Towards breeding of edible perennial lupins for Swedish conditions

Theoretical outlook and interspecific hybridization

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Supervisor: Kimmo Rumpunen, SLU, Department of Plant Breeding
Examiner: Cecilia Hammenhag, SLU, Department of Plant Breeding

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Abstract

With regards to current threats posed to society by climate change and lack of food sovereignty in Sweden and globally, the prospects of breeding perennial lupins for food and feed production were examined. This study consisted of a literature study focused on *Lupinus polyphyllus*, related species and hybrids thereof, and an experimental part on interspecific hybridization between *L. polyphyllus* and *L. mutabilis*.

Two lines of annual hybrids between *L. polyphyllus* and *L. mutabilis* developed by plant breeder B.S. Kurlovich were described and compared with *L. angustifolius* 'Boragine'. Attempted crosses between three lines of annual hybrids and naturalized *L. polyphyllus* conducted in 2020 and 2021 were described. However, no viable hybrid seeds from the crosses were obtained, proving that back-crossing these annual hybrids with *L. polyphyllus* being extremely difficult.

Previous and current breeding efforts regarding *L. polyphyllus* were studied, and the potential of *L. polyphyllus* as a perennial grain legume crop were evaluated according to selection criteria for candidate species. Impressive performance, valuable genetic material and important breeding work conducted on *L. polyphyllus* were highlighted as factors favoring a continued development and study of the species. The species habit of spreading and becoming classified as invasive, as well as the content of toxic quinolizidine alkaloids are two issues of importance in breeding and cultivation. Low alkaloid *L. polyphyllus* has the potential to emerge as a crop in the northern regions due to its reliable production, the nutritional qualities and its dual-purpose use as both grain and pasture/silage crop.

Keywords: lupins, perennial crops, plant breeding, grain legumes

Table of contents

List of tables

List of figures

1. Introduction

1.1 Background

Lupin crops are valued for their nutritional qualities, with high protein content, ranging roughly between 30–55 % dry matter (Taylor, De Angelis & Nelson, 2020; Baer, 2002). These nutritional values have earned the lupins their designation as a potential replacement for soy (McNaughton, 2011). Additionally, they have long been praised for soil enhancing properties due to their ability to thrive on infertile soils with low organic carbon content and low pH coupled with their nitrogen fixating capacities (Kurlovich, Earnshaw & Varala, 2007). In Europe in 2013, over 150 000 ha were cultivated with annual lupin crops (Lucas et al., 2015). These numbers include the three annual lupin crop species used in Europe: blue lupin, *L. angustifolius* L. (2n = 40)*,* white lupin, *L. albus* L. (2n =50) and yellow lupin, *L. luteus* L. (2n = 52). The world's largest producer is Australia, where mainly *L. angustifolius* is grown. A fourth annual lupin crop, pearl lupin *L. mutabilis* Sweet. (2n = 48), is to a lesser extent cultivated in South America, from where it originates. In Sweden, lupins are cultivated to a limited extent, grown on 185 ha in 2020 (Karlsson, 2020), these being of the *L. angustifolius*.

Large-leaved lupin, *Lupinus polyphyllus* Lindl. (2n = 48) is a cross pollinated, long-lived herbaceous perennial native to North America, with a natural distribution ranging from California to Alaska (Aniszewski, 1993). Perennial lupins, e.g. *L. polyphyllus* (but also *L. nootkatensis* and *L. perennis*) have become naturalized in many regions of Europe (as well as other parts of the world), and show great adaptability to northern regions with cold climates and short growing seasons (Gudjónsson & Helgadóttir, 1999; Kurlovich & Heinänen 2002). The value of perennial lupins as green manure, forage and for soil improvement and stabilization has been recognized for over half a century in Europe (Kurovich, 2006; Fremstad, 2010), and interest in low alkaloid forms of *L. polyphyllus* have been shown at least since 1958 (Hudson, 1958). Early domestication processes have been achieved with this species, and a few varieties of *L. polyphyllus* have been developed, e.g. 'Pervenec' and 'Truvor' for use as feed (Kurlovich & Heinänen, 2002). Furthermore, the existence of a related ancient annual crop, *L. mutabilis*, gives the possibility to use hybridization in order to introduce desired traits in *L. polyphyllus*. *L. mutabilis* and *L. polyphyllus* share the same chromosome number (2n = 48), though the annual lupin crop species are self-pollinated while *L. polyphyllus* is cross-pollinated. All lupins mentioned in the study rely on insects, mainly bees and bumblebees, for cross-pollination.

A perennial lupin crop, which is combining a satisfactory yield of high protein grain as well as vegetative biomass for pasture or silage, and soil improving qualities could provide valuable food while consuming little inputs with regard to fertilizer use and need for tillage or other soil management practices. This crop species may also have the potential to be well adapted to the growth conditions in all parts of Sweden, other northern countries and beyond, and be a valued component in future agricultural systems built on local recirculation of nutrients and low use of finite resources, for local and regional food supply and food sovereignty. Though studies aimed at promoting cultivation of annual lupin species are conducted in Sweden (Persson, 2022), no studies on perennial lupin crops have been conducted.

1.2 Aim

The aim of this study is to facilitate the breeding of perennial edible lupins that are adapted to the climate and soil conditions of Sweden. Experiments are combined with a literature review and discussion on the prospects, ongoing research, expected problems and trade-offs in different directions in potential breeding efforts. Such issues for discussion are for example: if plant breeding should opt for low or high alkaloid content in lupin seed; how to tackle or discuss invasiveness and potential problems of cross pollination between cultivated and invasive lupins.

The aims of the experiments are to describe the plant material at hand (*L. mutabilis* x *polyphyllus*) to sow and describe plants resulting from crosses attempted in 2020, and to conduct and describe the crossing procedure in the interspecific hybridization of *L. polyphyllus* and annual *L. mutabilis* x *polyphyllus* hybrids. A hypothesis was that it is possible to hybridize *L. polyphyllus* and annual *L. mutabilis* x *polyphyllus* in the field. Another hypothesis was that the resulting seedlings should show a range of useful characters derived from both parents. A third hypothesis was that the plant material tested and described should reveal novel characteristics.

2. Methods

2.1 Literature review on the outlook of perennial lupin breeding

The literature used was found through search engines like SLU Primo, Web of Science and Google Scholar, as well as through recommended literature from plant breeder Professor Boguslaw Kurlovich. The literature review will be limited to study the species of main concern in the experiments, i.e., *L. mutabilis* and *L. polyphyllus*, and other species of the subgenus *Platycarpos* may be more briefly discussed. The more commonly cultivated annual species of the subgenus *Lupinus* will be largely excluded from the study.

2.2 The experiments

All experiments were conducted during 2020 and 2021 on the author's property in north-central Scania at 115 m above sea level. The mean precipitation is about 802 mm per year (Climate-Data, 2021) and the soil is a granite-based moraine with high content of sand. The temperature is mild in winter and relatively hot during summer, with relatively high precipitation throughout the year, see table 1.

Statistical analyses were conducted using one-way ANOVA, Tukey's method with 95 % confidence in Minitab.

Table 1. The monthly mean precipitation (P), monthly mean sun hours per day (Sun) and monthly mean temperature (T) in Osby climate station, roughly 11 km to the east of the site of the experiments (Climate-Data, 2021).

	Jan.	Feb.	Mar.	Apr.	May	Jun.	July	Au.	Sep.	Oct.	Nov.	Dec.
P, mm	61	51	48	48	66	84	95	85	68	70	62	62
Sun h	1.8	3.0	4.9	7.9	9.5	10.1	9.7	8.2	5.9	3.6	2.1	1.7
T. °C	-0.6	-0.6	1.7	6.8	11.7	14.9	17.3	16.4	13	8.3	4.3	1.1

2.2.1 Description of the base material

The annual *L. mutabilis* x *polyphyllus* hybrids used in the experiments have been developed by plant breeder Professor Boguslaw Kurlovich, a long-time expert and developer of the knowledge and nomenclature of the *Lupinus* genus. As explained in an earlier stage in Kurlovich, Stoddard & Earnshaw (2008), interspecific crosses between *L. mutabilis* and *L. polyphyllus* were conducted in 1990 — 2007, and produced a variety of annual as well as perennial hybrids. Since then, however, the perennial hybrids proved less viable, and only annual hybrids from these trials remain (Kurlovich, personal communication, 2019). In the autumn of 2019, seeds from three lines of annual hybrids from Kurlovich were received by the author. These annual hybrids were used in the experiments under the pretext that these reportedly are earlier and more adapted to the climates of northern Europe, in comparison with the pure *L. mutabilis* native to South America. Additionally, to hybridize these annual hybrids with *L. polyphyllus* would essentially constitute back-crossing, which was hypothesized to potentially result in more stable lines being more homozygous and resulting in more similar offspring in coming generations, than hybrids obtained through crossing pure species.

In the autumn of 2019, the following seeds were received from plant breeder Professor Boguslaw Kurlovich: low alkaloid (<0.02 %) *L. polyphyllus*, and three stable annual lines of *L. mutabilis* x *polyphyllus,* numbered #1, #2, and #3, based on their relative seed size, with the largest seeds found in #1. Each line originated from a different hybridization event. The lines were subsequently cultivated in the south of Sweden in 2020, and naturally self-pollinated (obtained through isolating plants under fine-meshed plastic nets) and open pollinated seeds were collected from the annual hybrids. To use as comparison, the commercially cultivated and thus relatively more well-known lupin crop *L. angustifolius* 'Boragine' was obtained from a farmer in the region.

On April 30th, the obtained lupin seeds were sown in the field for description (growth speed, habitus and flowering form), comparison (height, time of flowering and maturation, seed size

and seed yield) and for hybridization with *L. polyphyllus*. In total 15 plots, 2.5 m x 0.75 m were prepared and sown with 50 seeds per plot, in three rows 25 cm apart, with 15 cm between the seeds. Two sites were used, site A on low elevation and site B 40 m away on 5 m higher elevation with dryer conditions. Both sites had sandy soil and low pH. On site A, the previous crop was buckwheat, while site B was previously unplowed for over 40 years and was covered with red clover and grasses. The plots were weeded manually and watered in the first two months.

Three plots on each site (A4-A6, and B3, B5, B6) were used for the purely descriptive part of the study (Table 2 and 3). These were sown with either open pollinated #1 or #3, or *L. angustifolius* 'Boragine'. Similarly, three plots per site (A1-A3, and B1, B2, B4) were designated for the hybridization attempts, and were sown with self-pollinated seeds from the three lines received from Professor Boguslaw Kurlovich. Since lupins are insect-pollinated, the self-pollinated seed were produced in 2020 by cultivating the plants under fine-meshed nets, effectively excluding all pollinating insects. Additionally, three plots (A7 — A9) were used to evaluate the seeds from three of the most productive and promising plants of annual lupins grown in 2020. These where two selections of line #3 (A7 and A9), and one selection from #1 (A8). Due to a small number of seeds, this was only done in site A.

Table 2. The field outline of the nine plots of site A, their seed source and their use in the study.

