# Biological and ecological trade-offs of seed oil content in alpine species

Authors: C. Espinosa del Alba1, A. Mondoni2, E. Fernández-Pascual1 & B. Jiménez-Alfaro1.

Affiliations:

1- Biodiversity Research Institute, IMIB (Univ. Oviedo-CSIC-Princ. Asturias), 33600 Mieres, Spain.

2- Dipartimento di Scienze della Terra e dell’Ambiente, University of Pavia, 27100 Pavia, Italy.

Abbreviations:

TAGs: triglycerides; FA: Fatty Acids; UFA: Unsaturated Fatty Acids; SFA: Saturated Fatty Acids

## Summary

## Introduction

Seed importance for evolution and plant regeneration, seed traits are understudied specially from wild plants.

### Seeds reservoirs: oil content

Seeds act as reservoirs of energy principally accumulating three types of macromolecules: proteins, lipids or carbohydrates (Levin, 1974; Baud and Lepiniec, 2010). The quality of these reserves is directly related to plant fitness (Westoby et al., 1992). Lipids, majorly in the form of triglycerides (TAGs, an ester of glycerol plus three fatty acids (FA) groups) (Voelker and Kinney, 2001), and carbohydrates, accumulated as starch, are the predominant reserve forms for angiosperms (Bretagnolle *et al.*, 2016). Carbons in FAs are highly reduced and, through beta-oxidation, they release more than twice as much energy as the oxidation of starch or proteins on a per g basis of dry weight (Levin, 1974; Baud and Lepiniec, 2010) (Luttge, 2012;). Consequently, plants mostly rely on lipids (hereafter “seed oil” for simplicity) for energy and they can account for up to 60% of seed weight (Ellis, 2006; Theodoulou and Eastmond, 2012). Seed oil is mainly stored in the cotyledons and endosperm (Ellis, 2006) but also in the radicle and hypocotyl (Li *et al.*, 2006). Although oil content variation is found within and among genera of the same family (Levin, 1974; Bretagnolle *et al.*, 2016) it is also highly constrained by phylogeny and subject to evolutionary change (Levin, 1974).

### Seed oil composition

In seeds, most FAs range from 10 to 22 carbons in length and the carbons may be joined by single or double bonds. FAs with one or more double bonds are referred to as unsaturated (UFA, here referred to as both monounsaturated and polyunsaturated) and FAs without double bonds are referred to as saturated (SFA) (Ellis, 2006). SFAs and UFAs have differential biochemical properties [41, that influence the responses to biological and ecological trade-offs. On a per-carbon basis, UFAs cost more to produce and yield less energy when oxidized than SFAs [41,(Linder, 2000). Moreover, the storage of UFAs needs the storage of antioxidant molecules to prevent damage due to the higher potential for oxidative damage Benson 1990). Hence, a maximal storage strategy for seeds should maximise SFA storage instead of UFA (Linder, 2000). However, the relative abundance of UFAs and SFAs highly varies in angiosperms (Voelker and Kinney, 2001) and, contrary to the expectation, many species synthesise a very low amount of saturated FAs (Linder, 2000) SFA also have higher melting points than UFA, with lower melting points (Benson 1990) Sanyal and Decocq, 2016 and influences the availability of these energy resources. Most abundant FAs found in seeds are the saturated palmitic (16:0, PA) and stearic (18:0, SA) acids and the unsaturated oleic acid (18:1n-9, OLA), linoleic acid (18:2n-6, LA) and a-linolenic acid (18:3n3, ALA) (Voelker and Kinney, 2001; Ellis, 2006; Baud and Lepiniec, 2010).

### Importance

Oil content and composition have profound effects on seed responses (Hoekstra, 2005) influencing seed dormancy and viability, germination potential (Westoby et al., 1992) and success (Linder, 2000) as well as the emergence and establishment of a plant [Bewley 1994]. Consequently, seed oil content and composition are crucial for plant adaptation (Sanyal and Decocq, 2016) and validate the adaptive hypothesis (Linder, 2000), also suggesting that selection could be acting simultaneously on multiple seed traits, like seed mass (Sanyal and Decocq, 2016). Both oil content and composition vary in response to environmental, geographical location and maternal genotypes (Linder, 2000; Ghebretinsae *et al.*, 2008). Seed oil content and oil seem to be under strong selection by temperature at both micro- and macro-evolutionary levels (Sanyal and Linder, 2013; Sanyal and Decocq, 2016). Although its importance, very few studies on native species address and analyze seed oil content and composition. A lot of specific studies in commercial species (cotton, brassica, soybean) gene expression, and irrigation effects (focused on plant breeding programs).

### Seed mass and oil content

One of the most studied seed traits is seed mass and as previously mentioned oil weight can contribute up to 60% of seed mass (Ellis, 2006). There is a consensus in the literature that seed mass and oil content are negatively correlated while stored seed energy and oil content are positively correlated (Bretagnolle *et al.*, 2016) (Levin, 1974). Large seeds generally store less oil than small seeds, whereas small seeds have higher oil content. Hence, a small oily seed can release as much energy as a starchy seed that is twice as heavy. Nevertheless, it has been reported a huge variation of oil content in small seeds although such variation in oil content decreases in large seeds (Bretagnolle *et al.*, 2016) (corroborated in our preliminary results). Such a relationship suggests that as oil synthesis is energetically costly relative to carbohydrates, and thus oil synthesis could be an advantage only for small seeds which can store energy in a smaller volume (Bretagnolle *et al.*, 2016).

