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D 1.10. Agronomic interventions (best management practices) for different European conditions, for each species defined

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Dissemination Level		
PU	Public	X
PP	Restricted to other programme participants (including the Commission Services)	
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0. Summary

The activities on best agronomic practices that aim to increase the sustainability of production of the different protein crops investigated in the project, are reported in this report and decribed by country and by crop.

In Italy, a 3-year (2016-2017-2018) field trial of sowing date and a 2-year (2015, 2018) field trial of sowing density were carried out to evaluate the effect on yield parameter and growth of different quinoa varieties under rainfed conditions. The results showed that the early spring sowing date and sowing density of 100 000 plant ha^{-1} were the most productive agronomic interventions for quinoa.

Also a 2-year (2017-2018) field trial was conducted in Vitulazio (CE), Italy, to evaluate the effect of rotation of winter fava bean with quinoa and amaranth.The rotation of fava/quinoa and amaranth did not however have a positive impact on seed yields. During three growing seasons, abiotic stress trials were conducted on both quinoa and amaranth. The deficit irrigation and water quality (abiotic stress) treatments had no significant effect on yield and yield component for quinoa. The water irrigation level only had an effect on the vegetative development of both crops. The N fertilization trial on amaranth and buckwheat was also carried out and is still on going. Winter and spring sowing dates were also evaluated for three fava bean and three lupin varieties, cultivated under rainfed conditions in two types of soils. Winter and spring sowing dates had no effect on fava bean and lupin when grown in clay soil, whereas, in sandy soil, winter sowing date was noted as the best treatments for fava bean.

For the European Nordic region, the experiments addressing agronomic interventions in protein crops were: sowing date trial; trial with two type of soils and different commercial cultivars of three species; an intercrop trial on lentils and oats; two sowing date trials with 11 accessions of winter faba beans; leaf harvest trial under controlled/semi-controlled and field conditions on six varieties of Amaranth, and lastly a quinoa fertilization x sowing density trial under organic production. For the intercropping combination trial, reported benefits were weed reduction and oats providing a support for lentil harvest. The trial with two type of soils showed that yields of lupin were approximately double on clay soil compared to sandy soil. The grain size and yield were significantly higher in clay than sandy soil in three tested cvs of faba bean, whereas in one of the materials tested, the protein content was slightly lower in the clay soil trial. No significant differences were found in yields of quinoa grown on sandy and clay soils. The sowing date trial showed that lentils produce higher yields when sown earlier. Generally there was no change in yields of lupins sown in May compared to April, whereas faba beans produced higher yields when sown early. Under the Danish climate conditions, sowing of buckwheat is recommended in May rather than April. The results for quinoa showed that the best sowing date depends on the cultivar. On the contrary, no significant differences in yield was recorded for amaranth when grown at three different sowing dates. The trial testing three quinoa cultivars, at two fertilizer levels and two plant densities, has shown that there were genotypic differences in response to fertilization and plant density.

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The same experimental trials carried out in northern and southern Europe. In the Netherlands, crop protection (fungicides and insecticides) trials were carried out on white and blue lupin, spring and winter faba bean, as well as soya bean in several locations and many years. Statistical analysis of the yields was performed separately for each year and location. The application of fungicides in lupin was tested over three years. In 2015, fungicide treatment resulted in a significant interaction between varieties and the use of crop protection. One white lupin variety (Amiga) had a significantly higher yield when fungicides were applied; the other varieties showed no significant effects. In 2016, there was no significant difference in yield for lupin with the trials applied with and without crop protection. In 2017, on the other hand, the yields were significantly different. The average yield of spring faba bean with crop protection (fungicides, and in one experiment both fungicides and insecticides) was significantly higher than in trials without crop protection. This was the case in 2 out of 6 experimental trials. With winter faba bean, average yields were significantly higher with two trials when crop protection was used. In the third trial, the average yield was higher without crop protection, but this was caused by poor winter survival of herbicide-treated plants. One trial was carried out with crop protection in soya bean, which did not result in significant yield differences.

All the results gained in this study can be used by farmers in decision-making process regarding decisions on using protein crops in their cropping rotation, decisions on intercropping, sowing date, stress tolerance, as well as how to enhance quality of plant proteins for the market and food processing industry. Due to sake of clarity, the work reported in this deliverable is divided by country, ie. beneficiary, consisting of Italy (CNR-ISAFoM), Denmark (UCPH- PLEN) and Netherlands (Luis Bolk).

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1 Introduction and objectives

To meet the increasing global demand for high quality, protein-rich food produced in a sustainable way, improving agronomic practices could help reducing the existing yield gaps. Cultural practices refer to all the operations carried out in farms, right from the beginning of the farming season to the end. Every crop has a yield potential that can be expressed in a sustainable way, throughout adoption of specific crop practices.

The aim of this deliverable is to describe the best agricultural practices in aim to introduce varieties of protein crops to different European environments. The aim is to optimize the yields while reducing the extensive use of natural resources.

2 Activities for solving the task(s)

Several field trials were carried out by each WP1 partner to evaluate specific agronomic practices that could help to increase seeds yield of specific protein crops under different european pedoclimatic conditions (Denmark, Italy, Netherland).

2.1 Italy (CNR-IAFOM)

2.1.1 Crops and agronomic practices

We focused our attentions on newly introduced crops in Italy, in particular on high qualitative protein crops like quinoa (*Chenopodium quinoa Willd*.), amaranth (*Amarantus* spp) and buckwheat (*Fagopyrum aesculentum* Moench), as well as high quantitative protein crops of white lupin (*Lupinus albus L*) blue lupin (*Lupinus angustifolius L*) and fava bean (*Vicia faba L.*). We evaluated the best sowing date and sowing densities that represent the main agronomic interventions in evaluating the adaptability of a crop in a new environment. We also evaluated the best irrigation strategies for crops tolerance to abiotic stresses (quinoa and amaranth), crop rotation and N fertilization.

Quinoa: Is a herbaceous plant belonging to Chenopodiaceae family, like sugar beets, spinach and ribs. Originally, from South America, particularly the Andean highlands, this plant (the Latin name Chenopodium quinoa) is catalyzing the interests of a growing number of farmers in Italy and around the world. Many places in Italy, quinoa is sown in early April, with a wheat seeder, while harvesting takes place in August. Yields therefore vary significantly, from the type of variety used and from the environmental and agronomic factors. Quinoa Real is the most requested variety in the international markets, but in Italy it does not grow well due to the photoperiod. The Titicaca and Vikinga varieties are the most promising in Italy, with the latter having a low saponin content. The evaluation given of Regalona variety, which did not perform well in the plains, was more uncertain, but it provided good results in the hills.

Amaranth: Plant native to Central America, and widespread all over the world, amaranth adapts well to any soil, but prefers soils rich in organic matter, and the warm climate. Amaranth is sown in April in southern Italy, and in May in the north since it fears the cold. Seeding in seedbeds allows cultivation

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to begin one month before. Amaranth is tolerant to abiotic stresses so we decided to evaluate the best irrigation strategies in this study.

Buckwheat: Buckwheat is a very sensitive plant to the cold (minimum temperature + 6 °C); it fears late frosts and early autumn colds, but due to its rapid development the crop manages to profit from the limited warm seasons of the Alpine regions where it is mostly cultivated in Italy. *Fagopyrum esculentum* develops mainly in fresh soils, adapting to acid soils, but it does not tolerate clayey and moist soils. In Northern Europe, buckwheat is often a main crop, while in Italy it is considered an interlayer and is sown after harvesting wheat or rye. In Italy, the cultivation of buckwheat is widespread especially in the north, and in particular in Bolzano and Sondrio. At the moment, the number of hectares cultivated with buckwheat plants in Italy is not yet registered by the main Italian, European and world institutions. However, it appears that the production in Italy is about 3 thousand quintals for an area of about 300 hectares (Cricca L., 2019).

Lupin: Lupin is a legume and widespread since the ancient times in the Mediterranean Basin and in the Middle East. Lupin is known to have remarkable adaptability to the most ungrateful, acid and lean environments, due to its power to improve fertility of the soil and for its ability to produce grains rich in proteins (over 35%). In Italy, the culture of lupin collapsed following the depopulation of the marginal areas. The Italian regions where lupine is most widespread are Calabria, Lazio, Puglia and Campania.

Fava bean: Fava has been used as a food plant in the Mediterranean and Middle Eastern areas since ancient times. In Italy, the fava bean production surface has fallen below 50.000 ha, located mainly in southern and island regions. In ancient times, throughout the Middle Ages and up to the last century, dried broad beans cooked in various ways constituted the main protein base food of many populations, especially those of southern Italy. In recent times, the consumption of dry seeds has been reduced, while fresh, preserved canned or frozen grains are still used widely. The harvest period is mid-June in southern Italy, the end of June in central Italy, and mid-July in northern Italy with spring sowing. The production of dry seeds, could theoretically exceed 5 Mg ha^{-1}, but in practice it is much lower: 2-3 Mg ha^{-1} are the most frequent average productions in Italy, with a high risks of resulting in even lower yields in certain years due to biotic abiotic factors (cold, drought, rust or aphid attacks, virosis).

2.2 ***Denmark (UCPH)***

2.2.1 Crops and agronomic practices

This section of the report present activities, results and information for D1.10 from the UCPH-PLEN partners, that are applicable for the European Nordic region. Field trials related to this project deliverable are presented in this report alongside a summation of existing research about the various crops studied in the project. Results in this report cover the period from 2015-2018 (with a last replication still ongoing, and not therefore reported in this document). The tested crops were:

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Pea: The biggest pea producers globally are Canada, Russia and China. European pea production represents 37% of global production. World yields average 1990 kg/ha, European yield 2586 kg/ha, and Danish yields 4463 kg/ha of dry peas (FAOStat, 2019). Peas are a high protein crop, which are grown as animal feed, and dried or fresh as food. The seeds contain more protein (18-36%), fibre (6%) and less starch than cereals. They can be grown in crop rotation, where they improve soil nutrition by fixing N.

Lentil: Lentils contain a high level of protein (20-30%) and has been reported as tolerant to high temperatures and drought (SEGES, 2018). There are many varieties available which are attractive for eating, with many sizes and colours. Currently, lentils cultivation is almost non-existent in Denmark. World lentil grain yields average 1179 kg/ha, and in Europe yields average 1076 kg/ha. Canada and India are the biggest world producers of lentils, while Europe produces only 3.6% of lentils global production (FAOStat, 2019).

Lupins: Lupin is a legume crop which can be used for food and feed, with protein levels up to 51%. They can also be used as green manures and they have great ornamental value. Global lupin production is now dominated by Australia, which produces 77% of all lupins, with yields averaging 2004 kg/ha (FAOStat, 2019). Global yields average 1731 kg/ha, while in Europe yields average 1613 kg/ha (FAOStat 2019). The main species used in Europe are *Lupinus albus* (white lupin) and *Lupinus angustifolius* (narrow-leaf lupin).

Fava bean: Faba beans have a historic tradition of legume production and consumption that has been changing with time in the northern Europe. The introduction of chemical fertilizers reduced its production by the end of the 1970s and more recently (2010) farmers started to foresee it as a profitable crop. In Denmark, faba beans had a cultivated area of 6,979 ha in 2015, increasing to 24,878 ha in 2018 (Landbrugsstyrelsen, 2018). In 2018, 39% of this production was organic. Under organic systems, this crop can produce between 1.7 to 2.1 t/ha with the lowest yields in warmer years (Alandia et al, 2019).

Soy bean: Soybeans are mainly produced in South America and China. In Europe, they are produced in Italy, France and Austria (FAO, 2019). The world yield reached 2756 kg/ha and the yield in Europe is reported as 2081 kg/ha (FAO, 2019). Protein content ranges from 30-45%, under Danish conditions it can contain 40-42% (Petersen, 2010). This legume grain is mainly used for feed, but increasingly also for food (dry and fresh seeds, flour, protein extracts). Soybeans are rarely grown in Denmark.

Buckwheat: Buckwheat contains 12-14% high quality protein. Production in Denmark is only 20-50 ha, and global production is mainly located in Russia and China (SEGES, 2018). It has a high amino acid and mineral content (SEGES, 2018). Due to the long flowering time of buckwheat, it is beneficial to bees and favoured in encouraging biodiversity. Buckwheat itself is dependent on bee pollination to ensure good yields (Jacobsen, 2015).

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*Quinoa***:** Quinoa is a high quality protein seed grain which is new to Denmark. Almost all production is in Latin America, mainly Peru and Bolivia. In Europe, the main areas of production are France, Spain, Denmark and the Netherlands. Breeding in Europe had the focus of reducing saponin content, increasing grain size, obtaining coloured seeds (red, black, brown), increasing yield, improving resistance to downy mildew and increasing earliness (Jacobsen, 2017).

Amaranth: Amaranth is a close relative of quinoa, also originating from Latin America. It has a protein content of 12-17%, with a composition similar to that of egg white (SEGES, 2018). There is little data available for amaranth production in Europe. It is cultivated in Central American, African regions and in the US. However, in Denmark and the North of Europe, this crop is not yet produced commercially and it is often seen as a weed rather than a crop.

Chickpea

Chickpeas were tested in the first two years of the project with unsuccessful adaptation to Danish conditions. Therefore, in the remaining years, it was not considered in the rest of the trials, and results are not presented in this report.

The experiments addressing agronomic interventions on protein crops for the European Nordic region are briefly described below:

- **Screening trial:** Over 100 accessions of nine species have been tested (2015-2019), in experimental units of 17 m^2 with three rows per accession and one replication per year. The objective was to test the performance of different genetic materials of protein species during the Danish spring growing season. Crops were grouped and arranged randomly, considering crop and pest management aspects (e.g. legumes were grouped together in order to protect them with electric fences).
- **Sowing date trial:** Eight species were evaluated to study the response to three sowing dates at 15-day intervals (S1, S2, S3) from mid-April to mid-May. The trial was carried out from 2015-2017. In the last year of replication (2017), further accessions of quinoa, amaranth and lupin were tested. Experimental units (same size as in the screening trial) were arranged in a split plot design with sowing date as main factor and three replicates arranged in blocks to reduce plot variation.
- **Genotype x Environment interaction trial**: Relevant results of one year (2018) are included in this report: the trial tested two soil environments (clay and sandy soil) and different commercial cultivars (cvs) of three species: quinoa (3 cvs); faba bean (4 cvs); and lupin (4 cvs). The experimental design corresponded to a split plot design with type of soil as main plot and genotype as sub-plot with four replicates arranged in blocks.
- **Intercropping: lentils and oats** were tested in an intercrop trial (2017-2019). Including three intercropping ratios in a replacement series (66/33; 50/50; 33/66) + two monocrop treatments (100% oats and 100% lentils). The experimental units were distributed in a random block design with four replicates per treatment.
- **Winter faba beans trials**: Winter faba beans have been tested from 2015-2018 in a sowing date trial with 11 accessions (cvs and breeding lines) and two sowing dates (winter and spring) distributed in a random block design with four replicates.

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- **Amaranth leaf harvest trial**: under controlled, semi-controlled and field conditions, we tested the effects of leaf harvests at various intensities and frequencies (0, 25, 50, 75 and 100% defoliation; 1, 2 and 3 times) on six varieties of Amaranth (2016-2017). Details of experimental designs are described in Hoidal et al. (2019).
- **Quinoa fertilization x sowing density trial under organic production**: we tested three cultivars of quinoa (Titicaca, Puno, Vikinga); two sowing densities (5 kg/ha; 10 kg/ha); and four nitrogen (N) organic fertilization levels (0; 50; 100; and 150 kg N/ha). These fixed factors were arranged in a split-split plot design with genotype as main plot, sowing density as subplot and fertilization rate as sub-subplot with four replicates.

2.3 Netherlands (Luis Bolk)

2.3.1 Crops and agronomic practices

Lupin

Crop protection in white and blue lupin

In the Netherlands, crop protection measures with fungicides were tested in white and blue lupin in several locations and years. The main disease problems in lupin cultivation in the Netherlands are fungal diseases. Many of these diseases are seed-borne, and could start from contaminated seeds. The most aggressive disease is anthracnosis (caused by *Colletotrichum lupine*). This disease can spread quickly through the trial, once a couple of plants are infected. In this way, contaminated seed of one white lupin variety ruined the entire trial in 2016. Therefore, in 2017 sowing material was screened at the start of the growing season for contamination with anthracnosis. In 2017, this resulted in the early preventive removal of the white lupin variety Dieta from the trial in Klazienaveen. Other fungal diseases that affect lupins in the Netherlands are grey leaf spot (*Stemphylium botryosum*), brown leaf spot (*Pleiochaeta setosa*) and Fusarium wilt (*Fusarium oxysporum* f.sp. *lupini*). Insect damage in lupins is rare, and the lupin aphid (*Macrosiphum albifrons*) seems to prefer bitter over sweet lupins. Damage to seedlings by hares or roe deer may occur as well. Trial fields were protected against hares by fences.

Sowing density trials in white and blue lupin

Sowing density of lupin has been investigated as an agronomic intervention. Both white (*L. albus*) and blue (*L.angustifolius*) lupin species contain indeterminate and restricted branching varieties. In varieties with an indeterminate growth habit, the main stem terminates in a racemose inflorescence. From the axils of a number of leaves on the main stem grow first order branches. These also terminate in a racemose inflorescence and bear a second order branch in some of the axils, and so on (Dracup and Tomson, 2000). Our hypothesis was that restricted branching types of both narrow-leafed and white lupins would benefit more from an increased sowing density than indeterminate growth types. As the first ones are unable to correct for a lower plant density by either increasing the number of axils from which branches grow, or the number of orders. In order to test this hypothesis, in 2015 two blue lupin varieties were compared at location Klazienaveen: restricted branching Primadonna, and indeterminate variety Iris, which were sown at 60, 80, 100 and 120% of their recommended sowing

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densities. In 2016 and 2017 the trials were repeated, with sowing densities of 50, 75, 100 and 125% of the recommended sowing densities, for restricted branching variety Regent, and indeterminate variety Iris.

Spring and winter faba bean

Crop protection in spring and winter faba bean

Faba bean is affected by several diseases in the Netherlands. Insect damage is mainly caused by black bean aphid (*Aphis fabae*) and pea leaf weevil (*Sitona lineatus*). Aphids may spread several viral diseases, such as the Bean Yellow Mosaic Virus. Fungal diseases which affect faba bean are mainly chocolate spot disease (*Botrytis fabae*), faba bean rust (*Uromyces viciae-fabae*) and Sclerotinia stem rot (*Sclerotinia sclerotiorum).* Crop protection trials in faba bean were directed at fungicide use, and performed at several locations and years. If the potential damage of aphids or pea leaf weevil became too large, insecticides were used in all treatments. Herbicides were applied to all plots as well, with the exception of pre-emergence herbicides, which were only applied in autumn 2017 in plots with crop protection.

Sowing depth and sowing density trials in winter faba bean

Sowing depth is an important factor in increasing the winter hardiness of autumn-sown legumes. Sowing depths of 10-12 cm may improve survival, as plants sown at 5-6 cm may be frost-heaved. Frost tolerance increases at prolonged exposure to low temperatures (2-5°C). De-hardening takes place at temperatures above 7°C and accelerates at temperatures above 10°C. In the 2016-2017 growing season, experiments were carried out with both sowing depth and sowing density in winter faba bean. Effects on yield of sowing depths at 5, 10 and 15 cm was investigated in cultivar Tundra at the Oostwold (marine clay) location, in combination with different sowing densities (10, 20 and 30 seeds/m2). At the Klazienaveen location (humic sandy soil), different cultivars were tested at three different sowing depths in a pilot design without replicates.