Plot tag	Seed source	Function
A1	Self-pollinated #1	Hybridization
A2	Self-pollinated #3	Hybridization
A3	Self-pollinated #2	Hybridization
A4	Open pollinated #3	Description
A5	*L. angustifolius* 'Boragine'	Description
A6	Open pollinated #1	Description
A7	Selection nr. 1 #3	Evaluation
A8	Selection nr. 1 #1	Evaluation
A9	Selection nr. 2 #3	Evaluation

Table 3. The content of the six plots of site B, their seed source and their use in the study.

Plot tag	Seed source	Function
B1	Self-pollinated #3	Hybridization
B2	Self-pollinated #1	Hybridization
B3	Open pollinated #3	Description
B4	Self-pollinated #2	Hybridization

| B5 | *L. angustifolius* 'Boragine' | Description |
| B6 | Open pollinated #1 | Description |

All plots were inspected regularly according to different objectives as the season progressed. 19 days after planting (DAP), emergence was measured. Starting 55 DAP, at the end of June when the plants began their elongated growth, the height was also measured weekly by approximating the mean height using a yardstick. 65 DAP, flowering began and the number of flowering plants was also counted weekly. The inspection of the flowering was detailed to record the primary, secondary, tertiary and eventually fourth flowering rushes. While the growth habit of *L. angustifolius* made it possible to harvest the whole plant when mature, the annual hybrids have an indeterminate flowering like that of *L. mutabilis*, and thus flowered more or less continuously until they were killed by either frost or pathogens. Therefore, the mature spikes matured successively and were harvested continuously when matured. Like the description of the flowering, the harvesting was also detailed to record the maturation of the different flushes of pods. However, the plots designated for hybridization were not harvested, as the first month of flowering – which has the best chance of maturing – were either hybridized or removed.

On the 12th of October, 165 DAP, the experimental part was terminated after a hard frost with many plants already dead due to fungal diseases. No further maturation of seeds was expected at that point.

After having dried completely indoors, each harvested component (primary or secondary spikes for the hybrids and whole plant for the *L. angustifolius*) were threshed and the seeds counted. The harvested seeds of the hybrids were divided into primary and secondary flowering, while the seeds of the whole plants of *L. angustifolius* were pooled. The division of the harvest of the hybrids' seeds were made this way since pods of the primary and secondary flowering mature at different times, while those of the *L. angustifolius* largely mature more simultaneously and are difficult to separate in the same way.

2.2.2 Report on the seeds resulting from hybridization attempts in 2020

In the summer of 2020, hybridization of the annual hybrids with local naturalized as well as low alkaloid *L. polyphyllus* was attempted from the beginning of July to the end of August similarly as described below in section 2.2.3. The result was 18 seeds.

The 18 seeds resulting from crosses of 2020 were sown on the 19 of April in a tray. 24 seeds each of the *L. mutabilis* x *polyphyllus* annual hybrids and *L. angustifolius* 'Boragine', and 72 seeds (to compensate for the more irregular germination of this undomesticated species) of low alkaloid *L. polyphyllus* were sown in the same way at the same time. All seeds from a perennial mother plant, i.e., the *L. polyphyllus* and two seeds from the crosses of 2020, were scarified with 150 grit sandpaper. The seeds were soaked for 3 hours prior to the sowing. The trays were kept indoors In 16°C temperature for 10 days, when they were placed outdoors under a protective frame with chicken wire and fiber cloth. At that time, the annuals had largely emerged and required more sunlight to avoid becoming spindly.

2.2.3 The conducting of interspecific hybridization between *L. polyphyllus* and *L. mutabilis* x *polyphyllus* 2021

In the attempt to hybridize the annual *L. mutabilis* x *polyphyllus* hybrids with wild *L. polyphyllus* in 2021, the former was used as mother plants, and the latter as pollen source. In total six plots of lupins, three at both of the two aforementioned sites, were reserved for making crosses (Table 2 and 3). During time used for crossing, all plants on these plots were used indiscriminately regardless of their performance or health. Prior to the maturation of the flower, the petals and stamens were removed using tweezers (Figure 1). As lupins depend on insects for cross-pollination, and the absence of petals that attract and guide the insects to the right position to pollinate the emasculated flower, no mesh bags were used. However, there remained a small chance of either accidental self-pollination or less likely, accidental cross-pollination by insects. Pollen was collected from mature flowers of *L. polyphyllus* growing wild within 100 m of the field with the experimental plots just prior to its usage.

On the 30th of June, 61 DAP, the first hybridization attempt was perfomed, as almost-mature flower buds began to form in the 6 plots designated for hybridization. On close to every day during the period of hybridization, every plot designated for hybridization was carefully combed for close-to mature flowers to be emasculated (Figure 2). All flowers with mature stamens were removed, to avoid any self-pollinated pods to develop in these plots in order to avoid confusion during harvest. The pollen used in the hybridization attempt was collected from naturalized *L. polyphyllus* growing in the immediate surroundings. The pollen was placed on the pistil of all emasculated flowers. The number of flowers emasculated and manually crossed increased from around 25 per day in the beginning to 75 per day towards the end of the period. On the 1th of August, 93 DAP, the hybridization attempts ended. Strings were tied on the flower spikes that had been crossed to avoid confusion in harvest time, since more secondary and third flowering that would not be crossed was to come.

Figure 1. A: Display of the layers of petals and stamens of an immature flower to be removed during emasculation. **B:** Emasculated and hand pollinated flowers of an annual *L. mutabilis* x *polyphyllus* hybrid, part of the hybridization attempts of 2021.

3. Results

3.1 Literature review on the outlook of perennial lupin breeding

3.1.1 Previous and current effort of breeding of perennial lupins for production

3.1.1.1 Pure species breeding

Several lupin species have been domesticated, the earliest being *L. albus* in the Mediterranean area over 4000 years ago, and *L. mutabilis* in the Andes roughly 3000 years ago (Kurlovich, 2006; Taylor, De Angelis & Nelson, 2020). Later, the other major lupin species used, *L. luteus* and *L. angustifolius,* were domesticated and developed in Europe and *L. cosentinii* has also been domesticated in Australia. These five annual lupin species have followed the same domestication pattern, for varying periods of time and in different parts of the world. Crucial traits selected through his homologous domestication are traits commonly associated with the domestication syndrome (Allaby, 2014): non-dehiscent pods to decrease losses at harvest, permeable seed coats and removal of seed dormancy to enable fast and even germination (Kurlovich, 2006; Taylor, De Angelis & Nelson, 2020) and improved nutritional value (Kurlovich, 2006). Development of varieties of these annual species with low alkaloid content, so-called sweet varieties, have been achieved rather late

in the breeding process, starting in 1927–1932 in Germany and Russia. These traits are also critical with regards to the domestication of perennial lupin species.

Since *L. polyphyllus* is a mainly cross-pollinated perennial, unlike the annual domesticated species that to a large extent are self-pollinated, breeding is more complicated (Kurlovich, Stoddard & Earnshaw, 2008). The perennial life-cycle with uneven and at least belated flowering in the first year translates into longer regeneration times. In the selection of certain plant traits, the breeder might have to take the performance of the plant over several seasons into consideration, which slows breeding efforts additionally. In species that are cross-pollinated, it is more difficult to isolate and stabilize useful traits. Although *L. polyphyllus* is not an obligate cross-pollinator, this still holds some implication with regards to breeding of this species.

Breeding efforts concerning pure species breeding of *L. polyphyllus* have focused on the species potential as a soil enhancing feed crop. Wild type (high alkaloid) forms of *L. polyphyllus* have already had the use as feed, e.g., in high country New Zealand for grazing (Black et al, 2014). Black et al. (2014) reported good survivability of the lupins after grazing. Their results demonstrated that lupin was comparable but inferior to lucerne as feed regarding livestock performance. The sheep's acceptability of the lupin leaves is low in the summer due to the alkaloid content (Scott, 1989). However, Scott & Tesfaye (2000) found no difference in sheep acceptability for grazing of low alkaloid and high alkaloid accessions. Additionally, they found no season-dependent difference in acceptability. While sheep weight gain on lucerne pasture significantly outperformed lupin-grass stands in New Zealand lowland area with moderately fertile soil (Black et al., 2015a), Merino sheep performance in experiments on low fertilizer high country conditions were comparable but inferior to grazing on lucerne (Black et al., 2014). Black et al. (2015b) support the use of *L. polyphyllus* in high altitude areas of New Zealand where lucerne cannot thrive, and Scott (2015) further substantiates its value as soil enhancer in N-deficient soils and report that it outperforms other pasture species, especially in "acid-, aluminum- moist soils under low allowable fertilizer rates" (pg. 31). Scott & Tesfaye (2000) evaluated 46 accessions of *L. polyphyllus* for use as sheep pasture crop in low fertility areas in New Zealand during 8 years, and identified 31 elite plants.

Efforts to domesticate *L. polyphyllus* have been ongoing mostly in eastern Europe for roughly 50 years, however with varying intensity (Kurlovich & Heinänen, 2002). According to Aniszewski (1992), alkaloid-poor forms of *L. polyphyllus* were developed by plant breeder Voronov as early as 1974 in Russia, but these forms had difficulties in growing back after being cut. Important breeding efforts are built upon the resources of the N.I. Vavilov Institute of Plant Industry, St. Petersburg, Russia, which holds 150 accessions of the species, originating from 8 countries (Kurlovich & Heinänen, 2002). As described by Kurlovich & Heinänen (2002), observation of lupins grown in several countries during 1973–1996 resulted in the acquisition of high yielding *L. polyphyllus* with low alkaloid content.

A few cultivars and varieties are mentioned in the literature, although little information is to be found on them (sweet cultivars have an alkaloid content of <0,02 %, while semi-sweet have reduced alkaloid content but higher than 0,02 %): 'Truvor', a low alkaloid fodder lupin adapted to the Ukraine. 'Pervenec', a semi-sweet cultivar adapted to northwest Russia. 'Pushkinsky', a bitter cultivar (Kurlovich, Stoddard & Earnshaw, 2008). 'Plarre', a sweet

cultivar (Baer, 2002). 'SF/TA', a semi-sweet variety developed in Finland from crossing lupins found in the roadside (Aniszewski, Kupari & Leinonen, 2000; Aniszewski, 1993).

Sweet forms of *L. polyphyllus* with alkaloid content of <0,02 %, have the reported uses as silage for cows and horses in Finland with good results (Kurlovich, Stoddard & Earnshaw, 2008). It can obtain fresh green matter yields of 40–50 ton/ha, while out-competing weeds (Kurlovich & Heinänen, 2002). Kurlovich, Earnshaw & Varala (2007) state seed yields of 1000–1600 kg/ha under favorable conditions in Finland. Scott (1989) estimates New Zealand wild type to yield 500 — 2000 kg/ha (sown at spacing of 30 cm between rows and 16 cm within rows) with regards to seed production, being harvested manually.

In a study of pot grown *L. polyphyllus* var. 'SF/TA' by Aniszewski (1993), a yield of 16 g per plant ranging from 10–27 g/plant is reported, which (disregarding the difficulties entailed in estimates of field performance based on data from pot grown plants) with 10 plants per m^2 translates to 1000–2700 kg/ha. In a ten-year experiment with the semi-sweet variety SF/TA in Finland (the first year was only a growing year without seed harvest), the seed harvest per plant remained rather stable at about 50 g per plant (Aniszewski, Kupari & Leinonen, 2001). However, seed count decreased while seed size increased from average 20 mg per seed to 50 mg per seed in the last year. In this experiment, seed size began to increase with the sixth year. Moreover, Aniszewski, Kupari & Leinonen (2001) identified four different seed types having different seed coating and sizes, which may correlate to different chemical composition including alkaloid content, to be produced within the same plant. The composition of the different seed types also changed over the experiment period.