### Storage behaviour and oil content.

Oil content has profound effects on storage behaviours because it influences seed viability (Hoekstra, 2005) and longevity, i.e. the ability of seeds to remain viable over certain storage periods (Bewley et al., 2013). Lipid oxidation generate free radicals and reactive oxygen species (ROS) ; Hendry, 1993; Bailly, 2004), which can cause detrimental effects on membrane integrity (Priestley and Leopold, 1979) (Kranner and Lutzoni, 1999; Kranner et al., 2002) and leads to mitochondrial dysfunction, enzyme inactivation, membrane perturbation and genetic damage (Coolbear, 1995). Specially the oxidation of UFAs is highly contributing to free radicals’ production and subsequent attacks on other macromolecules (Benson 1990). To prevent the deleterious effect of lipid oxidation, oily seeds also store antioxidants, and supporting findings show a positive correlation between antioxidant levels and the relative proportion of UFA (Kamal-Eldin & Andersson, 1997; Sattler et al., 2004; Falk & Munn\_e-Bosch, 2010). Thus, not only oil content but also the relative proportions of UFAs and SFAs (i.e. oil composition) affect the storage behaviour of seeds (Walters et al. 2004; Volk et al. 2006; Walters et al. 2015).

Accordingly, some studies have found oily seeds to be more sensitive to ageing (Nagel and Börner, 2010; Neto *et al.*, 2019). Detailed studies including oil composition have also shown that a higher proportion of UFA are associated with shorter longevity (Ponquett et al 1992) (Hoekstra, 2005). However, in general, the correlation between seed oil content and longevity has been described as weak (Nagel and Börner, 2010) Priestley et al., 1985; Walters et al., 2005). Even in other cases, no significant effect of oil on seed longevity has been reported (Probert, Daws and Hay, 2009; Gardarin *et al.*, 2010) (Mederios et al 1998). Further investigations on the effects of seed oils (content and composition) on longevity are desirable (Pritchard and Dickie, 2003).

It is noteworthy that seed longevity is partly determined by an intricate network of genetic factors, and taxonomic differences (Walters, Wheeler and Grotenhuis, 2005), but also by environmental factors. Several authors have found that seeds of species from cold and temperate climates have shorter longevity than seeds of species from hot and arid climates (McDonald, 1999; Kranner et al., 2010; Walters et al., 2010 (Kochanek 2010(Probert, Daws and Hay, 2009)). Additionally, according to Mondoni et al. (2014) a relationship between seed longevity and the environment was considerably strong and greatly affected by maternal genetics, Waterworth 2010 showed that prolonged exposure of A. thaliana seeds to low temperatures resulted in reduced viability and vigour, due to increased oxidative stress under those circumstances. Warmer parental growth environment leads to a better seed provisioning in S. vulgaris through multiple effects, which promote and increased tolerance of the offspring seeds to heat stress. Although other authors have reported the association between environmental conditions to be weakly associated with seed longevity Merritt et al. (2014b).

**Alpine species longevity or particularities in general?? Or keep for discussion??**

Differences between alpine and lowland seed lots are reflected at the genetic level by the rate or rearrangement of DNa and antioxidant responses during ageing (Donà et al 2013). Mondoni 2011 suggested that reduced longevity of seeds of alpine plants exposed to controlled ageing was caused by the low selection pressure for seed resistance to ageing, simple because seeds buried in the alpine soil are normally exposed to lower temperatures and therefore the rate of ageing are expected to be lower.

### Global seed oil content patterns

Few studies have tried to describe global seed oil content patterns. In 1974, (Levin, 1974) found that significant seed oil content variations depending on habitat type (increased with woodiness and shade tolerance), and life-form (lower in herbs than in shrubs than in trees) but no geographical differences between temperate, subtropical and tropical regions. However, a more recent study by (Sanyal and Decocq, 2016) found that seed oil content to be significantly higher in tropical plants compared to temperate plants, probably because seeds need higher energy to survive first life stages with high competition and/or low illumination (Levin, 1974) (Salisbury 1942). In the same study also found higher proportions of UFA (oleic and eicosenoic) with increasing latitude (Sanyal and Decocq, 2016) in concordance with previous studies (Linder, 2000; Sanyal and Linder, 2013), with the relative proportions of SFAs decreasing by 0.1% for each degree of latitude (Sanyal and Linder, 2013). Additionally, due to UFA and SFA different melting points, at lower latitudes or altitudes with higher temperatures, seeds with higher proportions of SFA would be favoured because they would have more energy for growth without delaying or slowing germination (Sanyal and Decocq, 2016). At higher latitudes and thus cooler temperatures, seeds that have a higher proportion of UFA may germinate earlier and/or more (Linder, 2000). Rich UFA seeds could then germinate faster and earlier than rich SFA seeds in cold conditions, providing a competitive advantage where cold temperature regulates seed germination (Linder, 2000).

|  |  |  |  |
| --- | --- | --- | --- |
| Table 1 Fatty acids in the triacylglycerols and phospholipids of Arabidopsis thaliana | | | |
|  |
| Fatty acids | Location | Symbol | Melting point (ºC) |  |
| Palmitic | TAG | 16:00 | 62.9 |  |
| Palmitoleic | PL | 16:01 | −0.1 |  |
| Stearic | TAG | 18:00 | 69.5 |  |
| Oleic | TAG | 18:01 | 13.4 |  |
| Linoleic | TAG | 18:02 | −5 |  |
| Linolenic | TAG | 18:03 | −10 |  |
| Arachidic | TAG | 20:00 | 75.3 |  |
| Eicosenoic | TAG | 20:01 | 25 |  |
| Eicosadienoic | TAG | 20:02 | 0 |  |
| Erucic | TAG | 22:01 | 33.5 |  |

FROM sanyal 2013! Use ranges?

