tested this in a boreal forest ecosystem where *Vaccinium* dominates the forest floor. Bird scats and seedlings were both more likely to occur on stumps than on the forest floor. Scats contained viable *Vaccinium* seeds. Not all stumps were used by birds nor were they equally effective as recruitment windows, highlighting the dependence of seeds arriving at the right stump at the right time. Scats were more common in forest with heterogeneous vertical structure and on stumps with moderate amounts of bare wood. Seedlings were more likely on larger stumps with established bryophytes or alternatively on smaller stumps with available bare wood. Directed endozoochory appears to be a vital component in the life cycle of berry-producing ericaceous plants. Bird-mediated dispersal towards decomposing stumps allows ericaceous plants to arrive at spatially unpredictable microsites within the temporal window of suitable conditions. Seedling establishment and distribution is thus related as much to the

activity of endozoochorous dispersers (i.e. getting to the right place) as it is to microsite availability.

3.0.2 Climate and microhabitat effects on the importance of endozoochory along an elevational gradient in southern Ecuador

ORAL

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Seed dispersal is a key process shaping the distribution of plant species. Particularly in tropical rainforests, endozoochory, the dispersal of seeds by frugivores, is the most dominant mode of seed dispersal. Understanding how abiotic factors shape the degree of endozoochory in tree communities is, however, largely understudied. Here, we investigated the effects of elevation, climate and microhabitat conditions on seed dispersal modes of plant communities located along an elevational gradient in the tropical Andes in southern Ecuador. Over one year, we quantitatively measured seed rain with 162 seed traps on nine 1-ha forest plots located at 1000 m, 2000 m, and 3000 m asl. We also recorded climatic conditions (i.e., temperature, rainfall) and microhabitat conditions (i.e., leaf area index, soil moisture, soil properties) to test how abiotic factors influence the dominance of endozoochory in the tree community. We recorded around 330,000 seeds belonging to 329 plant species. An average of 82.1% of species in the community was endozoochorous while 17.9% had other seed dispersal modes. Elevation had significant effects on the proportion of endozoochory in the tree community. Variation in the biomass of endozoochorous seeds was associated with variation in rainfall. In turn, the number of endozoochorous plant species decreased with decreasing temperature and simpler canopy structure (i.e., low leaf area index). Our analyses suggest that changes in climatic conditions and canopy structure may potentially alter the dispersal modes in plant communities and thus, the distribution of plant species in these mountains.

3.0.3 Role of seed dispersal by animals on secondary succession in Cantabrian mountains: implications for rewilding and forest restoration.

ORAL

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Rewilding of Cantabrian montane forests involves the recolonization of open anthropogenic habitats (pastures with variable cover of rocks and shrubs) by tree species. This ecological succession process depends, at a large extent, on the dispersal of tree seeds by wild vertebrates and on the facilitation of tree seedling recruitment by shrubs. Frugivorous birds (thrushes, warblers and jays) and mammals (carnivores) establish, through seed dispersal, the early template of forest regeneration at the landscape scale. Animal diversity impacts positively in the magnitude of seed dispersal service, due to the between-species trophic and spatial complementarity that leads to the deposition of different seed species on different sites across the landscape. Forest loss and fragmentation condition largely the activity of seed dispersers, hampering seed deposition in deforested areas. Nevertheless, the occurrence of isolated remnant trees within pastures buffers this dispersal limitation, by anchoring seed dispersers and promoting nucleation foci for recruitment. When established under shrubs, tree seedlings and saplings frequently avoid browsing and trampling by cattle and wild ungulates, overcoming the regeneration bottleneck. Rewilding of Cantabrian montane forests depends, thus, on three functional components: (1) remnant forest patches, by acting as seed sources; (2) isolated trees and shrubs in pastures, by nucleating seedling establishment and fostering long-term recruitment; and (3) animal seed dispersers, by moving seeds from source areas to deforested habitats. The interconnection between these three components represents, therefore, a systemic mechanism of resilience which should be considered a tool of passive restoration of Cantabrian forests.

3.0.4 Are large vertebrates redundant in seed dispersal functions and dung beetle attraction in the Atlantic forest?

ORAL

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Understanding the extent to which vertebrates play equivalent roles within communities, known as functional redundancy, is underlying to estimate the potential to offset ecological functions previously played by a disappearing species. The services provided by threatened species are broad, and include forest regeneration through primary seed dispersal processes, and also the influence on secondary seed dispersal by defining associations with invertebrates, such as dung beetles, with their feces. Based on the potential of megafrugivores to overlap in functions, we present a snapshot of a complex of tri-trophic interacting partners within a conserved scenario of the Brazilian Atlantic forest (AF). Using empirical field observations of seed dispersal by vertebrates and dung beetles attracted to their feces, we present the first documented food web encompassing plants, vertebrates and dung beetles. We (a) described patterns of interactions among plants and three large fruit-consuming vertebrates still inhabiting conserved remnants of the AF: tapirs (*Tapirus terrestris*), muriquis (*Brachyteles arachnoides*), and howlers (*Alouatta guariba*); (b) described patterns of interactions between dung beetles and feces; (c) evaluated vertebrate's redundancy in terms of plants dispersed, and as dung resource for beetles; (d) delineated the food web of interacting partners; and (e) evaluated the stability of the food web facing a potential scenario of vertebrate loss. We found: (1) moderate redundancy in terms of primary seed dispersal; (2) low redundancy in terms of the potential influence on secondary dispersal; (3) the tri-trophic food web was characterized by low connectance, linkage density and modularity; (4) as a consequence, the loss of a single vertebrate potentially harmed the whole community structure.

3.0.5 Drivers and functional consequences of an individual-based seed-dispersal network along a natural regeneration gradient

ORAL

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Plant-animal interactions are shaped and reconfigured by the ecological context where they happen, with impact on their functional consequences. Of current interest is the role of mutualistic interactions in plant range shifts due to the rapid changes that natural and human systems are currently facing. Interactions with seed-dispersing frugivores are key to the colonization process of many plant species. Understanding how seed dispersal interactions are shaped at the intra-population level across a natural regeneration gradient and their functional outcomes are key to comprehend the underlying colonization processes and range shift drivers. We analyzed the individual-based frugivory network of *Juniperus phoenicea* along a natural expansion gradient in Doñana National Park, SW Spain. We combined molecular analysis (DNA-Barcoding feces identification) with camera-trap surveys to sample frugivore visits at 105 focal junipers over two seasons. We used a modularity network metric to analyze the configuration of frugivory interactions along the regeneration gradient. Finally, we estimated the propagule contribution of each individual plant taking into account frugivore features (feeding rates/visit and seed treatment), and dispersal traits of individual plants (seeds/cone and seed viability). We found an individual interaction network structure concordant with the expansion gradient. We identified both modules composed of frugivore species and individual plants dominating the mature part of the gradient, as well as modules of plants and frugivores belonging to the colonization front. In addition, the individual context of each plant (both its intrinsic and extrinsic attributes) affected module composition, confirming the relevance of this individual-based approach. The contribution of individual junipers to the seed rain was

found to be maximal at the colonization front, and strongly determined by the number of animal visits. Our results provide empirical support for plant expansion processes being actually favored by interactions with seed dispersers at the colonization front.

3.0.6 Dense herbivore populations influence the spatial patterns of seed dispersal and seed predation in the Mediterranean dwarf palm

ORAL

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The spatial distribution of adult plants and their seed dispersal pattern provides significant information about the underlying processes of plant-frugivore interactions. Thus, the frugivores' assemblage (among other factors) could determine these spatial patterns and influence other plant-animal interactions, such as post-dispersal seed predation. We used spatial data of Chamaerops humilis adult palms and their dispersed seeds (Seed Dispersal Units, SDUs), collected during 2019 and 2020 in two different plots in Mallorca (Balearic Islands). These plots differed in the frugivores' assemblage since feral goats were absent in one of them (the "defaunated" plot), but they were highly abundant in the other (the "overfaunated" plot). Moreover, we recorded whether SDUs were predated by insects and the intensity of predation. We addressed, using spatial point pattern analysis (SPPA), the following objectives: 1) to compare the spatial distribution of adult palms and SDUs between the "defaunated" and the "overfaunated" plots, 2) to assess the spatial relationship between the distribution of adult trees and the seed rain generated, 3) to describe the spatial patterns of seed predation by insects and, 4) to find out whether insect-predated seeds tended to be aggregated around adult palms. Adult palms and SDUs showed a double-clustered distribution with more isolated points than expected under null model in both the "defaunated" and the "overfaunated" plots. However, the bivariate analyses revealed a strong spatial association between adult palms and SDUs only in the "defaunated" plot. Interestingly, seed predation showed significant spatial patterns, but only in the "defaunated"

plot. Seed predation happened more frequently and intensely within aggregated SDUs, and predated SDUs tended to be associated to adult palms. Our research disentangles for the first time the spatial patterns of seed predation by insects, and it calls for future studies on the effect of dense herbivore populations on plant spatial ecology.

3.0.7 Seed-dispersal limitation as a driver of biodiversity deficits in chronically fragmented landscapes

ORAL

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Human-induced landscape changes have transformed forest ecosystems into fragments of woodland remnants embedded in a matrix of anthropogenic habitats. Both habitat fragmentation and habitat disturbance within fragments can lead to local extinctions and, therefore, to biodiversity deficits. For most plant species, seed dispersal constitutes a key process for the (re)colonization of woodland fragments once disturbance regimes have ceased. However, seed dispersal might be limiting because the distances from source populations to unoccupied fragments can be higher (several kilometers) than those at which most plant species disperse their seeds. Thus, dispersal limitation may explain contemporary 'dark diversity', that is, the set of species that are absent from a particular fragment but present in the surrounding region under similar ecological conditions. Previous studies have used seed sowing experiments to assess dispersal limitation, showing that it is a widespread phenomenon across forest ecosystems. Yet, we know little about its prevalence in chronically fragmented Mediterranean woodlands. In this study, we used sowing experiments to infer dispersal limitation for ten Mediterranean woody species (five barochorous and five vertebrate-dispersed species) in woodland fragments using the following rationale: if seedling recruitment and establishment from sown seeds are similar between fragments where species are present and those where they are absent, we infer that dark diversity (i.e. species' absences) is mainly explained by dispersal limitation. Conversely, if recruitment and establishment are higher in fragments where the species is present than in those where it is absent, we infer that dark diversity is mainly due to environmental filtering driven by abiotic (e.g. water and soil conditions) or biotic factors (e.g. natural enemies). The results from this study have direct implications for management because local biodiversity could be enhanced

by direct restoration actions assisting (re)colonization, like sowings or plantings, whenever dark diversity is explained by seed-dispersal limitation.

3.0.8 Understanding long-distance seed dispersal by sea currents: first results of experiments on Juniperus, Daucus, Ferula and Pancratium spp. from the Mediterranean Basin

ORAL

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To increase their evolutionary success, plants developed several adaptations to spread as widely and quickly as possible. Among them, the dispersal by water or hydrochory is a widely recognized but often overlooked effective syndrome; if propagules are also saltwater resistant, these can be dispersed through sea currents and break the barriers of insularity. The Mediterranean Basin, and especially islands, are natural laboratories of sea hydrochory (thalassochory) as main or complementary syndrome. We performed experiments with dispersal units of four genera native to the Mediterranean (*Juniperus*, *Daucus*, *Ferula* and *Pancratium*) including species differing in their distribution and possible dispersal ability: the coastal *Juniperus macrocarpa*, *Daucus rouyi*, *Ferula arrigonii* and *Pancratium maritimum* vs. the preferably inland *J. turbinata* and *J. oxycedrus*, *F. communis* and *D. carota*; and the endemic inland *P. illyricum*. We tested their period of dispersal units' buoyancy and seed viability after floating to hypothesize their colonization distances. Our preliminary results suggest that some species show specific thalassochory syndrome while others could be dispersed by sea, although they probably evolved primarily for other dispersal types. *Pancratium maritimum* shows synchrony in floatability and vitality, suggesting a strong interconnection between the two traits. The congeneric *P. illyricum* shows poor floating ability and resistance to saltwater, which might explain its limited distribution. The genera *Daucus* and *Ferula* showed as well poor floating ability, but they can germinate after seawater exposition. Differently, all *Juniperus* species –all spread through insular and continental Mediterranean territories– show a less floatability than *P. maritimum*, while their viability remains high even after sinking, confirming that *Ju-*

niperus species are particularly adapted to other dispersal types, such as endozoochory, although they are occasionally or stochastically able to be long dispersed by sea. This study contributes to explaining the overlooked but potentially crucial ability of vascular plants to colonize Mediterranean coastal ecosystems.

3.0.9 Variation among plant species in seed retention time inside frugivores' gut: relevance and underlying traits

ORAL

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Endozoochorous seed-dispersal by frugivorous animals is a mutualistic interaction that plays a pivotal role in the configuration of plant communities across most terrestrial ecosystems. In this mutualism, animals obtain food resources from the nutritive pulp of fleshy fruits, while plants benefit from the movement of their seeds away from the mother plant. That is, a reduction in competition together with a potential expansion to suitable habitats. The process consists of three major phases: initiation, transport, and seed deposition. Once fruits have been ingested, the movement patterns of animals and the retention time of seeds inside their guts will determine seed-dispersal distances. Thus, information on gut retention times (GRT) is essential to estimate the dispersal capacity of fleshy-fruited species. Experimental studies of GRT have been conducted in mammals, birds and reptiles, and multiple analyses have revealed positive allometric relationships between frugivore size and GRT: the larger the body size of the frugivore, the longer the retention time of the dispersed seeds; thereby, the higher the capacity for long-distance seed-dispersal. However, the potential effect of plant traits, as seed size and pulp nutrient content, on GRT has been largely overlooked despite these are highly variable among plant species and can influence the speed of seed ejection. In this study, we focused on variation in GRT among plant species when they are dispersed by a given disperser species. To do so, we conducted a set of experiments in which we measured GRT for more than 30 fleshy-fruited species dispersed by the song thrush (a key migratory seed-disperser in the Palearctic). Our results unveil substantial differences among plant species in GRT and a major role of seed and fruit traits in determining such variability. Thus, our findings can be

interpreted in terms of variation among plant species in their intrinsic potential for long-distance seed-dispersal.

3.0.10 Plants on the ride: tracking the research diversity globally on endozoochory

POSTER

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Animal-mediated seed dispersal (zoochory) is one of the essential ecosystem services for plants that help them find new habitats. Considering this, studying zoochory has become an important research area among ecologists for several decades. However, we have no comprehensive information about the diversity of zoochory studies, particularly on endozoochory at the global level. Here, we assessed when, where, and which taxon of plants or animals endozoochory was studied. We specifically aimed to find disparities/biases related to studied taxon, ecosystems, and geography. A systematic literature survey narrowed 162 peer-reviewed studies on endozoochory published in the last five decades. Our result highlights a continuous growth in endozoochory studies from 1971 onwards with considerable imbalances at the geographical and ecosystem level; >80% of studies were conducted only in the temperate regions with a prominent focus on the forest ecosystem (37%). Among animals, 91% of the study focused on wild animal species. Poaceae (covering 90 genera/226 species) reported the most endozoochorously dispersed plant taxa. Birds and antelopes were the most studied animal groups, covering 60 and 36% of studies. Although studies targeting domestic animals were low in number, they were found to disperse more plant species than wild animals. Despite the growth in endozoochory studies, considerable knowledge gaps still exist, mainly in the global south, where natural and human-altered landscapes still harbor a high diversity and abundance of wild and domestic herbivores without any grazing restrictions. Thus, the study suggests the need for more research from the global south having complex human- and animal-populated fragile ecosystems to generate more in-depth knowledge on endozoochorous seed dispersal.