3.1.1.2 Interspecific hybridization

There has been a couple of different efforts to hybridize *L. polyphyllus* with *L. mutabilis*, both with 2n = 48 (Kurlovich, Stoddard, & Earnshaw, 2008) and of the subgenus *Platycarpos* (Kurlovich & Stankevich, 2002).

Building on the aforementioned resources of the Vavilov Institute, both the bitter cultivar 'Pushinsky' and semisweet cultivar 'Pervenec' were used in crosses with bitter (line k-2770) and sweet ('Inti' – a cultivar with 0.0075 % alkaloid content developed in Germany with selection for early maturation (Jacobsen & Mujica, 2008)) *L. mutabilis* in 1990–2007 (Kurlovich, Stoddard & Earnshaw, 2008). The crosses yielded both annual and perennial lines of hybrids. The annual hybrids have improved adaptability to the northern climates, high vigor and frost resistance–- the annual hybrids used in the experiments of this study come from these lines. The perennial hybrids had larger seed, although these lines later failed (Kurlovich, personal communication 2019).

Baer (2002) crossed sweet *L. polyphyllus* 'Plarre' with *L. mutabilis* sweet cultivar 'Inti' around 1987 and could identify differences in the F3 in 1990 regarding several important traits such as seed size, precocity, frost resistance, alkaloid content, seed composition and shattering resistance. Like Kurlovich, Stoddard & Earnshaw (2008), annual hybrids that were more early maturing, about 7 days earlier than 'Inti', and additionally, a more concentrated flowering (Baer, 2002). Annuals backcrossed with *L. mutabilis* (line SCG9) produced plants with larger seed size than that of the parental material (455 mg as compared to 221 mg for

SCG9 or 22 mg for *L. polyphyllus*). Another backcrossed line displayed increased protein content as compared to parental material. A perennial line with frost resistance and larger seed size was made, but due to its decreased homogeneity and density as compared to annuals, its development was not followed through.

To date, annual *L. mutabilis* x *polyphyllus* lines have achieved more success than their perennial counterparts, as *L. mutabilis* x *polyphyllus* 'Gris' and 'Negro' are cited as named samples of the diverse gene pool of the American species of value for agricultural use (Baer, 2019). Here, it is the traits of *L. polyphyllus* such as early maturation and frost resistance and the value of hybridization for vigor and diversity that are utilized – rather than the traits of *L. mutabilis* being crossed into the *L. polyphyllus* growth form. Another example of the use of *L. polyphyllus* as a donor of useful traits is the efforts of Godjónsson & Helgadóttir (1999) in crossing *L. nootkatensis* with low alkaloid *L. polyphyllus* in order to create low alkaloid forms of the *L. nootkatensis* for agricultural use in Iceland.

3.1.2 Reviewing *L. polyphyllus* for strengths and shortcomings

Schlautman et al. (2018) developed criteria for the selection of candidate species for breeding of herbaceous perennial grain legumes. *L. polyphyllus* and its hybrids apparently already has been selected for domestication and further development by several plant breeders (e.g., Kurlovich & Heinänen, 2002; Baer, 2002; Aniszewski, 1993; Godjónsson & Helgadóttir, 1999). Still, it may be worthwhile to use the criteria against *L. polyphyllus* and other perennial lupins in order to identify what strengths to utilize and shortcomings to tackle in any further projects to develop perennial lupins for use as feed and food.

The criteria, with four headings, *Agronomic Potential, Seed Quality, Ecosystem Services* and *Ease of Breeding*, each with three to five subheadings, is listed and the potential of the perennial species *L. polyphyllus* against these criteria is discussed.

3.1.2.1 Agronomic Potential

Crop Establishment
This subheading concerns the ease of establishment, a relevant factor as many wild legumes has tough seed coats that are impenetrable to water which often require scarification to enable germination (Schlautman et al., 2018). While the annual *L. mutabilis* is soft-seeded and germinate without effort, this can prove to be of concern for *L. polyphyllus*. Even though the seeds were scarified, only 26.4 % of the low alkaloid *L. polyphyllus* sown in trays germinated in this experiment. The low germination percentage could be attributed to aging seeds or insufficient scarification. Kurlovich, Stoddard & Earnshaw (2008) report that numerous recessive mutants, including those with water-permeable seed coats have been isolated from 30 years of self-pollination. However, to introduce these qualities reliably into an improved cultivar with other crucial traits is another task. (Kurlovich, Loxton & Naughton, 2019), in a trial with low-alkaloid *L. polyphyllus* still describe emergence as "painfully slow and inconsistent".

Another crucial aspect of successful establishment of lupin crops, which also holds true for many other legume crops, is the occurrence of the right symbiotic bacteria for efficient nitrogen fixation. In the case of lupins, if lupins have not been cultivated on the site

previously, this is best done through inoculation with improved strains of *Bradyrhizobium sp. (Lupinus)* (Kurlovich & Heinänen, 2002)*.*

Field Management
Field management in this case concerns the ease of which different management practices can be conducted in the relevant agronomic setting with respect to the growth habit of the species (Schlautman et al., 2018). Indeterminate vegetative and reproductive growth makes for difficult management for mechanical harvest, but may be suitable for certain small-holder and subsistence practices where manual harvest is feasible. The growth habit of both *L. polyphyllus* (and *L. mutabilis*) is rather erect, even and predictable, as compared to e.g., wild type *Phaseolus sp*. which allows for mechanical field scale management.

Harvestability
Harvestability concerns the ease of harvest under the conditions of the relevant agronomic system, in this case mechanical harvesting (Schlautman et al., 2018). The *L. polyphyllus* flowering form being terminal racemes that ripen at around the same time and usually placed high above the vegetative biomass is promising for the applicability of mechanical harvest. Furthermore, non-shattering forms of the low alkaloid *L. polyphyllus* have been developed (Kurlovich, Stoddard & Earnshaw, 2008). The harvest of the seeds occurs in July to the beginning of August, which is earlier than cereal grains (Kurlovich, Earnshaw & Varala, 2007). *L. mutabilis* meanwhile, exhibits an indeterminate growth form at least in northern climates, as it sets flowers and seed continuously until frost or succumbing to diseases. In order to suit mechanical harvesting in northern regions, interspecific hybrids between the two species need to retain a growth form much like that of *L. polyphyllus.*

Yield Potential
Kurlovich, Earnshaw & Varala (2007) state yields of 1000–1600 kg/ha under favorable conditions in Finland. As cited in 3.1.1, there are also few estimates of the yield potential of *L. polyphyllus* that are not based on field trials. Scott (1989) estimates New Zealand wild type to produce seed yields of 500–2000 kg/ha, while Aniszewski (1993) estimates grain yield potential of the semi-sweet variety 'SF/TA' to be 1000–2700 kg/ha based on data from pot grown plants yielding an average of 16 g seeds per plant. This is on par with the reported yields of *L. mutabilis* in the Andean region, which range from 800–2800 kg/ha (Jacobsen & Mujica, 2008). However, Aniszewski, Kupari & Leinonen (2001) later report 'SF/TA' in plots of 2 m^2 with four replicates in Finland to produce seed yields of slightly above 50 g per plant consistently over a ten years period. Kurlovich and Heinänen (2002), in describing populations of low-alkaloid *L. polyphyllus* in Finland created through different measures as well as a productive high-alkaloid populations used in crossing, reported yields from low-alkaloid forms of 61 g per plant on average at the most.

Adaptability
Adaptability here refers to the species ability to adapt to different climates, soils and water conditions. As Schlautman et al. (2018) remind us, adaptability may be of even larger importance in perennial crops than in annual crops, due to their hopeful longevity. This means that not only a few months during the growing season, but also cold winters and sometimes unpredictable springs or autumns, must be tolerated by the crop. With a natural range from California to Alaska, and proven to thrive and reproduce without risk in central, east and northern Europe as well as New Zealand, the adaptability of *L. polyphyllus* to

different climates is well documented. Considering the lack of reports regarding the effect of day time length on the growth or flowering of *L. polyphyllus,* this does not appear to be an important aspect of the adaptability of the species. To some extent, this large wild and naturalized occurrence also implies adaptability to soil and water conditions, although it does best on sandy, slightly acid soils 5.5–6.5 pH (Kurlovich, Earnshaw & Varala, 2007) with precipitation of around 600 mm per year (Fremstad, 2010; Black et al., 2014) and its native habitat is described as "shady, moderately dry, well drained, sandy-loam soils" (Fremstad, 2010).

3.1.2.2 Seed Quality

Nutritional Profile

The nutritional profile of the lupin seed is one of its clear strengths and an important factor for the interest given for its domestication and use as both food and feed. The component of primary interest is the protein which is very high in lupins, often comparable to that of soybean (Keeler & Gross, 1980). Oil content is another important component, which though more variable, is also comparable to that of soy. Kurlovich, Stoddard & Earnshaw (2008) report around 43.9 % protein in semi-sweet *L. polyphyllus* 'Pervenec' and slightly higher, 45.8–46.5 % in *L. mutabilis* line k-2770 and the annual and perennial hybrids, although the annual hybrids exhibited a relative high variation in protein content, see table 4. The oil content was significantly lower in 'Pervenec' (4.2 %), than *L. mutabilis* line k-2770 (14 %). Baer (2002), reported protein contents of 45,4 % and oil content of 9,5 % in sweet *L. polyphyllus* 'Plarre', and 43.3–53.6 % protein and 13.6–28.9 % oil in annual *L. polyphyllus* x *mutabilis* hybrids, as compared to 38–41.3 % protein in two *L. albus* cultivars. Aniszewski (1992) reported protein content of 41.9 % and 9.7 % oil in a sweet form of *L. polyphyllus*, while bitter wild type contained 43.1–46.2 % protein.

Table 4. The protein, oil and fiber contents of some analyzed lupin accessions and their references.

	Protein (%)	Oil (%)	Fiber (%)	Reference
Sweet *L. polyphyllus* 'Plarre'	45.4	9.5	-	Baer, 2002
Semi-sweet *L. polyphyllus* 'Pervenec'	43.9	4.2	-	Kurlovich, Stoddard & Earnshaw, 2008
Sweet *L. polyphyllus*	41.9	9.7	21	Aniszewski, 1992
Annual *L. polyphyllus* x *mutabilis*	46.1	13.2	-	Kurlovich, Stoddard & Earnshaw, 2008
Perennial *L. polyphyllus* x *mutabilis*	45.8	7.5	-	Kurlovich, Stoddard & Earnshaw, 2008
Wild type *L. polyphyllus*	43.1–46.2	8.5–8.8	15.9–17.7	Aniszewski, 1992

Sweet *L. mutabilis* 'Inti'	50.8	17.1	-	Baer, 2002

Anti-Nutritional Factors

Occurrence of anti-nutritional factors in legumes is common, and an important restriction to their broader cultivation and use historically and contemporary (Schlautman et al., 2018). The content of alkaloids is a central issue in the breeding of perennial lupins, which demands a thorough method to deal with it regardless if one opts for high or low alkaloid content. While offering protection against pests and diseases, the alkaloids are neurotoxic compounds that need to be removed before human consumption (Keeler & Gross, 1980).