In the 2017-2018 growing season, trials with three sowing densities (20, 30 and 40 seeds/m^2) were performed with two winter faba bean cultivars (Diva and Tundra) at the Oostwold location. Sowing densities were higher than in the previous trial, as a "minor" type French variety (Diva) was included, besides the larger "equina" type (Tundra).

Sowing date trial in winter faba bean

In the growing season 2017-2018, an experiment was carried out with winter faba bean variety, Honey, in order to assess the management choice to re-sow a winter faba bean variety after harsh winter conditions have severely reduced winter survival. In Bellingwolde, during the variety experiments, harsh winter conditions occurred at the end of February, with temperatures until -6 degrees, combined with hard wind and sand storms. Dehardening of the plants was already taking place, as in the previous weeks temperatures had been relatively high. The re-sowing experiment was carried out with the English winter faba bean variety Honey.

Soya bean

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Crop protection in soya bean

Crop protection in soya bean was directed at fungicide use, and took place in 2017 at the location Klazienaveen (KL). Sclerotinia stem rot (*Sclerotinia sclerotiorum*) is the main fungal disease affecting soya bean cultivation in the Netherlands.

Soya, lupin and fava bean Crop rotation trials

One of the aspects of crop rotation, is the host plant status of crops for nematodes. Crops may either stimulate or suppress the development of plant-pathogenic nematodes in soil. This determines their positioning in the crop rotation. In four field trials the effect of the inclusion of grain legumes in rotation schedules on plant-pathogenic nematodes was tested in naturally infected arable fields. Additional bioassays were carried out to assess the host status of several varieties of grain legumes for the root-knot nematodes Meloidogyne chitwoodi and Meloidogyne hapla, and for the beet cyst eelworm, Heterodera schachtii, as they are important pathogens in the rotation of arable crops in the Netherlands.

Nematode host status: field trials

In 2015 experimental trials were performed at two locations in the Netherlands: a sandy soil in Holten (HO) and a humic sandy soil in Klazienaveen (KL). Grain legumes included were white and blue lupin and faba bean. The natural development of plant-pathogenic nematodes during the growing season was compared with the development in spring wheat and black fallow. Nematode data were log-transformed before statistical analysis.In 2016, plant-pathogenic nematodes communities were determined at the start and end of the growing season in experimental field trials (Klazienaveen) with white and blue lupin, faba bean, quinoa, soya bean and buckwheat. The development of nematodes in spring wheat and black fallow were used as a reference. Initial population (Pi) was determined by combined samples at block level, final population (Pf) was determined per plot. The reproduction factor (Rf) for each plot was calculated (Pf/Pi) using the average of the three initial samples for calculation of the Pi. Nematode data were log-transformed before statistical analysis.

In 2017 field trials were carried out with white lupin, blue lupin, summer faba bean and soya bean. The fields at location Jipsingboertange had a natural, moderate infection with the Northern root-lesion nematode (Pratylenchus penetrans) (on average 380 nematodes/100 ml soil) and a light infection with Columbia root-knot nematode (Meloidogyne chitwoodi) (35 nematodes/100 ml). The initial infection of M. chitwoodi is not equally divided over the plots, and plots with barley and Andean lupin have a significant higher Pi (64 nematodes/100ml) than the other crops tested (23 nematodes/100ml). A very light infection of Paratrichodorus (8 nematodes/100ml) is only present in the plots with Andean lupin, soya bean, sugarbeat and barley. Reference crops in the field trials were sugarbeet (a poor host for P. penetrans, and very poor host for M. chitwoodi) and barley (a good host for both nematodes). Two weeks before sowing, the number of plant pathogenic nematodes was determined in each of the plots (Pi = initial population). After harvest, at the end of September population density (Pf = final population) was determined in the same way. The reproduction factor (Rf = Pf/Pi) was calculated for each of the plots. Statistical analysis was performed with log-transformed data.

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Nematode host status: bioassay

In 2017, pot trials have been carried out with blue lupin (7 varieties), white lupin (3), Andean lupin (4), summer faba bean (6), winter faba bean (6), and soya bean (4 varieties). In three separate trials, 10 plants/variety were inoculated with 600 larvae/plant of the nematode species *Meloidogyne chitwoodi*, *M. hapla* and *Heterodera schachtii*. After 7 weeks, the number of cysts (*Heterodera schachtii*) or egg masses (*Meloidogyne* species) were counted and plant root weight was determined. Oilseed radish has been used as a reference crop in the trials.

3 Results and discussion

3.1Italy

3.1.1 Experimental site and climate

Field trials to evaluate specific agronomic practices were carried out in three different locations characterized by different soil textures. More specifically, from 2015 to 2019, quinoa, amaranth, fava bean, buckwheat and lupin were tested in a clay soil, and two locations with sandy soil were added to evaluate fava bean and lupin from 2017 to 2019.

<u>*Vitulazio (Caserta, Italy)*</u>

Field experiments were carried out in Vitulazio (Caserta, Italy) at the experimental research station of CNR-ISAFoM (41°12’ N and 14°20’ E, 23 m above the sea level), during five growing seasons: 2015, 2016, 2017, 2018 and 2019. The climate is typically Mediterranean sub-humid, characterized by an average annual rainfall of 888 mm, mostly concentrated in autumn and winter months (October to March). The annual reference evapotranspiration (ET0 estimated by Penman–Monteith equation according to Allen et al. 1998) in the region is an average of 1077 mm reported in the period 1976–2018. The weather regime, in terms of cumulative precipitation (P), reference evapotranspiration (ET0), and average temperatures (Tavg.) during each month are given in figure 1 for the four growing seasons (2015 to 2018), compared to the reported historical means (1976-2018). The mean air temperature is resorded at 17.08°C in autumn, 8.66°C in winter, and 13.74°C in spring. The average minimum and maximum annual temperatures are 10.80°C and 20.52°C, respectively.

The main weather parameters for the study, including solar radiation, air temperature, relative humidity and precipitation, were obtained from a standard agro-meteorological station (iMetos ag, mod. IMT 280, Pessl Instruments, AT), which is located about 30 m from the experimental field. The soil at the site is a clay-loam texture (clay, sand and silt; at 46.1%, 30.2% and 33.7%, respectively) and defined as Mollic Haplaquept (USDA, 2006). Chemical and physical characteristics of the soil at the beginning of the experiments (April 2015) were the following: pH 8.05, Kjeldahl total N 1.81 g kg^{-1}, organic C 9.1 g kg^{-1}, electrical conductivity ECe = 0.23 dS m^{-1}, and bulk density 1.28 kg dm^{-3}.

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The volumetric soil water contents at field capacity was 0.38 $m^3 m^{-3}$, while the permanent wilting point was 0.13 m^3m^{-3}.

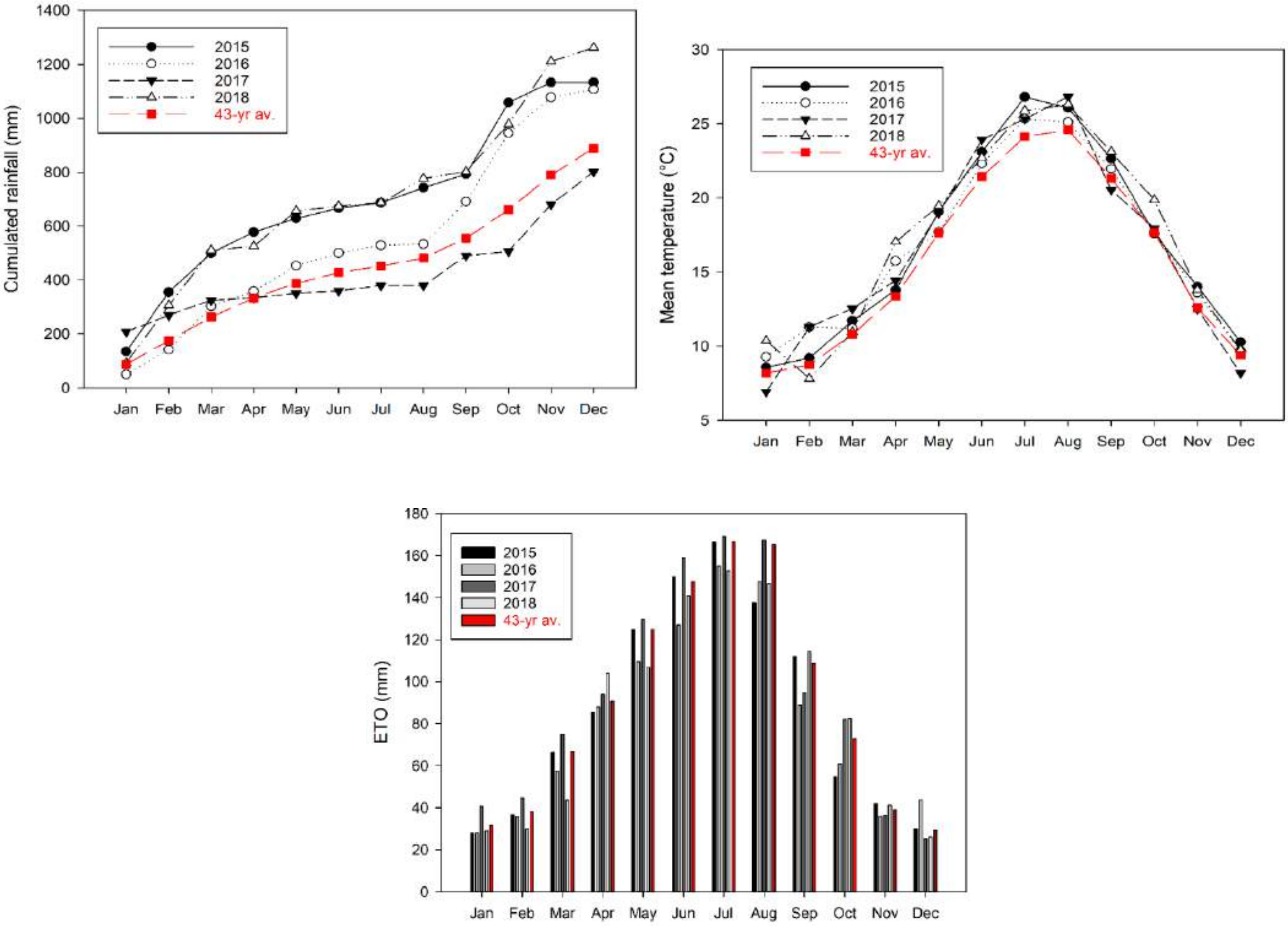

Figure 1 Cumulative monthly rainfall, mean air temperatures and reference evapotranspiration at the experimental site during the four growing seasons (2015, 2016, 2017 and 2018); 43-yr (1976-2018).

After computing the deciles index (DI) on seasonal scale (Figure 2), by applying one of the most widely used drought indices designed by Gibbs and Maher (1967), the first (2015) and second (2016) growing seasons were classified as normal (DI ≥ 5 for winter, spring, summer and fall season). Whereas, the spring and fall of the third season (2017) were classified as extremely and weak drought, respectively (DI = 1 for spring 2017 and DI = 4 for fall 2017). The spring in 2018 was weak drought index with DI = 4.

The analysis of the main climatic parameters during the fourth field trials (2015 to 2018, Figure 1), showed that the first, second and the fourth growing seasons were characterized by warmer and more wet than the long-term 43 year recorded mean: mean air temperature was 1.08, 0.91 and 1.43°C, respectively, higher than the average mean, and the rainfall was 27.6, 24.6 and 42%, respectively, greater as compared with the long-term 43 years (888.4 mm). The relatively warm weather conditions

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that prevailed during the fourth seasons increased seasonal ET0 compared to the long-term mean. Consistently, average annual ET0 was in the first year (2.83 mm), the second (2.67 mm), and the fourth trial (2.78 mm) lower than the long-term mean (2.95 mm) but was higher in the third year (3.05 mm) The rainfall distribution during the growing season greatly affected the behavior and the response of crops to water deficit. The trial carried out in the third season (2017) was characterized by a very dry spring season (March–May) compared to the other three growing seasons, with the cumulative rainfall 81 mm lower than the values recorded during spring season in the other growing seasons of 2015, 2016 and 2018.

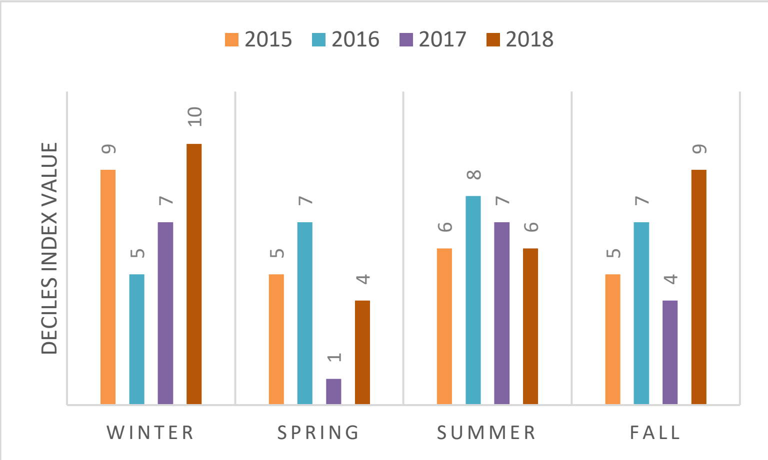

Figure 2 Deciles index (DI) on seasonal scale for the four growing seasons (2015, 2016, 2017, 2018). DI=1 (Extreme drought); DI=2 (Severe drought); DI=3 (Moderate drought); DI=4 (Weak drought) and DI>=5 (No drought).

Acerra (Naples, Italy)

An open-field experiment was carried out during 2019 growing seasons, at the experimental farm of "Arca 2010" located in Acerra-Naples, South Italy (40°57'N, 14°25'E, 26 m above sea level). The deep soil developed on volcanic material has a sandy-loam texture with 58.8% sand, 30.9% silt and 10.3% clay, and it is characterized by high chemical and physical fertility. For a depth up to 30 cm, the soil was characterized by a bulk density of 1.07 g cm^{-3}, 216 mg kg^{-1} (P_2O_5 available), 2173 mg kg^{-1} (K_2O), 0.14% (w/w) N and 2.02% (w/w) organic matter. The volumetric soil water contents at field capacity was 0.21 m^3m^{-3} while the permanent wilting point was 0.09 m^3m^{-3}, measured at soil matric potential (Ψ_m) of −0.03 and −1.5 MPa, respectively. The climate is typically Mediterranean; during the spring and summer seasons, 20-year average (1999-2018) monthly rainfall, air temperature and relative humidity were 75 mm, 16,5°C and 70%, respectively (data not shown). Data of both the average 20-year period and measurements in 2019 were collected from an agrometeorological station belonging to the Se.S.I.R.C.A. − C.A.R. of the Regione Campania (Italy), located 120 m from the experimental site.

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In Acerra (Na) the climatic trend was different from the reference mean data (1999-2018), especially in the case of volume and distribution of rainfall. Figure 3, indicates the monthly mean temperature and rainfall during the leguminous growing season (2018-2019), compared with long-term values (1999-2018). The rainfall during September to June was higher in years 2018-2019, 1140.8 mm compared to the long-term 20-year average of 859.1 mm. Over the leguminous growing season (2018-2019) the average mean temperature (14.6°C) was slightly higher than the long-term average (14.5°C) (Figure 3).

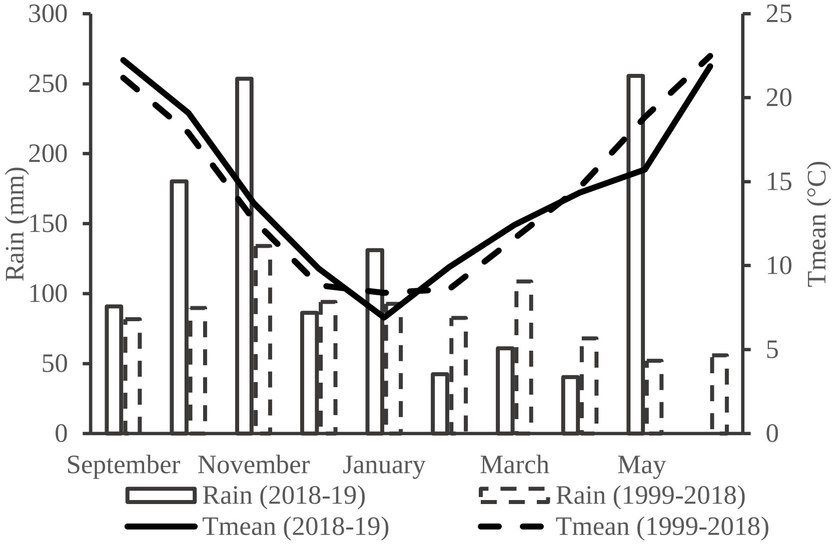

Figure 3 Monthly rainfall and average air temperature (Tmean) at the study site (Acerra) for leguminous growing season (2018-2019), compared with long-term values (1999-2018).

Ponticelli (Naples, Italy)

A field trial in 2018 was carried out in Ponticelli (40°86'N, 14°33'E) (Naples), characterized by the Mediterranean climate with warm dry summers and mild wet winters. The soil texture is a loamy fine sand due to its volcanic origin, classified as Andosols (USDA. 2006), which physical and chemical characteristics are: sand (2 > Ø > 0.02 mm) 80%, silt 12%, clay (Ø < 2 μ) 8%; pH 7.1, saturated paste extract electrical conductivity (ECe) 0.15 dS m^{-1}, soil moisture at field capacity 16.31 cm^3 cm^{-3} and at wilting point (−1.5 MPa) 7.69 cm^3 cm^{-3}, Bulk density 1.37 g cm^{-3}, Kjeldahl total N 1.81 g kg^{-1} and Organic matter 2.54 g kg^{-1}.

The following meteorological variables were recorded daily throughout the crop growing season: average air temperature, average air relative humidity, rainfall, wind speed, reference evapotranspiration (ET0) using a data logger (CR10, Campbell Scientific, USA), located approximately 50 m from the experimental field. Meteorological data recoreded were typical for Mediterranean environment (Table 1).

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Table 1: The main agro-meteorological variables, including rainfall, air temperature, relative humidity, wind speed and solar radiation recoreded at the experimental site.