3.0.11 Are seeds of wind-dispersed species really more oily? - The revised relationship between dispersal syndromes and seed nutrients.

POSTER

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Dispersal of all living organisms is a heterogeneous but, at the same time, very complex process that is driven by a combination of individual characteristics and environmental effects. In plants, seed dispersal is one of the main mechanisms of gene flow within but mainly between populations. Understanding the dispersal mechanism is crucial for answering the questions related to plant invasions and/or restoration processes. Different seed traits are considered to be connected with the dispersal spectra, but the evidence is usually not convincing. A relationship between dispersal spectra and seed mass was hypothesized, but found connection is weak. It was emphasized that not seed mass per se but the ratio of weight to the surface area of seeds is more important for dispersal, especially in the case of wind-dispersed species. It is thought that switching between carbohydrates and oils storage is the way how to manipulate this weight to surface area ratio because oils are more energy-rich compared to carbohydrates. On the other hand, oils are more energy demanding for their synthesis than carbohydrates. Therefore, there should be a balance between the costs of oils synthesis and the benefits of lighter seeds. Nevertheless, the existing evidence for higher seed oil content for wind-dispersed species in the literature suffers from unbalanced design, absence of phylogenetic correction, rough dispersal classification, and not distinguishing between a structural and non-structural part of seeds. Therefore, we used dispersal syndromes from the D3 database, which indicate a species-specific potential for a given dispersal vector instead of strict categorical classification. Further, we used seed oil and non-structural carbohydrate content data to distinguish the energy storage part of seeds that serve as nutrition and the structural part that mainly serves for embryo protection. We investigate

the connection between the oil/carbohydrates ratio in seeds reserves and dispersal syndromes more precisely.

3.0.12 The evolution of plant dispersal syndromes and seed traits: evolutionary cues and diversification.

POSTER

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Seed dispersal is a key trait for the reproductive success of plant species affecting the distribution and evolution of species across space and time. Different evolutionary pressures on plant movement have resulted in an enormous variety of seed dispersal mechanisms in seed plants. The broad range of dispersal syndromes (DS) can be summarized into four main groups: zoochory, anemochory, anemo-zoochory, and unspecialized. As expression of selective pressures, exerted by dispersal agents, we hypothesized a directional change from less specialized to more specialized syndromes. Moreover, we asked how dispersal ability affects species diversification rates in seed plants. Lastly, as seed traits play a specific role in each DS, we hypothesized that seed mass, terminal velocity, and floating capacity should show distinct evolution in each syndrome. We used stochastic character mapping (SIMMAP) to infer the ancestral state of DS and investigate their evolution in seed plants. We used the multistate speciation and extinction model (MuSSE) to test how DS influenced net diversification rates. The effect of dispersal ability on species diversification was assessed through quantitative state speciation-extinction models (QuaSSE). Lastly, we fit different evolutionary models (OUwie) to each seed phenotypic trait using 100 randomly sampled DS histories obtained through SIMMAP. The results show how the most specialized DS (anemo-zoochory) exhibit the highest speciation and diversification rates, confirming that the increase of possible disperser spectrum is an evolutionary advantage. Species with mean dispersal ability have the highest diversification rates, which may be explained by two main reasons: high dispersal ability could inhibit speciation events and colonization ability in plants is not strictly correlated with dispersal

ability. Contrasting evolutionary rates in seed mass and floating capacity and different optima in terminal velocity were observed, suggesting that these traits play a distinct role in each dispersal syndrome.

3.0.13 Unmasking the perching effect of the pioneer Mediterranean palm *Chamaerops humilis* L.

POSTER

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Seed arrival is a crucial necessary ecological process during the (re)colonization that can be enhanced by the use of the so-called "perch plants". Little is known about how spatial aspects of "perching" affect their effectiveness in disturbed habitats. To evaluate several spatial aspects of "perching" effect, including whether the seed arrival via frugivorous birds is associated to the spatial distribution of the perch plants, we used a spatially explicit approach in two disturbed plots within the Doñana National Park (SW Spain). Specifically, we chose as study system the pioneer Mediterranean dwarf palm *Chamaerops humilis* L., which is often used as perch by a variety of frugivorous bird species. A total of 289 *C. humilis* individuals were sampled in search of bird feces (N = 2998) and dispersed seeds (N = 529). Recorded seeds belonged to six different woody species from five different families. GLMs analyses indicated that taller males *C. humilis* with high richness of beneficiary woody species received more dispersed seeds. We detected a random spatial structure of bird feces and dispersed seeds in one study plot, while a nonrandom spatial structure was found in the other one, where isolated *C. humilis* received a higher number of bird feces and dispersed seeds than expected under spatial null models. The difference in spatial patterns between both study plots could relate, among other factors, to their different state of development in the ecological succession. Most of dispersed seeds were concentrated in a small number of *C. humilis* individuals that acted as "hotspots" of seed arrival. The fact that frugivorous birds visited most often isolated *C. humilis* questions the aggregated spatial structure of revegetation designs typically used in restoration projects. This study reveals novel spatial aspects of the "perching" effect which could be helpful in the restoration of human-disturbed habitats worldwide.

3.0.14 Seed dispersal by ungulates – from seed traits to landscape level

POSTER

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Free-ranging wild herbivores are a potential dispersal vector which can move seeds and fruits from mother plants to new localities connecting fragmented habitats. However, different species of herbivores provide dispersal for a different set of plant species due to the variability in animals' foraging strategies, digestive tracts and body size. Furthermore, not all plant species are equal when it comes to survival in the guts. We conducted a thorough experiment with almost forty plant species and four herbivores to test for the survival of seeds after passage through the herbivore digestive system. Furthermore, we compared the survival of tested species with their frequency in the dung samples and in the landscape. We ask: (i) which seed traits and other characteristics influence the survival of seeds in the digestive tract? (ii) How does the survival of species from the feeding experiment correspond to germination rates from field-collected dung samples? (iii) How does it correspond to the frequency in the landscape?

Chapter 4

Theory and methods in seed trait ecology

4.0.1 Identifying conservation and knowledge gaps in cultivated Brassicaceae: characterization of functional traits in crop wild relatives

ORAL

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Wild species hold a key source of diversity to improve and help adapt our traditional crops to deal with future climate scenarios. Those wild species with a close genetic relationship to a crop, named crop wild relatives (CWRs), are likely to possess useful traits due to greater genetic variability and adaptability compared to crops, making them of paramount importance for research and conservation. Previous studies have focused on major crops such as pulses, cereals and forages, and their respective CWRs, but relatively little work has been done on oil crops, vegetables or fruits, such as in the family Brassicaceae. Brassicaceae CWRs have a wide diversity in morphology and genetics, and host desirable traits such as drought and salt tolerance. In this study, we investigate potential CWRs of crops in Brassicaceae, collating information published from different databases (e.g., Genesys, USDA-GRIN), with the objective of understanding the quality and diversity of the accessions available and identifying conservation priorities and knowledge gaps. We will investigate 12 crops and more than 500 wild relatives of Brassicaceae, of which 260 species are found in only one location. Conservation assessments are urgently needed, since around 50% of the CWRs are Data Deficient or not assessed for their global threatened status, and more than 50% have little or no information available about their traits nor DNA data available. A target list of key CWRs will be obtained to prioritize seed conservation and explore potential traits.

4.0.2 Towards a tropical seed and germination trait database

ORAL

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A broad understanding of the ecology and evolution of seed traits depends critically on the availability of trait data from tropical regions, given their unique biodiversity and evolutionary history. Here, we introduce two recent initiatives to compile tropical seed functional trait databases: "MelastomaTRAITS" and "Rock n' Seeds". MelastomaTRAITS is a database of 67 functional traits, including 27 seed traits, for 2,569 species distributed in 161 genera from all 21 Tribes of the pantropical Melastomataceae – the 9th largest plant family with more than 5,800 species. Melastomataceae species occur in a large diversity of habitats, growth-forms, functional groups, geographic distribution, reproductive, pollination, and seed dispersal systems. Therefore, the family provides a promising starting point for testing a wide variety of ecological and evolutionary questions. In turn, "Rock n' Seeds" is a database of seed functional traits and germination experiments from the Brazilian rock outcrop vegetation, recognized as outstanding centers of diversity, endemism and refugia. "Rock n' Seeds" includes data for 16 seed functional traits containing the major axes of the seed ecological spectrum for 383 species from 149 genera, 50 families and 25 Orders. Raw data for 48 germination experiments for a total of 9,779 records for 280 species is also provided. Notably, 8,250 of these records include daily germination counts. Given the widespread occurrence rocky outcrop vegetation, "Rock n' Seeds" paves the way for comparative seed functional ecology at the global scale. As a result, both of our databases can contribute to improving our understanding of trait-based seed ecology across levels of organizations, plant-animal interactions, regeneration ecology, and support conservation and restoration programs.

4.0.3 Looking for the orthogonality of germination indexes: minimalism meets seeds functional ecology

ORAL

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The advancement of seed functional ecology relies on the proper characterization of the axis that composes the seed ecological spectrum. Since germination is one of these components, seed ecologists must seek to describe it in the most complete and concise fashion. Different methods have been developed to describe the germination axis, including many seed germination indexes. While it is true that a single index is insufficient to describe the germination behavior completely, there is still no consensus on a minimum set of indices that should be used to provide a complete description of this complex phenomenon. A further complication arises from a pervasive lack of consistency in the terminology to describe germination, the apparent redundancy of indices available, and the development of software that allows the calculation of several indexes with ease. In this research, we assessed the covariation of the 44 germination indices available at the germinationmetrics R package using a database of germination experiments from Brazilian rock outcrop vegetation in order to produce an orthogonal set of indices that characterize the different dimensions of germination behavior. The indices representing potentially different germination behavior dimensions, namely germinability, time, rate, synchrony, and uniformity, were calculated for a set of germination experiments under optimal, suboptimal, and supraoptimal temperatures. When tested for linear correlations, most indexes were found to be highly correlated between them. After systematically selecting from indexes different dimensions with little correlation between each other, we were left with only four indices: germination percentage, t50, coefficient of variation of germination time and germination uncertainty. These four indices arise as a promising group for characterizing the multiple dimensions of seed germination behavior across lineages and biogeographic regions.

4.0.4 An image analysis tool for automated extraction of diaspore morphological traits

ORAL

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The field of comparative morphology of diaspores provides basis for the characterization and identification of taxa, and for understanding how traits are linked to functions, processes, and ecological strategies. However, the description of diaspore traits relies mainly on manual measurements of few parameters, such as length and width, and on human classification of structures, both of which can be error prone and time-consuming. Image analysis applications address these shortcomings by offering an alternative approach for an objective characterization of diaspores. Several applications have been developed to extract quantitative traits from images, but these were tailored mainly for crop species, and do not provide a full automated process that works for diaspores in a variety of sizes, surface structures and colors. Here, we present a protocol and software for the measurement of diaspore morpho-colorimetric traits from images captured with flatbed scanners. The tool uses unsupervised segmentation to automate the separation of diaspores from high-contrast backgrounds, without the need for manual fine-tunning or thresholding. Next, the software determines diaspore size and shape descriptors, including length, width, area, perimeter, aspect ratio, circularity, diaspore surface structure, and solidity. Two types of colorimetric measurements are also extracted: values suitable for human recognition purposes and data independent of any particular animal visual system. To determine the application and accuracy of the software, we assessed morpho-colorimetric traits and performed exploratory analyses with a diverse diaspore collection composed of 1,477 taxa belonging to 97 plant families from Central Europe. The protocol is a highly automated solution that allows for rapid and reproducible measurement of diaspore traits of native species. This approach facilitates the acquisition of data that are readily comparable across different taxa, opening up new av-

enues to explore functional relevance of morphological traits and to advance on tools for diaspore identification.

4.0.5 The seed trait handbook

ORAL

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The need to understand the mechanisms behind any biodiversity pattern, vegetation process or threat have led to a functional approach using plant traits which reflect a species' response to its environment or its strategy to cope with. The first overview on functional traits by Weiher et al. has now culminated in the TRY database, actually in the fifth version with nearly 12 million trait entries. A deeper look, however, shows that only few seed ecological traits are presented, such as seed morphology, seed size, mass, dispersal mode, seed longevity and seed germination stimulation with by far most entries on seed size and dispersal mode. The limited functional knowledge on seed traits is also reflected by the second edition of the handbook for standardized measurements of plant functional traits by Pérez-Harguindeguy et al. Only six seed traits were included – dispersal mode and potential, dispersule shape and size, seed mass and seedling morphology. It is hence timely to increase knowledge on seed functional traits by providing a collection of standardized measurement methods, to motivate studies along new axes of functional ecology of seeds and to enable more reliable comparative works across different studies. Taking this gap as an opportunity, we organized a workshop in Perth, Australia in September 2016, which first outcome was to define a research agenda for seed-trait functional ecology.

We also worked out a list of 60 reproductive traits concerning flowering and fertilization (gametophyte traits), fruit and seed attraction and defense, seed dispersal and dispersal potential, seed persistence, seed dormancy and germination as well seedling emergence and establishment. Each trait was defined and its functionality, source of variability and the methodology (how to measure) were described. An overview of the seed trait handbook will be presented at this talk.

Chapter 5

Seed functions in plant communities

5.0.1 The role of seed functional diversity in buffering plant communities from species colonization

ORAL

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Species traits considerably contribute to species assemblage in plant communities. However, species traits may differ between adult and reproductive phase, thus different rules can drive species assemblage during each phase of plant life cycle. We analyzed if the colonization of a foredune plant community depended on the functional diversity of resident species, measured on both seed traits (reproductive phase) and vegetative traits (adult phase). We also accounted for phylogenetic and taxonomic diversity. In a dataset of 84 plots x 19 species, we classified species as "residents" or "colonizers", according to their fidelity (Phi coefficient) and frequency values for the foredune plant community. We associated to each species seed traits, namely the germination percentage under different temperature and light regimes, seed mass and shape, and vegetative traits, i.e. leaf traits (SLA, LDMC, LA), life form, and clonality. For each plot, we measured the Functional Diversity (FDis index) of residents, on both seed traits ("FDseed") and vegetative traits ("FDveg"). We also measured phylogenetic ("PD") and taxonomic diversity ("TD"). Through GLM we investigated if the cover percentage of colonizers per plot depended on FDseed, FDveg, and PD of residents. TD was excluded because it was correlated to FD. The cover percentage of colonizers increased at increasing FDseed, FDveg and PD, although significantly only for FDveg. Foredune plant communities with high FD and PD did not counteract species colonization, suggesting that the functional trait space was not saturated. FD during reproductive phase

can have a weaker influence on species colonization than FD of adult species, suggesting that colonizing species may take advantage from entering plant communities during early phases of plant life cycle. We therefore underline the importance to analyze patterns of both seed and vegetative functional diversity in plant communities to thoroughly understand the process of community assembly.