The alkaloids present in lupins are largely consisting of quinolizidine alkaloids, which mostly occur in *Fabaceae*, and of which there are eight structural classes; lupanine, lupinine, angustifoline, sparteine, multiflorine, aphylline, anagyrine, and cytisine (Frick et al., 2017), while over 150 quinolizidine alkaloid compounds have been identified (Wink, 1990). Quinolizidine alkaloids give the bitter taste of high-alkaloid forms of lupins, and can result in acute anticholinergic toxicity. The estimated lethal dose of quinolizidine alkaloids for children is 11–25 mg per kg body weight. No lethal poisonings of adults have been reported. When present in feed, anagyrine can cause congenital deformities such as crooked calf disease (Keeler & Gross, 1980). However, this alkaloid does not occur in neither the annual lupin crops, nor *L. polyphyllus* or *L. perennis* (Keeler & Gross, 1980), and is together with cytisine overall rare in the *Lupinus* genus (Frick et al., 2017).

L. mutabilis has been cultivated for human consumption for roughly 3000 years despite its high alkaloid content (Kurlovich, 2006; Taylor, De Angelis & Nelson, 2020). The alkaloid content was dealt with through leaching of the grain in sacks placed in streaming water (Keeler & Gross, 1980). Keeler & Gross (1980) found that cooking and leaching the grains of high alkaloid *L. mutabilis* decreased alkaloid content from 3.17 % to 0.003 % dry weight. However, 25–60 % of dry matter was simultaneously lost. If these methods are to be used, the yield gained through the improved protection in bitter cultivars must be weighed against yield losses in post-harvest management. Modern processing of bitter lupin grain can separate fractions of pure protein, dietary fibers, lipids, alkaloids, and residues (Wink 1990).

The industry threshold of quinolizidine alkaloid content for human consumption is 0.02 % dry weight (Frick et al., 2017). As stated by Aniszewski (1993), not only the total quinolizidine alkaloid content but also the composition of the alkaloid compounds is crucial in the determining of the safety of lupin seeds for food and feed purposes. Lupanine and sparteine are reported to be the most toxic quinolizidine alkaloids to humans and animals (Frick et al., 2017).

As stated by Kurlovich et al. (2016), "it is very important not only just to have low alkaloid (sweet) forms, but also to maintain their genetic integrity". In all low alkaloid lupin crops, bitter forms reappear and spread in the population, due to the dominant characteristic of bitter forms (Kurlovich et al., 2016; McNaughton, 2011). This problem is deepened in *L. polyphyllus* due to its perenniality and because it is cross pollinated (Kurlovich et al., 2016). In order to produce pure seed for low alkaloid forms of *L. polyphyllus*, all plants should be tested with Dragendorff reagent (a reagent used to detect presence of alkaloids in vegetative

matter) prior to flowering, and all wild type lupins be removed in the adjacent area within pollinators reach (Kurlovich & Heinänen, 2002). Then, the perenniality of the species can be used as a benefit, as the assured low alkaloid lupins can produce seed for several years.

The alkaloid content in the seed of the semi-sweet variety SF/TA, which had a total alkaloid content of 0.0226–0.0366 %, consisted to 97.2 % of lupinine, 2.1 % angustifoline, 0.69 % sparteine and 0.01 % gramine (Aniszewski, 1993), an indole alkaloid (Frick et al., 2017). Additionally, the piperidine alkaloid ammodendrine is a large component of *L. mutabilis* (Frick et al., 2017)*.* The alkaloid content is reported to vary significantly both in bitter wild-type lupins and sweet low-alkaloid lupins (Aniszewski, 1992). In this study, decreased alkaloid content was also correlated with a change in the "raw protein, fibre, fat, fatty and amino acids as well as sugar and macro- and micronutrient content'".

With the clear toxicity of these alkaloids for mammals, they hold an important role in the defense of the plant (Frick et a., 2017). Wink (1990) found that low alkaloid lupins were eaten by rabbits while bitter plants were left unharmed. The effect is not limited to protect against mammals either: Sparteine hinders multiplication of some virus and bacteria, while lupanine protects against fungal disease caused by *Erysiphe graminis* (Wink, 1990)*,* as well as being the quinolizidine most effective against aphids (Frick et al., 2017). Other insects as well as snails are also deterred by high levels of alkaloids in plant tissues (Wink, 1990).

Obviously, no protection is complete and numerous species have overcome this plant defense and tolerates these alkaloid compounds to different degrees. This results both in the fact that e.g., sheep can graze vegetative parts of e.g., *L. polyphyllus*, and that there are several pests and diseases that target bitter lupins as well as sweet forms – though problems can increase in sweet forms (Frick et al., 1990; Kurlovich, Loxton & Naughton, 2019). In a study on the qualitative effect of alkaloid content on the degree of pest damage, Kurlovich, Loxton & Naughton (2019) find a clear relationship: On average, plants with 'no symptoms' have an alkaloid content of 4 %, while plants with 0.02 % alkaloids showed 'very severe' damages. Meanwhile, 2 %, 0.2 % and 0,09 % resulted in 'slight', 'Intermediate' and 'severe' damages respectively. This offers useful implications for identification of low-alkaloid plants in breeding efforts, while it amply demonstrates the value that the alkaloids present for the health of the plants. Wink (1990) finds that plant defense in sweet forms of lupins is made up of other compounds such as flavonoids, isoflavones and phenolics.

It is estimated that approximately half of the quinolizidine alkaloid content in the seeds are produced in the seed while the other half is produced in other aerial tissue and subsequently translocated to the seed (Frick et al., 2017). One theoretical breeding aim is therefore to target the mode of translocation of the alkaloids to the seed, in order to decrease alkaloid levels in the grain while retaining the plant protective functions of the alkaloids in the vegetative parts (Wink, 1990; Frick et al., 2017). Mutations in the genes for the responsible carrier proteins are expected to occur naturally, and traditional breeding may thus be used (Wink, 1990), though this has proven difficult to achieve.

Alkaloid content is most rapidly screened for using Dragendorff reagent (Kurlovich, Stoddard & Earnshaw, 2008), which reacts to contents of >0.5 %, thus still above the industry standard of 0.02 % (Frick et al., 2017). However, this does not explain what genes are involved in the reduced alkaloid content. Mutations resulting in reduced alkaloid content are

recessive, and there are a number of different mutated alleles identified in different *Lupinus* species (Frick et al., 2017). The development in the fields of genetics and genomics, and greater understanding of the biosynthetic pathways may enable a more stable approach to tackle the problems of alkaloid content. Genetic markers linked to two low alkaloid loci in *L. angustifolius* have been identified, which can facilitate the selection process.

As stated by Frick et al. (2017) the use of Crispr/Cas9 would be another possibility with which to target specific genes to knock out production of certain alkaloids or carrier proteins which transport them to the seeds. This technique is allowed for research purposes, howeveras a practical plant breeding technique it is not feasible since plants produced using Crispr/Cas9 are regulated as GMO:s and not allowed in either Europe which is the focus of this study, nor in Australia where most lupin production is currently situated. Additionally, the technique can also bring unexpected changes in the genome, through off-target site mutations (Zhang et al., 2015). This could bring side-effects which are difficult to assess, especially considering the relatively understudied nature of *L. polyphyllus* and its genome.

Other than genetic factors, the alkaloid content is affected by environmental factors (Frick et al., 2017). Lower soil pH, lower temperature, potassium deficiency and high phosphorus levels are all linked to increased content of quinolizidine alkaloids in *L. angustifolius*.

High-Value Products
This section concerns compounds that could give added value to the grain legume, such as unique flavor, health benefits or uses in industrial processing (Schlautman et al., 2018). While no reports of unique flavor in *L. polyphyllus* have been made, nor other health benefits except those of the high nutritional value (however, see Li et al. 2020, for a review on alkaloids as candidates for medicinal value), some processing uses have been suggested. Aniszewski (1992) raises the possibilities of processing the quinolizidine alkaloids of high-alkaloid *L. polyphyllus* for medicative uses, as well as biological pesticides for organic farming, a possibility also raised by Wink (1990). The pigment and odorous compounds of sweet *L. polyphyllus* are suggested for potential use in perfumes and body oils, while fatty acids could be used in soap. Furthermore, the symbiotic bacteria are raised for their possible use in seed vaccines.

3.1.2.3 Ecosystem Services

Resource Acquisition and Retention
An important reason for focusing on legumes are the promised possibilities to promote resource efficient and low-input agriculture through biological nitrogen fixation and efficient capture of nutrients through a deep and perennial root system (Schlautman et al., 2018). *L. polyphyllus* is reported to biologically fixate 250 – 300 kg N per ha and year (Kurlovich, Earnshaw & Varala, 2007). As described by Schlautman et al. (2018), deep rooted perennial legumes, a description that fits *L. polyphyllus*, also have an increased efficiency in its retention of fixed nitrogen, as mineralized nitrogen from decaying plant parts can be taken up by the deep roots under a longer period than its annual counterparts. Its value of improving soils with low organic carbon content also increases the efficiency of applied mineral fertilizers (Kurlovich & Heinänen, 2002).

Pollinator Resources

As Schlautman et al. (2018) describe, 30 % of the world's food production is estimated to depend on bees as pollinators, and while this constitutes a weakness as disturbance to pollinator populations would result in dire consequences, agricultural models that provide important resources to pollinators are crucial for the overall picture. *L. polyphyllus* does not produce nectar, but supplies pollen to pollinators, mainly bumblebees (Haynes & Mesler, 1984), and honeybees (own observation).

Dual-Purpose Legumes

Schlautman et al. (2018) state that species that also produce large quantities of biomass that can be used as feed or for bioenergy have increased likelihood of being used more widely, as this might compensate for potential inconveniences of incorporating a perennial grain legume into farming systems. As indicated in 3.1.1.1, *L. polyphyllus* can be used both as a grain legume and for production of fresh biomass for fodder use, or as feedstock for bioenergy purposes. Again, these potential uses relate to the alkaloid content of the vegetative parts. While bitter wild type *L. polyphyllus* have been trialed as a forage crop for sheep in New Zealand, acceptability is naturally decreased by the presence of alkaloids (Scott, 1989). As early low alkaloid *L. polyphyllus* are described to be ill-adapted to being cut (Aniszewski, 1992), an issue apparently resolved in later years, the dual-purpose function should be considered in the breeding process.

When used solely for this purpose, low alkaloid *L. polyphyllus* can yield 40–50 tons of fresh green matter per ha in Finland (Kurlovich & Heinänen, 2002), while Kurlovich, Earnshaw & Varala (2007) report average fresh green matter yield of 60.2 ton per ha in trials at Vavilov Institute of Plant Industry in St. Petersburg, Russia. Of these, 39.1 ton per ha were from the first harvest and 21.1 ton per ha in the second harvest. Since the first harvest is done around the time when the plants start to flower, other methods are required for dual-purpose cultivation. An issue may be decreased quality of the aged green matter in August, when the seed has matured.