### The alpine environment

Specialits vs generalists

(species optimal microclimatic conditions)

### Aims, questions and hypotheses.

The goal of the study is to explore the pattern of seed oil content and composition (proportion of unsaturated fatty acids, UFA and saturated fatty acids, SFA) in alpine plants and understand their biological and ecological trade-offs. This kind of data is barely available for most wild plants and especially for alpine species. Although oil content is known to influence a wide range of biological processes, few studies have investigated biological and ecological correlates. The trade-offs identified in the literature have not been consistent across showing divergent patterns. Our study is structured in 3 main sections/questions:

1. Exploration and description of seed oil content in alpine species. Does oil content and composition in alpine species follow global patterns?
2. Seed oil content biological trade-offs. How do oil content and oil composition (SFA vs UFA) correlate with other seed traits? Explore seed mass, seed longevity (p50) and t50 (time to reach 50% germination)
   1. H2.1. Higher seed mass will correlate with less oil content (corroborated \*) and less UFA/SFA ratio (corroborated but marginal).
   2. H2.2. Less longevity will correlate with higher oil content (corroborated \*) and a higher UFA/SFA ratio (trend but NS). optimize space for more energy reserves
   3. H2.3. Faster germination (lower t50) will correlate with higher oil content (opposite trend and NS) and higher UFA/SFA ratio (opposite trend and NS).
3. Ecological trade-offs. How does oil content and composition (UFA/SFA) correlate with the ecological optimal of the species/community?
   1. H3.1. Strict alpine species (specialists) will have less oil content (opposite and NS) and a higher UFA/SFA ratio (opposite and NS).
   2. H3.2. Species living preferentially in warmer places (+GDD) will have higher oil content (trend but NS) and lower UFA/SFA ratio (opposite trend but NS).
   3. H3.3. Species living preferentially in colder places (+FDD) will have lower oil content (NS) and higher UFA/SFA ratio (opposite trend NS).

We will use a combination of techniques from laboratory based and physiological seed traits and other ecological traits. Seed traits like oil content could be a potential predictors for many biological responses.

## Methods

### Study system

We focused on alpine species from grassland communities in the Cantabrian mountains (Northwestern Spain). These grassland communities are continuously distributed along the mountain range, occupying reduced areas above the treeline and around mountain tops, between 1750 and 2500 meters (figure 1). Grassland communities are mostly dominated by *Poaceae* and *Cyperaceae*, the main lifeforms are Hemicryptophytes and Chamaephytes and local richness ranges from 4 to 28 species. Grazing impact is restricted to wild populations of Cantabrian chamois (*Rupricapra pyrenaica parva*). The mountain hub is a transitional region between the Eurosiberian and the Mediterranean biogeographical regions and concordantly there is a clear climatic gradient from northern slopes (temperate) to southern slopes (Mediterranean).

Gráfico

Descripción generada automáticamente con confianza media

Figure 1. Distribution of plot data stored in a database on alpine grasslands in the Cantabrian Range, and mountain areas (M1, M2) selected for field studies. Grey areas show altitudes >1600 m. Mapa con capa de precipitación/temperauras? + cross design? Variation of temperatures measured by iButtons?.

### Species data

We established eight sampling sites, four in the northeastern sector and 4 in the southwestern sector. To cover spatial variation of community composition, in each sampling site we established 20 additional plots (1m2), five in each cardinal direction with a 10 m separation (cross design, fig x). In total, we established 160 vegetation plots (8 sites x 20 plots) where we collected floristic and community composition data, for a total of 128 alpine grassland species.

All species were classified between specialist (strict alpine) and generalist distribution. We identified plant specialists as those that are significantly associated with the target vegetation type, using the Indicator Values (IndVal) in the indicspecies R package (REF). The calculations were based on 12,000 vegetation plots of grasslands stored in the SIVIM database for the Cantabrian Mixed Forests ecoregion (paper classification). From the preliminary list of indicator species for the studied vegetation, we removed species with median values of elevation below 1800 m, most of them characteristic of subalpine or nitrophilous habitats. We finally identified 40 plant specialists which are widely recognized as characteristic species of alpine and subalpine grasslands.

### Oil content and composition.

the analysis of 360 A. thaliana accessions from diverse geographical locations has shown that their seeds contain identical fatty acids, even though some variations in the relative proportions of those fatty acid species can be observed [9,10].