5.0.2 Easy to grow: assessing the role of regeneration traits in global urban trees selection

ORAL

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Urban trees provide important ecosystem services to city dwellers and are considered one of the efficient nature-based solutions for mitigating the adverse impacts of rapid urbanization. The 3-30-300 rule for urban ecosystem suggests everyone should see at least 3 trees from their home, live in a locality with 30% canopy cover, and be within 300m proximity of ample green space. Similarly, to achieve high tree diversity, the 10-20-30 rule advocates that the composition of one species, genus, and family in an urban ecosystem should not exceed 10, 20, & 30%, respectively. However, implementing these thumb rules and using urban trees as a nature-based solution may fail if human's selection of tree species is based solely on the species' aesthetic, functional utilities, and ease of establishment. Here, we quantified how regeneration traits influence the selection of urban tree species. Specifically, we asked, are the current global urban tree selection skewed towards easily reproducible species? We compiled data on seed storage behavior, dormancy, and clonality for 4734 species (c. 8% of the globally known tree taxa) listed currently in the Global Urban Tree Inventory. Results indicate that seeds of most (75%) urban tree species have a low probability of recalcitrance, signifying their orthodox storage behavior. Seed dormancy information was available for 12% (593) species, of which 77% produce dormant (mainly physiological) seeds. Clonality data for 11% (547) species indicate that 92% of them can reproduce through vegetative means. Surprisingly, most species with dormancy also had clonality traits. In addition to aesthetics and utilities, the ease of handling species regeneration (through

vegetative propagation, desiccation tolerance, or dormancy-alleviation during storage) tends to be another driving force for urban tree selection. It will be worth studying more regeneration traits of the urban trees to know how human choices have shaped community assembly in urban ecosystems.

5.0.3 Experimental germination responses to different temperature and light regimes and species spatial distributions in a guild of Western Australian annuals

ORAL

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Annual species have evolved sets of germination cues that are presumably predictive of the post-germination environment. In naturally patchy environments, germination microsites often vary considerably in the amount of light they receive and in the diurnal temperature fluctuations they experience. It is therefore possible that species' differential germination responses to temperature and light may be related to their spatial patterns of occurrence, but this remains largely untested. We investigated if species' germination responses to experimental temperature and light treatments are associated to their occurrences along local gradients of overstory and litter cover in Western Australian winter annual communities. We first surveyed species' occurrences in 150 quadrats across gradients of overstory and litter cover. Nineteen species recorded in this survey were then included in a germination experiment that manipulated (1) Light vs. Dark (12h light or 24h dark) approximating seeds near the soil surface versus those covered by litter and (2) Cold vs. Warm temperature regimes (7/18 °C and 7/24 °C) approximating diurnal fluctuations experienced in shaded versus sun-exposed microsites, respectively. In binomial models of occurrences in the field, one species was positively, and one was negatively associated with overstory cover. For litter cover three

species were positively and 4 negatively associated. In the germination experiment, six species had highest germination probabilities in the Light treatment (regardless of temperature), five in Cold + Light, one in Warm + Light, two were indifferent to the treatments and four did not germinate at all. Overall, species' experimental germination responses did not correspond with their spatial distributions along overstory and litter cover gradients. Our results suggest that either our experimental treatments did not accurately mimic microsite differences in the field, or that patchy germination is associated with factors other than light and temperature regimes, such as soil moisture, pH and salinity.

5.0.4 Inferring community assembly processes from functional seed trait variation along temperature gradient

ORAL

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Assembly of plant communities has long been scrutinized through the lens of trait-based ecology. Studies generally analyze functional traits related to the vegetative growth, survival and resource acquisition and thus ignore how ecological processes may affect plants at other stages of their lifecycle, particularly when seeds disperse, persist in soil and germinate. Here, we analyzed an extensive data set of 16 traits for 167 species measured in-situ in 36 grasslands located along an elevational gradient and compared the impact of abiotic filtering, biotic interactions and dispersal on traits reflecting different trait categories: plant vegetative growth, germination, dispersal, and seed morphology. Abiotic filtering impacted mostly the vegetative traits and to a lesser extent on seed germination and morphological traits. Increasing low-temperature stress towards colder sites selected for short-stature, slow-growing and frost-tolerant species that produce small quantity of smaller seeds with higher degree of dormancy, high temperature requirements for germination and comparatively low germination speed. Biotic interactions also filtered certain functional traits in the study communities. The benign climate in lowlands promoted plant with competitive strategies including fast growth and resource acquisition (vegetative growth traits) and early and fast germination (germination traits), whereas the effects of facilitation on the vegetative and germination traits were cancelled out by the strong abiotic filtering. The changes in the main dispersal vector from zoochory to anemochory along the gradient strongly affected

the dispersal and the seed morphological trait structure of the communities. Stronger vertical turbulence and moderate warm-upwinds combined with low grazing intensity selected for light and non-round shaped seeds with lower terminal velocity and endozoochorous potential. We clearly demonstrate that, in addition to vegetation traits, seed traits can substantially contribute to functional structuring of plant communities along environmental gradients. Thus, the ‚hard' seed traits are critical to detect multiple, complex community assembly rules.

5.0.5 Seed germination responses to microclimatic conditions in alpine communities

ORAL

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Topographic roughness of alpine landscapes creates a mosaic of microhabitats ranging from open areas subjected to freeze-thaw cycles (fellfields) to long-term snow-covered sites (snowbeds). Although such microclimatic heterogeneity is a key driver of plant distributions in alpine ecosystems, it is largely unknown how the seed regeneration niche of co-occurring species differ in the same community. We conducted a move-along seed germination experiment mimicking fellfield and snowbed environments. Experimental conditions were based on field measurements of soil temperatures and they were set as weekly temperature regimes with monthly photoperiod modifications but without water limitation. In total, we tested the germination response of 53 co-occurring species in two alpine communities, calcareous and siliceous, of the Cantabrian Mountains (Spain). Both study systems showed different dormancy patterns, with 50% and 80% of species germinating before winter in calcareous and siliceous bedrocks, respectively. At the local scale, we found that germination responses varied greatly between microhabitats, with the same species germinating better (higher and faster rates) in fellfield than in snowbed conditions. Nevertheless, we also observed intraspecific variation with germination differences between populations ranging from 0 to 65%, on average they differed more in fellfield (14.78%) than in snowbed (9.85%), calculated from 35 species. These results suggest specific germination adaptations of alpine plants to microhabitat conditions at both regional and local scales. While climate warming is expected to accelerate germination rates and to lengthen the growing season, the shrinkage of snowbeds will likely have negative effects on the species adapted to

this microhabitat. The combination of higher temperatures and autumn precipitations could trigger early germination, letting the seedlings vulnerable to winter temperatures. Nevertheless, further studies are needed to test the role of water availability on regulating germination traits in water-limited alpine communities, where low summer precipitation could prevent autumn germination and avoid winter's adverse conditions.

5.0.6 Identifying seed functional traits contributing to seed survival and plant regeneration in fire-prone ecosystems.

ORAL

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Regeneration from seed is a primary means of plant population persistence for many species in fire-prone environments. For plants to re-establish successfully following fire, their seeds must survive the fire event, be at a depth within the soil profile that allows for their emergence (i.e., not too deep) and be situated in a location that provides suitable access to light, water, and temperature for germination. Over the past seven years, we have been examining how seed traits influence seed survival and seedling emergence behavior in species from across Australia. Here we synthesize the results from our experimental fires in the field, and our controlled glasshouse and laboratory experiments. Seed lethal temperatures are strongly influenced by seed moisture content during exposure, and that seed size influences seed survival and seedling emergence. We have also identified three distinct functional responses based on lethal temperature thresholds and maximum seedling emergence depth that describe the varying sensitivity of species to fire

severity. 1) Seeds that can emerge despite high soil temperatures. 2) Seeds that can only emerge because they are able to emerge from deep within the soil. 3) Seeds that have limited emergence depth but also die at low soil temperatures. Our current research is focused on the understanding critical thresholds in seeds that possess physical dormancy (PY). Collating data from 50 species from across Australia we have quantified the response of seeds to exposure to temperatures between 40 °C and 150 °C. From this we can understand the traits that seeds possess to survive through fire, germinate, and emerge, which can provide a deeper understanding of post-fire recovery processes and inform how changing climate and fire regimes may influence post-fire vegetation composition. Future work will examine the biochemical mechanisms that underpin seed survival including small heat-shock proteins and the role of fatty acids in seed coats.

5.0.7 Cracking the Code: Seed Coat Chemistry in Physically Dormant Seeds

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Plant species in fire-prone ecosystems maintain dormant seeds in the soil which germinate in response to fire cues, such as heat shock, thus ensuring seeds are available to germinate post-fire to replace killed individuals. Seeds of physically dormant species in fire-prone ecosystems have varying temperature thresholds to overcome dormancy, ranging from 40 ºC to 120 ºC. Variation in dormancy temperature thresholds both between and within species is likely to be an important driver of community assemblage, as fire intensity and severity will determine which seeds germinate and thus define the post-fire diversity. However, the mechanism

behind this variation is not clear, despite the importance of seed dormancy in timing germination and ensuring seedling recruitment. The seed chemistry of plants in fire-prone regions is an understudied subject that may help in understanding what controls these dormancy mechanisms. This study tested the hypothesis that different lipid compositions in Faboideae seed coats are correlated with dormancy-breaking thresholds through applying analytical chemistry techniques to plant species from fire-prone and fire-free ecosystems. Seed coat lipids were identified through gas chromatography/mass spectrometry, and a positive correlation between lipid melting points and dormancy temperature thresholds was found. Additionally, overall lipid compositions were found to be distinct between species from fire-prone and fire-free habitats, establishing that the chemistry of the seed coat is seemingly under selection pressure in fire-prone systems. These findings contribute to our understanding of what drives the variation in dormancy-breaking thresholds, giving us better insight into how different species regenerate after fire. As future environmental conditions under climate change will result in more intense and frequent fires, understanding the processes driving plant population dynamics in fire-prone regions is imperative.

5.0.8 Burned fruits and heated seeds: are seeds still able to germinate?

ORAL

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In several flammable ecosystems, fire is responsible for triggering germination in different species. Smoke can stimulate germination in species with permeable seed coats, while heat shock can break dormancy of hard-coated seeds. However, less is known about what happens to seeds that are inside the fruits during fires: will the fruits be enough to protect seeds from being damaged by the flames? Will the heat stimulate seeds to germinate? Thus, we investigated if seeds inside the fruits during fire can germinate. We collected seeds from species with both permeable and impermeable seeds. Seeds were sampled before and after fire, from four Cerrado species and put them to germinate (27ºC, 12/12/ hs light, 30 days). Germination and viability percentages, and mean germination time was evaluated for all species. Although viability decreased from 98% to 54%, germination percentages were higher in seeds sampled in burned than in unburned fruits (57% and 11%, respectively) for Mimosa leiocephala (physical dormancy). Species with permeable seed coats showed different results: Jacaranda decurrens showed similar germination and viability percentages in both treatments, while Kielmeyera rubriflora showed a decrease in germination and viability for burned fruits. Our results showed that germination of seeds inside fruits during fire events may vary depending on seed coat permeability: species with physically dormant seeds were less damaged by fire, with an increase in germination percentages, while seeds with permeable coats had a decrease in both germination and viability. Thus, fire affect germination not only when seeds are in the seed bank but also before dispersion, showing the complexity of fire-responses to germination of Cerrado species.

5.0.9 In situ seed storage affects germination responses to fire-related cues in Cerrado species

POSTER

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Fire-related cues are known to influence germination and seedling establishment processes in fire-prone ecosystems. However, most tests use fresh seeds and the role of seed storage in soil seed banks in shaping response to fire-related cues remains unappreciated. We aimed to investigate whether in situ seed storage (buried in the field) would affect seed germination responses to heat shock and smoke. We collected freshly dispersed seeds (beginning of the dry season) of five Cerrado species. For each species, seeds were sorted and separated into four batches. Fresh seeds were exposed to germination experiments following seed collection (Fresh T0) and the remaining seeds were buried in the field (1cm depth) in six different plots in Cerrado vegetation. Batches were unearthed after three (T3), six (T6), and nine (T9) months. Seeds from each treatment (T0, T3, T6, and T9) were exposed to heat shock (100ºC and 200ºC for 1') and smoke experiments (1:1 smoke solution, 24 hr). Then, seeds were put to germinate in germination chambers at controlled conditions (12h/12h light and dark, 27 °C) for 30 days. Our results showed that seed storage time affected germination responses to fire-related cues in all tested species. Three species with permeable seed coats had an increase in germination when exposed to smoke after three and six months buried

in situ. Most of them were recruited when the rainy season started at T6. The two physically dormant species, Sida linifolia and Waltheria communis had an increase in germination percentages of at least three-fold at 200°C according to storage time with the highest germination at T9 (70±20.82% and 71.67±6.45%, respectively). Therefore, in situ seed storage affects germination response to fire-related cues, suggesting that recruitment from seeds in fire-prone tropical savannas is more complex than previously thought.

5.0.10 TESTA EXTENSIONS IN ORCHID SEEDS: MECHANISMS TO ATTACH THE BARK OF TREES

POSTER

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In Orchidaceae, testa extensions have been observed in obligate epiphytes, usually the so-called "twig orchids". These species are restricted to grow on the ultimate branches of their hosts, characterized by a short diameter (2,5 cm or less), with a limited source of epiphytic nutrients, such as decomposition of bark, debris from the host, and animal excreta. However, other taxa are common on the bark of trunks and thick branches. To-date, the testa extensions have been found in three subtribes within the subfamily Epidendroideae (Oncidiinae, Aeridinae, Angraecinae), the first distantly related to the other two. During our research on seed micromorphology in tropical orchids, we have analyzed the qualitative characters in the seed coat, checking an important variability in the traits, such as the testa extensions. To check the presence of testa extensions in orchid seeds, we obtained samples from mature capsules of specimens housed in the herbaria. An average of 10 seeds was analyzed under scanning electron microscope and 30 under light microscope. The aims of this research are to describe the typology of testa extensions and its relationship with the seed shape, and to check if these extensions are related with the type of branches in which the orchids grow.