Minimizing Ecosystem Dis-Services

Ecosystem dis-services can both come in the form of high requirements of environmentally damaging pesticides or other disturbances to the local ecosystem through competition for water and other resources (Schlautman et al., 2018). Additionally, a significant issue is the potential of candidate species of becoming invasive; the longevity, vitality and capacity to efficiently fixate nitrogen that are required for a candidate species to be of relevance for the criteria also constitutes traits that may enable the species to establish themselves in off-cultivation areas and outcompete native flora – and thus also affect native fauna.

L. polyphyllus has been described as an invasive species in several countries after its introduction in Europe in 1826 (Fremstad, 2010). In Europe, naturalized populations are recorded in Denmark, Norway, Sweden, Finland, Estonia, Latvia, Lithuania, Poland, Germany, Belgium, Netherlands, UK, Austria, Italy, Czech Republic and parts of European Russia (Fremstad, 2010; Ramula, 2015). Additionally, it has been established in Chile, Australia, New Zealand and parts of Asia (Ramula, 2015). Though they have several specialized herbivores in their natural range, these were largely absent (an exception may be the lupine aphid, *Macrosiphum albifrons*) in their new habitats (Wink, 1990). The habitats include managed and natural/semi-natural areas, e.g., roadsides, agricultural lands, forests,

grasslands and wetlands (Ramula, 2015). Its broad distribution demonstrates its potential to adapt, which as Ramula (2016) shows in a study on the performance of plants from different localities in Finland, is due to phenotypic plasticity, rather than local adaptation. *L. polyphyllus* mainly spreads through seeds, which are reported to remain viable for over 50 years, as well as through creeping rhizomes (Fremstad, 2010).

According to Ramula (2015), the largest naturalized populations of *L. polyphyllus* stem from the planting of the species for soil reclamation projects as well as use for agricultural and forestry purposes. Its ornamental use also gives rise to garden-escapes (Fremstad, 2010).

As indicated by Schlautman et al. (2018), the nitrogen fixating capacity of *L. polyphyllus*, together with its ability to thrive in nutrient poor, sandy soils with low organic matter, is an important factor in making it an invasive species. These traits allow it to utilize nutrient resources unavailable to many other species, and subsequently out-compete most species through its vigor (Scott, 2015). Another factor weighing in on its invasive character is the alkaloid content which largely discourages both damage from insects and browsing herbivores, although damage from slugs and aphids, as well as roedeer can occur (Ramula, 2015). Valtonen, Jantunen & Saarinen (2006) identifies negative impact on lepidopteran species by *L. polyphyllus* in roadsides in Finland. Fremstad (2010) states that eradication is not necessary in most locations, due to limited impacts of the plants on other species. In areas where they pose a threat to native flora and fauna, grazing for a couple of years, mowing for several years (twice a year, prior to flowering and two months later), or use of chemical pesticides, can be used as control methods.

3.1.2.4 Ease of Breeding

Reproductive Biology

The reproductive biology of perennial legumes, including cross-pollination or self-pollination, flower structure, requirement of specialist pollinators and lifecycle, highly affects the breeding and the time required (Schlautman et al., 2018). Although *L. polyphyllus* does not seldomly flower in its first year, it is not a reliable producer of viable seed until the second year, which obviously increases the time required for each generation cycle. *L. polyphyllus* is a non-obligate cross-pollinator, pollinated by several generalist bee and bumblebee species. As described in the experimental part of this study, the flowers of *L. polyphyllus*, *L. mutabilis* and their hybrids are highly vulnerable to damage during the emasculation process, and numerous flowers fall off even without interference, likely due to failed pollination.

As a cross-pollinated species, breeding efforts are more demanding, as controlled pollination is required for each generation step. In the case of *L. polyphyllus*, this includes artificial pollination and controlled self-pollination through tipping of flowers (Kurlovich, Stoddard & Earnshaw, 2008). For multiplication of seeds, all plants must be tested for the selective traits, and naturalized wild type lupins must be controlled.

Genome Structure

As Schlautman et al. (2018) states, development in the field of DNA-sequencing has made genomic assisted breeding, such as marker-based selection, affordable and possible also for non-standard crops. Here, the structure of the genome affects the ease of use of these techniques, where smaller, diploid genomes are less demanding than large polyploid

genomes. Though there exist tetraploid variants of *L. polyphyllus* (Ramula, 2015), the standard variant that is used in current breeding efforts is diploid, 2n=2x=48 (Kurlovich, Stoddard, & Earnshaw, 2008).

As of yet, no uses of marker-based selection in *L. polyphyllus* appear to have been conducted. Perhaps the most decisive trait, alkaloid content, is usually screened for using a rapid Dragendorff reagent test, which shows presence of alkaloids in plant tissue (Kurlovich, Stoddard, & Earnshaw, 2008). However, this does not state which genes are responsible for the low alkaloid content, of which there are several, which if combined results in more stable sweet lines.

Available Genetic Resources

The plant genetic resources available are the foundation of any breeding program, and the diversity of accessions sets the possibilities to identify critical traits for domestication (Schlautman et al., 2018). However, many candidate species in the list made by Schlautman et al. (2018) have low numbers of accessions in germplasm collections. On the contrary, there are a large number of accessions of *L. polyphyllus*, in the aforementioned Vavilov Institute of Plant Industry, and elsewhere, ranging from New Zealand to European collections, breeding material and naturalized populations, to South American resources and hybrids with *L. mutabilis*, and the original native populations in North America. Additionally, there are a number of related lupin species with the same chromosome number, which can be used in interspecific hybridization to further increase the diversity of the selection material.

3.2 The experiments

3.2.1 Description of the base material

Growth habit and flowering

After being sown on the 30th of April, the final plant count was close to being reached by three weeks, with most seeds to germinate having done so. During the first month and a half, the hybrid plants grew quite slowly, making a rosette-like form below 10 to 15 cm rather than putting on height. Starting in the second half of June, rapid elongated growth began, with many plants reaching a meter within a few weeks, see table 5. The plants of *L. angustifolius* 'Boragine' maintained a more even growth pattern during the first months, with no distinctive beginning of elongated growth. While the numbers on height for these plants are skewed due to browsing damage from wild hares, some plants had reached their final height two months after sowing; 50–70 cm in site A and 40–55 cm in site B (Table 5. As shown in table 6, line #1 of the annual hybrid, which has the largest seed size, also has the largest plants, reaching 80–130 cm A6 and 90–130 cm in B6 at full height at the times indicated in table 5. Meanwhile, line #3 of the annual hybrids reached 90–110 cm in A4 and 60–90 cm in B3 (table 5) at their full height as shown in table 5.

The annual hybrids exhibit a distinctive flowering pattern of several flushes of flowering, the first inflorescence emerging directly from the main stem, the second comes from branches of the main stem, and the third flowering descends from secondary branches that emerge from the first branches. Typically, the plant will have three primary branches, and each of those

will have three secondary branches later on. While the number of new inflorescences thus increase rapidly with time, they have fewer and fewer flowers. The flowering of primary/secondary or secondary/tertiary flowering often overlaps. The first few hybrids began their first flowering on the 30th of June, 61 DAP, while it took three weeks until all flowered. Generally, the secondary flowering began roughly two weeks after the primary, and the tertiary about two weeks after the secondary. The *L. angustifolius* began their first flowering one week earlier than the annual hybrids, and the plants were highly synchronized in their development.

Figure 2. A: Plot A6, planted with *L. mutabilis* x *polyphyllus* line #1, on July 4, 65 DAP. **B:** Plot A5, planted with *L. angustifolius* 'Boragine', on June 4, 35 DAP. **C:** Plot A4, planted with *L. mutabilis* x *polyphyllus* line #3, on June 4, 35 DAP

Table 5. A schematic overview of the development of the plots designated for description over time, with approximate dates for the entering into new developmental stages. A4 and B3 are self-pollinated annual hybrids of line #3, A5 and B5 are *L. angustifolius* 'Boragine', and A6 and B6 are self-pollinated annual hybrids of line #1. S = seeding. EG = elongated growth. PF = primary flowering. SF = secondary flowering. TF = tertiary flowering. FH = *Full height*. PM = Primary maturation. SM = Secondary maturation. M = maturation (used in the case of *L. angustifolius*, since they were harvested whole).

	30/4	24/6	30/6	4/7	11/7	16/7	25/7	1/8	8/8	16/8	30/8	8/9	15/9	22/9	28/9
DAP	0	55	61	65	72	77	86	93	100	108	122	131	138	145	151
A4 #3	S	EG		PF		SF	FH	TF				PM		SM	
B3 #3	S	EG			PF		SF		FH	TF	PM				SM
A5 *L. a*	S	PF	SF	TF		FH							M		
B5 *L. a*	S		PF	SF	TF				FH					M	
A6 #1	S	EG	PF			SF	FH	TF			PM		SM		
B6 #1	S	EG		PF		SF		FH	TF		PM			SM	

As visualized in Table 5, the plants in site A were generally one week ahead in development as compared to the plants in site B. The annual hybrids of line #1 was slightly faster to flower than those of line #3. However, due to the problems posed by a fungal disease, the possibilities to get accurate dates of the harvest were negatively affected. As mentioned, the *L. angustifolius* 'Boragine' were comparatively fast to flower, while maturation was affected similarly to the annual hybrids.

Pests and diseases
The first pest damage observed was loss of seedlings due to larvae, which would be expected since the land had been unplowed for several decades. Click beetle larvae, *Agriotes* sp., are known to be a problem the first years after plowing grassland (Nilsson, 1995), and may be the damage-causing species in this case.

On the 17th of June, virtually all of the *L. angustifolius* plants experienced browsing damage from hares to some degree. While no plants died, their height and photosynthetic capacity was obviously reduced. The plants bounced back rapidly, but likely became lower and more branched as a result. Interestingly, only the *L. angustifolius* plants were browsed, while only one or two hybrids were nibbled. No other plants close by, including peas, lettuce and root vegetables received damages.

The lupine aphid, *Macrosiphum albifrons*, was found on some of the hybrid plants from 6th of July until maturation (Figure 3 A). Occurrence appeared sporadic, with a few plants per plot

infested. Plants with early infestation received heavy damages and suffered premature death. This was possibly partly exacerbated by viruses transmitted by the aphids, although no confirmed documentation of viruses was made. Aphids infected by parasitoid wasps were also seen on the lupin plants as shown in Figure 3 A. The widespread occurrence of naturalized *L. polyphyllus* likely served as a source of the aphids, which could attack the cultivated plants. That the lupins and the aphids were established in the area also resulted in the presence of the parasitoid wasps that might help curb further damages to the crops. Aphids were not found on the *L. angustifolius* plants.

The by far most damaging organism for the lupins during the experiment was a fungal disease, likely anthracnose, *Colletotrichum lupini*, (Kurlovich, personal communication, 2021) whose first visible symptoms were gray-brown lesions on the stem (Figure 3 B). These infections were seen from the 7th of August and onwards. The lesions could occur anywhere on the plant stems, including the inflorescense, and cut off transportation in through the plant, killing it partly or completely. The infected stems can rot completely later in the season, and easily break during heavier rains. Seeds were also spoiled due to the fungi, and likely also by secondary infections that could thrive off the weakened plant parts. Since the pathogen cut off transportation in the stems, the supply of energy and nutrients to the forming pods and seeds were also negatively affected, resulting in smaller and more shrivelled seeds even when the seeds per se were uninfected. While the stems of *L. angustifolius* were not visibly affected, the seeds of this species were affected in the same way. The occurrence of this pathogen was significantly more widespread in site A, which is wetter since it is closer to the ground water table.