Mounth	Rainfall *mm*	Temperature *°C*	RH *%*	Ws *m s⁻¹*	Solar radiation *MJ m⁻²d⁻¹*	ET0 (PM) *mm day⁻¹*
October_17	30.99	16.89	73.57	0.96	12.34	1.56
November_17	176.79	12.15	80.07	0.94	7.38	0.97
Dicember_17	122.43	8.75	75,62	1.35	5.21	0.76
January_18	87.38	11.01	82,61	0.95	6.28	0.71
February_18	203.20	7.74	78.19	0.94	6.54	0.91
March_18	152.90	11.90	77.81	1.26	9.69	1.44
April_18	20.57	17.07	67.98	0.78	17.96	2.82
May_18	66.55	19.91	76.21	0.49	18.16	3.14
June_18	62.23	23.55	66.25	0.73	22.86	4.84

PM = Penman-Monteith

3.1.2 Crops

3.1.2.1 Quinoa

Sowing date

A 3-year field trial on quinoa (2016-2017-2018) was conducted in Vitulazio experimental farm to evaluate the effect of sowing date on yield parameter and growth of different quinoa verities under rainfed conditions. In particular, we tested the effect of an early (d1) and late (d2) spring sowing date. Were tested quinoa varieties with a short cycle length that can be cultivated under the selected pedoclimatic conditions. In 2016, Vikinga, Puno, Titicaca and Regalona were sown on 7th April and 24th May. In 2017, the quinoa varieties Titicaca, Puno, Salcedo and Vikinga were sown on 31st March and 9th May, and in 2018, Vikinga, Regalona and Titicaca were sown on February 1st and 24th April. The seeds were manually sown in plots with row spacing of 0.50 m and a theoretical density of 200 000 plants ha^{-1}. The experimental design was a randomized block design with 3 replicates. During all the experimental years, the plants were fertilized using a total amount of 150 N units, and divided in two equal rates at pre-sowing and pre-flowering stage. At harvest, the main yield components and some phenotypic parameters like plant height and diameter were evaluated. The trial for Titicaca, Vikinga and Regalona has been replicated in 2019 and is still ongoing. The data for 2019 will be analyzed and reported in the Final Periodic Report at the end of P2F project.

Some problems occurred in 2016 and 2018 due to low seed germination; in 2016 only Regalona plants reached maturity; no data for Titicaca, Vikinga and Puno were collected due to low germination in both sowing dates. Data from 2017 showed that early sowing date can significantly increase seeds yields for quinoa; Puno and Titicaca produced more respect Vikinga. No significative differences between d1 and d2 plants for height and diameter were found. Vikinga was not significantly affected by sowing date (Table 2). There are significante differences between treatments for the interaction cultivar (C) x sowing date (Sd), relatively to grain yield (Table 2 and Figure 4).

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Table 2 Yield and yield components as affected by cultivar and sowing date under quinoa experiment on 2017

Source of Variation	**Grain Yield**	**AGB**	**HI**	**Height**	**Diameter**
	g Plant^{-1}	*g Plant^{-1}*		*cm*	*cm*
Cultivar (C)	***	ns	***	*	**
Puno	7.55±7.45 a	33.12±26.29	16.19±8.21 a	90.00±15.49 a	8.13±2.40 a
Titicaca	6.29±4.42 a	28.92±11.75	18.29±9.36 a	97.50±15.04 a	9.38±1.24 a
Vikinga	1.49±1.23 b	22.29±18.75	7.25±3.21 b	59.08±26.70 b	5.17±1.75 b
Sowing date (Sd)	****	**	****	ns	ns
Sd_1	8.71±4.90 a	40.57±15.83 a	21.26±8.17 a	88.39±30.66	8.03±3.06
Sd_2	1.11±0.50 b	13.71±4.10 b	8.86±3.27 b	76.00±18.24	7.08±1.90
C x Sd	**	ns	ns	ns	ns

*, **, ***, **** *indicate respectively differences at P≤0.05, P≤0.01, P≤0.001 and P≤0.0001; ns indicates not significant difference. Means followed by different letter in each row are significantly different according to the Duncan test (P=0.05). AGB, HI, Sd1 and Sd2 represent, respectively: Dry above ground biomass, harvest index, "31 March" and "9 May".*

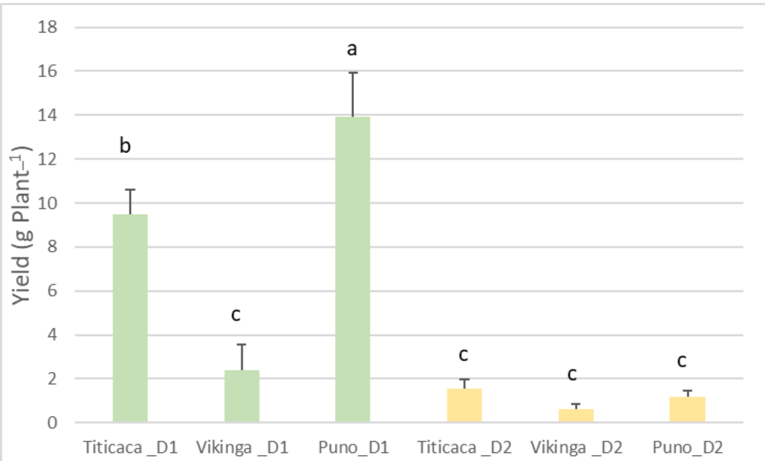

Figure 4. Yield as affected by cultivar and sowing date under quinoa experiment on 2017. Vertical bars indicate standard deviation.

In 2018, only plants from first sowing date reached maturity, due to air temperature higher than 30° C during vegetative and flowering period that affected negatively plant growth and seed formation. Titicaca produced more seeds in respect to Vikinga also in 2018 (Table 3).

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Table 3 Yield and yield components as affected by cultivar under quinoa experiment on 2018 at early spring sowing date.

Treatment	Yield *g.Plant*$^{-1}$		AGDB *g.Plant*$^{-1}$		Weight 1000 seeds *g*		Harvest index *%*		Height *cm*		Diameter *cm*	
	F - test	Means	F - test	Means	F - test	Means	F - test	Means	F - test	Means	F - test	Means
	*		NS		*		*		NS		NS	
Regalona		2.35±0.85 ab		14.58±2.42		1.68±0.32 b		16.48±6.79 ab		69.00±24.11		5.00±0.87
Titicaca		3.64±1.38 a		15.54±6.93		2.21±0.15 a		24.24±2.99 a		78.00±16.12		5.67±1.04
Vikinga		1.05±0.33 b		17.68±7.73		1.44±0.04 b		8.34±5.54 b		50.13±0.88		5.25±1.75
S.E.		0.75		0.92		0.23		4.59		11.38		0.17
CV (%)		55.18		9.96		22.18		48.62		30.76		54.39

** indicate respectively differences at P≤0.05; ns indicates not significant difference. Means followed by the different letter in each column are significantly different according to the Duncan test (P=0.05).*

Rotation

A 2-year (2017-2019) field trial was carried in Vitulazio experimental farm to evaluate the effect of rotation with winter fava bean and quinoa; the idea was to cultivate fava bean for fresh consumption from October to March and after fava bean was harvested then quinoa was sown. Quinoa varieties with a short cycle length of Puno and Titicaca were tested after cultivation of a commercial fava bean. The trial was conducted under rainfed conditions using a randomized block design with three replicates.

At harvest the main yield components and some phenotypic parameters such as plant height and diameter were evaluated. The trial was repeated in 2018-2019 but failed due to specific climatic conditions. In 2019, for instance, the rainfalls occurred after fava bean harvest delayed the seedbed preparation for quinoa until middle of June; and then June was unsuitable for quinoa growht due high temperatures. The rotation of fava bean/quinoa did not have a positive impact on the yield of quinoa seeds.; In fact, seed yield was significatively higher for quinoa grown without rotation. In general, the effect of a rotation between pulses and other herbaceous plants should be evaluated during a longer period (Table 4).

Table 4 Results of the Scheirer-Ray-Hare test concerning the Yield and its components for first annual trial (2017-2018) in Vitulazio. The values are referred to a dimensionless rank.

Source of variation	Grain Yield	1000 Grain Weight	AGB	HI	Height	Diameter
Experimental (E)	**	ns	**	**	**	ns
Rotation	3.5 b	5.75	3.5 b	3.5 b	3.67 b	4.92
Without_rotation	9.5 a	7.25	9,5 a	9.5 a	9.33 a	8.08
Cultivars (C)	ns	*	ns	ns	ns	ns
Tititcaca	6.5	9 a	6.5	6	5	6.5
Puno	6.5	4 b	6.5	7	8	6.5

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E x C	ns	ns	ns	ns	ns	**

*ns and *, ** Not significant or significant at P ≤ 0.05 and P≤0.01, respectively.* ***Means Rank*** *followed by different letter in each column are significantly different according to the Dunn-Bonferroni pairwise comparisons test (P= 0.05)*

Sowing density

A 2-year (2015, 2018) field trial was carried in Vitulazio experimental farm to evaluate the effect of sowing density on yield parameter and growth of different quinoa verities under rainfed conditions. We tested 2 theoretical sowing densities of respectively 100000 (d2) and 200000 (d1) plant ha^{-1}. In 2015, Vikinga, Puno, Titicaca, Regalona and Riobamba were tested, and in 2018 the trial was repeated for Vikinga, Puno, Titicaca and Regalona. The seeds were sown manually in plots with row spacing of 0.50 m. The experimental design was a randomized block design with 3 replicates. During both experimental years the plants were fertilized using a total amount of 150 N units, and divided in two equal rates at pre-sowing and pre-flowering stage. At harvest, the main yield components and some phenotypic parameters like plant height and diameter were evaluated.

In both years, plants sown with a d2 density showed a positive trend in the yield component data collected. In 2018, d2 plants showed higher values of seed yield and biometric parameters in respect to d1 plants, and the plans sown with a lower density value (d2) grew more and produced more. The Danish quinoa varieties Titicaca and Puno showed higher values for all collected parameters in both experimental years. The sowing density d2, in particular, had a positive impact on plants of the Titicaca variety (Table 5 and 6). In 2018, There were significant differences between treatments for the interaction cultivar (C) x sowing density (Sd), relative to grain yield (Table 6 and Figure 5).

Table 5 Yield and yield components as affected by cultivar and sowing density under quinoa experiment in 2015

Source of Variation	Grain Yield	AGB	HI	Height	Diameter
	g Plant^{-1}	*g Plant^{-1}*	*%*	*cm*	*cm*
Cultivar (C)	ns	ns	**	**	ns
Puno	3.75±2.95	11.03±11.19	25.49±6.28 b	83.17±10.98 a	7.13±3.01
Regalona	3.76±1.66	19.53±9.70	20.92±4.99 bc	86.81±4.73 a	7.75±1.71
Riobamba	2.27±1.11	20.11±13.07	10.85±5.64 c	87.75±4.63 a	9.00±0.58
Vikinga	1.65±0.24	7.02±2.24	25.17±7.71 b	58.75±11.03 b	6.50±1.87
Titicaca	5.17±0.63	11.81±1.92	43.99±2.19 a	97.13±6.05 a	6.25±0.96
Sowing density (Sd)	ns	*	ns	ns	ns
Sd_1	2.70±1.63	9.48±6.19 b	25.31±13.25	80.25±9.24	7.40±2.02
Sd_2	3.93±1.92	18.32±10.44 a	25.24±12.23	77.70±16.25	7.25±1.90
C x Sd	ns	ns	ns	ns	ns

**, ** indicate respectively differences at P≤0.05 and P≤0.01; ns indicates not significant difference. Means followed by different letter in each row are significantly different according to the Duncan test (P=0.05). AGB, HI, Sd1 andSd2 represent, respectively: Dry above ground biomass, harvest index; "Sd1"; "Sd2".*

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Table 6 Yield and yield components as affected by cultivar and sowing density under quinoa experiment in 2018

Source of Variation	Grain Yield	1000 Grain Weight	AGB	HI	Height	Diameter
	g Plant^{-1}	*g Plant^{-1}*	*%*	*cm*	*cm*	*g Plant^{-1}*
Cultivar (C)	****	****	*	**	***	*
Puno	5.09±0.63 b	1.37±0.07 c	28.48±5.24 a	19.08±4.20 ab	100.13±7.08 a	8.50±1.10 a
Regalona	2.25±0.84 c	1.61±0.22 b	18.54±6.24 b	15.52±7.87 bc	67.25±14.26 b	5.92±1.32 b
Titicaca	6.31±2.24 a	2.22±0.12 a	22.69±3.88 ab	26.84±4.19 a	93.13±6.94 a	7.50±2.32 ab
Vikinga	1.55±0.74 c	1.31±0.15 c	17.08±6.76 b	10.85±6.76 c	59.06±14.25 b	6.50±2.27 b
Sowing density (Sd)	**	ns	ns	ns	*	*
Sd_1	3.06±1.66 b	1.69±0.39	19.77±7.63	17.67±7.42	74.66±24.72 b	6.36±2.12 b
Sd_2	4.51±2.63 a	1.62±0.41	24.03±5.79	19.78±8,68	85.13±14.70 a	7.95±1.44 a
C x Sd	*	ns	ns	ns	*	*

, **, *, **** indicate respectively differences at P≤0.05, P≤0.01, P≤0.001 and P≤0.0001; ns indicates not significant difference. Means followed by different letter in each row are significantly different according to the Duncan test (P=0.05). AGB, HI, Sd1 andSd2 represent, respectively: Dry above ground biomass, harvest index; "Sd1"; "Sd2".*

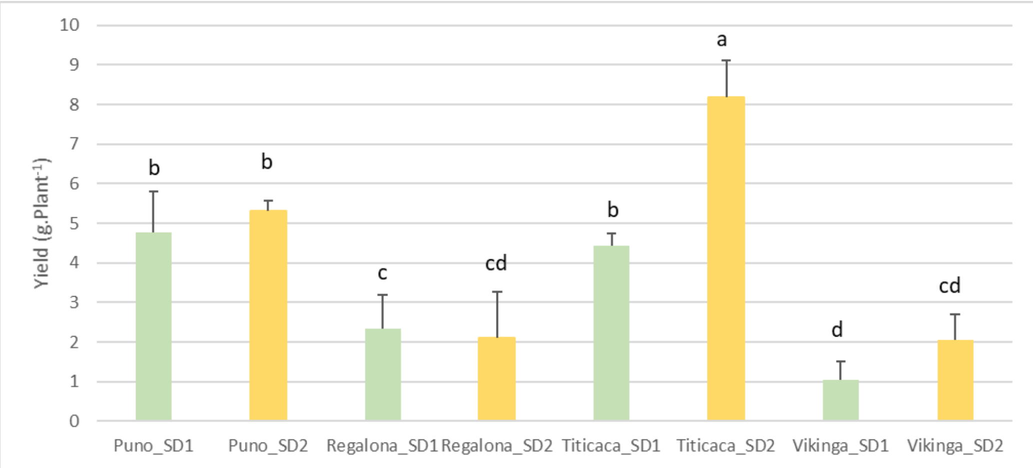

Figure 5 Grain yield as affected by cultivar and sowing density under quinoa experiment in 2018

<u>Abiotic stress trial</u>

The field experiments on abiotic stress were carried out during three growing seasons of 2015, 2016 and 2017 with the Danish quinoa variety "Vikinga". Twelve treatments derived from a factorial combination of two irrigation regimes (I100 and I33), two water qualities [freshwater (N=0.8 dS m^{-1}) and saline water (S=22 dS m^{-1})] and three phenological stages (Fi, D1 and D2). I100 corresponding to a restitution of 100% (full irrigation) of irrigation water to replenish 40 cm soil layer to field capacity (F.C.), and I33 to a restitution of 33% of full irrigation. Fi was the treatment irrigated during the whole growth cycle, D1 the treatment irrigated from sowing to flowering, D2 from flowering to harvest. All the combinations of treatments (I100 and I33; S and N; Fi, D1 and D2) were arranged in randomized complete block design with three replications. Each experimental unit consisted of 10 rows, 4 m in length.

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Saline water was obtained with salts ($NaCl$, $CaCl_2$, KCl, $MgCl_2$ and $MgSO_4$) and added to the groundwater in same stechiometric quantities as seawater (For details see Table 1 in D1.8). The solution conductivity achieved was about 22 dS m^{-1}. The irrigation system consisted of two main supply pipes connected to a network of pipes placed along the plots with drip lines along plant rows (0.5 m). The nominal flow rate was of 4 L h^{-1} at the operating pressure of 0.1 MPa. Irrigation was carried out at fixed weekly intervals and was started the day after sowing (DAS) on days 49, 12, and 56 for the growing seasons of 2015, 2016 and 2017, respectively.

In 2016, there were lower irrigation requirements in comparison to 2015 due to more favorable rainfall patterns. In 2017, the seasonal irrigation amount was lower than 2016 because the crops required less irrigation supplies during the growing season (For details see Tables 2, 3 and 7 in D1.8). During each growing season, to measure the soil water content in each plot at depths of 0-0.20, 0.20-0.40 and 0.40-0.60 m, the gravimetric method (Qiu et al., 2001) was used. Volumetric moisture measurements were carried out before and 24 hours after each watering, as well as after a rainfall of 5 mm or more. Before sowing and at the end of crop cycle, the electrical conductivity of the soil (EC) was measured at the same soil depth used to measure soil moisture. The harvest was made by hand at physiological maturity. The total yield, the 1000 seed weight and the above-ground biomass were determined on three plants per elementary plot. The harvest index (HI) was calculated as a ratio between yield and total above-ground biomass. Seed samples of both species and of each treatment, were then chemically analyzed to evaluate the principal qualitative components.

Quinoa was sown on day (DOY) 111, 168 and 102 of the year 2015, 2016 and 2017, respectively. The crop cycle length ranged from 110 to 117 days during the three experimental years (see Table 3 in D1.8). The difference in crop cycle length between the years was due to the different thermic sum. The ET0 demand during the crop cycle ranged from 466 mm (2016) to 531 mm (2015). Differences in seasonal irrigation volume between the three years were also reported due to differences in the evapotranspiration demand. The final ECe value was significantly higher compared to the ECe initial values for the two applied saline irrigation treatments (33S and 100S). The winter rainfall prevented salt accumulation in the first two layers of soil whereas the underlying layer presented a behavior that is more constant over time, with a tendency towards a gradual increase in ECe values (see Table 4 in D1.8).

The statistical analysis of main yield components (yield, dry biomass, harvest index), that were measured during the three experimental growing seasons, were analyzed by year, and for single effects of irrigation level (Wr), saline treatment (S), phenological stage (ph) and for all their interactions. Generally, for all yield components there were no significant differences in the statistical results in the three growing seasons. Only in 2016 growing season, there are significant differences between treatments for the interaction of Phenological stage (ph) x Water regime (Wr), in comparison to Plant height, yield and Y IWUE. For the interaction of Water regime (Wr) x Salinity (S) was significant only for the Y IWUE parameter (Table 7). Since no differences were detected for the simple effects of ph and S, but only for the simple effect of irrigation level on plant height ($P<0.05$), this indicates that the water irrigation level only have an effect on the vegetative development without

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any influence on simple effects of yield. In the table 7, only the Multi-way ANOVA results related to the significant results discussed are reported, reserving a detailed description in the next deliverable 1.11.

Table 7 Multi-way ANOVA results on plant height, yield and Y-IWUE of quinoa in 2016

Source of Variation	Plant height cm		Yield $g\ plant^{-1}$		Y-IWUE $kg\ ha^{-1}\ mm^{-1}$	
	Pr.	Means	Pr.	Means	Pr.	Means
Phenological stage (ph)	NS		NS		NS	
Fi		62.1±6.62		0.33±0.14		0.14±0.07
D1		62.33±9.24		0.29±0.12		0.17±0.08
D2		59.42±6.82		0.35±0.22		0.15±0.12
Water regime (Wr)	**		NS		NS	
I_100		64.54±8.06 a		0.35±0.13		0.14±0.08
I_33		58.03±5.71 b		0.30±0.19		0.16±0.10
Salinity (S)	NS		NS		NS	
N		62.53±8.65		0.37±0.14		0.16±0.11
S		60.04±6.48		0.29±0.18		0.14±0.06
ph x Wr	*		*		*	
ph x S	NS		NS		NS	
Wr x S	NS		NS		*	
ph x Wr x S	NS		NS		NS	

**, ** indicate respectively differences at P≤0.05 and P≤0.01, ns indicate not significant difference*
Means followed by the different letter are significantly different according to the LSD test (P=0.05)

3.1.2.2 Amaranth

Rotation

A 2-year (2017-2018) field trial was carried in Vitulazio experimental farm to evaluate the effect of rotation of winter fava bean with amaranth; the experimental protocol and design was the same carried out for quinoa rotation. Were tested the accession 12 and 14 of grain Amaranth received from University of Copenhagen, after cultivation of a commercial fava bean. The rotation fava/amaranth did not had positive impact on amaranth seeds yield; grain yield value was significantly higher for amaranth grown without fava rotation (Table 8). As for quinoa, this trial should be repeated for at least another two years.