5.0.11 Alien and native seed germination performance might shape the species assembly of temperate grasslands under a global warming scenario

POSTER

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Global warming is rapidly altering our ecosystem in terms of biodiversity, functions and services loss. The rising temperatures have often shown the ability to enhance biological invasion by facilitating spread and growth of alien species in the new lands. The success of plant invasion requires the overcome of multiple biological barriers. Among the crucial life stages, seed germination greatly contributes to the final species assembly of plant community. Several studies have already suggested that alien plant success is likely linked to their high seed germination rates and longevity. We hypothesized these traits to be further enhanced by future temperature rise. Among terrestrial ecosystems, temperate dry grasslands are considered important hotspot of biodiversity, but also among the most prone ecosystems to biological invasions. In these habitats, Asteraceae is one of the most represented family, in terms of abundance and number of species, for both alien and native plants. In this scenario, we designed a germination test experiment at two temperatures (i.e., 22 °C and 28 °C) including common Asteraceae grassland species, considering native and alien species (split into archaeophyte and neophyte). Our aim was to test the relationship between seed germination, temperature, and their interaction with alien status. The test was performed on both separated and mixed pools of species by using a full-factorial orthogonal design. We expect to find a decrease/delay in native species germination compared with alien seeds with the increasing temperature. We also suppose alien

species germination to affect the final species assembly of mixed germination plates, by inhibiting local species germination, also in the light of plausible allelopathic interactions. These results will give important information on future management actions aimed at curbing alien plant invasions, by improving our knowledge on seed-bank response and interactions mechanisms of common species occurring in natural disturbed areas or restoration sites.

Chapter 6

Seed-based restoration and conservation

6.0.1 Addressing seed needs for reforestation in the western United States

ORAL

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Climate change has increased the frequency and intensity of disturbance regimes in the Western United States, negatively impacting native conifer forests. High intensity wildfires are driving the loss of critical tree populations from which wild-type seed can be sourced for restoration. In parallel, there are many regional initiatives calling for reforestation en masse as a pathway to carbon sequestration and stabilization of these habitats. Although a mature forest management industry exists in the region, its seed collection and processing capability is limited and largely reliant on genotypes derived from orchards and tree improvement programs developed for timber production. Wild seed collection is both an art and a science, requiring knowledgeable practitioners to survey for mast and rely on a detailed understanding of myriad conifer species' seed biology to develop a collection strategy. Today skilled seed collection practitioners are few, as academic or professional institutions have not maintained training programs to support this sector in much of the United States. To address non-industrial reforestation needs and establish genetically resilient and structurally diverse forests, significant innovation and investment in scaling up responsible and genetically appropriate seed collection efforts from wild populations is needed. This presentation will provide an overview of the current practices of seed collection, cleaning and banking, as well as highlight examples of scaling up collection efforts to meet new reforestation needs in the Western United States. We will feature Silvaseed - a 130 year old historic regional seed provider - as an example of a legacy operation that is innovating processes and products to meet seed needs for ecologically relevant reforestation in the era of climate change.

6.0.2 Mediterranean seed-based ecological restoration: selecting native species with regeneration traits adapted to future climate scenarios

ORAL

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Seed-based restoration is an effective tool to address biodiversity loss as a consequence of climate change. However, knowledge of regeneration traits is still very limited compared to that of vegetative plant traits. Therefore, if we advance in the understanding of the functional ecology of regeneration traits, we can improve the efficiency of projects aimed at biodiversity conservation. In the Mediterranean basin, climatic dynamics are even more challenging when it comes to regeneration from seeds under a warming climate. Seed germination will be likely affected by warmer winters as it is limited by "typical" Mediterranean cold signals and the release of dormancy is reduced in those species that usually germinate in spring. In addition, hotter and drier summers will compromise the survival of plants by affecting their seedling establishment phase. Taking into account these predicted scenarios, we will identify native species from the Mediterranean region that are pre-adapted to these expected climatic changes and can resist extreme environmental conditions during their regeneration stage. We will use the species from the EUNIS Mediterranean habitats to compile a database that will include both regenerative primary data and vegetative traits, as well as information on their potential interest related to their contribution to the associated ecosystem services for private companies and public bodies involved in ecological restoration (e.g. species with horticultural, gardening or pollinator interest). We will then carry out a meta-analysis of

regenerative primary data that will allow us to identify species that resist extreme environmental conditions. In conclusion, the gathered data will provide information based on regenerative traits that can be used in effective seed-based conservation strategies in the Mediterranean basin.

6.0.3 Greening Iberian olive groves with native seeds

ORAL

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Agricultural intensification of Mediterranean woody crops (vineyards, olive groves and fruit trees) has dramatically changed traditional landscapes that were relatively sustainable before the twentieth century. Current agroecological practices suggest the need to move towards the establishment of herbaceous ground covers. However, we do not know which species are suitable for restoration, and how seeds of these species can be produced on large scales. Here, we present an overview of major challenges for greening olive groves in southern Iberia and recent initiatives that combine applied research with seed production in Andalusia, Spain. We developed a comprehensive framework for selecting regionally adapted species for restoration that also emphasizes considerations for seed production. A major finding was that research on ground covers for regenerative agriculture has largely overlooked native species at the expense of commercial and ill-suited varieties. Our assessment of native annuals showed that 85% of the evaluated grasses and forbs exhibit a suite of ecological and production traits that can be tailored to meet the requirements of farmers, seed producers and environmental agencies. Moreover, seed experiments conducted with natural populations of suitable species allowed us to describe the seed ecological niche of preferential grasses and forbs to predict the natural regeneration of established populations. The contribution of scientific data and the trends in agroecological practices have strengthened the production of native seeds by specialized producers and the early growth of a seed market for greening Spanish olive groves. Together with other initiatives and trials conducted on experimental olive groves, it seems we are facing a change of paradigm in the sustainability of these agroecosystems. Nevertheless,

we are still far from a healthy seed market coupled with national-based policies on native seed production.

6.0.4 Selecting native seeds for the revegetation of coastal dunes altered by Carpobrotus species

ORAL

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The RIVALSA project aims for an integrated plant and fungal approach to the revegetation of coastal dunes altered by the invasive alien species Carpobrotus edulis and C. acinaciformis, introduced in Mediterranean Europe to stabilize sandy areas and for ornamental purposes. In addition to their ability to grow and spread, their litter has an allelopathic effect, reducing germination and establishment rates of native species. Among the objectives of the project, here we focus on the evaluation of seed germination in the presence of allelopathic substances, to select a pool of wild plant species whose seeds can tolerate the presence of such substances in the soil. Seeds of 9 species were collected in invaded or non-invaded dunes of Latium (central Tyrrhenian Italy). To evaluate the allelopathic effect, three different leaf extracts were prepared for both Carpobrotus species using water, 70% ethanol, and acetone as solvents. Germination tests were performed using the optimal protocol for each species, with increasing concentrations of the extracts (0, 0.3, 0.66, 1 and 2 mg/ml). Preliminary results showed different sensitivity of seeds from various species to the Carpobrotus extracts. For example, Anthemis maritima showed a higher germination rate in the control (64%) than in the presence of the water extract of C. acinaciformis (37% at the highest concentration). Besides, a comparison of populations of species growing in non-invaded or invaded areas, such as Centaurea sphaerocephala, revealed that the latter plants were less or not sensitive to allelopathic

extracts (96% vs 89% for the control and the highest concentration, respectively), demonstrating how plants adapt to chemicals from the invader. Thus, it may be possible to identify seeds from native species not inhibited by Carpobrotus extracts and, together with a selected fungal consortium, use them for the recovery of the native dune vegetation after the eradication of Carpobrotus plants.

6.0.5 Germination protocol for herbarium-stored seeds to recover extinct plants

ORAL

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Herbarium specimens have been proposed to be valuable sources of viable diaspores (pollens, spores and seeds), which can provide native germplasm for recovering extinct plant species and their genetic variation lost. Diaspores taken from herbarium specimens or similar collections can remain viable for decades, and therefore may have potential for recovering extinct species (de-extinction s.l.). Although the lack of documented experiments of plant de-extinction using herbarium material, some attempts with locally extirpated species demonstrate the feasibility

of this approach, which is supported by a general consensus of conservationists and curators. Indeed, a protocol for herbarium specimens' seeds germination was not developed so far. Already existent protocols and guidelines, like germination standard protocols developed by the MSB (Millennium Seed Bank Partnership and Royal Botanical Garden, Kew) can be applied. Our research is dedicated to find germination protocols for trying plant de-extinction from seeds preserved in herbarium specimens. To this aim, we chose 26 species congeneric to worldwide extinct species, collecting more than 20,000 seeds from circa 400 herbarium specimens held in 8 Institutions (BR, RO, FI, PAV, FER, MSNM, MSPC, and Roma Tre University). Criteria for seed collection were specimens' age, seed ripening, number of specimens, abundance of seeds per specimens, and ad hoc curators' guidelines. In addition, we tested freshly harvested seeds to countercheck. Several germination protocols are tested, including different pre-treatments, sterilization techniques, priming and germination conditions (e.g. temperature, light cycle, substrate). To date, we obtained more than 200 germinations of seeds taken from herbaria, from the 19th century until 2016, and we expect more germinations from the ongoing tests. This project represents the first documented and systematic development of germination protocols for old seeds from herbarium collections for conservation purpose, which lays the basis for the next attempt to the first herbarium-based seed plant de-extinction.

6.0.6 Fit for the future: alpine plant responses to climatic stress after 20 years of seed bank conservation

ORAL

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Climate warming is occurring in alpine environments faster than the global average, making cold-adapted alpine specialists particularly vulnerable to population decline and extinction. Ex situ seed conservation is of growing importance but little is known about how plants from genebank stored seeds will respond to climatic changes. We grew plants from seeds of the artic-alpine specialist Viscaria alpina, collected from the same location at different time points and held under seed bank conditions over the last 20 years. The site has experienced a warming trend of 0.3 °C per decade, with large interannual variation in temperature and precipitation. For six accessions (three old and three recent) we exposed juvenile plants to the stresses of heat and/or drought for one month. Plant biomass and leaf traits (leaf area, specific leaf area, leaf dry matter content) were measured as proxies of fitness under the different treatments. Interestingly, the temporal origin of the seeds did not have a significant effect on plant traits. However, we found differences in the stress response that were linked to inter-annual variation of the climate at the collection site. Specifically, plants from seeds produced in wet/cold years had significantly lower biomass and smaller leaf area when exposed to heat/dry and heat/wet stresses than those from seeds produced in drier/warmer years. As a result, these latter plants showed increased fitness when exposed to similar stress experienced by the mother plant. Our results demonstrate that 20 years of storage produces viable plants of V. alpina, but it may be important to collect seeds frequently as those produced in different years respond differently to stress. Using temporally separated accessions to study climate change

and stress response, we highlight a lack of contemporary evolution but observe environmentally induced plastic responses that may have important implications for V. alpina survival under climate change.

6.0.7 Frost resistance of pedunculate oak seeds and the implications for their storage

ORAL

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Effective seed storage is extremely problematic for short-lived pedunculate oak (Quercus robur L.) seeds, categorized as recalcitrant. We examined the impact of seeds cold acclimation to -7 °C as well as decreasing of seeds moisture content on their frost resistance and further seedlings growth. Simultaneously, we investigated the effect of acorns thermotherapy (the pathogen elimination procedure, 40°C/3h) and storage temperature of acorn in terms of the production of reactive oxygen species (ROS) in their embryonic axes. Results show that fresh pedunculate oak seed can survive freezing temperature down to -10°C and produce good quality seedlings. Temperature around -11° to -13°C is near-lethal to seeds and significantly reduces their viability. Overall, desiccation does not increase their frost resistance, thus acorns should be stored in a highly hydrated state. Thermotherapy did not increase the level of three types of ROS (H2O2; O2-• and •OH). The temporal heat stress of thermotherapy (40°C/3h) resulted in slightly reduced levels of H2O2, indicating activation of the antioxidant systems in acorn preparation for storage. The effect of constant storage temperatures (-3, -5, -7 °C) and their combinations (cold acclimation: -3 → -5 °C or -3 → -5 →-7 °C) on ROS levels and seed viability showed that the highest ROS levels were detected in acorns stored at -7 °C, whereas three-step cold acclimation was beneficial for reducing ROS levels. Thermotherapy and cold acclimation of acorns can improve their viability after storage by decreasing ROS levels.

6.0.8 Inter-population variability in seed germination, reproductive ecology and genetic structure of a rare chasmophyte (Campanula raineri, Campanulaceae): insights for conservation purposes

ORAL

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Campanula raineri Perp. is a stenoendemic chasmophyte of the Italian Prealps. A multidisciplinary approach combining seed germination tests and other reproductive ecology traits (e.g. pollen quality, the trade-off between sexual and vegetative reproduction) across populations, population genomics and past-to-future Species Distribution Models (SDMs) will highlight potential threats for this species and support conservation strategies involving plant material produced from seed. Scattered populations at different elevations across the entire distributional range of C. raineri provide data and material (e.g. seeds for in vitro germination tests and pollen for quality assessment) for the analyses. Population structure is currently being defined using a 2b-RAD genotyping approach followed by bioinformatics analyses of SNPs. SDMs are being elaborated to combine presence/absence data with ecological information (bioclimatic variables, elevation and substrate).Seed germination rate and pollen viability exhibited strong positive relationships with elevation, while flower production decreased slightly. Initial results regarding population structure suggest extensive outbreeding across populations and scarce nucleotide diversity within populations. This genetic uniformity hinders the species ability to cope environmental changes, making it particularly threated. Preliminary models combining only species occurrence localities and bioclimatic variables correctly predict the current distribution

area of C. raineri, and are being integrated with elevation and substrate information and extended in time to describe past and future potential distributions. Preliminary genomics results suggest the need of conservation actions for C. raineri. Projections of future species distribution will identify populations at most risk of climate change, and suitable areas for survival and colonization. In vitro production of plants can easily provide material for population reinforcements or reintroductions, even transferring material between populations -suggested by the high gene flow naturally occurring between populations-, taking into account that high-altitude populations produce less but higher-quality flowers.

6.0.9 Seed ecology of candidate species for restoration of humid forest in New Caledonia: the case of Koniambo Massif

ORAL

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New Caledonia has the important mission to reconcile its exceptional biodiversity with the rapid development of human activities. One of the most important threats is habitat fragmentation especially due to mining exploration on ultramafic soils. Over the past 40 years, revegetation has been developed to reduce such impacts but ecological restoration concept capability and concepts have only been developed in the past 15 years. It involves knowledge of ecological succession and the study of seed ecology for restoration. According to the obligation of KNS mining company, as part of compensation, we investigated on how to accelerate the evolution of para- (MPF) and pre-forest (PF) towards a dense humid forest (F). We first identified forest zones to be studied and reinforced on their edge on the Koniambo massif. We then realized species inventories in the forest and in surrounding vegetation to evaluate species composition. This step permitted to reinforce our database dealing with 41 life-traits such as the reproductive type, the adult height, the type of fruit, the size and weight of seeds, the dispersion type, the germination and dormancy types. It also led to a list of candidate species that can participate to the acceleration due to their presence in all type of vegetation from MPF to F, but also their capacity to close the vegetation and to attract dispersers. From this list, seeds were harvested and studied regarding their structure, germination, dormancy breaking, and conservation capacities in relation with their environment. This also allowed producing plantlets we used for a plantation of 1 hectare both in MPF and PF. An evaluation of success according to the planted vegetation and

species was made. An analysis of all these data will be presented and will led to recommendations to provide to managers for implementing ecological restoration projects in forest environment.