Figure 3. A: Occurrence of lupine aphid, *Macrosiphum albifrons,* feeding on the pods of an annual *L. polyphyllus* x *mutabilis* hybrid. Among these are aphids infected by parasitoid wasps, these aphids being beige in color. **B:** Infection of the fungal disease anthracnose on the lower stem of an annual *L. polyphyllus* x *mutabilis* hybrid.

Maturation and harvest

The first mature spikes from the annual hybrids were harvested on the 30th of August, 122 DAP, and were all resulting from the primary flowering. Generally, two weeks after the first harvest of primary pod spikes, the first of the secondary pod spikes were harvested. An insignificant number of tertiary pod spikes matured and were therefore excluded from further analysis. Harvest of annual hybrids were highly spread out much due to the continuous flowering, continuing until the hard frost of the 12th of October, 165 DAP. The maturation of the *L. angustifolius* 'Boragine' was more even, due to their more constrained flowering period, but in some cases still failed to mature completely before the frost. A problem in both species was the occurrence of pod dehiscence, which was found in a few plants in some plots. It was found to be most common in plot A5 and A9.

Table 6. The total seed number, size, harvest from the plant of the different plots at the two sites A and B.

Plot	Height (cm)	Plants harvested	No. seeds	Thousand kernel weight (g)	Seed harvest per plot (g)	Seed harvest per plant (g)
A4. *L. polyphyllus* **x** *mutabilis* **#3**	90–110	25	1922	39.5	76	3
B3. *L. polyphyllus* **x** *mutabilis* **#3**	60–90	21	993	40.3	40	1.9
A5. *L. angustifolius* **'Boragine'**	50–70	40	697	146.3	102	2.6
B5. *L. angustifolius* **'Boragine'**	40–55	23	651	172	112	4.9
A6. *L. polyphyllus* **x** *mutabilis* **#1**	80–130	30	1476	53.6	79	2.6
B6. *L. polyphyllus* **x** *mutabilis* **#1**	90–130	31	1688	55.7	94	3

As shown in Table 6, the seeds of the *L. angustifolius* 'Boragine' are roughly three to four times larger than those of the annual hybrids used in the experiment. It should be noted however, that the seed size for all plots, but especially for those in site A were negatively affected by the fungal disease described above. The same was true for the fungal disease's impact on the yield; the seed harvest per plot was highest and most consistent in the *L. angustifolius* 'Boragine', while a higher yield per plant was found in B5. The lowest seed harvest both per plot and per plant was found in B3, while seed size in this plot was the same as in A4.

Table 7. The number of harvested spikes (spikes with only spoiled seeds excluded) from the primary and secondary flowering of the annual hybrids.

Harvest type	No. spikes	No. seeds	Mean harvest per spike (g)	Seed harvest per plot (g)	Thousand kernel weight (g)
A4. *L. polyphyllus* x *mutabilis* **#3** <u>Primary harvest</u>	25	573	1	25	43.6
A4. *L. polyphyllus* x *mutabilis* **#3** <u>Secondary harvest</u>	86	1349	0.59	51	37.8
B3. *L. polyphyllus* x *mutabilis* **#3** <u>Primary harvest</u>	21	488	1.05	22	45.1
B3. *L. polyphyllus* x *mutabilis* **#3** <u>Secondary harvest</u>	26	505	0.69	18	35.6
A6. *L. polyphyllus* x *mutabilis* **#1** <u>Primary harvest</u>	30	838	1.6	48	57.3
A6. *L. polyphyllus* x *mutabilis* **#1** <u>Secondary harvest</u>	68	638	0.46	31	48.6
B6. *L. polyphyllus* x *mutabilis* **#1** <u>Primary harvest</u>	31	903	1.94	60	66.4
B6. *L. polyphyllus* x *mutabilis* **#1** <u>Secondary harvest</u>	50	785	0.68	34	43.3

Table 7 shows the differences between the primary and secondary pod sets of the annual lupin plants, while *L. angustifolius* was excluded from the comparison due to its aforementioned flowering pattern of less distinctive flushes of flowering. The seed harvest per spike was overall lower in the secondary harvest than in the primary harvest, on average 47.2 % lower. However, the difference was higher in the line #1 annual hybrids of plots A6 and B6 (61.7 % to 64,5 % lower respectively) than in the line #3 annual hybrids of plots A4 and B3 (37 % to 25.4 % lower respectively). No clear difference in yield per plot for primary and secondary harvest could be made, as plot A4 clearly stood out from the rest with regards to the trend that the primary harvest was slightly larger than the secondary. This may be explained by higher lateral branching and therefore higher number of secondary flower spikes in A4 stimulated by the fact that the early germination and survivability of the seedling in A4 was low in comparison with its counterpart A6 (52 % compared to 68 %), coupled with the smaller and bushier plant size of the line #3 compared to line #1 as described above. For all plots, the data shows that the seeds produced from the primary

flowering were larger than those produced by the secondary flowering, on average 28 % larger, ranging from 15.3 % for A4 to 53.3 % for B6.

The number of seeds per spike differed significantly between some of the harvest types, see table 8. In three of four analyzed plots, the secondary harvest had a significantly lower number of seeds per spike which was most evident in site A, while B3 showed no statistically significant difference between the first and second harvests (see figure 4). The difference between the two sites may again be explained by the increased prevalence of the damaging fungal disease anthracnose, which decreased seed yields massively. The comparison between primary and secondary harvests were conducted in order to determine whether the secondary harvest was of a significant size, which has implications on at what time the crop should be harvested for maximal seed yield. This in turn has implications for the geographical range where it the crop can successfully be cultivated.

Table 8. Grouping of statistically significant different yields of seeds of the primary and secondary harvests of the annual *L. polyphyllus* x *mutabilis* hybrids using the Tukey method and 95 % confidence. Mean value refers to the seed number per spike.

Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean	Grouping		
B6 Primary harvest	31	29,13	A		
A6 Primary harvest	30	27,93	A		
B3 Primary harvest	21	23,24	A	B	
A4 Primary harvest	25	22,92	A	B	
B3 Secondary harvest	25	20,20	A	B	C
B6 Secondary harvest	50	15,70		B	C
A4 Secondary harvest	86	15,69		B	C
A6 Secondary harvest	68	9,38			C

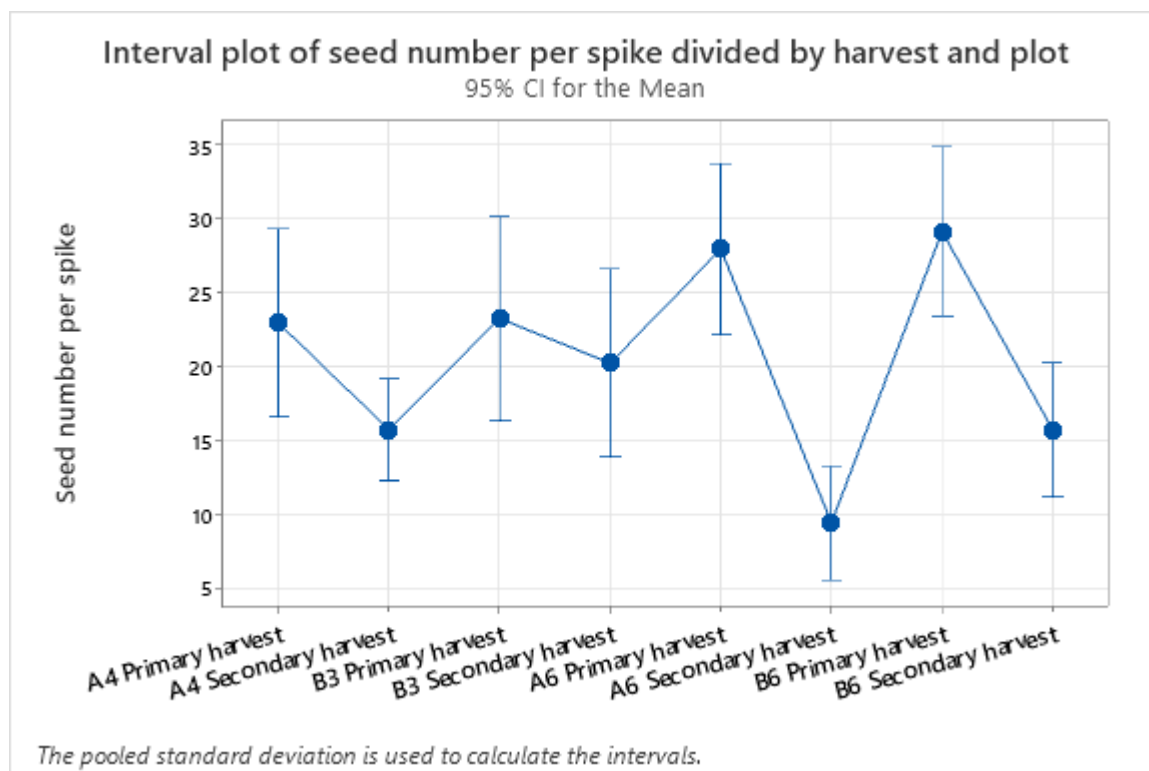*Means that do not share a letter are significantly different.*

Figure 4. Interval plot displaying the mean and interval values of seed number per spike for the plots of annual *L. polyphyllus* x *mutabilis* divided by primary and secondary harvests.

3.2.2 Report on the seeds resulting from hybridization attempts in 2020

Out of the 18 seeds received from the hybridization attempts of 2020, only three germinated. Of these, two were from annual *L. polyphyllus* x *mutabilis* hybrids mothers, while the third was from a perennial *L. polyphyllus* mother plant. Although an even smaller sample size, the germination rate of the crosses with annual mother plants had a much lower germination rate (12.5 %) than that of the annual hybrids (71 %), see table 9. While still regarded as a failure, this may indicate that some of the seeds initially were a success in that they were true hybrids, but failed to germinate due to genetic errors. However, the low germination rates may also be due to other factors such as insufficient scarification, seed dormancy or damages to the seed during development or scarification.

The two seedlings of the 2020 crosses with an annual *L. polyphyllus* x *mutabilis* hybrids mother plant followed the identical development as the annual hybrids, both with regards to time and physical traits. As no differences could be discerned, they were there regarded not to be successful crosses.

The seedling from the 2020 crosses with a perennial *L. polyphyllus* mother plant initially stood out, as it emerged slower than the seedlings of the low alkaloid *L. polyphyllus* that were sown for comparison. However, the seed that the plant emerged from was smaller than usual, and after some weeks of slower growth, the seedling picked up pace with the other *L. polyphyllus* seedlings and there were no physical differences between them. In conclusion, this seedling was not the consequence of a successful cross.

As shown in table 9, the *L. angustifolius* has a faster and more even germination compared to the annual *L. mutabilis* x *polyphyllus* hybrids, of which three outliers germinated after roughly a month as compared to the first half which germinated within 9 days. The low alkaloid *L. polyphyllus*, although germinating evenly, had a low germination percentage, which might be caused by older seeds or improper scarification of the seed coat.