Table 8 Results of the Scheirer-Ray-Hare test concerning the amaranth Yield and its components. The values are referred to a dimensionless rank.

Source of variation	Grain Yield	1000 Grain Weight	AGB	HI	Height	Diameter
Experimental (E)	ns	ns	ns	ns	ns	ns
Rotation	5	6.33	6.5	5	5.17	7
Without_rotation	8	6.67	6.5	8	7.83	6
Cultivars (C)	**	**	**	**	*	**
A12	3.5 b	3.5 b	3.5 b	3.5 b	4 b	3.5 b

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	A14	9.5 a	9.5 a	9.5 a	9.5 a	9 a	9.5 a
E x C		ns	ns	ns	ns	ns	ns

*ns and *, ** Not significant or significant at $P \leq 0.05$ and $P \leq 0.01$, respectively.* ***Means Rank*** *followed by different letter in each column are significantly different according to the Dunn-Bonferroni pairwise comparisons test (P= 0.05)*

Fertilization

One amaranth field experiment was replicated in Vitulazio during 2018 and 2019 with 6 N rates ranging from 0 to 300 N kg ha^{-1}. The N (as NH_4NO_3) rates indicated in table 9 (except 0) were divided in 3 equal parts (at pre-sowing, vegetative development and pre-flowering stages) and applied to the experimental plots arranged in a randomized block design with 3 replicates. At harvest, the main yield parameters and biometric traits were evaluated. The accession A12, characterized by a short cycle length (4 months), was sows in 2 May. The field trial of 2019 is still ongoing at the time of writing the deliverable.

Grain yields showed increasing values correlated with the increasing N amount applied (Table 9). The highest grain yield values were recorded for plants fertilized with a total N amount of 300 kg ha^{-1}. The seed production was not related to biomass development and biometric parameters. Data from 2019 trial will be later collected and analyzed.

Table 9 Yield and yield components as affected by fertilization under amaranth experiment in 2018

Treat.	Yield *g Plant^{-1}*			Biomass *g Plant^{-1}*		1000 seeds *g*		Harvest index *%*			Height *cm*			Diameter *cm*		
	F	Means		F	Means	F	Means	F	Means		F	Means		F	Means	
	****			ns		ns		*			**			***		
A0		5.24±1.47	d		141.56±72.59		0.67±0.02		14.38±1.38	ab		199.50±2.50	a		17.00±0.00	a
A100		9.93±0.46	c		162.85±13.62		0.67±0.02		17.89±0.73	a		179.5±0.50	ab		13.67±1.53	cd
A150		10.03±0.58	c		226.84±38.98		0.65±0.02		9.51±0.95	b		168.50±2.50	ab		17.50±0.50	a
A200		13.32±2.74	b		178.40±25.72		0.67±0.02		21.38±1.09	a		170.67±3.06	ab		16.00±0.00	ab
A250		15.11±1.23	b		214.97±25.33		0.65±0.02		20.56±3.41	a		173.00±3.00	ab		14.50±0.50	bc
A300		23.39±0.56	a		255.07±114.80		0.64±0.00		21.04±5.89	a		143.00±22.00	b		12.00±2.00	d
S.E.		2.52			17.48		0.01		1.92			7.46			0.86	
CV (%)		48.12			21.78		2.02		26.95			10.60			13.95	

, *, **** *indicate respectively differences at $P \leq 0.01$, $P \leq 0.001$ and $P \leq 0.0001$; ns indicates not significant difference. Means followed by the different letter in each column are significantly different according to the Duncan test (P=0.05).*

Sowing density

A 2-year (2015, 2018) field trial was carried in Vitulazio experimental farm to evaluate the effect of sowing density on yield parameter and growth of different amaranth accessions (A5, A7, A12, A14) under rainfed conditions. We tested 2 theoretical sowing densities of respectively 100000 (d1) and 200000 (d2) plant ha^{-1}. In 2018, the trial was repeated only for A5, A14 and A7. The seeds were

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manually sown in plots with row spacing of 0.50 m. The experimental design was a randomized block design with 3 replicates. During all experimental years the plants were fertilized using a total amount of 150 N units, divided in two equal rates at pre-sowing and pre-flowering stage. At harvest, the main yield components and some phenotypic parameters, such as plant height and diameter, were evaluated.

In both years, no significant differences were recorded for yield component values (Table 10 and 11). This confirms that grain amaranth has ability to compensate for in-row planting density. No significant interaction between cultivar and sowing date (C x Sd) was found.

Table 10 Yield and yield components as affected by cultivar and sowing date under amaranth experiment in 2015

Source of Variation		Grain Yield	AGB	HI	Height	Diameter
		g Plant^{-1}	*g Plant^{-1}*	*%*	*cm*	*cm*
Cultivar (C)		ns	ns	ns	**	ns
	A12	16.15±8.68	58.84±9.37	26.71±11.18	155.88±10.14 a	19.00±1.73
	A14	11.54±3.01	62.26±29.43	19.93±5.02	142.75±5.87 ab	18.13±1.75
	A5	13.43±3.58	83.15±52.09	19.99±9.48	116.75±9.43 c	16.13±3.42
	A7	13.71±4.46	55.79±13.73	24.14±3.63	129.5±10.40 bc	19.13±3.15
Sowing date (Sd)		ns	ns	ns	ns	ns
	Sd_1	10.79±3.53	46.85±8.71	23.05±5.83	136.63±17.56	17.38±2.15
	Sd_2	16.62±4.95	83.17±32.94	22.33±9.65	135.81±17.94	18.81±3.06
C x Sd		ns	ns	ns	ns	ns

*** indicate respectively differences at P≤0.01, ns indicates not significant difference. Means followed by different letter in each row are significantly different according to the Duncan test (P=0.05). AGB, HI, Sd1 andSd2 represent, respectively: Dry above ground biomass, harvest index; "Sd1"; "Sd2".*

Table 11 Yield and yield components as affected by cultivar and sowing date under amaranth experiment on 2018

Source of Variation		Grain Yield	AGB	HI	Height	Diameter	1000 Grain
		g Plant1)	*g Plant^{-1}*	*%*	*cm*	*cm*	*g*
Cultivar (C)		*	ns	ns	ns	ns	*
	A5	37.71±20.93 ab	212.89±59.81	16.97±6.04	194.92±43.56	22.70±3.23	0.95±0.02 a
	A14	30.48±5.23 b	146.79±48.48	21.06±6.01	184.92±23.33	22.20±1.04	0.90±0.07 b
	A7	59.69±5.30 a	239.25±90.51	17.94±4.60	189.83±24.65	23.30±1.75	0.89±0.05 b
Sowing date (Sd)		ns	ns	ns	ns	ns	ns
	Sd_1	44.52±14.04	204.99±85.43	20.42±5.18	195.06±30.96	23.00±0.94	0.92±0.06
	Sd_2	38.40±20.94	184.59±63.73	16.90±5.64	184.72±30.41	22.33±3.25	0.90±0.06
C x Sd		ns	ns	ns	ns	ns	*

** indicate respectively differences at P≤0.05, ns indicates not significant difference. Means followed by different letter in each row are significantly different according to the Duncan test (P=0.05). AGB, HI, Sd1 andSd2 represent, respectively: Dry above ground biomass, harvest index; "Sd1"; "Sd2".*

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Sowing date

The effect of 2 different sowing dates on yield components and biometric parameters of 3 amaranth accessions (A14, A7 and A5) was evaluated; the experimental field trial was carried out in Vitulazio experimental farm. The seeds were manually sown in plots with row spacing of 0.50 m. The experimental design was a randomized block design with 3 replicates. During all experimental years the plants were fertilized using a total amount of 150 N units, divided in two equal rates at pre-sowing and pre-flowering stage. The first and second sowing date were respectively 8 of April and 24 May 2018.

In 2016, collected data showed an increasing trend from an early sowing date (d1) to a late sowing date (d2), even if there were no significate differences between d1 and d2 examinated values (Table 12).

Table 12 Yield and yield components as affected by cultivar and sowing date under amaranth experiment on 2016

Source of Variation		Grain Yield	AGB	HI	Height	Diameter
		g Plant^{-1}	*g Plant^{-1}*	*%*	*cm*	*cm*
Cultivar (C)		ns	ns	ns	ns	ns
	A14	7.96±3.42	63.83±30.03	15.51±9.27	163.75±8.56	19.00±1.22
	A7	7.88±0.44	82.90±9.79	9.56±0.60	167.33±9.57	18.13±2.48
	A5	10.68±10.19	91.88±79.99	12.79±8.26	167.33±31.53	20.38±4.73
Sowing date (Sd)		ns	ns	*	ns	ns
	Sd_1	11.56±7.25	84.60±67.36	16.92±7.57 a	172.89±20.89	20.45±3.59
	Sd_2	6.11±1.74	74.48±12.92	8.32±2.28 b	159.39±12.51	17.89±1.80
C x Sd		ns	ns	ns	ns	ns

** indicate respectively differences at P≤0.05, ns indicates not significant difference. Means followed by different letter in each row are significantly different according to the Duncan test (P=0.05). AGB, HI, Sd1 andSd2 represent, respectively: Dry above ground biomass, harvest index; "Sd1"; "Sd2".*

Abiotic stress trial

The experimental site, climatic conditions and experimental design of abiotic stress trial for amaranth were the same as used for quinoa. Amaranth was sown at DOY (day of the year) on days 111, 147 and 107, respectively in years 2015, 2016 and 2017. The crop cycle length ranged from 120 to 144 days during the three experimental years. The ET0 demand during the crop cycle ranged from 565 mm in 2016 to 659 mm in 2017. The total amount of applied irrigation was higher for full irrigated trials with fresh water treatments, in respect to the saline and drought treatments. This is probably due to the different transpiration rates caused by different plant development. The average ECe values (0-0.6 m) indicated an increasing trend over the three years for the saline treatments (see Table 7 in D1.8). The climatic patterns of the three experimental years created statistical differences between treatments for the interaction of Phenological stage (ph) x Water regime (Wr), and for the interaction ph x Salinity (S). No statistical differences were detected for the three ways interactions (ph x Wr x S) for the yield and the other productive parameters.

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The average pattern of the values recorded in the three years show the higher values of yield for the treatments irrigated at 100% of FC with not saline water and until flowering. More details wil be underlined in the coming specific deliverable (D1.11). The HI values show the opposite trend due to a higher vegetative growth of the well-irrigated treatment with no salt added. Furthermore, no differences were recorded between treatments irrigated during completely growing cycle and those irrigated until flowering (Table 13).

Table 13 Yield, biomass and HI of amaranth as mean of the three experimental years for the two-way interactions

Source of Variation	Yield *g plant^{-1}*	Biomass *g plant^{-1}*	HI *%*
Phenological stage x Water regime			
FI 100	4.20	52.4	7.97
FI 33	3.77	43.0	8.24
D1 100	4.29	51.5	7.61
D1 33	3.22	37.9	8.68
D2 100	3.35	49.4	7.80
D2 33	2.69	36.9	6.58
Phenological stage x Salinity			
FI N	4.54	49.0	9.51
FI S	3.36	45.5	6.63
D1 N	4.64	42.9	9.87
D1 S	2.86	47.0	6.27
D2 N	3.65	46.8	7.58
D2 S	2.41	40.4	6.89

3.1.2.3 Buckwheat

Fertilization

The effect of 6 N rates ranging from 0 to 300 N kg ha^{-1} on the Buckwheat var Panda, was evaluated in 2018 at the experimental farm located in Vitulazio; the trial was further replicated in 2019. The N (as NH_4NO_3) rates indicated in table 14 were divided (except 0) in 3 equal part (at pre-sowing, vegetative development and pre-flowering stages) and applied to the experimental plots arranged in a randomized block design with 3 replicates. At harvest, the main yield parameters and biometric traits were evaluated. The Buckwheat var Panda was sown in 10th May 2018. The field trial of 2019 is still ongoing.

In 2018, grain yield showed increasing values with the increasing N amount applied. The highest grain yield values were recorded for plants fertilized with a total N amount of 300 kg ha^{-1} (Table 14). The seed production was not related to biomass development and biometric parameters.

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Table 14 Yield and yield components as affected by fertilization on buckwheat in 2018

Treatment	Yield Mg ha^{-1}			Biomass Mg ha^{-1}			Weight 1000 seeds g			Harvest index %	
	F -test	Means		F -test	Means		F -test	Means		F -test	Means
	***			****			****			NS	
B0		0.08±0.02	cd		0.59±0.18	c		19.40±0.28	d		2.61±1.16
B100		0.05±0.02	d		0.77±0.06	c		21,68±1.07	c		4.73±0.99
B150		0.06±0.00	cd		1.28±0.09	a		24.20±0.24	a		5.94±1.09
B200		0.10±0,04	bc		0.62±0.11	c		23,25±0.54	ab		4,80±3.73
B250		0.13±0.04	ab		1.38±0.12	a		22.52±0.68	bc		6,49±0.71
B300		0.16±0.01	a		1.04±0.10	b		22.60±0.04	bc		5,59±1.60
S.E.		0.02			0,14			0.67			0.56
CV (%)		43.73			35.67			7.36			27.14

****, **** indicate respectively differences at P≤0.001 and P≤0.0001; ns indicates not significant difference. Means followed by the different letter in each column are significantly different according to the Duncan test (P=.05).*

3.1.2.4 Fava bean

Sowing date

Winter and spring sowing dates were evaluated for three fava bean varieties (Tiffany, Fuego, Taifun) cultivated under rainfed conditions. A field trial was carried out in 2017-2018 and replicated in 2018-2019 in clay soil (Vitulazio) and sandy soil (Ponticelli and Acerra) conditions. In the first trial year, the three varieties were sown on 6th December 2017 (d1) and 30th January 2018 in Vitulazio and Ponticelli. In the second trial year, the sowing dates in Vitulazio and Acerra were 13 November 2018 and 28 February 2019, respectively. At harvest, the yield components, plant height and diameter were evaluated. The trials were arranged in a randomized block design with 3 replicates.

Data collected during 2018 in Vitulazio (NA, Italy) showed no significant differences for all evaluated parameters in the Cultivar (C) x Sowing dates (Sd) interaction and for the simple effect of Sd and C. Significant differences were recorded only for the plant diameter values between Cultivars (Table 15).

Table 15 Yield and yield components as affected by cultivar and sowing date under clay soil (Vitulazio) in 2018.

Source of Variation	Grain Yield	AGB	HI	Height	Diameter
	Mg ha^{-1}	*Mg ha^{-1}*	*%*	*cm*	*cm*
Cultivar (C)	ns	ns	ns	ns	*
Tiffany	5.97±2.11	8.18±1.20	74.84±30.31	113.83±5.53	10.67±1.29 a
Fuego	5.81±2.28	6.91±1.07	82.09±35.09	105.58±11.99	9.58±1.07 ab
Taifun	4.76±1.60	7.07±1.71	60.91±20.50	107.00±8.83	8.58±0.97 b
Sowing date (Sd)	ns	ns	ns	ns	ns
Sd1	6.23±2.17	7.21±0.87	80.26±33.13	110.56±10.44	9.72±1.52
Sd2	4.80±1.56	7.61±1.72	64.96±23.47	107.06±8.43	9.50±1.28
C x Sd	ns	ns	ns	ns	ns

Means followed by different letter in each row are significantly different according to the Duncan test (P=0.05). AGB, HI, Sd_1 and Sd_2 represent, respectively: Dry above ground biomass, harvest index; "6th December"; "2nd February".

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In Ponticelli, under sandy soil conditions, significantly higher values of grain yield and 1000-grain weight were recorded for plants sown in late autumn (d1) respect to plants sown in February (d2). The variety Fuego produced more in respect to the Tiffany and Taifun varieties (Table 16).

Table 16 Yield and yield components as affected by cultivar and sowing date under sandy soil conditions in 2018.

Source of Variation		Grain Yield	AGB	HI	1000 Grain Weight
		Mg ha⁻¹	*Mg ha⁻¹*	*%*	*g*
Cultivar (C)		ns	**	ns	ns
	Tiffany	2.97±2.70	6.38±4.07 b	43.11±11.37	435.76±127.31
	Fuego	5.11±3.63	11.23±6.24a	41.39±12.73	432.43±153.46
	Taifun	3.61±2.56	7.54±4.43 b	44.55±8.48	432.37±76.02
Sowing date (Sd)		***	****	ns	***
	Sd_1	5.90±3.01 a	11.84±5.11 a	47.32±10.72	526.11±79.09 a
	Sd_2	1.90±0.80 b	4.92±1.87 b	38.72±8.62	340.93±54.05 b
C x Sd		ns	ns	ns	ns

, *, **** indicate respectively differences at P≤0.01, P≤0.001 and P≤0.0001; ns indicates not significant difference Means followed by different letter in each row are significantly different according to the Duncan test (P=0.05). AGB, HI, Sd_1 and Sd_2 represent, respectively: Dry above ground biomass, harvest index; “Sd_1 late autumn”; “Sd_2 February”.

3.1.2.5 Lupin

Sowing date

Winter and spring sowing dates were also evaluated for three lupin varieties (Boros, Butan, Tennis) cultivated under rainfed conditions. For this reason, a field trial was carried out in 2017-2018 and replicated in 2018-2019 in clay soil (Vitulazio) and sandy soil (Ponticelli and Acerra) conditions. In the first trial year, the three varieties were sown on 6th December 2017 (d1) and 2 nd February 2018 (d2) in Vitulazio and Ponticelli. In the second trial year, the sowing dates in Vitulazio and Acerra were 13 November 2018 and 28 February 2019, respectively. At harvest, yield components, plant height and diameter were evaluated. In 2019, two blue lupin varieties, Primadonna and Iris, were added in Acerra field trial. The trials were arranged in a randomized block design with 3 replicates.
The data collected in 2018 in Vitulazio showed no significant differences to Cultivar or Sowing date nor to interaction C x Sd, for all evaluated parameters except Kernel weight (Table 17). The grain yield data were too low compared to data from literature; this maybe indicates a low adaptability of lupin varieties Boros and Butan under the soil conditions of Vitulazio.

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Table 17 Yield and yield components as affected by cultivar and sowing date under lupin experiment on 2018 in Vitulazio (clay soil).

Source of Variation		Grain Yield	AGB	HI	1000 Grain Weight
		Mg ha⁻¹	*Mg ha⁻¹*	*%*	*g*
Cultivar (C)		ns	ns	ns	ns
	Boros	0.72±0.30	1.93±0.83	36.39±6.04	276.00±24.91
	Butan	0.96±0.43	2.05±0.83	45.91±8.38	260.00±27.97
Sowing date (Sd)		ns	ns	ns	*
	Sd1	0.76±0.46	1.78±0.99	40.84±12.13	283.20±26.92 a
	Sd2	0.92±0.29	2.20±0.54	41.46±3.71	252.80±16.67 b
C x Sd		ns	ns	ns	ns

** indicate respectively differences at P≤0.05; ns indicates not significant difference Means followed by different letter in each row are significantly different according to the Duncan test (P=0.05). AGB, HI, Sd_1 and Sd_2 represent, respectively: Dry above ground biomass, harvest index "6 December"; "2 of February".*

In Ponticelli some problems occurred due to birds that destroyed cotyledons of d1 plants. Only data from d2 were therefore collected (Table 18). In this case, the grain yield data is comparable with data from literature (Sellami et al., 2019).