6.0.10 Dormancy release and germination of selected forest species under controlled conditions

ORAL

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To maintain the ambitious plan for wood production and forest conservation, adopted under the 2015 Paris Agreement, effective seedling production is crucial. However, the most economically important forest species in Europe produce dormant seeds, which can affect the final seedlings outcome. Species like European hornbeam (Carpinus betulus L.), mountain ash (Sorbus aucuparia L.), Norway maple (Acer platanoides L.), or common yew (Taxus baccata L.), can differ in their dormancy level and easily induce secondary dormancy. In the case of European hornbeam, viable but non-germinated seeds can consist of ca. 20% of sowed seeds. As a result, not all viable seeds can germinate and eventually converse to seedling. This can be even more important in the case of valuable seeds stored in genebanks used for restoration purposes. The common practice of Autumn sowing in nurseries can result in erratic and unpredictable germination due to the risk of predation and weather conditions during winter and early spring. The stratification and germination under controlled conditions allows for minimizing losses and sowing the seeds to the soil at the optimal time. To delay germination already stratified seed, it can be stored below 0°C for a short time which is commonly used in beech (Fagus sylvatica L.), common ash (Fraxinus excelsior L.), yew and many other species. In this way, it is possible to sow seeds that are fully prepared in advance in the nursery, to avoid their premature germination and minimize the risk of secondary dormancy and unify germination time. In a laboratory, good practice for seed germination capacity test in many forest tree species test is applying temperature cycles of 3°/20°C at 16/8 h intervals, which limits the risk of induction of secondary dormancy and/or allows dormancy release in those seeds in

batch, with have its deeper level. Better understanding of environmental control of dormancy release and germination in many forest species allow to produce higher amounts of quality seedling vital in any reforestation programs.

6.0.11 Understanding germination processes can enhance restoration: a role for the woody endocarp of Morella parvifolia (Benth.) Parra-Os from the south Andes of Ecuador

ORAL

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The Andes of Ecuador are a highly diverse yet threatened biodiversity hotspot. The urgency to recover native biodiversity here is growing, as is the need for knowledge to better inform conservation efforts. We present novel information on temporal patterns of imbibition and germination for endocarps and seeds of Morella parviflora (Myricaceae), a native Tropical Andean shrub ideal for initial stages of restoration. Endocarps and seeds extracted from fresh fruits of M. parvifolia did not express physical or physiological dormancy. Imbibition was completed within 12 hours. However, imbibition rate was about 1.2-fold greater in seeds compared to endocarps. Germination occurred readily at ambient temperatures after 7 (seeds) and 14 (endocarps) days. Interestingly, although seeds tended to germinate more rapidly, endocarps displayed 2.0-fold higher final germination compared to seeds. Seeds displayed high levels of fungal contamination by day 30. Alternatively, contamination did not exceed 1% for endocarps throughout the 60-day germination trial. Fruit storage for 4 or 8 months had a significant effect on imbibition and germination. Imbibition was relatively unchanged in endocarps, but germination lag increased. In seeds, imbibition was reduced while germination lag increased after 8 months of storage. Germination did not exceed 26% for any type of unit after 4 to 8 months of storage. Fresh endocarps contained about 11 mg of residual wax. However, residual wax increased 2.6-fold by 8 months of storage. This may contribute to an osmo-regulatory mechanism in drupes and when combined with

protection against pathogen attack may support a functional role for the endocarp. We suggest that large-scale propagation of M. parvifolia is feasible when using mature, fresh drupes processed within 72 hours of collection. Processing should include the complete removal of the waxy flesh to enhance imbibition. Extracting seeds from endocarps, however, is not recommended for large scale propagation.

6.0.12 Seed Treatments Improve Plant Recruitment in Dryland Environment

ORAL

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The restoration of terrestrial ecosystems is a recognized global priority. While large-scale restoration efforts rely on direct seeding, plant recruitment from seed is most severely limited during the germination and emergence phases of plant development. Seed treatments may promote plant establishment in degraded landscapes, particularly in drylands where severe disturbance and moisture limitations critically curtail natural recovery. The goal of this study was to determine whether seed treatments would increase seedling recruitment of a keystone Australian endemic species (Triodia pungens) in a harsh post-mining restoration scenario. We evaluated seeds treatments (removal of floral appendages, priming in a karrikinolide [KAR1] solution, and seed coating) in their effectiveness to relieve physiological seed dormancy and to promote plant recruitment. We also examined the germination, emergence, and morphological traits of seedlings exposed to a range of moisture conditions typical of the Pilbara Bioregion of northwestern Australia. Seed dormancy and low soil moisture limited seedling recruitment. Combining floret removal with KAR1 priming increased germination up to 67% compared to untreated seeds (7%) or untreated seeds retained in florets (<1%). Seed coating reduced emergence slightly, but priming seeds prior to coating diminished the negative effects of coating and in some cases improved seedling growth in certain moisture conditions. Our study indicates that removing key germination restrictions through seed treatment (floret removal

and KAR1 priming) prior to sowing can enable higher recruitment in moisture-limited conditions and may contribute to greater recruitment of difficult to establish native plant species in highly degraded dryland environments.

6.0.13 Seed biopriming and pelleting with indigenous microbes for restoring native plants in Australian ecosystems

ORAL

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Terrestrial ecosystems worldwide are being seriously affected by degradation processes. Developing cost-effective large-scale solutions to restore these landscapes is crucial to preserve biodiversity and achieve ecosystem functionality and sustainability. In seed-based revegetation, enhancing seed germination and seedling establishment is essential to ensure restoration success. Indigenous soil bacteria, including cyanobacteria from soil biocrusts, have shown promise as bio-fertilizers given their ability to promote seedling growth of native plants. However, these bio-inoculants have not yet been fully exploited in seed-based ecological restoration. In this presentation, we will showcase our most recent research on (i) the effects of bio-priming seeds with indigenous bacteria and cyanobacteria on plant growth of native plants used in ecosystem restoration and (ii) the development of novel methodologies for targeted delivery of functional bacteria and cyanobacteria (i.e., via seed bio-encapsulation in extruded pellets). Our results have shown that both bacteria and cyanobacteria promote germination and seedling growth of native Australian plants, including hummock grasses, Acacia trees and shrubs. We also found that microbial inoculation can increase the levels

of soil carbon and microbial diversity and promote seedling growth of native plants under abiotic stress conditions, i.e., salinity and drought. These microbial-based approaches are now ready to be applied in large-scale ecosystem restoration programs.

6.0.14 Enhancing direct seeding with unmanned aerial vehicles, operational capacity, and seed technology in the western US and beyond

ORAL

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DroneSeed is a Seattle-based startup developing software, hardware, and infrastructure for land surveying, and aerial seed deployment in forests and rangelands. Wildfire and other large-scale ecosystem disturbances are increasing in frequency and severity. Constraints to post-disturbance revegetation include accessibility to remote areas, difficulty with precise distribution of limited seed supply at scale, biotic and abiotic conditions (e.g. invasive species, seed predation, and moisture availability), and associated costs (e.g. labor). DroneSeed provides post-disturbance recovery programs that include surveying and distribution of native seed that has been manufactured into a vessel to deliver substrates and amendments to protect seed and promote germination. DroneSeed is trialing this technology for public, private, and philanthropic land management organizations throughout the US, with hundreds of hectares of trials underway in New Zealand and Canada as well. The presentation will provide an overview of the company's technology and innovations to revegetation processes, introduce select projects, and highlight the research and development supporting our data-driven approach. We will focus on efforts from 2018 to 2022 to collect greenhouse and field data on the efficacy of seed enablement technology in direct seeding post-fire and post-harvest landscapes across several biomes and ecotypes. We will present the seedling establishment results of project sites with conifers, grasses and forbs, as well as tropical hardwoods. We will share next steps and how this seeding technology can play a role in restoration and address challenges we still face while building towards scalable solutions for ecosystem restoration.

6.0.15 The project Life SEEDFORCE, an integrated approach for plant translocation

ORAL

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Developing successful plant conservation projects is a challenging task and requires an inclusive and multi-disciplinary approach, putting together diverse expertise to address all critical issues for a successful outcome. An integrated in-situ and ex-situ approach can yield numerous benefits and offer opportunities to showcase to the public the actual plants that need protection, building participation and support from the local community. The Life+ SEEDFORCE project aims at improving the conservation status of 29 plant species from Italy, Malta, Slovenia and France, reported in bad conditions according to art. 17 of the 92/43/EEC Habitats Directive. The project includes best practice preparatory actions to assess the genetic make-up of the target species and populations, analyse the current species climatic envelope and project possible future scenarios, taking into account trophic decencies in target species. The outcome of such preparatory actions will be used to prepare the propagation mix best adapted for each site to be

use for reintroduction and population reinforcement. Threats to each species will be first identified and removed. The genetic make-up and the ecological niche will support the identification of the appropriate donor populations and the development of joint protocols for species propagation and population reinforcements or re-establishments in extinct sites. A series of engagement activities have been devised to gain public support and to involve farmers that in most cases can influence the long-term conservation of many plant species in secondary habitats. The transnational partnership, covering all the natural distribution range of the selected species will allow a fruitful exchange of experience to better investigate the plant biology, aiming at understanding differences in species conservation status and management among neighboring countries.

6.0.16 Pairing population genetics with seed and seedling traits to quantify inbreeding impacts on an endangered species

POSTER

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The long-term viability, and a potential limiting factor, of threatened plant species is often related to seed production and subsequent seedling vigor. Understanding such limitations can be difficult, however coupling knowledge of the mating system and population genetic kinship provides a powerful approach for quantifying this. Using the critically endangered shrub Hibbertia spanantha as a case study, we confirmed that high kinship (relatedness) can reduce quantity and weight of viable seeds. Further, these impacts continue to be seen in seedling vigor both in the nursery and when planted to augment wild populations. This research has far reaching implications for how we select plants for ex situ conservation and seed storage and emphasizes the need to understand plant mating systems and utilize genetics as part of planning conservation of threatened species.

6.0.17 Warm treatments break the dormancy of stored seeds of Carex radiata (Wahlenb.) Small

POSTER

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Seed longevity and vigor assessment are crucial to the effective management of seed conservation collections in genebanks because it validates the selection of viability re-test intervals and, therefore, recollection strategies. As well, determining the germination requirements of a species is essential to ensure seed quality during storage. However, knowledge on breaking dormancy and germinating seeds of wild species stored under seed bank conditions before they lose significant viability is still unknown for a high number of species in the USA. We investigated the germination requirements and seed longevity of Carex radiata seeds collected from temperate forest habitat in the Midwestern USA in a seed bank using the move-along technique. Germination of 15 and 3 year-old seeds stored at -20C following drying to 20%rH in the Dixon National Tallgrass Seedbank (Illinois, USA) was determined by incubating them for 4 to 16 weeks at several temperatures that simulate the different seasons in a natural habitat. The results showed that germination of the 3-year-old collection was highest (up to 69%) when conditions were above 77/59oF (25/15oC), which occurred primarily between 4 and 16 weeks in this temperature. Similar germination was observed when seeds were exposed to 16 weeks of cold stratification (41oF/5oC) and then placed into incubation at 59-68oF (15-20oC). This also occurred mainly between 4 and 16 weeks after being placed in incubation. In contrast, germination of the 15-year-old accession was extremely low (maximum of 7% germination under any treatment condition). Thus, the most significant germination values were in the warmer incubation temperatures. Long-

term (15 years old or over) stored seed of Carex radiata may not remain viable following standard seed bank protocols.

6.0.18 The impact of ozone treatment on seed germination and pathogen infections in wheat, barley, triticale and rapeseeds

POSTER

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Seeds of agriculture crops can be contaminated by fungal species that affect the germination rate of seeds and the final yield of crops. Seed treatment plays an important role in sustainable agriculture and therefore alternatives to chemical seed treatment are being sought. Physical seed treatment of seeds could be one of environmentally friendly technology. The objective of this study was to assess the efficacy of O3 treatment on seeds germination (germination rate, root and germ length) and fungi infection in four agriculture crops – spring wheat (cultivar Registana), winter barley (cv. KWS Meridian), winter triticale (cv. Tulus) and spring rapeseed (cv. Ovace). The seeds were divided into control groups (no O3 gas) and treated groups (3 ppm), which were exposed for 5, 10 and 60 min (maximum length treatment). The ozone-treated samples of both variants (5 and 10 min) were vigorous, and very healthy. However, the initial development of the ozone treated seeds was slower compared to the control. After seven days of germination, the ozone treated samples showed values comparable to or higher than the control, the response was quite individual according to the crop species tested.

Interestingly, the application of a 60 min ozone dose did not affect germination parameters. The control seeds (no O3 gas) and ozone treated seeds (5 and 10 min) of four crops were cultivated on potato dextrose agar to monitor microscopic fungi. Statistical analysis proved important differences between tested crops and between the ozone exposure times on the seeds. The highest number of CFU (colony forming units) of microscopic fungi was detected in non-treated samples (no O3 gas) of all crops. The highest effect of ozone treatment was observed in barley and wheat samples treated for 10 min. The most frequent genera of cultivated fungi were Penicillium, Aspergillus and Alternaria.

6.0.19 Seed lipid thermal fingerprints of Mediterranean terrestrial orchids in relation to their ecology and ex situ conservation

POSTER

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Ex-situ seed storage offers a potential solution to mitigate the extinction risk of threatened plants. However, dry seed longevity under conventional seedbank conditions (-20°C) is known to be extremely variable, with some families tending to be relatively short-lived, like Orchidaceae. Seed lipids have been thought to be a determinant of seed ageing with lipid composition impacting differing susceptibility to oxidation and variation in thermal behaviour: e.g. unsaturated fatty acids are more prone to oxidation during ageing at high temperatures but they seem to provide larger thermal stability (than saturated fatty acids) during -20°C storage. Understanding the balance of lipid composition and thermal characteristics of their melting and crystallization behaviour could prove a useful tool for identifying longer-term cold storage problems in lipid-rich seeds, helping to inform storage practices. Terrestrial orchid seeds are orthodox in storage behaviour and have lipid as the main storage compound. Only very few data are available on the thermal properties of orchid seeds. We have used differential scanning calorimetry to explore potential links between poor storage performance, lipid composition and lipid thermal fingerprints of seeds of 15 Mediterranean terrestrial orchids. We have determined the temperature spread and complexity of the lipid melt, the peak temperature and enthalpy of the main peak, and some aspects of the short-term crystallization kinetics. We aimed to test the hypothesis that Mediterranean orchid seeds, as typical for temperate environments,

have polyunsaturated fatty acids with melting temperatures around -30°C as the main storage lipids. We wanted to test whether species presenting short lifespans present lipid compositions that tend to show multiple melting events at -20°C and high temperature melting ends, as typical in the saturated fatty acids present in many tropical seeds. Data are interpreted in relation to the ecology of the species and the risk of lipid crystallization during cold storage.