We preliminary explored within-accession seed oil content variation in 5 species analysing 3 subsamples for each of those (also to ensure high precision of methodology), and we could not detect any statistical differences between subsamples. Consequently, a single sample for each species seed accession was analysed, so that within-species seed oil variation was not explored. Fatty acids (FA) analysis (oil content and composition) requires a sample of 200 mg of dry seeds we collected from the sampling sites. We collected enough seeds from 36 species, many of the data obtained about FAs content and composition of these species were not known before. We sent the samples to an external analytical laboratory (USTA-CSIC) where they used a gas chromatographer with a Flame Ionization Detector (Agilent 7820A, EZChrom Elite software). Samples were manually ground with liquid nitrogen, then fatty acids were transformed to fatty acid methyl esters (FAMEs) using a mix of sodium methoxide 0.5M in methanol and acetyl chloride in methanol (1:10 v/v) (table xx in appendix with complete list of Fame’s possible to identify). As a result, we obtained the oil content on a percentage of dry-weight bases and the percentage of each FAME’s type relative to the total of oil content.

Check methodology details in Sanyal 2013!

### Seed trait data

Seed mass data was calculated for all species weighting 5 replicates of 50 dry seeds. Earliness of germination (T50 trait) is calculated as the time (days) to reach 50% germination and obtained from a phenology germination experiment (Espinosa del Alba et al. 2024), is available for 28/36 all species. under snow germ trait 36/36??.

Longevity data (raw germination scores and p50) is available for 20 of those 36 species. We applied a standard comparative longevity protocol (Probert, Daws and Hay, 2009; Davies *et al.*, 2016) optimised for short-lived species (Davies *et al.*, 2016) like alpine species (Mondoni *et al.*, 2011). The artificial accelerated ageing protocol allows the measurement of p50 value (amount of time for seed viability to drip to 50%). The initial RH value was 30-35% (Hygropalm 3 display unit; Rotronic Instrument UK Ltd, Crawley,UK). Before the start of the ageing protocol, all species were tested to ensure germination above 85% and for those species from the Cistaceae family were physically scarified with sandpaper. Seed samples (200 seeds per species) were first rehydrated to 47% RH at 20ºC for five days in a non-saturated LiCl solution (Hay et al., 2008) in crystal petri dishes and kept in in a 300 x 300 x 130 mm sealed electric enclosure box (Ensto UK Ltd, Southampton, UK) before moving the vials into the ageing conditions. The ageing conditions consisted in a temperature of 45°C, a RH of 60% and darkness. A subsample of 42 seeds was withdrawn after 2, 10, 15 and 30 days in ageing conditions and consequently sowed in petri dishes 1% agar with 250 ml/L of GA3 (Kew Royal Botanic Garden Technical Information sheet\_13a). For those species which required a cold stratification period (*S. oppositifolia*), after removing from ageing conditions were put into a refrigerator at 5ºC and darkness for a month before starting germination experiments (same procedure followed by (Satyanti *et al.*, 2018). In some species, the number of seeds for subsamples was reduced due to the lack of seeds. Germination conditions for the species were set at 22-12ºC alternating temperatures with a 12/12 h photoperiod or for those whose germination required colder temperatures a second germination chamber was set at 15/5ºC alternating temperatures with a 12/12h photoperiod (see table xx for details). After the sowing, the seeds were checked once a week for one month. Germination was scored when there was a visible root (>1.5 mm) and then removed from the Petri dishes. After the end of the 28 days germination test, the ungerminated seeds were cut-tested under the binocular loupe to visually assess the state of the embryo. We considered seeds with white and firm embryos viable, i.e. potentially germinable (Baskin and Baskin, 2014) and we removed empty or infected seeds from further analysis.

Longevity analysis have been widely used by seed bank managers based on the viability equation: v = Ki – (p/σ), where v is viability in NED (Normal equivalent deviates), p are the days of ageing, Ki is the initial viability, σ is the standard deviation of the distribution of deaths over time.

### Species ecological preferences

To calculate species ecological optimums (following the methodology (Jiménez-Alfaro *et al.*, 2024), we measure microenvironmental gradients using an iButton datalogger, buried at 5 cm deep, in each vegetation plot (Thermochron, iButton, Newbury, UK; accuracy: +/- 0.5 °C from -10 °C to +65 °C, resolution: 0.5 °C, records every four hours) which recorded temperatures across 11 months. In basic plots, the iButtons recording period went from 1st October 2018 to 31st August 2019 (330 days) while in acidic vegetation plots, the recording period for the iButtons went from 12th July 2021 to 29th May 2022 (321 days, all raw data available in GitHub repository).

From the environmental data, we calculated a series of bioclimatic indices per plot following (Jiménez-Alfaro *et al.*, 2024). We calculated bioclimatic indices based on standard variables used by WorldClim (Fick & Hijmans 2017), together with other variables with a relevant function on alpine topographic gradients. The selected variables were: (1) bio1 = annual mean temperature; (2) bio2 = mean diurnal range, i.e. the mean of the monthly differences between maximum and minimum temperatures; (3) bio7 = temperature annual range; i.e. the difference between the maximum temperature of the warmest month and the minimum temperature of the coldest month; (4) snow = the number of days of snow cover, when temperature is around 0 ºC, calculated for the period in which the maximum temperature was < 0.5 ºC and the minimum temperature was > -0.5 ºC; (5) FDD = freezing degree days, i.e. the sum of daily mean temperatures for days in which the mean temperature was below 0 ºC (Choler 2018); and (6) GDD = growing degree days, i.e. the sum of daily mean temperatures for days in which the soil mean temperature at five cm deep was above 5 ºC (Körner 2021). For FDD, we transformed the values from negative to positive, so higher values represent more freezing. Then we use the presence and abundance of each species in each plot to estimate an adult optimal ecological niche. For each species we considered those plots with more than 1% of abundance and….CHECK!!!