Table 9. The germination rate in percentage (in brackets are seeds germinated and seeds sown), and mean and median germination time of the seeds sown in trays indoors of the different species, stable hybrids and crosses of 2020. The sample size of the germinated crosses of 2020 was too small for calculation of mean and median germination time.

	L. angustifolius 'Boragine'	*L. mutabilis* x *polyphyllus* line #3	Low alkaloid *L. polyphyllus*	Crosses of 2020: Annual mother	Crosses of 2020: Perennial mother
Germination rate	91.7 % (22/24)	71 % (17/24)	26.4 % (19/72)	12.5 % (2/16)	1/2
Mean germ. time (days)	6.1	12.8	7.8	-	-
Median germ. time (days)	6	9	8	-	-

3.2.3 The conducting of interspecific hybridization between *L. polyphyllus* and *L. mutabilis* x *polyphyllus*

The majority of the emasculated flowers fell off within a few days, while some fell off after having begun forming a pod. A high early loss of the flowers used for hybridization attempts was to be expected as many flowers are naturally culled also on the plants used for the descriptive part of the study. However, even more flowers were abscised early, likely due to the stress inflicted upon the flower during the emasculation process. This was to be expected as Kazimierski (1961) reports that the flowers of *L. polyphyllus* and *L. mutabilis* are highly vulnerable to mechanical injuries and often fall off, more so than *L. albus* and *L. luteus.*

The remaining pods continued developing seemingly normal (Figure 5 A) until around the time when maturation of the seeds was expected. However, they remained empty or containing only shriveled or ballooned seed coats (Figure 5 B), their embryos likely having been aborted at a later stage. Unfortunately, no viable seeds were collected from the attempts in 2021.
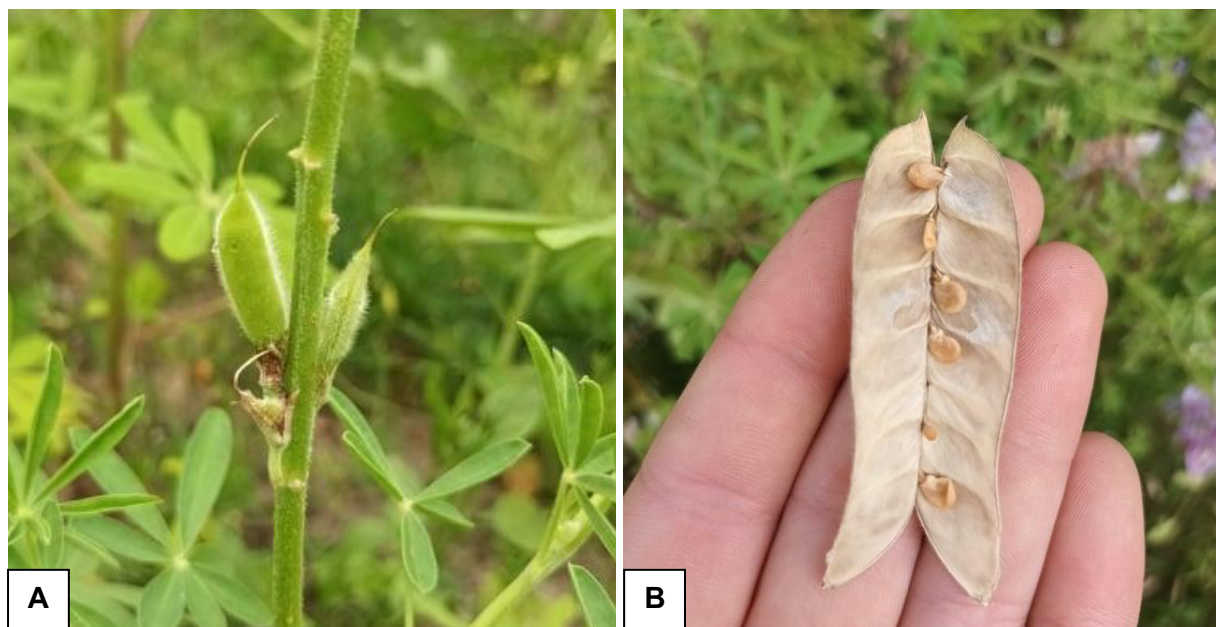
Figure 5. A: Pods forming from flowers of annual *L. polyphyllus* x *mutabilis* hybrids that were part of the hybridization attempts. **B:** A representative sample of the pods resulting from the attempts to cross annual *L. polyphyllus* x *mutabilis* hybrids with wild local *L. polyphyllus*, showing shrivelled and empty ballooned seeds in a mature pod.

4. Discussion

4.1 Implications from literature review

In reviewing the potential of *L. polyphyllus* for breeding, two factors which required the most consideration due to their complexity and significant implications for any breeding program on *L. polyphyllus* or related species and hybrids thereof, were the alkaloid content, and the invasiveness of lupin species. These topics will be discussed further, but first, the fundamental issues of the approach of targeting a perennial crop rather than the already domesticated and broadly cultivated annual *Lupinus* species, are considered and discussed.

4.1.1 Trade-off? Adaptability, yield and perenniality

In the case of the novel perennial crop intermediate wheatgrass 'Kernza' and its annual counterparts in wheat, *Triticum* sp., the tradeoff between high yield and perennial growth form is made clear. Indeed, the stark differences between potential novel perennial crop species and annual crops having undergone domestication and breeding for several thousands of years may cause reason to suggest a universally applicable truth on the superiority of annual crops with regards to production potential, while perennial crops may hold attractive features with regards to resource utilization and soil health. However, as this may hold true in theory, the world is far more complex: Results may differ in different parts of the world, where different growth factors, social aspects and political conditions shape the opportunities and restrictions against different farming systems.

With focus on plant breeding for conditions in northern Europe, where a short and relatively cold season sets other conditions than those commonly explored with regard to 'Kernza' and annual wheat. As recognized by Sengbusch (1938), *L. polyphyllus* is the species capable of being cultivated furthest north, further than the annual species *L. angustifolius* which is hardier than *L. luteus*, which in turn is hardier than *L. albus.* In northern Europe, it is thus not possible to directly compare the yields of perennial lupin forms with the major annual lupin species, due to their unreliable production in the short season with high risks of damaging frosts. A perennial grain legume crop for northern climates must be viewed in other lights, and instead compared with e.g., ley crops and imported grain legumes.

Nevertheless, the reported gain yields of 1000–1600 kg/ha in Finland for low alkaloid forms of *L. polyphyllus* coupled with production of high protein pasture or silage may stand on its own against e.g., *L. angustifolius*, if additional breeding efforts are conducted. Indeed, in theory the breeding of perennial crops may follow an opposite direction of spread than that of the annual grains and grain legumes emerging from the middle east and Mediterranean regions: *L. polyphyllus* may gain its foothold in northern Europe, gaining attention enough to develop more and more, eventually being a viable option further south as the crop progresses and being able to compete with its annual counterparts.

4.1.2 Trade-off? Edibility and resistance

A key question faced in the process of breeding lupins for food and feed is how to manage the presence or absence of quinolizidine alkaloids in the seeds and vegetative parts of the plants. The domestication tactic used for all main lupin species has been to develop sweet forms, although bitter forms have been used traditionally. Both methods have their pros and cons, which holds true to the present day.

Sweet forms enable easier use of the harvest and avoids losses linked to the debittering process of bitter forms. Importantly for *L. polyphyllus*, low alkaloid content enables a broader use of vegetative parts of the plants for feed. This certainly connects with the dual-purpose model of perennial crops, which is expected to favor their broader use. The decreased natural protection of the lupin crop that is coupled with low alkaloid content poses new and important problems with pests and diseases. This calls for a range of pest management tactics, which if not due to other reasons than because of the insect-vectored cross-pollinated habit of *L. polyphyllus,* need to take insect populations into account. Grazing by large herbivores can cause large damages, not least in northern Europe which typically holds high wildlife populations, which also demand management efforts.

Conversely, the bitter forms need processing of the grain before use, which in modern technical processing plants separate the grains into different fractions, more suitable for industrial uses and food processing industries than for direct human consumption. Vegetative parts may have minor uses as post-grain harvest pasture, but can otherwise be used as feedstock in bioreactors. The plant protective qualities of the bitter forms may allow for a more extensive form of cultivation with less effort demanded for pest management strategies. Additionally, alkaloids separated during grain processing can be used as biological pesticides, perhaps in cultivation of sweet lupins.

Overall, sweet forms of *L. polyphyllus* can have an important role in an integrated livestock and grain agroecosystem, producing readily used high protein grains, while also producing feed for silage or grazing and providing soil enhancing functions through deep roots and efficient nitrogen fixation. Meanwhile, high-alkaloid form of *L. polyphyllus* may be more suitable as an industrial crop, demanding less care due to its natural protection which may enable it to be cultivated at larger scale (which does not necessarily translate to larger area), while producing for industrial processing facilities such as protein factories and bioenergy plants.

What may appear as the golden middle way, a plant with natural protection in its vegetative parts while containing low levels of alkaloids in the grain, naturally also has both pros and cons. There are clear advantages with offering higher protection against pests and diseases, while also producing readily used grain. However, high alkaloid content in vegetative parts again restricts use as animal feed. Additionally, the challenges in retaining genetic integrity (let alone achieve such forms of lupins in the first place) may prove too large to handle in practice, considering the difficulties posed keeping pure stands of the standard sweet forms of lupins.

4.1.3 Invasiveness of lupins

No doubt, *L. polyphyllus* exhibits strong invasive habits, spreading and thriving throughout much of Europe, as well as in other continents outside its native range of western North America. Neither is it the only lupin species that is classified as invasive or as a weed. For example, the closely related perennial species *L. nootkatensis* is widely spread in Iceland, while the annual crop species *L. cosentinii* native to the Mediterranean area is an environmental and agricultural weed in Western Australia (Weeds of Australia, 2016). The latter example also demonstrates that not only wild type lupins, but also domesticated species can become invasive. Though outside the scope of this report, the key to deal with invasive problems of the species may be in its utilization; grazing is prescribed as a way to control it. Here, increased understanding of *L. polyphyllus* that comes with its development as a crop may be useful in its management as an invasive species.

Since its spread is attributed to the use of the species in soil reclamation and for agricultural/forestry purposes as above its use for its ornamental value, further development and use of the species must take risks of further spread into consideration. Introduction of new vigorous lines into the gene pool of existing populations of naturalized *L. polyphyllus* may exacerbate existing problems. The high alkaloid content of wild type lupins is an important factor for making it a troublesome species and sweet cultivars are therefore preferable, as they themselves are less likely to become invasive. Furthermore, constant introduction of alleles for sweet forms, albeit a recessive trait, may theoretically affect the resistance in nearby naturalized populations through cross pollination. However, it is obvious that the opposite scenario is more likely which instead poses a problem; the introduction of genes for high alkaloid content in sweet cultivated lupins through cross pollination with local naturalized lupins.