6.0.20 Ex situ conservation guidelines for Australia's national plant treasures

POSTER

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The Australian Network for Plant Conservation recently released the third edition of Plant Germplasm Conservation in Australia: strategies and guidelines for developing, managing and utilizing ex situ collections. Known as the Australian Germplasm Guidelines, this publication is technical and practical, setting a benchmark for ex situ conservation of wild species into the future. The Germplasm Guidelines distils research on seed biology and ecology into practical actions for effective seed banking at various scales, with a workflow to address each step of acquiring, maintaining and utilizing genetically representative collections. An understanding of seed ecology informs sections of the Guidelines dealing with seed germination, seed storage behaviour and seed longevity in storage. The need to capture and utilize 'special' types of germplasm (material from ferns, mosses, liverworts), or taxa with 'special' life history stages or growing requirements (terrestrial orchids with mycorrhizal associations, carnivorous and parasitic plants) requires an understanding of species ecology. Ex situ collections such as those maintained by the Australian Seed Bank Partnership also enable ecological research

to be conducted on difficult-to-collect, rare or threatened species. The Germplasm Guidelines complements the 'Guidelines for the Translocation of Threatened Plants in Australia 3rd edition' and the 'Florabank Guidelines for native seed collection and use 2nd edition', published in 2018 and 2021 respectively. This trio of publications provides guidance on how to conserve and restore the biodiverse flora of Australia in the face of multiple threats. The approaches, techniques and technologies included in the Guidelines are broadly applicable to plants across the globe. These Guidelines were updated with funding from The Ian Potter Foundation, in collaboration with the Australian Seed Bank Partnership and the assistance of the restoration and agriculture sectors, botanic gardens, CSIRO and universities. Many contributors play a role in ecological restoration, seed conservation, ecological research, or related fields.

6.0.21 Best-practice guidelines for threatened plant translocation, native seed use, and plant germplasm conservation

POSTER

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Over the past few years, the Australian Network for Plant Conservation (ANPC) has led the revision and publication of the following three best-practice guidelines: (1) Guidelines for the Translocation of Threatened Plants in Australia; (2) Florabank Guidelines for native seed collection and use; (3) Plant Germplasm Conservation in Australia for ex situ conservation. These guidelines can be used separately or together to inform plant conservation and restoration in Australia and across the world. All guidelines support action towards restoring functional, self-sustaining plant populations by providing guidance on different aspects of this process. Each one brings together current literature and best practice to create an accessible, evidence-based handbook. They also link readers into international guidelines, with relatable Australian examples. The Germplasm Guidelines and Translocation Guidelines feature case studies that capture collaborative efforts and showcase innovative methods. All three guidelines are available for free download from the ANPC website https://www.anpc.asn.au/product-category/publications/.

6.0.22 Ex situ conservation experiences for seven threatened taxa: the case of the Life FLORANET project for the conservation of plant biodiversity in the Apennines (Italy)

POSTER

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The Life Floranet project has just ended, with the main objective of protecting and improving the conservation status of seven plant species of EU importance (Annexes II-IV to Directive 92/43/EEC) within the Natura 2000 areas of the Central Apennines (Italy): Maiella National Park, National Park of Abruzzo, Lazio and Molise and Sirente-Velino Regional Park. The selected species investigated in this project are: Cypripedium calceolus L., Adonis distorta Ten., Androsace mathildae Levier, Iris marsica I. Ricci & Colas., Astragalus aquilanus Anzal., Klasea lycopifolia (Vill.) Á. Löve & D. Löve, and Jacobaea vulgaris subsp. gotlandica (Neuman) B. Nord. In situ and ex situ conservation strategies have been adopted, as well as reducing the tourist impact, awareness campaigns among stakeholders. These strategies are essential to carry out concrete actions for effective species conservation. Specifically, for each species, we have performed: seed collection, in vitro propagation, seed reproduction, long-term conservation, in situ repopulation and creation of new populations. The project involved collecting seeds using ENSCONET protocols and thousands of seeds were obtained which were processed at the Maiella germplasm bank in the Plant Biodiversity Conservation Center. Part of the seeds were stored at -20 ° C (with duplicates at KEW's Millennium Seed Bank) and part was used to carry out germination tests. From these tests, four germination protocols were developed with very high percentages, and seedlings from all target species were

obtained. These seedlings through the nurseries of the Maiella National Park were made to grow and were used for some restocking interventions in nature. In this context, the Floranet project plays a crucial role in implementing conservation strategies and monitoring endemic or rare species, reducing their extinction risk. These actions strengthen the conservation role of the ex situ structures through an integrated conservation strategy combining in situ and ex situ activities.

6.0.23 Success of native hay-seed mix for dry grassland restoration: do not neglect the role of soil seed bank

POSTER

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Ecological restoration has been proved to be an effective strategy to contrast biodiversity loss due to land use change. Among terrestrial ecosystems, dry grasslands represent biodiversity hotspot strongly threatened by soil consumption. Passive grassland restoration, based on spontaneous succession, is highly unpredictable and, in some cases, unsuitable to re-establish native communities. The use of spontaneous seeds (hay seed) and or/ commercial seed mix might contribute to boost early restoration stages by triggering native plant colonization. A crucial stage for the success of any restoration action and for determining community composition is the seeds germination. In addition, the soil seedbank of restoration sites can exert main effect on the final plant assembly, even if it is often neglected. We designed a controlled environment experiment in which commercial grassland seed-mix and spontaneous hay seeds collected in local dry grassland were sown on commercial soil (seedbank less) and agricultural soil (with seedbank) with two seed density. We hypothesized soil, seed mix type and density to interplay in determining community diversity and composition. Commercial seed provided greater species abundance and rapid containment of seedbank species. The abundance of the seedbank ruderal species greatly contributed to the total biodiversity in the case of hay seeds. The number of individuals of species not belonging to the seedbank has always been higher if

commercial seed mix was sown; in the case of hay seeds this parameter was favored by agricultural soil probably due to the substrate or to positive interaction with seedbank species. The composition of functional groups (i.e. monocot vs dicot and life span) and species assembly were also influenced by the soil and seed mix type. Soil seedbank effect in determining the success of site restoration when operating seed sowing should not be overlooked, alternative management actions aimed at curb seedbank germination should be, hence, considered.

6.0.24 ECOLOGY AND PHYSIOLOGY OF COASTAL DUNE PLANT SEEDS AS A MODEL FOR THEIR CONSERVATION

POSTER

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Coastal dune vegetation is adapted to tolerate extreme environments of high salinity, high temperature, low precipitation, low nutrients, and burial on accreting beaches. This vegetation provides important ecosystem services, such as erosion prevention and control, protection against hurricanes, biodiversity refuge, and provision of forest resources. Nevertheless, most coastal dune ecosystems are endangered due to habitat degradation resulting from the exploitation of natural resources for housing, tourism, agriculture and industry, and other factors such as invasive species, sea level rise, and climate change. In Mexico, these ecosystems are undervalued and among the less studied, estimating more than 50 % of their 800,000 hectares have been lost. Studies on the use of native pioneer species have shown these represent a potential for successful dune stabilization, but more studies on dune management in restoration projects are required. Studies of physiology, ecology, and conservation of native seeds are needed in restoration projects to recover the health of these degraded ecosystems. To be successful, restoration programs must consider the establishment of different stages in their protocols, before and during revegetation, choosing optimal collection sites, properly collecting seeds, describing dormancy and germination cues to improve the production of native plant materials, and storing viable seeds in adequate conditions for each species, functioning as a gene bank. Gene banks are

an ex situ conservation option for storing dried seeds from wild populations at low temperatures. However, not all seeds can be stored in traditional seed bank conditions, so it is necessary to study each species physiology to propose better conservation options. The present work evaluates the ecological and physiological behavior of coastal dune seeds in order to find the adequate conditions of ex situ conservation by analyzing the germinative responses, dormancy break and desiccation tolerance of the main pioneer coastal dune species in two Southeast Mexican beaches.

6.0.25 Seed priming to optimize germination in Arthrocnemum Moq.: a prime halophyte candidate for agricultural and biotechnological uses

POSTER

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Seed germination and seedling growth constitute the first stage of a plant's life cycle for crop establishment. Arthrocnemum Moq. is a halophyte of the subfamily Salicornioideae (Amaranthaceae), which could be recognized in the foreseeable future as an emerging candidate in applied biosaline agricultural programs, mainly due to the large biomass it represents in coastal and inland saltmarshes, in addition to its interesting nutritional and pharmacological properties. However, to ensure their subsequent use as a crop, it is necessary to optimize their germination through appropriate seed priming treatments. The main goal of this work was to seek the optimization of Arthrocnemum germination process using different pretreatments (exposure to high concentrations of salt in the dark and its subsequent transferred to distilled water separately and together with the combination of pH, salinity, and iron conditions). The experiments were tested on six samples of two different species: A. meridionale (from Tunisia) and A. macrostachyum (from Spain). Salinity priming of seeds for 15 days in darkness improved germination percentages by almost 25% at 600 mM NaCl, in both Tunisian and Spanish species. However, keeping seeds at different salt concentrations for 30 days produced higher improvement percentages at lower concentrations in A. meridionale (100-200 mM NaCl), while in A. macrostachyum the highest improvement percentages were obtained at 600 mM NaCl (per-

centage improvement of 47%). When the dark time period is reduced to 5 days at higher salt concentrations, the greater germination percentages were reached in all the samples at the concentration of 800 mM NaCl, increasing the improvement of germination between 17% and 50%. Finally, the conditions of pH = 7, pretreatment in darkness at 800 mM NaCl and 400 µM or iron, turned out to be an effective medium for seed germination.

6.0.26 FLEURS LOCALES, un proyecto sobre la cadena de valor de la producción de semillas nativas en el sudoeste de Europa

POSTER

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El incremento de las restauraciones medioambientales, el paisajismos sostenible y la restauración de la biodiversidad útil en los agrosistemas de toda Europa, demandan cantidades crecientes semillas de especies silvestres con garantía de origen geográfico y genético. Esta demanda se ha cubierto en cada país de manera diferente dependiendo limitaciones administrativas y condiciones sociales entre otras razones. A pesar de ello este incremento en el uso de las semillas nativas ha generado un aún incipiente nuevo sector productivo de semillas nativas en todos y cada uno de los países de Europa. Bajo esta situación de desarrollo del sector y debido a las limitaciones técnicas, falta de experiencia y disparidad de situaciones en los países del Sudoeste de Europa, se hace necesario la adopción de estándares de calidad y una ética en la producción de las semillas, que garanticen la originalidad de la oferta en términos genéticos y permitan el desarrollo del sector de manera organizada. Para ello, el proyecto Europeo INTERREG-SUDOE FLEURS LOCALES, analiza la cadena de valor de la producción y uso de semillas nativas en el Sudoeste de Europa. Los siete socios principales y trece miembros asociados, distribuidos en tres países (Francia, Portugal y España), y en diversos sectores implicado en esta cadena (producción de semillas, usuarios profesionales, responsables políticos, asociaciones de agricultores, administraciones regionales y estatales,...) tienen como objetivo conocer la situación de la producción de las semillas nativas, sus limitaciones y posibilidades de desarrollo futuro. Durante los tres años del proyecto (2020-23) se trabaja en varios territorios piloto para experimentar a partir de métodos comunes de trabajo, donde cada socio

implementa cadenas de restauración que responden a retos locales específicos ya sean cubiertas vegetales en cultivos leñosos, praderas floridas o zonas dañadas por los incendios.

6.0.27 Reducing seed losses in commercial seed production through understanding of the genetic mechanisms controlling seed shattering

POSTER

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Seed loss due to unfavorable weather conditions close to the time of harvest or lack of uniform seed maturation poses a great challenge for the commercial seed production of several crops. Non-shattering phenotypes have been selected through domestication in crops such as rice and wheat, and the genetic mechanisms controlling this trait have been widely investigated. However, in less studied plants, such as parsnip, where no reference genome sequence is available and genetic resources are limited, understanding how seed shattering is controlled is crucial in order to develop strategies for the reduction of seed losses in a commercial seed production setting. We have developed a method for the assessment of seed shattering in parsnip and we screened a population of 33 genetically diverse parsnip lines for their ability to retain the majority of seed on the plant after commercial seed maturation. We have observed a great variation between these genotypes and identified lines with extreme phenotypes that will be used for further QTL mapping. In addition, we are using a reverse genetics approach to identify orthologues of known key regulators of seed shattering and study their function in parsnip. Our work will help to identify and develop markers of reduced seed shattering in parsnip that could be incorporated in breeding programs to reduce seed losses.

Chapter 7

Environmental control of seed germination timing

7.0.1 Smaller, lighter, later: effects of a future warmer and drier climate on alpine seeds and seedlings

ORAL

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Early plant life-history stages, such as germination and seedling establishment, are considered highly vulnerable to climate change and are important to species persistence because they could represent a bottleneck to future recruitment. The strong relationship between the changing climatic factors and early life-history stages suggests that they will be significantly affected. Here, we present the results of a field manipulative experiment where we looked at how (i) seed maturation, (ii) seed germination and (iii) seedling establishment of ten alpine species will respond to a future warmer and drier climate. For this, we established a two-factorial experiment at the Australian Alps. We created warmer and drier conditions using modified open-top chambers. Then, to determine the effects of our experimental treatments in the studied life stages, we: (i) collected seeds from plants inside the chambers and contrasted their mass, size, viability and cotyledons size and greenness against seeds collected in control plots; (ii) we buried seeds inside mesh bags and recorded their monthly germination; and (iii) we planted seedlings and measured their monthly growth and survival. Overall, we find negative and neutral effects of our experimental treatments in the studied life stages. (i) Seeds from plants inside the chambers were significantly lighter and smaller and had smaller and less green cotyledons in almost all species. (ii) The final proportion of germinated seeds was negatively affected by warmer and drier conditions in all species. More importantly, warmer and drier conditions also affected the germination time, where most germination occurred later in the snow-free season compared to control sites. (iii) Seedling survival and growth were negatively affected by warmer and

drier conditions. Finally, we discuss some of the potential implications of our findings and their significance to our understanding of future plant recruitment in alpine areas.