Table 18 Yield and yield components as affected by cultivar under lupin experiment for sowing date D2 on 2018 in Ponticelli (sandy soil)

Source of Variation		Grain Yield	AGB	HI	1000 Grain Weight
		Mg ha⁻¹	*Mg ha⁻¹*	*%*	*g*
Cultivar (C)		ns	*	ns	*
	Tennis	2.75±1.04	4.63±0.44 a	59.42±20.72	419.70±11.59 a
	Boros	1.08±0.15	2.08±0.27 b	52.11±3.68	236.91±21.62 b
	Butan	2.83±0.96	4.60±1.61 a	61.66±10.30	248.02±67.77 b

ns indicates not significant difference Means followed by different letter in each row are significantly different according to the Duncan test (P=0.05). AGB and HI represent, respectively: Dry above ground biomass and harvest index.

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3.2 Denmark - (UCPH – PLEN)

3.2.1 Experimental site and climate

The trials have been carried out at the experimental station of Højbakkegaard, Tåstrup, part of the Faculty of Science, University of Copenhagen (UCPH-PLEN). Geographically, the experimental station is located at 55°40´9´´ N, 12°18´35´´ E and 28 m above the sea level. Plots in the experimental station of the University of Copenhagen rotate annually; most trials had winter cereals cultivated before our trials (e.g. winter wheat, winter barley). The soil in most plots used was a sandy clay loam soil, with a pH range between 6 – 7 and organic matter content of around 2%.

The sowing of all genetic materials was done mechanically following the recommendations from Jacobsen (2015) for sowing depth, plant density and row distance for each of the studied species. Weeding was mainly done mechanically but also manually. After sowing, nets were used to protect lupin plots from hares. Aphids were present in faba beans and quinoa in the month of July and preventive control was used to keep low populations. No other significant pest and diseases were controlled during the study.

All experimental units from the field trials had a standard size of 17 m^2 with three rows spaced by 50 cm for mechanical weed control. Only the Genotype x Environment experiment had 12.5 cm row distance as stated in the project protocol for this experiment. Studied variables focused on yield components (grain yield, thousand seed weight (TKV), protein content in grain (%), ratio of carbon and nitrogen (C:N) in grain, protein yield and growth cycle in days). To evaluate the effect of the main fixed factors under study in each trial, ANOVA analysis was performed with linear mixed models fitted to each variable. Differences between the fixed factors of interest (e.g. genotypes) were tested with post hoc pair comparisons. All analysis used R version 1.1463 (R Core Team 2015). The weather conditions under which these trials were implemented are summarized in the table 19.

Table 19 Climate data in Taastrup 2015-2018

Year	Growing season	Temperature (°C) Mean	Min	Max	Precipitation* (mm)	Evapotranspiration (mm)
2015	1/04 – 30/09	12.2	-1.1	30.7	326	367
2016	1/04 – 30/09	14.5	-1.0	31.0	336	452
2017	1/04 – 13/09	13.5	-3.6	25.2	440	372
2018	1/04 – 18/08	14.9	-1.3	28.8	64	380

For most crops the project has been working with, the land preparation followed the general agricultural practices for production systems in Denmark. Rotations generally include three main crops which are barley, winter wheat and winter rapeseed. Currently, legumes are not included in rotations and fields are left fallow. Under organic systems, it is recommended to cultivate over winter to reduce weed populations. Land preparation starts in November under conventional systems, and in February under organic systems.

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3.2.2 Crops

3.2.2.1 Peas

Sowing date: Generally, in Denmark peas can be sown early. The crop is relatively cold tolerant, so early sowing can reduce aphid damage and water stress. However, trials at UCPH-PLEN in Denmark, under the weather conditions described in Table 1, have shown numerically, higher yields. The 3rd sowing date (mid-May) gave significantly higher TKV compared to the first and second sowings. Sowing one month later increased the grain weight (TKV) by 25 g. In our trials, mid-late May resulted the optimum sowing time.

Table 10. Results of sowing date trials for Peas at UCPH-PLEN from 2015-2017

		Sowing time	Yield (kg/ha)			TKV (g)			Protein (%)			Protein yield (kg/ha)			Growing time (days)		
			Mean	s.e		Mean	s.e		Mean	s.e		Mean	s.e		Mean	s.e	
Pea	Karina	S1	858	118	a	184	11	a	26	0.9	a	224	29	a	122.9	9.9	b
		S2	1097	176	a	186	11	a	25.9	1.1	a	277	43	b	105.9	8.7	a
		S3	1544	384	a	209	11	b	26.7	0.3	a	472	92	ab	98.5	8.2	a

Note: S1: mid-April; S2: beginning of May; S3: Mid-May. s.e.: standard error of the mean. Means followed by the same letters are not significantly different.

Yields: in the screening trial at UCPH-PLEN grain yield means for four years (2015 – 2018) ranged from 1.2 - 2.9 t/ha, with a protein range of 20-28%. The highest yielding cultivars include Eso (3.4 t/ha), Atlas (2.4 t/ha) and Nitouche (2.3 t/ha). High protein cultivars include Maxigolt (28.5%) and Utrillo (27.9%).

Table 21 Screening trial data for Peas in Taastrup, Denmark (2015-2018)

PEAS	Yield (kg/ha)			TKV (g)			Protein (%)			C:N			Protein Yield (kg/ha)			Grow time (days)		
	M	SE		M	SE		M	SE		M	SE		M	SE		M	SE	
Brun.Ært Nakskov	1977	611	ac	314	16	ef	22.7	0.4	cd	12.1	0.3	abcd	450	140	ab	115	10.7	a
Erindlev.Ært	1466	470	ab	185	15	a	22.8	1.3	cd	12	0.7	abe	315	92	abc	115	10.7	a
LollandskeRosiner	2141	719	ac	322	24	f	22.0	1.0	ac	12.4	0.6	ab	460	164	ab	115	10.7	a
Atlas	2412	754	bc	280	28	de	19.7	0.3	a	13.7	0.4	f	344	114	a	115	10.7	a
Eso	3409	1004	c	233	11	abd	18.8	1.2	a	14.1	0.9	f	426	80	ab	115	10.7	a
Karina	1182	559	a	198	10	ab	24.2	0.9	d	11.5	0.4	e	286	141	c	115	10.7	a
Maxigolt	1498	604	ab	233	28	ac	28.5	0.8	e	10.1	0.4	g	429	173	abc	115	10.7	a
Nitouche	2267	716	bc	254	14	cd	22.3	0.7	bcd	12.2	0.4	ac	503	164	a	115	10.7	a
Pinochio	1591	485	ab	206	14	ab	19.9	1.4	ab	13.6	1.1	cdf	234	85	bc	116	7.6	a
SvenskStorGråärt	1273	833	ab	345	19	f	23.9	0.0	cd	11.9	0.4	bd	305	200	abc	115	10.7	a
Utrillo	1711	661	ab	227	28	bc	27.9	0.7	e	10	0.2	g	466	176	ab	115	10.7	a

Note: M: Mean SE: standard error of the mean. Means followed by the same letters are not significantly different.

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*Conclusions***:** As a N fixer, pea is a beneficial crop to grow, and it gives high yields, as it is well suited to the Danish soils and climate. Pea production is already significant in Denmark, although mainly produced for feed and at a lesser scale for food, although with good potential. In 2018 there were 6657 ha dry peas, 3098 ha fresh peas and 6201 ha for whole seed silage (SEGES, 2019). Since 2015, Danish pea production has increased by 43%, and 30% of this production was organic in 2018.

3.2.2.2 Lentils

*Sowing date***:** Field trials at UCPH-PLEN (2015-2017) showed that lentils produce higher yields when sown earlier. Sowing four weeks after the beginning of the sowing season reduced yields by 484 kg/ha compared to the earliest sowing. Furthermore, earlier sowing resulted in bigger seed size, 2.3 g heavier in the first sowing compared to the last.

Table 22. Results of sowing date trials for Lentils at UCPH-PLEN from 2015-2017

		Sowing time	**Yield (kg/ha)**			**TKV (g)**			**Protein (%)**			**C:N**			**Protein yield (kg/ha)**			**Growing time (days)**		
			M	s.e		M	s.e		M	s.e		M	s.e		M	s.e		M	s.e	
Lentil	Pardina	S1	911	241	b	38	2	b	25.8	0.3	a	10.5	0.2	a	232	61	ab	135.9	22.6	b
		S2	757	199	ab	35	1	a	25.5	0.4	a	10.7	0.2	a	195	52	a	119.3	19.9	a
		S3	427	111	a	36	1	a	25	0.3	a	10.9	0.2	b	105	26	b	109.8	18.3	a

Note: S1: mid-April; S2: beginning of May; S3: Mid-May. M: Mean; SE: standard errors. Means followed by the same letters are not significantly different.

Yields: 32 accessions of lentil were screened in trials at UCPH-PLEN from 2015-2018. Yields ranged from 181 – 1128 kg/ha and seed protein ranged from 16.7 to 32.3 %.The 10 highest yielding cultivars (mean averages for the years tested), of a total of 32 tested, are shown in table 5 below. Those cultivars with a grain yield above 1t/ha are underlined.

Table 23. Screening trial data for Lentils in Taastrup, Denmark (2015-2018)

LENTILS	**Yield (kg/ha)**			**TKV (g)**			**Protein (%)**			**C:N**		**Protein yield (kg/ha)**		**Growing time (days)**		
	M	SE		M	SE		M	SE		M	SE	M	SE	M	SE	
Anissia	514	0	abc	26	0	abce	28	0	be	9.7	0.1	145	0	130	18	a
Delikatesse.linser.Herkulinsi	585	0	abc	23	0	abd	30	0	be	9.2	0.1	173	0	130	18	a
Dunkelgrüne.marmorierte	587	0	abc	24	0	abd	30	0	be	9	0.1	178	0	130	18	a
Eston	1091	244	bc	31	2	abc	27	0.7	bcd	10	0.2	290	61	130	16.4	a
Gotlandlins	724	0	bce	20	0	abc	31	0	ce	8.8	0.1	227	0	130	18	a
Morena	601	181	c	49	7	bce	27	1.1	bcd	10	0.2	160	49	130	16.4	a
Pardina	539	275	bc	29	3	abd	26	1.0	bc	10.3	0.1	144	73	132	16	a
Späths.Alblinse.I.Die.Grosse	822	0	bc	32	0	cdfg	33	0	de	8.3	0	269	0	130	18	a
Urtekrams.Beluga.Linser	683	0	bcd	17	0	ab	31	0	ce	8.7	0.1	214	0	130	18	a

Note: M: Mean; SE: standard errors. Means followed by the same letters are not significantly different.

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Intercropping lentils/oats trials at UCPH-PLEN from 2017-2018 (continuing in 2019) have found 71% less weeds in the lentil-oat intercrop compared to 100% oats. The land equivalent ratio (LER) for the grain yield was between 0.98 and 2.4 for the different intercrop ratios and years. When LER>1, the intercrop can be recommended. This was the case for a humid year (2017) with a 66 lentils/33 oats intercropping ratio, but not for a dry one (2018). An important aspect to consider though for this system is that seed sorting can be difficult due to the similar size of lentils and oats. Benefits of this intercropping combination are weed reduction and that oats provide a support for lentils harvesting.

Table 24 Relative grain yield (RY) and land equivalent ratio (LER) for a "lentil/oat" intercrop with five ratio treatments, 2017

Ratio L	Ratio O	Total yield kg/ha	Yield L	Yield O	RY L	RY O	LER
1	**0**	933.10	933.10	0.00	1.00	0.00	**1.00**
0.66	**0.33**	2058.90	208.38	1850.53	0.22	0.76	**0.98**
0.5	**0.5**	2336.08	191.10	2144.98	0.20	0.88	**1.08**
0.33	**0.66**	2340.08	167.65	2172.43	0.18	0.89	**1.07**
0	**1**	2448.40	0.00	2448.40	0.00	1.00	**1.00**

L=Lentils; O=oats; mean values (n = 4) ± S.E. Harvest was done at 147 DAS

Table 25. Relative grain yield (RY) and land equivalent ratio (LER) for a "lentil/oat" intercrop with five ratio treatments, 2018

Ratio L	Ratio O	Total yield kg/ha	Yield L	Yield O	RY L	RY O	LER
1.00	**0.00**	697.10	697.10	0.00	1.00	0.00	**1.00**
0.66	**0.33**	3402.83	1130.20	2272.63	1.62	0.78	**2.40**
0.50	**0.50**	3155.18	1190.03	1965.15	1.71	0.67	**2.38**
0.33	**0.66**	3078.50	993.25	2085.25	1.42	0.71	**2.14**
0.00	**1.00**	2922.70	0.00	2922.70	0.00	1.00	**1.00**

L=Lentils O=oats; mean values (n = 4) ± S.E. Harvest was done at 103 DAS.

Conclusions: Lentils are beneficial as they are N fixers and a good source of protein. Lentil yields varied year to year and were low compared to world and European average yields. However, with variety of selection, and in the future climate, they may be suited to cultivation in Denmark. Further intercrops could be tested using other crops in combination with lentils. There is no widely available data on the area of lentil production in Denmark currently.

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3.2.2.3 Lupins

Soil type: Trials at UCPH-PLEN 2017-18 found that in a dry year (2018), yields of lupin were approximately double on clay soil compared to sandy soil, with up to 1t/ha more yields in clay soil. Seed quality was also higher, and protein yields almost doubled in the clay soil.

Table 26 GxE trial data for Lupins in Taastrup, Denmark (2015-2018)

	Yield (kg/ha)					**Protein (%)**			
	Clay		**Sandy**			**Clay**		**Sandy**	
Acc	Mean	SE	Mean	SE		Mean	SE	Mean	SE
Boros	1210.3	*153*	497.6	*153*	**	38.1	*1.1*	39.7	*1.1*
Butan	1700.2	*153*	762.0	*153*	***	41.4	*1.1*	41.2	*1.1*
Iris	2746.4	*153*	1485.4	*153*	***	34.3	*1.1*	34.2	*1.1*
Primadona	2439.6	*153*	1267.1	*153*	***	32.3	*1.1*	32.5	*1.1*

*Note:SE: standard error. Stars represent significant difference between sandy and clay soils, *:p<0.05, **:p<0.01, ***:p<0.001.*

Sowing date: Lupins can be grown from the 1st of April, or as soon as the soil is ready. Soil temperature should be at least 5°C (Alandia *et al*, 2019). Trials at UCPH-PLEN 2015-2017 showed that generally there is no change in yields of Lupins sown in May compared to April. Although, in some cultivars this may vary. For example, Boregine showed better yields when sown later (yield is 1097 kg/ha higher when sown in S2 (beginning of May) compared to S1 (mid-April)), while Iris has higher TKV (14 g higher in S1 compared to S3 (mid-May)), and a trend of higher yields in early sowings.

Table 27 Results of sowing date trials for Lupins at UCPH-PLEN from 2015-2017

		Sowing time	**Yield (kg/ha)**			**TKV (g)**			**Protein (%)**			**C:N**			**Protein yield (kg/ha)**			**Growing time (days)**		
			M	s.e		M	s.e		M	s.e		M	s.e		M	s.e		M	s.e	
Lupin	Boregin*	S1	2574	424	a	168	3	a	29.4	2.2	a	8.5	0.7	a	784	177	a	152.8	10.1	a
		S2	3671	67	b	160	4	a	25.1	0.6	a	10	0.6	b	919	20	ab	144.6	9.7	ab
		S3	3571	177	ab	159	4	a	29.2	1.1	a	8.8	0.5	a	1046	88	b	133.3	9.1	b
	Iris	S1	2147	391	a	148	3	a	33.8	1.7	a	8.2	0.5	a	503	60	a	153.4	8.2	a
		S2	1932	452	a	135	6	ab	34.4	1.1	ab	8.2	0.4	a	639	141	a	140.2	7.7	ab
		S3	1764	529	a	134	6	b	33.5	0.8	b	8.4	0.4	a	564	160	a	131.8	7.3	b
	Primadona*	S1	2025	138	a	163	2	a	26.8	1.1	a	9.3	0.6	a	542	35	a	138.9	9.4	a
		S2	2497	161	a	154	2	a	27.5	1.3	a	9.2	0.6	a	685	47	a	123.8	8.6	a
		S3	2229	309	a	163	3	a	26.4	1.0	a	9.6	0.6	a	585	77	a	133.3	9.1	a
	Regent*	S1	3200	388	a	132	5	a	27.7	1.6	a	9.3	0.7	a	883	108	a	159.7	10.4	a
		S2	3912	1078	a	120	9	a	27.1	3.0	a	9.4	0.6	a	1058	244	a	144.6	9.7	ab
		S3	4082	1105	a	132	8	a	24.2	3.3	a	10.3	0.8	a	994	274	a	133.3	9.1	a

Note: M: Mean; SE: standard errors. Means followed by the same letters are not significantly different.

Screening: The highest yielding cvs in trials at UCPH-PLEN were Regent (PL), Boregin (DE), Dieta (UK). Highest protein levels were found in the *L. mutabilis* lines (27CA and 27CB),

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however these were lower yielding species, partly due to their long growth cycle. Seed weights varied widely between the different species – generally the *L.albus* with a larger seed size and the *L. angustifolius* with smaller.

Table 28 Screening trial data for Lupins in Taastrup, Denmark (2015-2018)

LUPIN			**Yield (kg/ha)**			**TKV (g)**			**Protein (%)**			**C:N**		**Protein Yield (kg/ha)**		**Grow time (days)**		
CVR	*Orig*	Spp.	*M*	*SE*		*M*	*SE*		*M*	*SE*		*M*	*SE*	*M*	*SE*	*M*	*SE*	
27CA	BO	*mutab.*	716	383	a	157	19	a	50.0	0.5	b	6.4	0	357	114	186.5	9.7	b
27CB	BO	*mutab.*	1006	383	ab	193	10	ab	48.4	0.2	b	6.6	0	486	99	188	9.7	b
Boregine	DE	*angust.*	2815	469	c	203	4	ab	30.2	2.5	a	9.7	0.7	850	64	148	12.2	a
Boros	PL	*albus*	2038	469	bc	227	75	ac	34.4	5.1	a	8.6	1	644	91	148	12.2	a
Boruta	DE	*angust.*	2363	663	bc	144	0	a	37.2	0.0	a	7.8	0.1	879	0	_	_	_
Butan	PL	*albus*	1539	663	ac	312	0	bc	37.7	0.0	a	8	0.1	580	0	_	_	_
Dieta	UK	*albus*	2588	663	c	172	136	c	33.0	3.9	a	7.7	0.1	456	382	_	_	_
HaagsBlue	DE	*angust.*	2177	663	ac	147	0	a	30.8	0.0	a	9.3	0.1	671	0	_	_	_
Iris	DK	*angust.*	2051	383	bc	150	5	a	32.2	1.6	a	8.9	0.4	638	130	147	8.6	a
Mirabor	DE	*angust.*	1887	663	ac	190	0	ab	33.3	0.0	a	8.6	0.1	628	0	_	_	_
Primadonna	DK	*angust.*	2086	383	c	152	11	a	35.4	2.4	a	8.1	0.5	756	144	147	8.6	a
Probor	DE	*angust.*	2365	663	bc	151	0	a	33.2	0.0	a	8.6	0.1	786	0	_	_	_
Regent	PL	*angust.*	2929	469	c	177	6	a	32.7	0.9	a	8.8	0.1	958	27	148	12.2	a

Note: M: Mean; SE: standard errors. Means followed by the same letters are not significantly different.