### Statistical analysis

We use GENSTAT software to apply a Probit analysis (Hay, Mead and Bloomberg, 2014) and calculate p50 values.

Phylogeny was included using a reconstructed tree for the 36 species (Supporting information Fig. **S1**), created with V.PHYLOMAKER R package (Jin and Qian, 2019) using phylosignal and phylobase R packages (Keck *et al.*, 2016; R Hackathon et al., 2020).

Exploratory PCA for FAME’s composition and total oil content (in percentage)

Our two response variables are total oil content (in percentage) and UFA/SFA ratio (both log transformed).

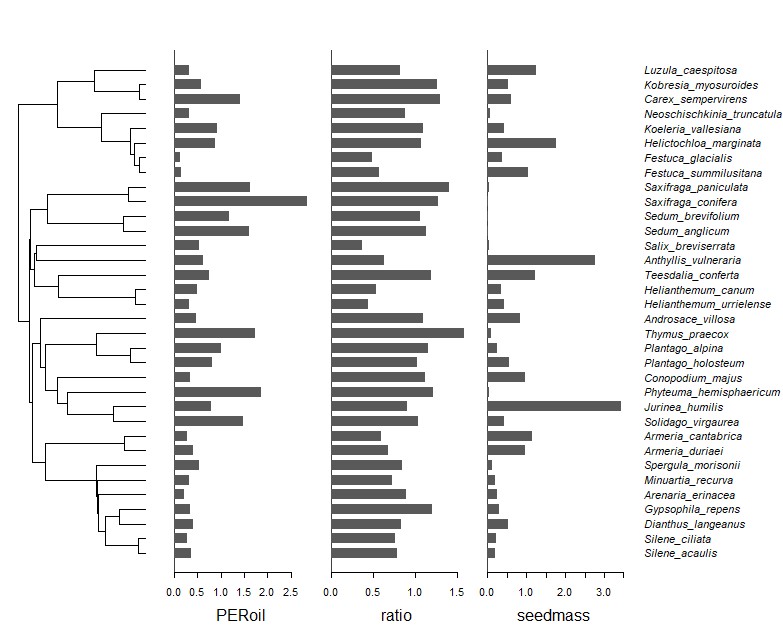
Biological trade-offs: seed mass, longevity (p50), earliness of germination (t50).

MCMC-GLMM: We analyzed the data by fitting Markov Chain Monte Carlo generalized linear mixed models (MCMCglmm) with Bayesian estimation using the R package MCMCGLMM (Hadfield, 2010). To model raw germination scores from longevity experiment, we used binomial MCMCglmms (family = multinomial2) while for the rest of traits we scaled the values and used gaussian MCMCglmms (family = gaussian). When needed traits were log transfomed to ensure normality of the data distributions. In all models we used weakly informative priors, with parameter-expanded priors for the random effects. Each model was run for 1,000,000 iterations, with an initial burn of 100,000 and a thinning interval of 100. From the resulting posterior distributions, we calculated mean parameter estimates and 95% credible intervals (CI).

Ecological trade-offs: species ecology, GDD, FDD

## Results

Congeneric species has very similar levels and proportions of FA and oil content (as stated in literature, and see figure 2)



### Seed oil content patterns in alpine species

The exploratory PCA showed relatively low explained variation within the first two axes (21.7 and 16.2 5 respectively). FAMEs of C22:1n9 and C22:2n6 are the ones contributing the most in PC1 and PC2 (around 11%) along with the percentage of oil content (11%) also contributing in PC2 (Fig 3A and 3B). Additionally, PCA revealed that oil content percentage is not highly correlated with any specific FAMEs type (table in appendix).

Within our species, oil content varies from 1.3% in *Festuca glacialis* (Poaceae) to 30.1% in *Saxifraga conifera* (Saxifragaceae) (Fig 3C). Most abundant FAME in alpine seeds were the unsaturated linoleic acid (18:2n-6, LA, 43.1 ± 16.1%), oleic acid (18:1n-9, OLA, 22.9±13.3%) and alpha-linolenic acid (18:3n3, ALA, 16.2 ± 19.3%); and saturated palmitic (16:0, PA, 10.6 ± 4%). These 4 represent a mean of 92.7% (SD = 52.75). The next with higher % were stearic acid (C18:0, 2 ± 0.9%, saturated) and c-linolenic acid (C18:3n6, 1.1±2.9%, unsaturated). Additionally, in our data set, Erucic acid (C22:1n9) had also high values but only in Brassicaceae species (Fig 3D). The mean frequency of saturated fatty acids (SFA is 14.7% with SD of 5.3) and the mean ratio between unsaturated fatty acids (UFA) and SFA is 6.8 (SD= 2.1), ranging from 2.7 in *S. breviserrata* up to 11.4 in *T. praecox*. In general, the Salicaceae (*S. breviserrata*) and Cistaceae (*H.canum* and *H.urrielense*) had low values of the ratio, indicating higher synthesis SFAs, while other families such as the Lamiaceae (T. praecox) or the Saxifragaceae (*S. conifera* and *S. paniculata*) were characterised by higher ratios showing a tendency to synthesise more UFAs (fig 3E).