7.0.2 Germination patterns under climate change in the plants of the Central Anatolian steppe

ORAL

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Climate change may affect plant community composition and dynamics by altering seed germination patterns in several ecosystems. In this study, we studied the germination response of 80 plant species from Central Anatolian steppes, an area falls within the Irano-Anatolian global biodiversity hotspot, to various incubation temperatures, cold stratification, and drought/salinity conditions. Seeds were collected from natural habitats in Central Anatolia, Turkey. The fixed and alternate incubation temperatures (10, 15, 20, 25, 30, 35, 20/10, 25/15, 30/20, 25/35 °C), a cold stratification treatment (4 °C for 30 days), and drought and salt stress with various osmotic potential levels (0, -0.25, -0.50, -0.75, -1.00 MPa of PEG 6000 and NaCl) were examined in the study. Temperature experiments were performed under complete dark and photoperiod (12h:12h dark/light) conditions, while drought and salinity tolerance tests were conducted under 20°C temperature and 12h:12h photoperiod conditions. Germination checks lasted for 40 days for each experiment. Germination data were analyzed using the analysis of deviance assuming a binomial distribution. Germination responses of the species to different incubation temperatures and cold stratification were idiosyncratic at the species level. The germination percentages decreased in some species as incubation temperature increased, but some species showed the opposite pattern. On the contrary, the germination percentage gradually decreased with increased PEG 6000 and salinity levels. However, the tolerance limit to drought and salt stress was also idiosyncratic as the

maximum osmotic potential that plant species can tolerate was different. Some species such as Bassia prostata, Eremophyrum triticeum, Fumana aciphylla, Salsola stenoptera, and Taeniatherium caput-medusae showed high germination percentages even at the highest level of incubation temperature and stress treatments. The results indicate species-specific germination response to temperature, drought, and salt stress and suggest that seed germination patterns in steppe plant communities of Central Anatolia can significantly be altered by climate change.

7.0.3 Adaptation of European beech (Fagus sylvatica L.) to the variable environments in the term of seed germination

ORAL

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Global climate change alters environmental conditions, and thus affects the reproduction of plants from seeds. The mechanism underlying climate adaptation is a key element in predicting the potential of species to face climate warming. One of the adaptations to environmental conditions is the dormancy phenomenon, which allows the coordination of seed germination and plant establishment with the environment. This work focuses on presenting the adaptation mechanism of beech to the various environments, with emphasis on the prospective role in adaptation to the changing climate. Beech seeds from 26 Polish climatic provenances were investigated in terms of seed dormancy depth and germination and seedling traits. Any correlations between these traits and the environmental conditions in the habitat of each investigated beech stand were investigated. Results showed that climatic characteristics of seeds provenances more strongly affect seed characteristics than seedling parameters. Variation was statistically significant between the two regions of seed origin. The group of northern populations started to germinate later (by 3 weeks), and had a longer average time to germination (by 2 weeks), but a shorter duration of germination time (by 1 week) than the group of southern populations. Beech seed dormancy and germination depend on latitude, longitude and precipitation seasonality. Especially germination capacity depended on the climatic conditions in the population stand, and differentiation in temperature and precipitation seasonality was the significant factor. All tested seedling traits differed among seed origins. The effect of seed characteristics on seedling traits

was observed. The observed link between seed germination, seedling growth and climatic conditions may imply that in the face of global warming, increasing temperature and decreasing precipitation can be the limiting factors for beech populations. This research was supported by Project No. 2019/33/B/NZ9/02660 funded by the National Science Centre, Poland, to Pawłowski T.A.

7.0.4 Multiple relations between seed traits and germination performance change with temperature

ORAL

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Global warming triggers multiple responses in plant populations and communities. Since seed functional traits are essential co-variates to understand germination timing but also survival during the seed bank stage it is crucial to know whether these relationships remain stable across -or vary over- environmental temperatures. Here we present the relationships between seed traits and germination speed at different exposure temperatures for 37 herbaceous species. At low temperatures (10°C) germination rates increase with shape, lightness, and ratio of embryo to seed. At intermediate temperatures (20°C), germination rates decrease with mass, but increase with embryo to seed ratio and terminal velocity. At high temperatures (30°C), germination rates decrease with seed shape. Our work demonstrates the importance of temperature for studying seed trait – performance relations and we suggest to proceed with studying seed-related processes at different temperatures in the seed environment.

7.0.5 Environment and polyploidy affect germination of rare shrub species

ORAL

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Lydia.Guja@environment.gov.au Ploidy and species threat status have both been linked to variation in phenotypic and phenological seed and seedling traits, including seed size, germination rate (speed) and seedling stature. We determined whether ploidy (the heritable condition of possessing more than two chromosome sets) and range size (related to threat status) are associated with variation in seed and seedling traits that might limit the regeneration performance of obligate seeders in fire-prone systems. We investigated Pomaderris, a genus of shrubs that includes many threatened species of conservation concern and both diploid and polyploid taxa. We experimentally quantified seed dormancy and germination using fire-related heat treatments and evaluated seedling performance under drought stress. We also examined the association of seed size with other seed and seedling traits; and investigated the temperature thresholds for dormancy alleviation. Polyploids had bigger seeds, a faster germination rate and larger and taller seedlings than diploids. There was a lack of any clear relationship between range size and seed or seedling traits. Dormancy alleviation thresholds appeared to be linked to habitat. The ploidy effects observed for many traits are likely to be indirect and associated with the underlying seed size differences. These findings indicate a higher potential competitive advantage in polyploid than diploid Pomaderris during regeneration, a critical stage in the post-fire environment. These insights to the regeneration phase can inform

conservation seed banking and may need to be considered when planning and prioritizing management of threatened species.

7.0.6 Molecular mechanisms of allelochemicals in germination and seedling establishment

ORAL

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How phytotoxic allelochemicals interfere with seed germination and seedling establishment is largely unknown. Knowledge of the underpinning molecular mechanisms is however decisive for the chemical ecology of plant competition and the dynamics of natural plant communities. In contrast to most synthetic phytotoxic chemicals (xenobiotics), natural compounds in general have multiple molecular targets and numerous bioactivities. We demonstrate here that the flavonoid myrigalone A (MyA), a rare dihydrochalcone and putative allelochemical in the fruit leachates of Myrica gale (bog myrtle), inhibits the seed germination and seedling growth of target species via multiple molecular pathways. Allelopathy assays using Lepidium sativum (garden cress) as target species combined with (eco)physiological, biomechanical and biochemical analyses revealed that MyA interferes with key processes which have endosperm weakening and embryo growth as target. Our recent work employed transcriptome (RNAseq) and hormone analyses which demonstrated that MyA triggers a phased detoxification programme and inhibits cress seed germination via multiple mechanisms and interference with several molecular pathways. Evaluation of MyA's proposed bioactivities reveals that it, in contrast to several other chalcones, does not act as bleaching herbicide and is not a p-hydroxyphenylpyruvate

dioxygenase (HPPD) inhibitor. MyA acts by interference with cell growth processes including by reducing the production of apoplastic reactive oxygen species. MyA acts by interference with the seed's hormone metabolism, signaling and/or transport targeting gibberellin, jasmonate, ethylene and auxin pathways. The interference with auxin homeostasis was associated with altered expression patterns of auxin transporter genes required for proper seed germination and seedling growth. Our comparative analysis of the MyA gene expression responses with other chalcones and several chemically distinct plant-derived phytotoxins revealed that most allelochemicals seem to interfere with auxin transport. They however differ in which auxin transporter genes they have as targets. The importance and ecophysiology of conserved and allelochemical-specific mechanisms will be discussed.

7.0.7 Ecophysiological and molecular mechanisms of blackgrass weed seed bank persistence

ORAL

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The sole use of herbicides for the control of the weed blackgrass (Alopecurus myosuroides) is becoming increasingly unsustainable due to the widespread diffusion of herbicide resistance in this species, making blackgrass the most herbicide-resistant weed in the UK. Estimation of the seedling emergence pattern of blackgrass in the field is becoming key in the development of alternative strategies to control the weeds persistence in the soil seedbank. Blackgrass is characterized by two emergence peaks, allowing it to compete against both winter and spring crops in the field. Blackgrass seeds can also enter secondary dormancy, a state which provides the weed with a survival advantage when environmental conditions for plant growth are unfavorable. Seed dormancy is also known to be an important factor to the persistence of seeds. While some ecological studies have been conducted to look at secondary dormancy and persistence of blackgrass seeds in the field, the molecular mechanisms underlying these processes have not yet been characterized. We investigated the mechanisms of blackgrass physiological dormancy by modelling seed responses to different temperatures as major environmental factor and by comparing distinct seed dormancy states. Transcriptome analysis (RNAseq) identified key hormone biosynthesis and signaling pathways genes involved in blackgrass dormancy in relation to temperature as environmental cue and the dormant state as intrinsic seed property. These findings will help provide a better understanding of the change in the physiological state of blackgrass

seeds in the soil seedbank in response to the environment. They will be further used to improve existing mathematical models of blackgrass emergence in the field, which can help identify more effective blackgrass control strategies.

7.0.8 Pericarp-elicited transcriptomic and hormonomic changes underpin early life history traits in the dimorphic diaspores of Aethionema arabicum

ORAL

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The dimorphic annual Aethionema arabicum is a Brassicaceae producing two distinct fruit and seed morphotypes. Smaller indehiscent fruits contain a single non-mucilaginous seed ('M-') dispersed, via abscission, enclosed in pericarps as fruit diaspores. Larger dehiscent fruits contain around four mucilaginous ('M+') seeds and dehisce on maturity dispersing bare M+ seeds as seed diaspores. The ratio of the two fruit morphotypes is affected by the environmental conditions, including ambient temperature, experienced by the mother plant, with M+ seed favored at high temperature. This plastic diaspore bet-hedging strategy may facilitate long-term fitness an Anatolian habitat where temperature and aridity extremes occur with growing in screes at different elevations. The two diaspores contrast in germination ecology. Pericarp-imposed dormancy restricts germination of M- seed-harboring fruits to lower temperatures compared to the M+ seeds. Our large-scale RNAseq experiment compared transcriptomes of M+ and M- seed from plants grown at high and low temperatures imbibed at four temperatures at multiple time points. Further, we assessed the effect of pericarp removal on the physiology, transcriptome and hormonome of M- seeds. We found the pericarp, which contains germination inhibitors, altered the transcriptome

of the M- seed increasing expression of dormancy-associated gene modules and repressing germination-associated gene modules. Fruits from parent plants grown in different environments differed in germination-inhibiting potency of the pericarp contents and the sensitivity of the M-seed to the germination inhibitors. We discuss the role of the pericarp in integrating temperature in the plant's diaspore dimorphism syndrome to define contrasting early life-histories of the two diaspore morphotypes in adaptation to its natural habitat.

7.0.9 Seed priming with gas plasma activated water in Ethiopia's "orphan" crop tef (Eragrostis tef)

ORAL

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Tef (Eragrostis tef) is a cereal grass and a major staple crop of Ethiopia and Eritrea. Despite its significant importance in terms of production, consumption, and cash crop value, tef has been understudied and its productivity is low. In this study, tef grains have undergone different priming treatments to enhance seed vigor and seedling performance. A conventional hydro-priming and a novel additive priming technology with gas plasma activated water have been used and tef grains were then subjected to germination performance assays and accelerated ageing. Tef priming increases the germination speed and vigor of the grains which leads to more uniform germination and better germination timing in the environment amongst other benefits. Priming with gas plasma activated water retained the seed storage potential after ageing, therefore presenting an innovative environmental-friendly seed technology with the prospect to address variable weather conditions and ultimately food insecurity. Seed technology opens new possibilities to enable precision agriculture that leads to increased productivity of tef crop farming to

achieve a secure and resilient tef food system and economic growth in Ethiopia by sustainable intensification agriculture beyond breeding. Beyond "orphan" crops innovative seed technologies such as priming with gas plasma activated water may also be used for improving seed quality in habitat restoration projects.

7.0.10 Impact of red:far red ratios on germination in Australia's tropical mountain cloud forests

ORAL

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In Australia, tropical mountain cloud forests (TMCF) are restricted to the Wet Tropics World Heritage Area of north-eastern Queensland and contain over 70 endemic plant species. Suitable TMCF habitat is predicted to decline or disappear for many of these species by 2085. However, virtually nothing is known about factors that determine the regeneration and distribution of these species. Regeneration via seed is determined by temperature, moisture and light conditions. Light quality, specifically the ratio of red to far-red light (R:FR; 660:730 nm), has been shown to influence seed germination of light-sensitive seeds, including many small-seeded tropical species. Canopy cover, leaf litter and cloud immersion all affect the R:FR that plants are exposed to, potentially permitting fine-scale discrimination of light conditions for seed germination. We investigated the impacts of R:FR using natural sunlight and green polyester filters. Seeds of six previously unstudied TMCF species were sown in a temperature-controlled glasshouse beneath a gradient of R:FR ranging from 0.1 (such as beneath leaf litter and a thick plant canopy) to 1.14 (unfiltered sunlight). Germination of Dracophyllum sayeri increased exponentially with increasing R:FR, including low germination in the dark. In contrast, Abrophyllum ornans, Melastoma

malabathricum subsp. malabathricum and Lenbrassia australiana required light for germination and germinated well in all R:FR. Similarly, light quality had no effect on germination of Dianella caerulea. Interestingly, germination of Gahnia sieberiana was greater in the dark than in unfiltered light but was inhibited by a low R:FR reminiscent of beneath a plant canopy or leaf litter. Findings provide insights into plant recruitment in situ, and the acclimation potential of these species. Such information can also inform the long-term management of TMCF flora.

7.0.11 Effects of fire season on the reproductive effort and seed resourcing of a post-fire flowerer

ORAL

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Fire is an integral part of many ecosystems and recent record-breaking fires in natural systems around the world are indicative of changes occurring to the fire regime. While fire severity and frequency are usually the focus of studies, fire seasonality also plays a major role particularly in the reproductive success of plant species. Post-fire flowerers are particularly susceptible to changes in fire seasonality, their seeds usually lack dormancy and are reliant on post fire conditions. We examined the effect of fire season on the reproductive effort and seed resourcing of the post-fire flowerer Doryanthes excelsa from eastern Australia. We conducted field experiments at sites burnt in different seasons and of different soil moistures. Reproductive success was measured through seed vigor, including seed germination and the percentage of lipids, carbohydrates, and crude proteins of seeds, as well as the percentage flowering and the number of seed pods. What we found was that sites burnt in summer increased germination, lipid levels in seeds, the percentage flowering and the number of seed pods, when compared to sites burnt in spring or autumn. Flowering was also delayed when burnt outside of summer. Post-fire flowerers are a major functional group within communities that frequently experience fire. Ongoing climatic changes driving shifts in the fire regime pose an imperative to consider fire season in management to conserve this important functional group.

7.0.12 Environmental thermal regulation of dormancy in Echinochloa crus-galli (L.) P. Beauv. seeds

ORAL

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Predicting weed emergence is of vital importance for weed management, since emergence is a key process in weed population dynamics and the seedling stage is the most effective for the application of control practices. In weeds species whose seeds present dormancy, as in Echinochloa crus-galli, predicting emergence depends on understanding how the environment regulates dormancy level. In the present work, we studied the environmental regulation of dormancy in E. crus-galli seeds. For this, E. crus-galli seeds were storage under different conditions: i) moist at 5, 10 and 15ºC (stratification) and ii) dry at 15 and 25ºC (dry after-ripening) for 80 days. During storage, the seeds were tested for germination at regular intervals under a wide temperature range (10-30ºC). The results showed that the changes in dormancy level were associated with a widening of the thermal range permissive for seed germination as consequence of a decrease in its lower limit temperature (Tl). This decrease in Tl was higher when the seeds were stratified at 10ºC than at 5 and 15ºC. Seeds exposed to dry after-repining showed a significant lower rate of dormancy release. Obtained results were used to establish functional relationships able to predict field emergence of E. crus-galli as a function of soil temperature.