Conclusions: Lupin is a high yielding crop in Denmark, with high protein content and N fixing properties. It can be beneficial in crop rotations as well as for feed and food. Further research, which is also under way in the Protein2Food project, is necessary to continue to develop food products with lupins. In Denmark in 2018, there were 346 ha of Lupin production, 79% of which was organic. This was a 53% increase compared to the lupin production area in 2015 (226 ha) (Landbrugsstyrelsen, 2015, 2018).

3.2.2.4 Faba Beans

Soil type: Field trials at UCPH-PLEN 2017-2018 found faba beans to increase yields significantly in different soil types: with three varieties (Taifun, Tiffanny, Fuego) yields were over 500 kg/ha higher in clay soil compared to sandy soils. The fourth variety tested (Sampo) did not give stable yield results. The grain size (TKV) was significantly higher in clay soil than sandy, in all four cvs tested. In one of the materials tested, the protein content (%) was slightly lower in the clay (Table 29). These trials continue in 2019.

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Table 29 GxE trial data for Fava beans in Taastrup, Denmark (2015-2018)

	Yield (kg/ha)					Protein (%)				
	Clay		Sandy			Clay		Sandy		
Acc	Mean	SE	Mean	SE		Mean	SE	Mean	SE	
Sampo	205.8	*153*	11.5	*153*		30.4	*1.1*	34.8	*1.6*	*
Taifun	731.9	*153*	211.9	*153*	*	27.5	*1.1*	29.1	*1.1*	
Tiffanny	937.2	*153*	212.8	*153*	***	26.4	*1.1*	29.3	*1.1*	.
Fuego	804.2	*153*	176.2	*153*	**	28.6	*1.1*	30.4	*1.1*	

*Note: SE: standard error. Stars represent significant difference between sandy and clay soils, *:p<0.05, **:p<0.01, ***:p<0.001.*

Sowing date trials at UCPH-PLEN (2015-2017) found that faba beans produced higher yields when sown early in S1 and S2 (mid-April and beginning of May), compared to S3 (mid-May). The earliest sowing gave yields 1457 kg/ha higher than the latest sowing. But the grain size was significantly higher at earlier sowing dates, 111 g higher in the first sowing date compared to the last (Table 12).

Table 30. Results of sowing date trials for Faba beans at UCPH-PLEN from 2015-2017

		Sowing time	Yield (kg/ha)			TKV (g)			Protein (%)			C:N			Protein yield (kg/ha)			Growing time (days)		
			M	s.e		M	s.e		M	s.e		M	s.e		M	s.e		M	s.e	
Faba bean		S1	2217	371	b	515	11	c	30.1	0.3	a	9.1	0.1	a	671	117	ab	145.1	5.8	c
	Colombo	S2	2669	217	b	451	13	b	30.3	0.4	a	9.5	0.2	b	795	73	a	129	4.9	b
		S3	1457	362	a	404	9	a	30.5	0.6	a	8.9	0.2	c	430	102	b	114.5	4.6	a

Note: S1: mid-April; S2: beginning of May; S3: Mid-May. M: Mean; SE: standard errors. Means followed by the same letters are not significantly different.

Yields: from screening trial at UCPH-PLEN (2015-2018) are shown in Table 31. Highest yielding cvs are underlined. As a reference, world yields for this crop are on average 1.8 t/ha, the yield in Asia reaches 2.1 t/ha and in Europe 2.7 t/ha (FAOStat, 2019).

Table 31. Screening trial data for faba beans in Taastrup, Denmark (2015-2018)

Faba beans Cv.	Yield (kg/ha) M	SE		TKV (g) M	SE		Protein (%) M	SE		C:N M	SE		Protein Yield (kg/ha) M	SE		Growing time (days) M	SE	
<u>Alexia</u>	2796	885	b	521	27	b	29.8	0.3	b	9.6	0.2	ab	834	266	a	139.5	8.35	ab
Colombo	2094	585	ab	501	16	ab	29.5	0.7	ab	9.3	0.3	a	633	194	b	129	8.03	a
Fuego	1879	597	a	473	34	a	27.3	1.6	a	10.7	0.6	c	526	181	b	152.5	8.73	b
<u>Gracia</u>	2757	990	b	511	31	ab	28.2	0.6	ab	9.8	0.2	bc	789	293	ab	139.5	8.35	ab
<u>Julia</u>	2782	906	b	481	31	ab	28.3	1.0	ab	9.8	0.3	abc	810	282	ab	139.5	8.35	ab

Note: Or: Origin; M: Mean; SE: standard errors. Means followed by the same letters are not significantly different.

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Winter faba beans

Trials at UCPH-PLEN (2015-2018) tested 11 accessions of winter faba beans sown in October and April. These survived temperatures of -12°C, and a drought year (2018). Winter sowing gave a yield gain of 118% for winter fava beans compared to spring beans. The first week of October was identified as the optimum sowing date in order to avoid aphid attacks. Flowering was 27 days earlier, and harvest was 26 days earlier in winter beans. However, bean seed beetle infestation was significantly higher in winter-sown beans.

Table 2 Average yields of winter faba beans tested in two sowing dates (Winter/Spring) in Denmark from 2015 to 2017

Season	Yield, t/ha	Year	Sowing date	Yield t/ha	SE
Winter	5.428	2015	Oct 12	5.151	1.29
		2016	Sept 19	5.153	1.07
		2016	Oct 07	6.317	1.31
Spring	2.490	2016	Apr 12	2.045	1.66
		2017	Apr 11	2.754	0.88
		2017	Apr 27	2.471	0.84

Note: SE=standard error to the mean.

Conclusions: Faba bean is the second most cultivated grain legume in Europe and it is highly cold tolerant, which gives it good potential for northern Europe. Green manure can be considered as a potential side stream. The grain is high in fiber and can have levels of protein in the range of 22-38%. Nowadays it is used in Denmark mainly for feed and has high potential to be re-introduced and promoted as food dry, fresh and as flour. Challenges for its production are mainly related to market development, plant protection management and breeding for lower anti-nutrient contents in the seed.

3.2.2.5 Soy Beans

Sowing date Three years of trials at UCPH-PLEN indicated that for the cultivar used (Regina cv), 15 and 30 days of difference after the start of the thermal sowing season did not have an impact on the production and grain quality variables analysed.

Table 33. Results of sowing date trials for Soy beans at UCPH-PLEN from 2015-2017

		Sowing time	Yield (kg/ha)			TKV (g)			Protein (%)			C:N			Protein yield (kg/ha)			Growing time (days)		
			M	s.e		M	s.e		M	s.e		M	s.e		M	s.e		M	s.e	
Soy bean	Regina*	S1	148	97	a	158	1	a	36.4	0.0	a	8.7	0.0	a	121	51	a	188	7.9	b
		S2	91	36	a	154	4	a	34.7	0.6	a	8.7	0.3	a	34	18	a	172	7.6	ab
		S3	172	70	a	163	4	a	34.0	1.3	a	9.2	0.4	a	131	1	a	161.3	7.3	a

Note: S1: mid-April; S2: beginning of May; S3: Mid-May. M: Mean; SE: standard errors. Means followed by the same letters are not significantly different.

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Yields: from screening trial from UCPH-PLEN (2015-2018) are shown below. Erica was the highest yielding cultivar.

Table 34. Screening trial data for Soybeans in Taastrup, Denmark (2015-2018)

SOYBEANS	Yield			TKV			Protein			C:N		Protein Yield		Growing time		
	M	SE		M	SE		M	SE		M	SE	M	SE	M	SE	
Abelina	606	39	ab	142	2	abc	31.6	0.7	ab	10.0	0.3	191	8	157.7	15.5	b
Bohemians.C1	382	130	a	175	18	bd	32.8	1.7	b	9.6	0.6	123	39	90.3	8.1	a
Erica	2720	2221	ab	177	37	abc	23.4	6.6	ab	10.0	0.3	241	47	_	_	_
Madina	1019	0	bc	134	0	abc	32.6	0.0	ab	9.3	0.3	332	0	_	_	_
Merlin	633	153	bc	120	32	b	31.4	2.8	b	10.9	0.4	201	52	92.3	8.2	a
Moravians.C1	540	217	ab	147	44	ad	33.9	2.5	ab	8.9	0.6	184	81	90.8	8.1	a
Regina	1100	27	c	176	5	cd	37.4	2.1	a	8.4	0.5	410	13	157.7	15.5	b
Royka	543	95	ac	202	17	abc	35.4	1.4	ab	9.3	0.3	196	42	_	_	_
SG.ANSER	399	173	ab	185	15	d	34.9	2.1	ab	9.0	0.4	129	51	91.8	8.2	a
Silesia.E	317	117	a	154	10	cd	34.2	1.1	ab	9.0	0.6	106	37	91.3	8.2	a
Vilshanka	606	39	a	142	2	bc	31.6	0.7	ab	9.3	0.3	191	8	89.8	8.1	a

Note: M: Mean; SE: standard errors. Means followed by the same letters are not significantly different.

The results of the screening trial between 2015 and 2018 showed very high variation between years. This variation is mainly explained by the difference of weather conditions as showed in Table 1 and by the difference in the number of materials that were included each year that generated an unbalanced model. Results of this trial showed mean yields that ranged from 0.3 to 1.1 t/ha. The best average yielding cvs were Erica (2.7 t/ha), Regina (1.1 t/ha) and Madina (1.0 t/ha). Yields were highest in warmer, drier years.

Conclusions: World and the European yield averages of soybean are markedly higher than the yields from our trials. As such, the adaptation of these materials to Danish conditions does not seem promising. Furthermore, the materials used had quite low protein contents (31-37%) compared to the potential this crop can have for protein production. Between 2015 and 2018, cultivation of Soybeans in Denmark has decreased from 17 ha to just 1 ha in 2018 (Landbrugsstyrelsen, 2018). This is in line with an increase in Danish faba bean production for feed. More breeding is therefore necessary to adapt soybeans to the northern European climate as they do have beneficial characteristics. Indeed, they are N-fixing, can adapt to calcareous and water logged soils, tolerate high temperatures and can be used as green manure. Aspects of crop management to take into account in further trials are soil fertility, proper weed and disease management as well as its sensitivity to drought during flowering.

3.2.2.6 Buckwheat

Sowing date: Trials at UCPH-PLEN tested two cultivars of buckwheat (Russian line in 2015 and in 2017; Mancan in 2016) and found that later sowing tends to give higher yields in both cultivars tested. In Mancan, a Canadian variety, S1 and S3 sowing dates gave highest yields. Russian gave yields 1300kg/ha higher in S3 than the S1 sowing date. Therefore, under Danish conditions, sowing is recommended in May rather than April.

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Table 35. Results of sowing date trials for Buckwheat at UCPH-PLEN from 2015-2017

		Sowing time	Yield (kg/ha)			TKV (g)			Protein (%)			C:N			Protein yield (kg/ha)			Growing time (days)	
			M	s.e		M	s.e		M	s.e		M	s.e		M	s.e		M	s.e
Buckwheat	Mancan*	S1	2202	455	ab	30.0	3.0	a	12.7	0.7	a	22.5	1.5	a	271	48	a	_	_
		S2	1498	155	a	36.4	0.3	b	10.7	0.1	a	26.9	1.4	b	161	16	b	_	_
		S3	2331	38	b	29.8	0.3	a	12.4	0.2	a	22.8	1.1	a	288	9	a	_	_
	Russian	S1	1285	400	a	31.6	0.1	a	14.9	0.9	a	18.5	1.6	ab	213	45	a	_	_
		S2	2103	240	b	32.8	0.4	a	14.6	0.9	a	17.8	1.4	a	303	32	b	_	_
		S3	2604	254	b	32.0	0.5	a	13.1	0.7	a	21	1.9	b	337	31	b	_	_

Note: S1: mid-April; S2: beginning of May; S3: Mid-May. M: Mean; SE: standard errors. Means followed by the same letters are not significantly different.

Screening trial at UCPH-PLEN 2015-2018. Yields from trials were high, up to 1.9 t/ha, while world and European yields were 0.97 t/ha and 1.08 t/ha respectively (FAOStat, 2019).

Table 36. Screening trial data for Soybeans in Taastrup, Denmark (2015-2018)

Buckwheat	Yield (kg/ha)			TKV (g)			Protein (%)			C:N		Protein yield (kg/ha)		Growing time (days)		
Acc	M	SE		M	SE		M	SE		M	SE	M	SE	M	SE	
Adja.Darja	1026	342	cef	32.1	2.4	f	13.7	0.3	ac	21	0.9	138	43	132	9.3	a
Kora	1212	167	eg	25.7	0.3	cd	13.3	0.2	ac	21	0.2	160	20	135	13	a
Mancan	1030	463	bcef	22.6	1.1	ab	13.4	0.1	c	20	0.3	206	30	132	9.3	a
Panda	1142	6	fg	28.2	1.1	def	13.7	0.1	ab	22	0.4	157	2	135	13	a
Polish.NN	1156	129	def	30.0	0.9	fg	13.7	0.1	ac	21	0.4	159	17	132	9.3	a
Russian	1292	402	deg	29.9	0.2	ef	13.7	0.1	c	20	0.8	178	57	132	9.3	a
Spacinska	1651	135	cef	23.3	0.3	bc	13.0	0.4	bc	20	1	217	25	132	9.3	a
Tartary.Z	1684	299	g	19.6	0.9	a	13.7	0.5	a	23	0.9	227	33	132	9.3	a

Note: M: Mean; SE: standard errors. Means followed by the same letters are not significantly different.

3.2.2.7 Quinoa

Soil type: Trials at UCPH-PLEN 2017-2018 found no significant differences in yields between quinoa grown on a sandy and clay soil.

Table 3 GxE trial data for Quinoa in Taastrup, Denmark (2015-2018)

	Yield (kg/ha)				Protein (%)				
	Clay		Sandy		Clay		Sandy		
Acc	Mean	SE	Mean	SE	Mean	SE	Mean	SE	
Titicaca	210.1	*262*	60.2	*262*	14.0	*1.3*	24.4	*1.3*	***
Vikinga	23.2	*262*	29.2	*262*	18.4	*1.3*	15.8	*1.3*	
Puno	13.0	*262*	24.4	*262*	17.9	*1.3*	16.3	*1.3*	

*Note: SE: standard error. Stars represent significant difference between sandy and clay soils, *:p<0.05, **:p<0.01, ***:p<0.001.*

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 635727.

<u>*Sowing date:*</u> <u>Trials at UCPH-PLEN 2015-2017</u> showed that there may be better yields and seed size at earlier sowing dates, depending on the cultivar. The three Danish cultivars were tested for this trial: Titicaca from 2015 to 2017; Puno and Vikinga in 2017.

Table 38. Results of sowing date trials for Quinoa at UCPH-PLEN from 2015-2017

		Sowing time	Yield (kg/ha)			TKV (g)			Protein (%)			C:N			Protein yield (kg/ha)			Growing time (days)		
			M	s.e		M	s.e		M	s.e		M	s.e		M	s.e		M	s.e	
Quinoa	Puno*	S1	1229	266	a	2.3	0.1	a	12.9	2.1	a	22.2	1.1	a	119	36	a	159	10.8	a
		S2	913	122	a	2.2	0.1	a	12.6	2.1	a	22.2	0.9	a	99	22	a	143	9.9	ab
		S3	917	116	a	2.2	0.1	a	12	2.1	a	23.7	0.1	a	120	17	a	131.7	9.3	b
	Titicaca	S1	1142	162	ab	3.6	0.1	a	14	1.2	a	20	0.6	a	160	20	ab	137.2	7.9	a
		S2	1394	208	a	3.4	0.1	b	14.6	1.2	a	18.9	2.2	ab	204	27	a	129.2	7.5	ab
		S3	865	137	b	3.2	0.1	b	13	1.2	a	21.9	0.5	b	112	19	b	122.2	7.2	b
	Vikinga*	S1	1631	421	a	3	0.1	a	13.1	1.0	a	21.6	1.0	a	173	48	a	138.3	9.7	a
		S2	927	354	ab	2.7	0.1	b	15.4	1.0	b	18.0	2.0	b	124	38	ab	143	9.9	a
		S3	423	106	b	2.7	0.1	b	14.1	1.0	a	20.2	0.4	a	71	16	b	131.7	9.3	a

Note: S1: mid-April; S2: beginning of May; S3: Mid-May. M: Mean; SE: standard errors. Means followed by the same letters are not significantly different

Fertilisation x Density trials under organic production at UCPH-PLEN 2016-2018 (continuing 2019)

The trial tested three quinoa cultivars, four fertilizer levels and two plant densities. There were genotypic differences in the response to fertilization and plant density. Titicaca was more sensitive to N fertilisation (in biomass, yield, foliar area and chlorophyll content) compared to the other two cultivars tested. Both Vikinga and Puno had a stable response to N variation (Figure 6). Puno was more sensitive to higher densities (10 kg/ha) with a higher fraction of intercepted light compared to the low plant density tested (5 kg/ha) (refer to Figure 7).

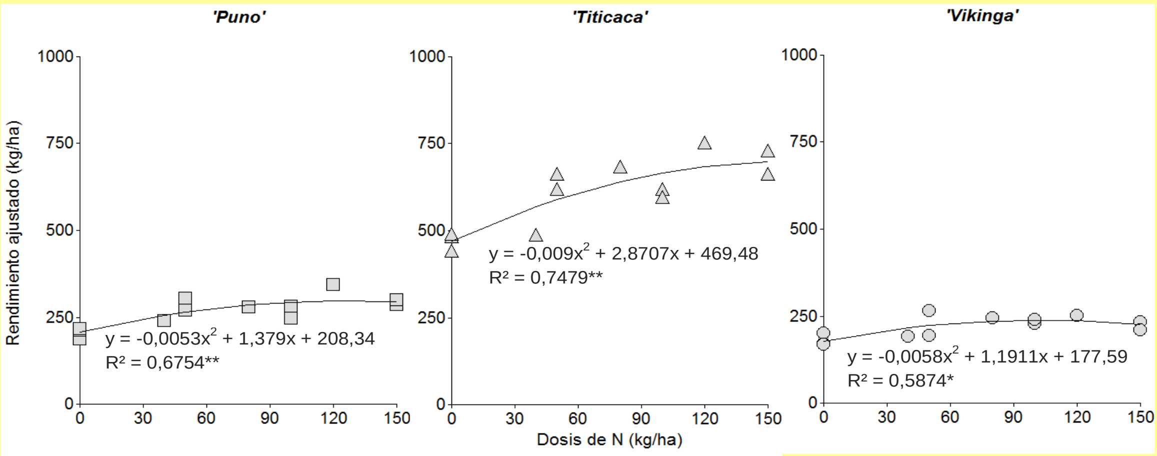

Figure 6 Adjusted yield of three quinoa varieties in relation to N fertilization doses, 2016, 2017 and 2018, in Taastrup, Denmark.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 635727.