Problems with cross pollination with bitter wild type lupins are especially important in setting up seed production, as well as in the vicinity of breeding stations. This points again to the importance of dealing with the problem of invasive lupins, preferably through its utilization as

sheep forage which may help foster the realization of the values and potentials of *L. polyphyllus* as a crop. However, extermination of wild type lupins in the vicinity of sweet *L. polyphyllus* cultivation for food and feed production should, while beneficial due to the decreased risks of introduction of self-seeded bitter lupins, not be necessary. Further, the on-farm production of seed should have a decreased importance as compared with that of annual counterparts, considering the decreased need for seed because of the perennial life cycle. Additionally, since the great adaptability of lupins to different growth conditions is attributed to phenotypic plasticity rather than genetic adaptation, the need for a wide variety of local varieties may be rather small.

4.2 The experiments

Literature cites interspecific hybridization of the pure species of *L. polyphyllus* and *L. mutabilis* as not being difficult (Kurlovich, Stoddard & Earnshaw, 2008; Baer, 2002), although successes vary from year to year depending on, e.g., the weather (Kurlovich, personal communication 2020).

The attempts in 2020 and 2021 to conduct crosses between the stable annual *L. mutabilis* x *polyphyllus* hybrids and local naturalized *L. polyphyllus* as well as low alkaloid *L. polyphyllus* failed to produce hybrid seeds. Considering the result of these attempts, and the literature on the hybridization of the pure species of *L. polyphyllus*, *L. mutabilis* as well as *L. nootkatensis* which describe the process as rather easy, this indicates that it is extremely difficult to conduct crosses between these lines of annual *L. mutabilis* x *polyphyllus* hybrids and *L. polyphyllus* (Boguslaw, personal communication 2021).

Obstacles for achieving viable crosses between the annual *L. mutabilis* x *polyphyllus* hybrids and local *L. polyphyllus* may be genetic. Wild and naturalized *L. polyphyllus* with different ploidy levels also exist (Ramula, 2015), which is a factor that remained unaccounted for in the experiment. It has been reported that successful crosses are more likely to be conducted if *L. polyphyllus* is used as the mother plant (Clements et al., 2005), which may be due to the importance of mitochondrial DNA. Despite this, the annual *L. mutabilis* x *polyphyllus* hybrids were consistently used as mother plants, due to the difficulties in otherwise timing the flowering to gain a useful number of flowers and pollen.

Other than genetic causes for the failure to obtain hybrid seeds, the success of hybridization can also be strongly affected by weather conditions. No doubt, the flowers were also highly vulnerable to damages during the emasculation process. The quality of the pollen sources may also be a factor at play in the failure to obtain successful crosses.

Possible solutions to the obstacles faced in achieving perennial hybrids of *L. polyphyllus* and *L. mutabilis* may include using *L. polyphyllus* as mother plant, by harvesting and storing pollen from *L. mutabilis* in a freezer in the season prior to new experiments. Use of pure lines of *L. mutabilis* as pollen parent instead of annual hybrids may result in higher success rates. Additionally, this may also prove more advantageous to the breeding efforts since desired genes found in *L. mutabilis,* such as seed size, may have disappeared from the annual lines of *L. mutabilis* x *polyphyllus* used in the experiment over the course of their stabilization. The production of the plots of *L. mutabilis* x *polyphyllus* during the experiment

ranged from 40 – 94 g per plot, each with 1.875 m^2. This constitutes a yield of 21.3 – 50.1 g/m^2 as compared to yields of 100 – 270 g/m^2 reported in studies on the seed yield of *L. polyphyllus*. While pathogens, notably anthracnose, significantly affected the vitality and performance of the used for hybridization and description in the experiment, this puts into question the logic of using these annual *L. mutabilis* x *polyphyllus* hybrids as parent material for improved lines of perennial *Lupinus* crops.

Consistent with the foundings of Wink (1990), all sweet lupins were grazed by hares, while bitter plants were left unharmed. This, as well as the other reported pathogens and insects, were practical demonstrations of the trade-offs between sweet and bitter forms of lupin crops.

5. Conclusion

Breeding of perennial lupin crops based on *L. polyphyllus, L. nootkatensis*, *L. mutabilis* and hybrids thereof, has been conducted for roughly 50 years, albeit at low intensity and consisting of a few different efforts rather than one coherent program. While a long-term coherent program like the one at the Land Institute in Kansas focusing on intermediate wheatgrass 'Kernza' may have been beneficial, the different programs conducted throughout the years and especially with the resources of the Vavilov Institute of Plant Industry, may provide a range of valuable elite material for further progress.

While the potential of introducing crucial traits for domestication in *L. polyphyllus* through hybridization with the annual crop species *L. mutabilis* in theory and at the onset also in practice may be highly promising, the fruits of previous interspecific hybridization between the two species has instead been improved annual lines. Here, it has rather been valuable traits found in *L. polyphyllus* such as frost tolerance and faster maturity that have been introduced in *L. mutabilis*. The difficulties in continuing the efforts of obtaining genetically stable improved perennial hybrids have been further strengthened in the experiments of this study, which demonstrate the obstacles found in later stages of interspecific hybridization, where genetic incompatibility may prove an important factor.

The review of the potential of *L. polyphyllus* through the criteria for candidate species for breeding of perennial grain legumes set up by Schlautman et al. (2018) affirms the importance of previous and current breeding efforts focused on the species, and calls for a strengthening and development of these. The species, which produced high protein grain at impressive quantities while offering dual-purpose use as pasture or silage, displaying great adaptability to different growing conditions, and of which there are significant available genetic resources, may obtain an important and interesting role in future food production if further breeding efforts are conducted. Nevertheless, the issues of its proven invasiveness, and the problems posed by either retaining the purity of sweet forms and the associated pest problems, or the need for further processing and debittering prior to use, need to be accounted for.

While bitter forms of *L. polyphyllus* may be suitable for industrial use in food processing industry, chemical industry and bioenergy plants, sweet forms are more suitable for the basic premises of the study in addressing social needs and environmental problems: It is more

suitable for small- and medium scale farms in the northern regions in the forest areas which have the potential to produce large amounts of food if the agricultural and economic policies allow it. In an agroecological farm system, sweet forms of *L. polyphyllus* may produce high protein grain used for direct human consumption or as feed supplements for e.g., poultry, while offering post grain harvest pasture or silage for livestock. Clearly, these crops are in need of further development and evaluation, and be made available to farmers.

Additionally, the viability of farmers to produce protein rich grain in competition with, e.g., imported soy, needs to be examined and take into consideration the current and future policies, such as multi- and bilateral free trade agreements, the internal market of the EU and the agreements of the World Trade Organization. Clearly, in a market overflowed with imported grain legumes sold at below the cost of production due to low tariff quotas and a globalized food trade system like the contemporary one, little incentive exists to breed or produce grain legumes domestically.

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Popular scientific abstract

With regards to current threats posed to society by climate change and lack of food sovereignty in Sweden and globally, the prospects of breeding perennial lupins for food and feed production were examined. This study consisted of a literature study focused on *Lupinus polyphyllus*, related species and hybrids thereof, and an experimental part on interspecific hybridization between *L. polyphyllus* and *L. mutabilis*.

Two lines of annual hybrids between *L. polyphyllus* and *L. mutabilis* developed by plant breeder B.S. Kurlovich were described and compared with *L. angustifolius* 'Boragine'. Attempted crosses between three lines of annual hybrids and naturalized *L. polyphyllus* conducted in 2020 and 2021 were described. However, no viable hybrid seeds from the crosses were obtained, proving that back-crossing these annual hybrids with *L. polyphyllus* being extremely difficult.

Previous and current breeding efforts regarding *L. polyphyllus* were studied, and the potential of *L. polyphyllus* as a perennial grain legume crop were evaluated according to selection criteria for candidate species. Impressive performance, valuable genetic material and important breeding work conducted on *L. polyphyllus* were highlighted as factors favoring a continued development and study of the species. The species habit of spreading and becoming classified as invasive, as well as the content of toxic quinolizidine alkaloids are two issues of importance in breeding and cultivation. Low alkaloid *L. polyphyllus* has the potential to emerge as a crop in the northern regions due to its reliable production, the nutritional qualities and its dual-purpose use as both grain and pasture/silage crop.

Populärvetenskaplig sammanfattning

Fleråriga lupiner, liknande de som växer förvildade i bland annat vägrenar, kan ha potential som odlad gröda för matproduktion i Sverige. Genom växtförädling av blomsterlupin *Lupinus polyphyllus* L., närbesläktade arter eller korsningar mellan olika lupin-arter finns det en möjlighet att ta fram en ny flerårig gröda som är anpassad till svenska förhållanden och som ger proteinrika frön som går att äta. Fleråriga lupiner som gröda skulle kunna vara av nytta för att öka matproduktionen i landet, genom att passa på marginalmarker som idag används i allt lägre grad, samtidigt som odling av fleråriga växter kräver mindre markbearbetning – och därmed bränsle – än ettåriga grödor.

Då blomsterlupin använts i växtförädling i drygt 50 år, främst i Östeuropa, fanns resultatet av tidigare och pågående försök att dra slutsatser kring. Det finns olika potentiella strategier för växtförädling av fleråriga lupiner att utforska, exempelvis gällande hur giftiga alkaloider som vanligtvis finns i oförädlade lupiners fröer ska hanteras: Det finns sorter med mycket låga halter som går att äta utan att fröerna behandlas, men då alkaloiderna är till för att skydda plantan är dessa mer benägna att angripas av skadedjur. En annan strategi är att förädla fram produktiva och lättodlade sorter av lupiner med högt innehåll av alkaloid, där fröna sedan lakas på det giftiga ämnet innan de används. En möjlig nackdel med denna strategi är att grönmassan då inte är lämpat som djurfoder, något som lupiner med lågt alkaloidinnehåll kan vara användbart som.

Att blomsterlupin är klassade som invasiv art i Sverige och flera andra länder är en annan relevant aspekt för fleråriga lupiners potential som åkergröda. Risken att potentiella nya lupinsorter förvärrar existerande problem med blomsterlupiner som invasiv art behöver hanteras, och den risken är mindre med lupiner med lågt alkaloidinnehåll, då dessa lättare angrips av bland annat sniglar, insekter och betande djur. Förekomsten av förvildade lupiner kan också innebära svårigheter för odling av blomsterlupiner med lågt alkaloidinnehåll, då generna för produktion av alkaloider kan korsas in i de odlade populationer. Detta gör det svårare att ta utsäde av lupiner med lågt alkaloidinnehåll, och innebär en risk att lupiner med högt alkaloidinnehåll kan etablera sig i fält.

I arbetet ingår ett fältexperiment där korsningsförsök med flerårig blomsterlupin och ettåriga lupinhybrider, korsningar mellan blomsterlupin och den ettåriga lupinarter tarwi (*Lupinus mutabilis* Sweet.), framtagna i Finland av växtförädlaren Boguslaw Kurlovich genomfördes 2020 och 2021. Att korsa blomsterlupin med tarwi, som i runt 3000 år odlats i Sydamerika, kan teoretiskt sätt vara ett sätt att introducera önskvärda egenskaper som större frön. Försöken resulterade dock inte i några lyckade korsningar, vilket bland annat kan ha att göra med genetiska hinder mellan plantorna. Ett sätt att nå bättre resultat kan vara om de fleråriga blomsterlupinerna används som moderplantor istället för, som i experimentet, pollendonatorer.

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