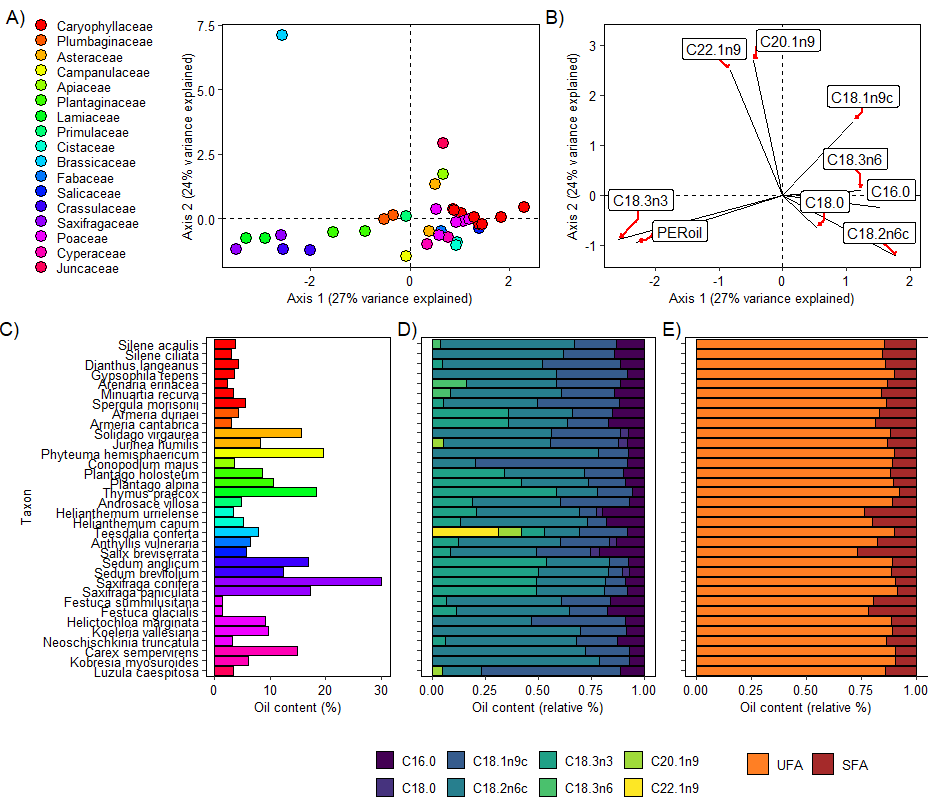


Fig 3: Alpine species seed oil content and composition exploration (n=34). A) Exploratory PCA species points and B) variables directions and contributions. C) Oil content per species in percentage, colors representing families as panel A. D) Seed oil composition of those FAME’s with more than 3% of relative abundance. E) Seed oil composition per species with FAME’s divided between Unsaturated Fatty Acids (UFA) and Saturated Fatty Acids (SFA).

### Biological trade-offs

#### Seed mass

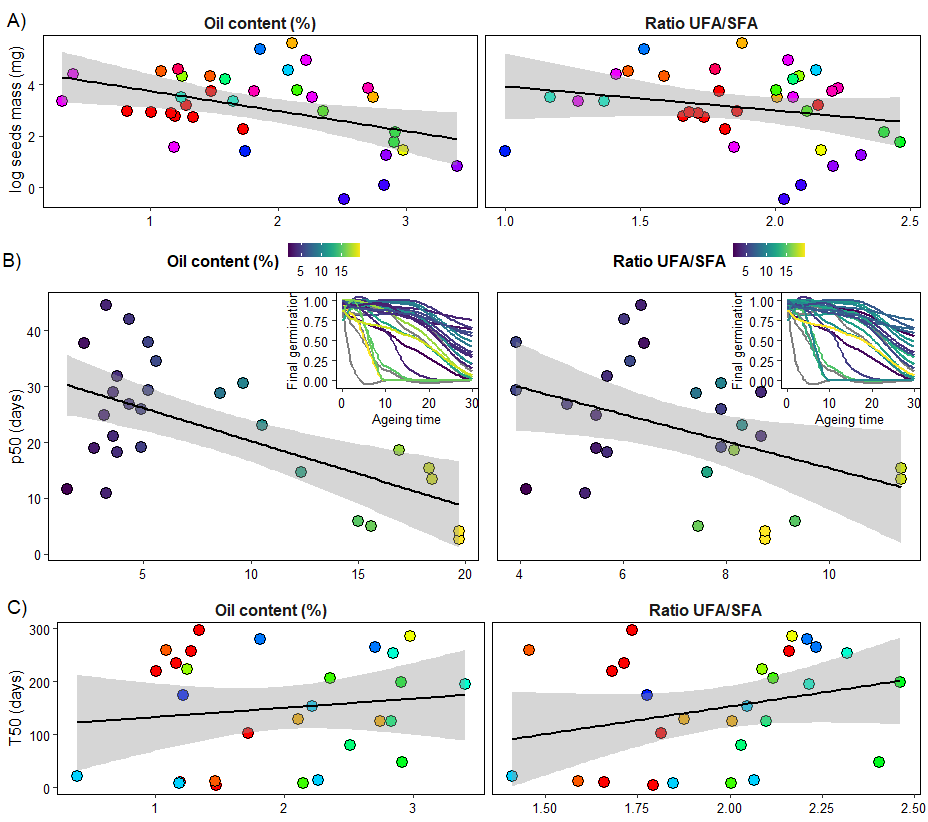
Seed mass values (dry weight 50 seeds) ranged from 0.6 to 268 mg, with a mean of 47.5 mg (sd=58.8). We observed a notable variation of oil content in small seeds, but its variability decreased in large seeds which at the same time have lower oil content. We found a significant negative correlation between seed mass and oil content (p<0.05, fig 4A, left panel) and a marginal negative relationship between seed mass and UFA/SFA ratio (p = 0.06, fig 4A right panel).