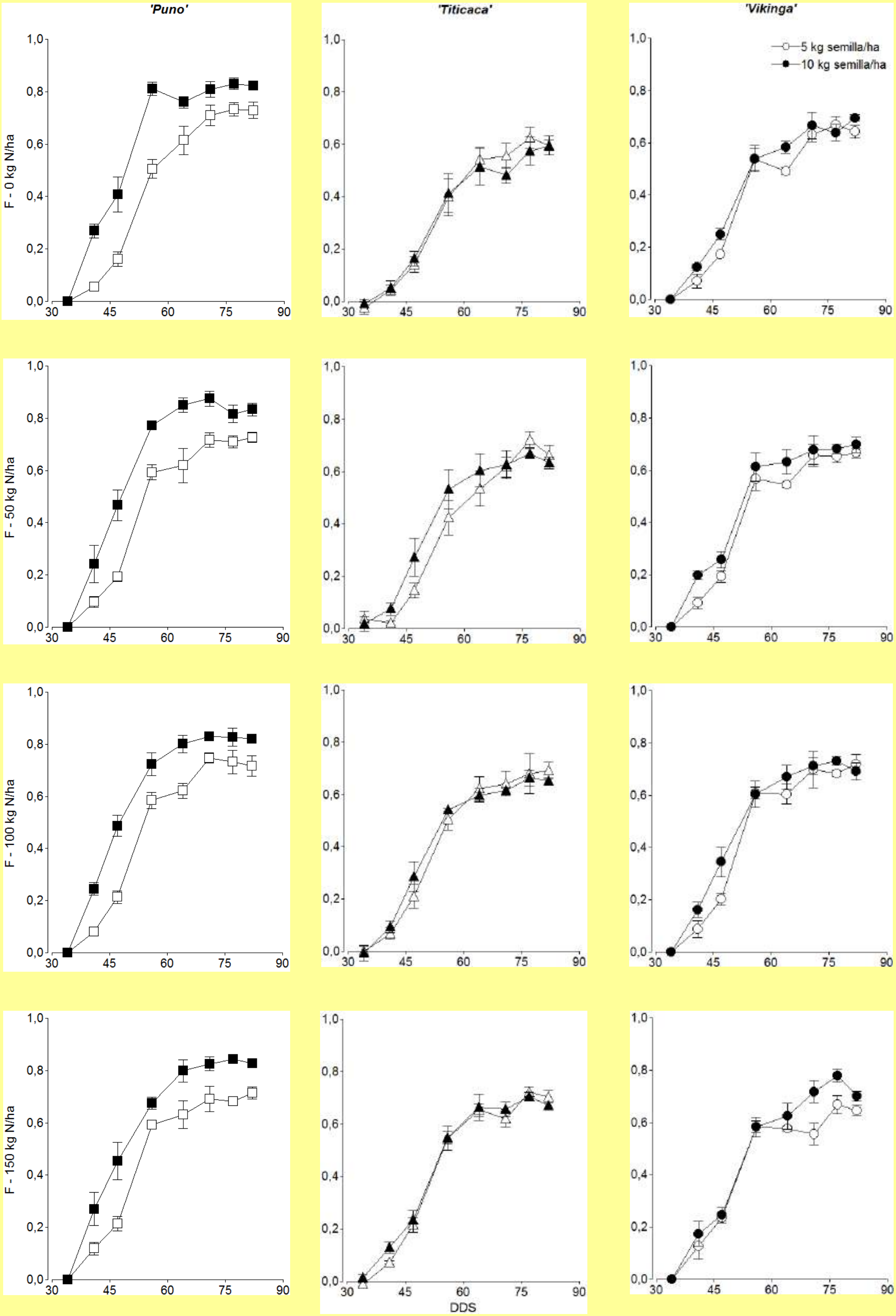

Figure 7 Intercepted fraction of photosynthetic active radiation (F) in relation to the days after sowing (DDS) in three quinoa genotypes and two sowing densities (5 and 10 kg/ha).

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 635727.

Yields: from screening trials at UCPH-PLEN are shown below. The trials gave yields up to 2.6 t/ha. In comparison, average world yields for quinoa are 847 kg/ha (FAOStat 2019).

Table 39. Screening trial data for Quinoa in Taastrup, Denmark (2015-2018)

Quinoa		Yield (kg/ha)			TKV (g)			Protein (%)			C:N			Protein Yield (kg/ha)			Growing time (days)		
		M	SE		M	SE		M	SE		M	SE		M	SE		M	SE	
Atlas	NL	2229	253	ab	2.5	0.3	c	12.1	0.5	b	18	1	c	272	36	abcf	184	8	d
ICBA.Q3	UAE	893	0	ab	1.4	0.0	abc	14.0	0.0	b	15.7	1	a	125	0	ab	151	12	abc
ICBA.Q4	UAE	1919	0	c	1.8	0.0	abc	13.3	0.0	b	18.4	1	c	254	0	d	194	14	d
ICBA.Q5	UAE	926	0	ab	1.5	0.0	abc	13.6	0.0	b	16	1	b	126	0	c	151	12	abc
Jessie	FR	2596	189	ab	2.5	0.3	c	16.0	0.7	b	18.1	2	abc	420	48	be	151	7	b
Pasto	NL	1981	185	b	2.1	0.1	bc	16.0	2.1	b	19	1	cf	321	58	abcf	184	8	d
Puno	DK	1054	195	ab	2.2	0.1	bc	13.7	0.6	a	24.3	1	d	141	23	abcf	155	7	b
Red.Quinoa	-	1187	0	bc	1.8	0.0	abc	14.9	0.0	ab	21	1	de	176	0	e	194	14	d
Riobamba	NL	353	191	ab	1.9	0.3	b	14.6	1.1	b	19.7	1	f	55	30	abf	176	8	cd
Titicaca	DK	400	192	ac	3.1	0.5	a	19.6	0.8	b	19.4	1	cef	73	35	ef	130	7	a
Vikinga	DK	692	348	ab	2.2	0.4	bc	18.6	0.8	b	18.1	2	abcf	123	64	a	150	7	b

Note: M: Mean; SE: standard errors. Means followed by the same letters are not significantly different.

Conclusions: Quinoa is adapted to different areas of cultivation, now including Danish climate and soil types. It can produce in dry and saline environments. It has a high protein content (14-18%, of which 73% can be absorbed), and all the essential amino acids (Jacobsen, 2015). However some cultivars contain high levels of saponin, therefore sweet varieties (<11% saponin) must be selected. There are some potential uses of quinoa side-streams – for example saponins for cosmetics, soaps etc. which are still to be investigated. However, quinoa is a highly productive crop which has shown itself to be well suited to Danish production and to the consumer market. Breeding efforts in Denmark have established varieties, which are suited to the day length and therefore are successful in this climate (Jacobsen, 2017). Quinoa production in Denmark increased from 6 ha in 2015 (100% organic) to 159 ha in 2018 (29% organic) (Landbrugsstyrelsen, 2018). This represents a 2550% increase in land area.

3.2.2.8 Amaranth

Sowing density, depth, row distance: Amaranth has been grown successfully at UCPH-PLEN in 50 cm rows, at a density of 100 plants/m^2 (around 0.2 g/m^2 when adjusting for germination and emergence). This sowing density matches the 'standard seeding rate' suggested by Myers (1996). These wide rows allow for mechanical weed control.

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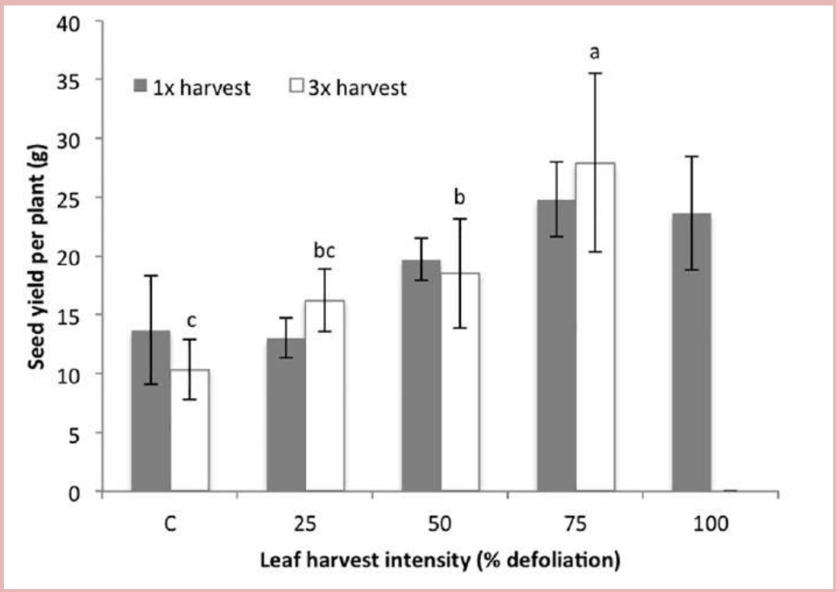
Amaranth leaf harvest trial

Trials at UCPH-PLEN in 2016-2017 tested six *Amaranthus* cultivars in a leaf harvest trial, removing 0, 25, 50, 75 and 100% of foliage at 42 days after sowing. Under multiple environments (controlled, semi-controlled and field conditions), the trial found that a one-time harvest of up to 50% did not negatively affect yields or seed quality. Furthermore, multiple harvests at low level (25% defoliation) did not reduce yields nor quality. This shows that amaranth has a tolerance to moderate levels of leaf harvest. Farmers can therefore use amaranth as a dual-use crop, benefitting from the high nutritional value of its leaves and seeds (more details in Hoidal *et al*, 2019).



Note: Lowercase letters correspond to the significance groups for 3x harvested plants (p < 0.05, Tukey test). 1x treatments did not result in significant yield differences, so significance groups are not included.

Figure 8 Seed yield (g/plant) in the growth chamber, following one or three consecutive harvests, at five defoliation levels.

Table 40 Crude seed protein content in seeds (%N * 6.25) and standard error (SE) in six amaranth varieties subjected to one defoliation event, at five harvest intensities, 2016.

Leaf harvest intensity	*A. cruentus*				*A. caudatus*		*A. hypocondriacus*					
	Benito		Françoise		Cecilia		Inessa		Katia		Maria	
	Yield	SE	Yield	SE	Yield	SE	Yield	SE	Yield	SE	Yield	SE
0%	12.64^{A}	0.25	12.87^{A}	0.21	13.38^{A}	0.08	13.48^{A}	0.24	13.22^{A}	0.14	12.15^{AB}	0.23
25%	12.99^{A}	0.18	12.87^{A}	0.08	12.64^{A}	0.23	12.26^{A}	0.23	13.10^{AB}	0.30	12.33^{AB}	0.16
50%	13.1^{A}	0.10	13.01^{A}	0.14	13.39^{A}	0.30	13.21^{A}	0.11	12.21^{B}	0.18	12.48^{AB}	0.43
75%	12.8^{A}	0.24	12.65^{A}	0.21	13.53^{A}	0.17	14.74^{A}	1.02	12.21^{B}	0.40	11.59^{A}	0.38
100%	12.62^{A}	0.25	12.88^{A}	0.26	13.44^{A}	0.15	14.08^{A}	0.28	13.76^{A}	0.23	12.90^{B}	0.43

Note: Letters represent significance groups of harvest intensity treatments within each variety (p < 0.05, Tukey test).

Sowing date: Trials at UCPH-PLEN 2015-2017 tested two different cultivars and found there were no significant difference in yields when grown at three different sowing dates. Total growing time was around one month less when sowing in mid-May compared to mid-April.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 635727.

Yields: screening trials 2015-18 are shown below (Tables 41 and 42).

Table 41. Results of sowing date trials for Amaranth at UCPH-PLEN from 2015-2017

		Sowing time	Yield (kg/ha)			TKV (g)			Protein (%)			C:N			Protein yield (kg/ha)			Growing time (days)		
			M	s.e		M	s.e		M	s.e		M	s.e		M	s.e		M	s.e	
Amaranth	Françoise*	S1	106	39	a	0.89	0.01	a	17.5	0.2	a	19.5	1.2	a	15	6	a	171	14.2	a
		S2	175	73	a	0.90	0.02	a	15.5	0.4	a	17.8	1	b	27	11	a	157.1	13.2	ab
		S3	278	30	a	0.91	0.01	a	19.7	0.6	a	19	1.3	a	38	2	a	147.4	12.5	b
	Maria	S1	892	229	a	0.88	0.01	a	16.7	0.8	a	18.2	1.2	a	113	30	a	174	13.1	a
		S2	1005	178	a	0.84	0.01	b	14.6	0.6	a	19.6	1.5	ab	127	22	a	156.1	11.9	b
		S3	1174	176	a	0.84	0.01	c	14.0	0.4	a	19.7	1.2	b	159	24	a	142.3	10.9	c

Note: S1: mid-April; S2: beginning of May; S3: Mid-May. Est: Mean estimates; SE: standard errors. Means followed by the same letters are not significantly different.: Material tested only one year (2017)*

Table 42. Screening trial data for Amaranth in Taastrup, Denmark (2015-2018)

Amaranth	Yield (kg/ha)			TKV (g)			Protein (%)			C:N			Protein Yield			Growing time		
	M	SE		M	SE		M	SE		M	SE		M	SE		M	SE	
A14	1075	16	a	0.78	0.01	bc	17.5	0.1	ab	16.3	1.5	ab	188	2	a	182	14	a
Cecilia	1131	293	a	0.61	0.05	a	15.5	0.7	a	17.8	1.1	b	176	47	a	184	8	a
Françoise	937	26	a	0.86	0.03	c	19.7	0.4	b	14.7	1.5	a	184	4	a	184	8	a
Inessa	1091	34	a	0.76	0.05	b	16.7	0.4	ab	17.2	0.9	ab	182	3	a	179	8	a
Katia	1596	143	a	0.83	0.04	bc	14.6	1.0	a	18.7	0.9	c	233	15	a	179	8	a
Maria	1591	46	a	0.84	0.03	bc	14.0	0.3	a	19.9	1.3	c	223	6	a	179	8	a

Note: M: Mean; SE: standard errors. Means followed by the same letters are not significantly different.

Conclusions: When producing amaranth, farmers may experience losses at harvest, particularly in humid weather. Breeding for more uniform maturation could improve the success rates of Amaranth. There is not yet a large market for amaranth; therefore, demand must be created before it can experience commercial success. Amaranth as a leaf vegetable may be of interest in high-end catering, although this could incur high labour costs.

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3.3 The Netherlands - Louis Bolk Institute (LBI)

3.3.1 Experimental site

This section of the report present activities and results from trials carried out for D1.10 by the Louis Bolk Institute. Field trials to define relevant management measures for different grain legumes have been carried out at different locations in The Netherlands. Different soil types as well as climatic conditions are present at these locations. The main location of the trials is located in Klazienaveen, on an anthroposol (former histosol), where peat has been dug off in the past, and the sandy subsoil has been mixed with the remaining peat layer. Organic matter contents and pH on different fields range from 5.8-12.0% OM, and a pH between 4.3 and 5.5. The locations in Lelystad and Oostwold are located on young marine clay soils, the locations in Bellingwolde and Jipsingboertange on sandy soils.

3.3.2 Crops

3.3.2.1 White and blue lupin

Crop protection in white and blue lupin

In 2015 three varieties of blue lupin (Primadonna, Iris, Regent) and three varieties of white lupin (Boros, Amiga, Volos) were sown in trial fields (sowing date April 14), with and without fungicides, at location Klazienaveen on a humic sandy soil. The variety Volos suffered from a low seed quality and average germination rate of 3% in the field, and was not been harvested. White lupins suffered from a high pressure of fungal diseases: grey leaf spot (*Stemphylium botryosum*), brown leaf spot (*Pleiochaeta setosa*) and Sclerotinia stem rot (*Sclerotinia sclerotiorum*) from the end of July onwards. Blue lupins suffered from Fusarium wilt (*Fusarium oxysporum* f.sp. *lupini*), and brown leaf spot (*Pleiochaeta setosa*). Crop protection was performed by treatment with 1,2 l/ha Caramba (metconazol) and 1,6 l/ha Signum (boscalid and pyraclostrobin) applied during the closing of the crop at the end of May. Statistical analysis of yields, showed that crop protection with fungicides resulted in a significant interaction between varieties and the treatment with/without fungicides (P=0.038). In Table 43 the yields of different varieties with and without fungicides are shown. The table shows that in the 2015 trial, only in white lupin variety Amiga, crop protection resulted in a significantly higher yield.

In 2016, trials with and without fungicides were carried out in white lupin at location Lelystad on a young marine clay soil. In the variety Amiga, an early infection with anthracnosis (*Colletotrichum lupini*) was found in the trial fields by the end of May, caused by contaminated seed. A fungicide (Switch) was sprayed twice (June 10 and 28) against foliar disease. This treatment was however too late to be effective against anthracnosis. At the time of harvest, lupin variety Boros could be harvested. The varieties Amiga, Feodora and Dieta had almost no pod development, and the few pods present were heavily infected with anthracnosis. Yield of Boros was 1.9 t/ha with fungicides, and 1.5 t/ha without fungicides, but differences were non-signicant.

In 2017, trials with and without fungicides were carried out in white and blue lupin at location Klazienaveen (humic sandy soil). Plots were slightly infected by fungal diseases (mainly

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Stemphylium), and variety Feodora suffered from a viral infection. Significant differences were found between varieties. The effect of crop protection was almost significant (P=0.055), with on average 2.13 t/ha (no fungicides) and 2.43 t/ha (with fungicides) (l.s.d. 0.305).

Table 43 Yields (t/ha) of white and blue lupins with (yes) and without (no) the use of fungicides (2015 and 2017 trials on humic sandy soil Klazienaveen; 2016 trial on young marine clay Lelystad). In the 2015 trial, letters indicate significant differences between yields (P<0.05) and can be compared between both columns and rows (l.s.d. 0.716). In 2016 there was no significant difference in yields with and without crop protection, in 2017 the yields were almost significantly different.

		2015		**2016**		**2017**	
		KL		**LE**		**KL**	
variety	**Species**	**yes**	**no**	**yes**	**no**	**yes**	**no**
Amiga	L. albus	2.9 cd	1.6 a	nd	nd		
Boros	L. albus	2.4 bc	2.1 ab	1.9	1.5	3.0	3.2
Butan	L. albus					3.2	2.8
Dieta	L. albus			nd	nd		
Feodora	L. albus			nd	nd	4.2	3.5
Volos	L. albus	nd	nd				
Boregine	L. angustifolius					2.2	1.2
Boruta	L. angustifolius					2.2	1.8
Heros	L. angustifolius					0.5	0.8
Iris	L. angustifolius	3.3 d	3.1 d			1.1	1.0
Primadonna	L. angustifolius	3.1 cd	2.9 cd				
Regent	L. angustifolius	3.0 cd	3.3 d			2.4	2.5
Wars	L. angustifolius					1.2	1.2
average		**2.9**	**2.6**	**1.9**	**1.5**	**2.4**	**2.1**
l.s.d. crop protection						0.305	
l.s.d. crop prot. * variety		0.716					

Sowing density trials in blue lupin

The hypothesis that restricted branching varieties could profit more from a higher sowing density than indeterminate ones was not confirmed by the yields of three years of testing, in which four sowing densities (50%, 75%, 100% and 125% of the recommended practice) were compared. Yields were significantly different during the 3 years, and between the four different densities. However, no significant effect of growth habit (indeterminate/restricted branching) or interaction between growth habit and sowing density was measured. Compared to the standard practice (100% sowing density), only the lowest sowing density of 50% produced a significantly lower yield. The high weed pressure in 2017 was expected to produce a more pronounced effect of sowing density on yield, but this effect was not significant (Table 44).