7.0.13 Germination thresholds and stress tolerance of Australian native species under a changing climate

POSTER

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Climate variability is projected to increase due to climate change, with changing temperatures, moisture availability and fire frequency. These variations will have a significant impact on plant recruitment from seed. Although global change is expected to have a greater impact on early life history stages, studies examining the effects varying environmental impacts will have on germination responses are still missing for many ecosystems. In Australia, climate change is interacting with existing environmental challenges (such as highly variable rainfall, high frequency of high temperature stress events, and numerous soil constraints, including low fertility, salinity, and sodicity) and this will have significant impacts on Australia's distinct biota. This study investigated seed germination traits of sub-tropical Australian native species when subjected to various environmental treatments with the aim of evaluating germination thresholds of species used in seed-based restoration. This information can be used to make reliable predictions on how seeds of native species will respond to changing environmental cues. Three Poaceae and five Fabaceae species were selected based on differences in seed attributes, plant form and restoration benefits. Control (untreated) and pre-treated seeds (gibberellic acid [GA3] for Poaceae and hot water scarification for Fabaceae) were germinated under different temperatures (four alternating and 10 different constant temperatures) and light conditions (light/dark). They were also subject to different stressors (osmotic, salt, pH and burial depth) to simulate possible climate change scenarios. The results demonstrate how global change can influence seed germination patterns, which will have significant impact on the functioning of natural ecosystems as well as for seed-based restoration. This information is

crucial for a better-informed prediction on how plant communities will respond to climate change and for guiding restoration efforts.

7.0.14 SEED ECOLOGY OF Atropa baetica Willk. AS A POSSIBLE EXPLANATION FOR ITS RARITY

POSTER

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There is currently a consensus regarding the existence of multiple causes to explain the origin of endemic plant taxa. Comparative studies contrasting the biology of rare taxa with common closely related phylogenetic congeners, which could be considered as "control species", are considered particularly valuable.With this objective, the germinative ecologies of Atropa baetica (endangered Ibero-North African endemism) and Atropa belladonna (wide Eurasian distribution) are compared in this study. Specifically, the following are analyzed: a) phenology of seedling emergence, b) germination of dry stored seeds tested in a wide range of temperatures (5, 15/4, 20/7, 25/10, 28/14 and 32/18ºC) in light and darkness, c) effect of different periods (between 1 and 4 months) of cold stratification (5ºC) on germination.During the first autumn no seedlings emerged, and after the first spring, 57% emergence was recorded in A. baetica (March-April) and 32% in A. belladonna (May). After 3 years, the accumulated emergence reached 78% and 65%, respectively. In dry stored seeds, germination was null at 5ºC and 15/4ºC for both species. In the remaining thermoperiods, A. baetica seeds increased germination with age up to close to 100%. However, A. belladonna seeds did not germinate at 20/7ºC and 25/10ºC for any age, but they did at 28/14ºC and 32/18ºC up to 50-70% for the highest ages. In both taxa, the photoperiod promoted germination. Cold stratification increased germination.Seeds of both species have non-deep physiological dormancy. This germination strategy explains the absence of seedling emergence during

the first autumn. The increase in germination with seed age and after a period of cold stratification has been verified in both species but with a delay between emergence of their seedlings. This way, seedlings of A. belladonna emerge later, increasing their chances of survival given the sensitivity of seedlings of these species to frost.

7.0.15 Comparative studies on seed germination in related Aquilegia species (Ranunculaceae)

POSTER

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Columbines (Aquilegia L.) represent a model system in evolutionary biology, however, the phylogenetic, taxonomic and functional relationships within this genus are still misunderstood, and proposed interpretive models are often conflicting. Even more challenging is the study of seed ecology in this genus. So far, only a few studies on seed dormancy and germination behavior in Aquilegia have been conducted, probably due to the complex thermal requirements necessary to overcome dormancy. Here, we investigate seed germination in multiple populations from 5 high mountain and 2 nemoral European species of Aquilegia. Seeds were exposed to different temperatures shortly after harvesting, while others were pre-treated either with warm stratification, cold stratification, or with a sequence of warm + cold stratification and complemented by a "move-along" experiment and periodic seed sections to monitor embryo growth over time. The seeds of all species are dormant at dispersal, with different dormancy degree depending on species relatedness and habitat ecology. However, requirements for dormancy removal remains to be better clarified. Seed sections highlighted a slow but progressive embryo growth in all populations during the "move-along" experiment. To fill the knowledge gap on germination mechanisms in Aquilegia we aim to perform a meta-analysis on seed germination by aggregating our own experimental data with the literature data available on germination ecology in this genus. This study will help to better understand the adaptive mechanisms underpinning seed germination in Aquilegia and better clarifying the contribution of these processes in species delimitation.

7.0.16 Timing of fire during summer determines seed germination in Cistaceae

POSTER

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Mediterranean-type climate ecosystems are fire-prone environments where species have evolved in presence of seasonal summer conditions and frequent fires. When a fire occurs, usually in summer, seeds in the soil suffer not only the heat shock of the high temperatures reached during the fire, but also the conditions throughout the summer days. Hardseededness is a trait developed by Cistaceae together other plant families, which prevents germination until some factor, such as fire and summer temperatures, breaks the imposed physical seed dormancy. We evaluated the effects of fire and summer temperatures on seed germination of 12 species of Cistaceae. Furthermore, we wondered if the timing in which seeds are exposed to a heat shock along the period of high summer temperatures affects to seed germination. A heat shock (100°C for 10 min) was produced before, after and before plus after, seeds were exposed to summer temperatures (50/20°C for one month). Heat shock increased germination of all species whatever the timing of heat shock when seeds were not exposed to summer temperatures. However, when seeds were exposed to summer temperatures, the timing in which the heat shock was produced, at the beginning or at the end of the summer period, was determinant for seed germination. Pre-summer heat shock had a significant negative effect on seed germination in comparison to post-summer heat shock, which was related with a higher proportion of hard coated seeds and might be explained by a cycling of sensitivity to physical dormancy break. Timing of fire during summer plays an important role controlling seed germination of Cistaceae so, both factors, fire and summer work together breaking physical seed dormancy.

7.0.17 Cone/seed morphometry and germination ecophysiology of Juniperus drupacea at the westernmost area of its global distribution (Mt. Parnon, Greece)

POSTER

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Juniperus drupacea Labill. is a relict, dioecious tree/shrub found in Turkey, Syria, Lebanon and Israel, while in Europe it is native only in Greece (in Mt. Parnon and in a small part of Mt. Taygetos). In Europe, according to the IUCN Red List criteria, J. drupacea has been characterized as endangered (EN), the most serious threats being grazing of saplings, timber overexploitation and climate change. The germination behaviour of the species in Greece and the morphometric characteristics of its cones and seeds are being studied for the first time. Seeds have been subjected to different periods of pre-chilling (0, 4, 8, 12, 16, 20, 24, 32 weeks) and then germinated at 15 oC and in darkness, while for the 16 weeks pre-chilling period, germination was also studied at 20 oC and both in the light (12/12h) and dark. The germination of the species seems unaffected by the light conditions while a prolonged pre-chilling period of at least 16 weeks is necessary for achieving the maximum, so far, germination percentage of 40% at 15 oC. Regarding cone and seed morphology (cone mass, sound seeds per cone and seed mass), 20 random, mature cones have been gathered from 11 sites of both northern and southern populations of Mt. Parnon, while seeds per cone were additionally counted in an extra batch of 829 cones. The average mass of a cone is 2810 mg and each cone contains 1-5 sound seeds (m = 2.3 seeds per cone) with a mean seed mass of 32.7 mg.

7.0.18 Physiological and molecular mechanisms of Lepidium sativum seed longevity and vigor

POSTER

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Seeds have a central role in almost all the food supply chains important to human and animal survival. Seed longevity and vigor are key quality-defining traits that determine plant species adaptability to changing environmental conditions and seed performance after storage. Seed vigor is a complex trait with genetic and environmental components determining the rate and uniformity of seed germination and seedling growth under harsh weather conditions and abiotic stresses. The mechanisms that regulate seed germination have been widely studied; however, little is known about the mechanisms related to seed longevity and vigor. Moreover, the relationship between seed pigmentation, dormancy and vigor, and their roles in environmental adaptation are poorly understood. Therefore, the objective of the present study is to investigate the physiological and molecular mechanisms underpinning the regulation of seed longevity and vigor in Lepidium sativum by using accelerated ageing techniques in combination with naturally aged lines. The results show changes in germination kinetics associated with the ageing treatments. Initial comparative transcriptomics analysis also indicates differences among the different treatments and lines. Moreover, differential expression patterns of key genes involved in genome integrity and stability processes were observed between the lines, indicating that this is a promising system for further analyses.

7.0.19 Ecophysiology seed traits of Devil's claw (Martynia annua; Martyniaceae)

POSTER

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Martynia annua is an invasive weed that carries many medicinal properties and is characterized by the presence of unique fruit with upwardly covered hooks that protrude from its woody endocarp. Despite being an invasive weed, its seed ecophysiology is poorly known. This study aimed to understand how this species' seed dormancy, germination, and storage physiology facilitate its invasion and survival. The endocarp of M. annua comprises four locules that bear mainly four seeds per fruit. Although none of the soil buried or laboratory incubated seeds germinated even after six months, woody endocarps of M. annua did not restrict water imbibition (seed mass without- and with-woody endocarp increased 61 and 104% within ten days of imbibition, respectively). Fully developed investing embryos present in seeds suggest that there is no morphological component to its dormancy. Seeds with- and without-woody endocarp germinated (>90%) only after being treated with 500 ppm GA3. The seed coat ratio of M. annua was <0.5, indicating a low probability of becoming a desiccation-sensitive seed. Moreover, dry-stored seeds retained >90% viability even after one year of storage. Our findings revealed that the removal of the endocarp failed to facilitate seed germination, indicating that both the endocarp constraint and the low growth potential of the embryo were responsible for the delay in seed germination. Thus, it confirmed that M. annua produces physiologically dormant and

desiccation-tolerant seeds, capable of dispersing longer-distance at spatial and temporal scales. These specific seed traits could help explain its wider distribution and invasion success globally.

7.0.20 What method is the best? Comparison between different smoke treatments on the germination of Cerrado species

POSTER

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Smoke produced during fire events may affect seed germination of species of fire-prone ecosystems. However, there are many ways of conducting smoke experiments, and this can affect species response to smoke. Therefore, we aimed to investigate the potential of different smoke methods to stimulate seed germination of Cerrado native species. We evaluated the effect of aerosol smoke (10 min of exposition, combustion of the biomass sampled in the study sites), non-commercial smoke water (seeds soaked for 24 hs in smoke water solution produced with biomass sampled in the study sites), and commercial smoke water (seeds soaked for 24 hs in 5% and 2.5% concentration of Regen 2000 ® smoke water) on the germination and on the mean germination time of 12 species from the Cerrado. After treatments, seeds were placed to germinate in distilled water. The germination experiments were conducted for 30 days, and viability tests were carried out with ungerminated seeds. Our results showed that germination responses varied among methods: higher germination percentages were achieved by using commercial smoke water (89.2%; 91.2%), independently of the concentration, followed by aerosol and non-commercial smoke water (78.83%; 66.67%, respectively). Moreover, species germinated faster by using commercial smoke water, independently of the concentration, followed by non-commercial smoke and aerosol. Commercial smoke water, besides being the method that most increased germination percentage and most decreased mean germina-

tion time, is the most practical method for large-scale use in situ and ex-situ. Finally, we conclude that the responses of Cerrado species can be different according to the smoke methods.

7.0.21 Seed color reveals the bet-hedging strategy in an endemic species of Central Apennines (Italy): Astragalus aquilanus

POSTER

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Astragalus aquilanus (Fabaceae) is an endemic species of Apennines (Italy). The seeds of this species have wide intraspecific plasticity. The seed color strongly differs among and within individuals and/or populations, and it varies from straw yellow to black. From explorative germination tests, it was highlighted as a "light" seed has geminated faster compared to a dark grey. Thus, we have disentangled how color can provide information about the bet-hedging strategies. To do so, we have split the seeds into color groups, by morpho-colorimetric analysis, from lighter to darker colors. Imbibition test was carried out for each color group, collecting both weight and area of seeds, at several time breaks (from 2h to 96h). The results highlight as the lightest color group shows fully imbibed seed and also the imbibition was faster compared to the others group. These results indicate that color distinct the severity of physical dormancy, suggesting as these traits can provide key information about bet-edging strategies. For these reasons, we carried out a preliminary analysis to test the effect of climate factor on the size and color of seeds in four populations of A. aquilanus. The results suggest as populations with higher temperatures and scarce rainfall show a greater number of dark and small seeds (presumably dormant). These differences among populations can be due to the maternal effect, which produces different proportional of light and dark seeds based on climatic factors. Dry sites are more frequently subjected to rainy events that cause germination but are not sufficient to allow the plants to ripen, probably favoring a high proportion of "dark" seeds. This method should

be tested in a wide number of populations and species, across a long-time span. This data could provide crucial information to better assess the effect of climate change on plants and to predict future extinction risks.

7.0.22 Acacia saligna and Vachellia karroo: germination ecophysiology of two invasive species in the Mediterranean Basin

POSTER

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In the framework of the project Interreg Italia-Francia Marittimo "ALIEM", two species of Acacia s.l. were selected to study some of their invasive traits in the Mediterranean Basin. We focused on seed germination ecophysiology of the phanerophytes Acacia saligna (Labill.) H.L. Wendl., native to Western Australia and Vachellia karroo (Hayne) Banfi & Galasso native to Southern Africa, both recorded as invasive in the Mediterranean Basin. Germination requirements of three populations of A. saligna (two from Sardinia and one from Corsica) and three of V. karroo (from Sicily, Sardinia, and Corsica) were evaluated on scarified seeds at constant (5, 10, 15, 20 and 25 °C) and alternating temperatures (25/10 °C), both in light (12/12 h) and in darkness (0/24 h), and under NaCl stress (0, 125, 250 and 500 mM). The recovery capacity of non-germinating seeds after salt stress were evaluated as well. Non-scarified seeds were tested at 15, 20 and 25 °C in light condition. A. saligna scarified seeds germinated at all tested temperatures (ca. 100%); V. karroo scarified seeds showed capability to germinate at all the temperatures (ca. 95%), except at 5 °C in which germination from 40 to 0% were recorded both in light and in total darkness. Non-scarified seeds in both species showed lower germination percentages than scarified seeds, with no significant differences among populations. The limit of tolerance to NaCl varied among the two species and populations. Seeds of A. saligna germinated up to 250 mM,

while V. karroo seeds germinated also at the highest NaCl concentration (500 mM). Both species showed a low capability of germination recovery after salt exposure, when stressed with 500 mM of NaCl. Our results represent a novel contribution in developing management plans for these alien species and for the control of biological invasions in coastal habitats.

Chapter 8

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