#### Seed longevity

Seed longevity was analysed using two different approximations, firstly using the raw germination data from the artificial ageing protocol (with MCMC-GLMM) but also using the p50 value (obtained from the probit analysis). P50 values ranged from 3 to 45 days, with a mean of 22 days (sd=11). Results were consistent using both data types, although raw germination scores had higher statistical power. Higher oil content significantly reduced seed longevity (p<0.05, fig 4B left panel) and higher UFA/SFA ratio (i.e. more unsaturated fatty acids) also decreased longevity, although the signal found in the ratio was only marginally significant (p= 0.08, fig 4B right panel).

#### Earliness of germination

We measured the earliness of germination (germination speed) using t50 value from Espinosa del Alba et al (2024), who worked with a similar dataset of species. T50 values ranged from 4 to 295 days with a mean of 149 days (sd=102). We did not find any significant relationship between t50 and oil content (fig4C left panel) o UFA/SFA ratio, although it appears to be a trend (+ ratio – t50 ) with the latter (fig 4C right panel).

Fig 4. Seed oil content and Ratio UFA/SFA biological trade-offs. A) correlation between seed mass (log-transformed) and oil content (% log-transformed) and ratio Unsaturated Fatty Acids (UFA)/ Saturated Fatty Acids (SFA), also log-transformed. Colours represent families as in Figure 3) P50: time for viability to drop to 50%, based on probit analysis (GENSTAT software) and raw germination curves across artificial accelerated ageing protocol in the lab, colour represent, oil content percentage (left panel) and UFA/SFA ratio (right panel). C)T50: time to reach 50% germination, data from germination phenology which uses the same species from the same area (Espinosa del Alba et al., 2024) depending on oil content (% log-transformed) and ratio UFA/SFA (log-transformed). Colours represent families as in Figure 3.

EXTRA???: will ecological preferences modify longevity? AS preliminary results from Giovanni tesis and IAVS congress. Significant only when using raw germination data.

Gráfico, Diagrama

Descripción generada automáticamente

### Ecological trade-offs

#### Species distribution

Within our 36 species, we had 24 classified as specialist (strict alpine) and 12 as generalist. We found no significant differences between these two groups in oil content or ratio UFA/SFA, nevertheless looking at the data specialist species appeared to have a wider range of values compared to generalists which have higher mean values of both oil content and ratio UFA/SFA (fig 5A).

#### Optimal ecological conditions

Species optimal ecological conditions in GDD ranged from 650 to 2295ºC with a mean of 1473ºC (sd=481). We found no significant relationship between GDD and oil content or UFA/SFA ratio, although it seems to follow a positive trend.

Species optimal ecological conditions in FDD ranged from 0 to 170ºC with a mean of 29ºC (sd=30). We found no significant relationship between GDD and oil content or UFA/SFA ratio, although it seems to follow a negative trend when looking at the ratio.

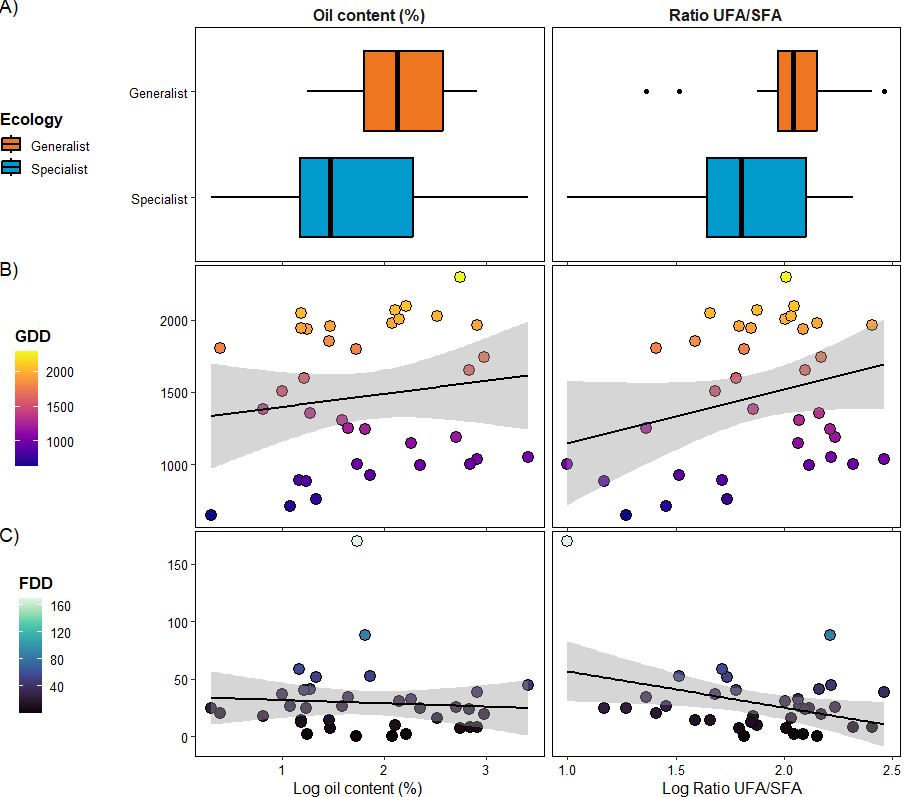


Fig 5. Seed oil content ecological trade-offs. A) Differences of oil content (%, log-transformed) and UFA/SFA ratio (log-transformed) between specialist and generalist species. B) Relationship between GDD (Growing Degree Days) and oil content (%, log-transformed) and UFA/SFA ratio (log-transformed). C) Relationship between FDD (Freezing Degree Days) and oil content (%, log-transformed) and UFA/SFA ratio (log-transformed).

## Discussion

Our study is xxx

### Comparison with global patterns

Sanyal 2016 found significant negative correlation between seed oil content and palmitic (C16:0) and linoleic acids (C18:2n6) and positive correlation with oleic (C18:1n9), arachidic (C20:0) and eicosenoic (C20:1n9). In our preliminary analysis no significant high correlations between total oil content and other components in either direction.

The oil content can vary from 1 % in Musa paradisiaca to 76 % in Chrysobalanus icaco [9]. Bretagnolle found a huge variation in seed oil content ranging from seeds with virtually no oil (Trifolium pratense L., 0%) to very rich seeds (Papaver rhoeas L., 54%) (Bretagnolle 2016). In our preliminary results oil content varies from 1.3 % (F. glacialis) to 30.1% (S. conifera).

According to Bretagnolle 2016 most FA found in seeds are the saturated palmitic (16:0, PA) and stearic (18:0, SA) acids and the unsaturated oleic acid (18:1n-9, OLA), linoleic acid (18:2n-6, LA) and a-linolenic acid (18:3n3, ALA). Corroborated in our results + 2 extra FAs erucic (C22 1n9, mainly from Brassicaceae) and eicosenoic (c20. 1n9, mainly from Juncaceae and Brassicaceae).

Bretagnolle 2016. the three most common fatty acids were palmitic acid (C16:0, PA), oleic acid (18:1n- 9, OLA) and linoleic acid (18:2n-6, LA). These three FAs represented a mean of 76.5% (SD = 20.5%) of the total FAs in the seeds of the species analysed and can represent more than 90% of the FA synthesized by the seed. In our case also linoleic (18:2n-6, 43.1 ± 16.1%), oleic (C18:1n9, 22.9±13.3%), alpha-linolenic (C18:3n3, 16.2 ± 19.3%) and palmitic (C16:0, 10.6 ± 4%). These 4 represent a mean of 92.7% (SD = 52.75)

Two other FAs were found highly represented: the a-linolenic acid (18:3n3, ALA) and the c-linolenic acid (18:3n6, GLA). These five FAs (PA, OLA, LA, ALA and GLA) represented 85.7% of the FAs (SD = 13.1%) among all the species analysed, and for the majority of the species, these five FA’s represented more than 70% of the seed oil content. In our case, the next with higher % were stearic acid (C18:0, 2 ± 0.9%), c-linolenic acid (C18:3n6, 1.1±2.9%).

According to Bretagnolle 2016 the mean frequency of saturated fatty acids (SFA) was 15.6% (SD = 8.8%) and the ratio between unsaturated fatty acids (UFA) and SFA was 7.1 (SD = 4.3). In general, the Asteraceae and Poaceae had low values of the ratio, indicating the synthesis of a high proportion of SFAs, while other families such as the Apiaceae or the Brassicaceae were characterised by a high ratio indicating a tendency to synthesise mostly UFAs. In our case the mean frequency of SFA is 14.7% with SD 5.3%, and the mean ratio between UFA and SFA is 6.8% with SD 2.1%. Ranging from 2.7 in salicacea up to 11.4 in lamiaceae.

‘unususal’ fatty acid species can be deposited in the seeds of particular angiosperm families that have evolved specific fatty acid modification and acylation pathways [8]. For instance, oil in Brassicaceae (Brassica napus, Arabidopsis thaliana) is rich in elongated acyl chains ranging from C20 to C24,(Baud 2010)

Erucic acid’s trend to lower levels at higher latitudes. In addition to having a relatively high melting point (33.5°C), erucic acid is also known to be unpalatable to many species of animals [29,42]. Since herbivory generally decreases at higher latitudes [43,44] selection might be stronger for higher levels of erucic acid at lower latitude.

### Biological trade-offs

Bretagnolle 2016 found a huge variation of oil content exists in small seeds, although such variation strongly decreases towards low oil values in large seeds (Corroborated in our preliminary results).

### Ecological trade-offs

Oil content and UFA/SFA ration seems not to have an important/detectable ecological trade-offs at local scale.

We report the first evidence supporting adaptive evolution of seed TAGs in A. thaliana on a latitudinal cline and the first evidence that the plastic responses of seed TAGs to growing temperature appear to be adaptive. (Sanyal and Linder, 2013)

## Acknowledgements

We are grateful to the Analysis Service Unit facilities of ICTAN for the analysis of Chromatography. We also want to thank Giovanni and Francesco for their assist in labwork.