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Table 44 Yields (t/ha, 15% moisture) of indeterminate and restricted branching varieties of Lupinus angustifolius in 2015-2017 at different sowing densities.

sowing density % of target	**pl/m2**	**growth habit**	**2015**	**2016**	**2017**	**average**
50%	60	restricted branching	2.76	2.36	2.19	**2.35 a**
	45	indeterminate	2.86	2.16	1.65	
75%	90	restricted branching	2.85	2.77	2.26	**2.54 ab**
	68	indeterminate	2.92	2.30	2.03	
100%	120	restricted branching	2.74	2.26	2.47	**2.61 bc**
	90	indeterminate	3.03	2.56	2.52	
125%	150	restricted branching	2.80	*	2.88	**2.86 c**
	112	indeterminate	3.15	2.56	*	
lsd year		0.255	**2.88 b**	**2.42 a**	**2.26 a**	
l.s.d. sowing density						0.306

Sowing density trials in white lupin

Trials with sowing density of white lupin were performed in 2016 and 2017. In 2016 indeterminate white lupin variety Feodora was completely removed from the field after infection with anthracnosis. Only restricted branching variety Boros could be harvested, but without representative yields due to high anthracnosis contamination (between 0.9 and 1.3 t/ha).

In 2017 the trials were repeated with a restricted branching (Boros) and indeterminate (Feodora) white lupin. Seeds were sown at 50%, 75%, 100% and 125% of the recommended sowing density. Yields were significantly different between the two varieties, and also between the sowing densities. There was however no interaction between growth type (indeterminate/restricted branching) and sowing density (Table 45). Also in this trial with white lupin, only the 50% sowing density resulted in a significantly lower yield than the recommended 100% sowing density.

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Table 45 Seed yield of indeterminate and restricted branching white lupin on humic sandy soil (Klazienaveen location) at different sowing densities (t/ha, 15% moisture)

sowing density % of target	pl/m² restricted branching/ indeterminate	restricted branching Boros	indeterminate Feodora	average
50%	45 / 25	2.3	3.0	**2.7 a**
75%	68 / 38	2.6	3.9	**3.2 b**
100%	90 / 50	3.2	3.6	**3.4 b**
125%	113 / 63	2.9	3.6	**3.3 b**
average		**2.8**	**3.5**	
l.s.d. growth type/variety		0.247		
l.s.d. target sowing density				0.349

3.3.2.2 Spring and winter faba bean

Crop protection in spring faba bean

In spring faba bean, trials with and without fungicides were carried out at the marine clay location in Lelystad (LE) in 2016, 2017 and 2018. In 2017, trials with and without crop protection were also performed on humic sandy soil in Klazienaveen (KL), and in 2018 in Bellingwolde (BW) (sand) and Oostwold (OW) (marine clay).

In 2016 at the LE location, two treatments with a fungicide (Switch) were performed, due to heavy infection with chocolate spot disease (*Botrytus fabae* and/or *Botrytis cinerea*). Insecticides were applied to all plots. A very early infection with virus(es) affected strongly the growth in the Lelystad trials, causing a high variation between plots. The virus was spread by black bean aphid (*Aphis fabae*) that infested the plants in early spring. Despite two spray treatments with insecticides of all the plots (also against the pea leaf weevil *Sitona lineatus*), spots with heavy infestations of viruses could not be prevented. Application of the fungicide resulted in a significant yield difference (on average for all varieties 3.9 t/ha without, and 5.0 t/ha with crop protection).

In 2017 at the LE location, there was a high infestation with chocolate spot disease, and the fields with fungicide treatment were sprayed twice (once with Prosaro, once with Rovral). At this location, all fields were sprayed once against black bean aphid at the end of June. The treatment with fungicides resulted in LE in a significantly higher yield (average 8.2 t/ha compared to 6.8 t/ha without fungicides). Also at the KL location, there was a high pressure of fungal diseases, both chocolate spot and faba bean rust (*Uromyces viciae-fabae*). Although the crop remained green for a longer period of time, the use of fungicides (Switch) did not result in significant yield differences (Table 4).

In 2018, crop protection trials with faba bean were performed at three locations. On March 9, spring faba bean was sown in Bellingwolde (BE), on March 19 in Oostwold (OW). The weather conditions

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were extremely dry and hot during summer, which resulted in low yields at the sand location (BE), due to the low drought tolerance of faba bean. In the beginning of June, a treatment with Prosaro was carried out against chocolate spot disease, but due to the dry weather, the disease came to a halt in all treatments. Yields were not significantly different in treatments with (3.3 t/ha) or without (3.1 t/ha) crop protection.

Two marine clay locations were included in the 2018 trials. At the OW location, one spray treatment against pea leaf weevil (*Sitona lineatus*) was carried out in all treatments. A treatment against faba bean rust was carried out at the beginning of June in the plots with fungicide use, but also this disease did not spread further. The average yield with (6.3 t/ha) and without (6.1 t/ha) crop protection were not different. Due to the higher moisture availability on the clay soils, yields were less suppressed by the hot and dry summer. At the second marine clay location in Lelystad (LE), treatments with and without fungicides (Prosaro and Switch) were carried out against chocolate spot disease. At this location, insecticides were used in all plots (both with and without crop protection). They were used against a heavy infection with black bean aphid (3 treatments), and with pea leaf weevil (2 treatments). The average yield in treatments with (6.6 t/ha) and without (6.5 t/ha) fungicides was not significantly different (Table 46).

Table 46 Yields of spring faba bean with and without crop protection (t/ha, 15% moisture). Trials were with 2 replicates in Lelystad (LE) or 3 replicates in Klazienaveen (KL), Bellingwolde (BE) and Oostwold (OW).

	2016		**2017**				**2018**					
	LE		**LE**		**KL**		**BW**		**OW**		**LE**	
variety	**yes**	**no**	**yes**	**no**	**yes**	**no**	**yes**	**no**	**yes**	**no**	**yes**	**no**
LG Cartouche			8.5	7.2			4.0	2.9			7.0	6.7
Divine	3.9	3.6										
Fanfare	5.1	3.6	8.3	6.8							7.4	7.3
Fuego			8.5	7.1	7.6	7.2	3.9	3.8	7.3	7.1	7.1	6.8
Honey							3.7	3.7				
Imposa	5.3	4.4	7.9	6.5	6.9	7.0						
Julia	5.1	3.7										
Lynx			8.5	6.8	7.2	6.7						
Pyramid			8.2	6.8	6.1	6.4						
Sampo							1.3	1.1	3.2	3.0	4.0	4.2
Taifun	5.1	3.9	7.5	6.3	7.3	6.9	2.6	4.0	7.0	6.7		
Tiffany	5.5	4.0	8.4	6.8	6.4	6.3	4,0	3.5	7.5	7.5	6.5	6.9
Trumpet											7.3	7.1
average	**5.0**	**3.9**	**8.2**	**6.8**	**6.9**	**6.8**	**3.3**	**3.1**	**6.3**	**6.1**	**6.6**	**6.5**
l.s.d crop prot.	0.4		0.14		0.24		0.70		0.29		0.6	

<u>Crop protection in winter faba bean</u>

In winter faba bean, trials with and without both fungicides and insecticides were performed in the growing seasons 2016-2017 and 2017-2018. In 2016-2017 in Klazienaveen, on a humic sandy soil. In 2017-2018 in Bellingwolde on a sandy soil, and in Oostwold on a young marine clay soil.

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Trials with and without crop protection in winter faba bean were sown in Klazienaveen on November 1, 2016. Low autumn temperatures delayed germination with about two weeks. In December and January temperatures fell down to -10 degrees, but as plants had developed sufficient winter hardiness, this had no effect on plant survival. Spring circumstances were favorable for crop development, but at the end of June, wet weather caused a rapid development of chocolate spot disease in all varieties. Organdy and Diva were affected the most by chocolate spot disease, Bumble and Tundra the least. Organdy was also early infected with faba bean rust. Early dying away caused less pod-filling and faster ripening in the affected crops. Yields were significantly lower (on average 4.8 t/ha) in fields without crop protection, compared to fields with crop protection (5.6 t/ha) (Figure 1).

Fig. 1 Winter faba bean variety Organdy on July 6, 2017. Left: untreated plots, heavily infected with chocolate spot disease. Right: fungicide treated plots.

In autumn 2017, trials with and without crop protection were sown at two locations: in Oostwold, on marine clay on October 27, and in Bellingwolde, on a sandy soil on November 2. Pre-emergence herbicides (Stomp and Centium) should have been applied to all plots, but were accidentally only applied in plots with crop protection. Winter faba beans suffered from two severe frost periods, but especially the second period at the end of February in Oostwold, with temperatures of -10 degrees, combined with strong wind and sand storms, caused plants to freeze back below the soil surface. Re-growth took place, but many of the plants that had been treated with herbicides, died soon after emergence. It has been confirmed that pendimethalin might be taken up by the growing tip and true leaves of faba bean, after re-emergence. Plant survival after emergence was 12% in plots with herbicides, and 22% in plots without herbicides. In Bellingwolde, frost was less severe, and depending on the variety, 44-95% of plants survived. The German variety Hiverna and the French variety Diva had a significantly better winter hardiness than the English varieties Bumble, Honey, Tundra and Wizard. Due to the harsh winter conditions, yields were extremely low in 2018 at both locations.

In Oostwold, spraying of insecticides (Decis) was applied against pea leaf weevil *Sitona lineatus* in early May, and fungicides (Prosaro) were applied against faba bean rust at the beginning of June. However, the negative effect of pre-emergence herbicides in combination with severe winter circumstances overshadowed the application of insecticides and fungicides, and resulted in Oostwold in significantly better yields in fields without crop protection (2.9 t/ha), compared to fields with crop

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protection (2.4 t/ha). In Bellingwolde, no serious problems with pea leaf weevil developed, but chocolate spot disease affected the plants at a very early stage. At the beginning of June, fungicides (Prosaro) were applied against chocolate spot disease. This resulted in Bellingwolde in significantly higher yields in the treatment with crop protection (2.4 t/ha) compared without crop protection (1.8 t/ha). Results for the different trials in winter faba bean with and without crop protection are summarized in Table 47.

Table 47 Yields of winter faba bean, harvested in 2017 and 2018 on different trial locations: Klazienaveen (KL), Oostwold (OW) and Bellingwolde (BW), with (yes) and without (no) crop protection. (t/ha, 15% moisture)

		2017		**2018**			
		KL		BW		OW	
variety	type	yes	no	yes	no	yes	no
Bumble	coloured	6.7	5.4	2.4	2.1	1.7	2.7
Diva	coloured	5.3	4.9	2.2	1.6	1.6	2.0
Hiverna	coloured	5.8	4.4	3.4	2.4	5.0	4.8
Honey	coloured			2.7	1.7		
Organdy	white	3.8	3.6				
Tundra	coloured	5.6	5.2	1.8	1.3	1.6	2.5
Wizard	coloured	6.4	5.4	2.1	1.6	2.1	2.5
average		5.6	4.8	2.4	1.8	2.4	2.9
lsd crop protection		0.43		0.33		0.39	

Sowing depth and sowing density trials in winter faba bean

At the Oostwold (marine clay) location, differences in sowing depth were almost significant in the 2016-2017 trials (p=0.061, l.s.d. 0.89), where the sowing depth of 15 cm resulted in a lower yield than sowing at 10 cm (Table 48). No severe frost occurred during the 2016-2017 winter. No significant differences between the three sowing densities (10, 20 and 30 plants/m2) occurred. At the Klazienaveen (humic sand) location, the average yield of the different cultivars at different sowing depth shows the same trend, with 15 cm resulting in the lowest yield (Table 49). In both locations this trend may be related to a later emergence at a sowing depth of 15 cm, and less time for hardening until the first frost appeared. In the 2017-2018 trials, the very harsh winter conditions (-10 degrees, combined with strong wind and sand storms) were combined with water stagnation in this part of the field trial, due to which hardly any plants survived. Yields were so low that a comparison of the different sowing densities was not meaningful.

Table 48 Yield of winter faba bean cultivar Tundra at different sowing depths and densities on marine clay in Oostwold (t/ha, 15% moisture) (2016-2017 trials).

	sowing depth			
sowing density (plants/m2)	**5 cm**	**10 cm**	**15 cm**	**average**
10	7.1	7.0	5.7	**6.6**
20	7.1	7.3	6.7	**7.1**
30	7.4	8.1	6.8	**7.5**
average	**7.2**	**7.5**	**6.4**	

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l.s.d. sowing depth	0.89

Table 49 Yield of winter faba bean cultivars at different sowing depth (location Klazienaveen) on a humic sandy soil (t/ha, 15% moisture). Trial without replicates.

	sowing depth		
variety	**5 cm**	**10 cm**	**15 cm**
Bumble	7.2	6.3	5.4
Diva	4.7	5.3	5.0
Hiverna	6.0	6.1	5.1
Organdy	4.1	3.8	3.4
Tundra	7.7	7.2	5.4
Wizard	5.6	6.4	7.1
average	**5.9**	**5.9**	**5.2**

Sowing date trial in winter faba bean

Winter faba bean trials were sown in Bellingwolde on November 2, 2017, aimed at a plant density of 25 plants/m2. Initial germination rate of autumn-sown faba bean variety Honey was over 100% (which might be due to an actual lower thousand kernel weight than provided by the seed supplier), but after the harsh winter conditions at the end of February, only half of the plants survived. At the same trial field, winter faba bean Honey was re-sown in spring, on March 9, 2018, with an aimed plant density of 30 plants/m2. Germination was high, resulting in a plant density of 30.9 plants/m2 at the end of April. At the same moment, plant density in winter-sown Honey was 13.3 plants/m2. In winter-sown faba bean, plants reacted with increased tillering on the lower plant density, with on average 1.8 stems/plant. In spring-sown faba bean, no tillering occurred. Autumn sown faba bean started flowering on the 5^{th} node, while spring-sown faba bean started on the 8^{th} node. The delay of first flowering is a known effect of the absence of vernalisation in winter faba bean. Autumn-sown faba bean reached a maximum height of 60 cm, with a total of 18 nodes, while spring-sown became 72 cm high, with an average of 20 nodes. Re-sowing of faba bean resulted in a significantly higher yield, of 3.7 t/ha, compared to 2.2 t/ha for autumn-sown faba bean.

3.3.2.3 Soya bean

Crop protection in soya bean

In 2017, trials with and without crop protection in soya bean have been performed in Klazienaveen. The use of fungicides (Switch) did not result in significant differences in yield with (2.6 t/ha) and without (2.7 t/ha) crop protection. Results are summarized in Table 50.

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Table 50 Yields of soya bean with and without crop protection on a humic sandy soil in Klazienaveen (t/ha, 15% moisture).

	2017	
	KL	
variety	**yes**	**no**
Abelina	2.8	2.9
Adsoy	2.3	2.1
Alexa	2.8	3.3
SG Anser	2.5	2.6
average	**2.6**	**2.7**
l.s.d. crop protection	0.27	

3.3.2.4 Crop rotation trials with soya, lupin and fava bean.

Host status of grain legumes – field trials

In 2015 field trials to assess the host status of different grain legumes (white and blue lupin and faba bean) were carried out at two locations in the Netherlands: in Holten (sandy soil) and Klazienaveen (humic sandy soil). In 2016 field trials were conducted in Klazienaveen, and in 2017 in Jipsingboertange. In the 2015 trials, nematode populations were only assessed at the end of the growing season, and compared with populations in black fallow and spring wheat. In the 2016 and 2017 trials, populations were assessed both at the beginning and at the end of the growing season. All crops were cultivated in three replicates in a completely randomized block design.

Holten 2015 trial

Grain legumes were sown at April 9, 2015 in Holten. At April 27/28 severe frost (-10 degrees) occurred, while faba beans were just emerging, and in a very vulnerable development stage. All faba bean varieties were frozen, and could not be included in the final measurement. Field plots in Holten were naturally infested with the root-lesion nematodes *Pratylenchus crenatus*, *Pratylenchus neglectus* and *Paratrychodorus pachidermus*, and with free living nematodes from the *Trichodorus* group 1 and 2, and Trichodorus viruliferus. No significant differences in nematode population development were seen between the different crops compared to black fallow (Table 51).

Table 51 Nematode population (nematodes/100ml) at the end of the growing season in 2015 field trials (means of three replicates) at location Holten. No significant differences exist between the different crop treatments. Pc = Pratylenchus crenatus; Pn = Pratylenchus neglectus; Pap = Paratrichodorus pachydermus; T1 = Trichodoridea group 1; T2 = Trichodoridea group 2; Tv = Trichodorus viruliferus

crop	**cultivar**	**root-lesion nematodes**			**free-living nematodes**		
		Pc	**Pn**	**Pap**	**T1**	**T2**	**Tv**
black fallow		85.3	4.7	2.3	3.3	1.3	3.0
blue lupin	Iris	417.7	31.3	0.7	1.3	0.7	3.7
white lupin	Boros	458.7	34.0	0.7	3.3	0.7	2.3
spring wheat		209.3	20.3	0.3	0.0	0.3	0.3

Klazienaveen 2015 trial

Field plots in Klazienaveen were sown at April 14. Night frost at the end of April was less severe than in Holten (-6 degrees), and faba been seedlings survived. The trial fields in Klazienaveen were

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naturally infested with the Northern root-knot nematode (*Meloidogyne hapla*), Columbia and false Columbia root-knot nematodes (*M. chitwoodi* and *M. fallax*), barley root-knot nematode (*M. naasi*), beet cyst eelworm (*Heterodera schachtii*) and potato cyst nematode (*Globodera sp.*). The Klazienaveen location contained significantly more *Pratylenchus crenatus* nematodes than the Holten location. At the end of the growing season, no significant differences in nematode population development were seen between the different crops, with the exception of potato cyst nematodes. Highest numbers of total cysts of *Globodera* were found in the wheat reference crop in Klazienaveen, with lower numbers (of only dead cysts) in white lupin, fallow and blue lupin. Larvae and living cysts of *Globodera* were only found in the wheat reference plot in Klazienaveen (Table 52). It is plausible that *Globodera* propagated on remaining potato plants in the wheat plots, as weed management is more difficult in grains.

Table 52 Nematode population (nematodes/100ml) at the end of the growing season in 2015 field trials (means of three replicates) at location Klazienaveen. Data were log-transformed before statistical analysis. Gc = Globodera sp. cysts; Gl = Globodera sp. larvae; Glc = Globodera sp. living cysts; Hs = Heterodera schachtii; Mc = Meloidogyne chitwoodii; Mf = Meloidogyne fallax; Mh = Meloidogyne hapla; Mn = Meloidogyne naasi; Pc = Pratylenchus crenatus; Pp = Pratylenchus penetrans; T2 = Trichodoriden group 2; fl = free living

crop	variety	cyst nematodes				root-knot nematodes				root-lesion		fl
		Gc	Gl	Glc	Hs	Mc	Mf	Mh	Mn	Pc	Pp	T2
black fallow		1.0	0.0	0.0	0.0	0.0	0.0	7.7	0.0	447	0.0	0.0
blue lupin	Iris	1.0	0.0	0.0	0.7	0.0	0.0	12.3	0.0	1164	0.3	0.0
white lupin	Boros	0.7	0.0	0.0	1.0	0.0	0.0	0.3	0.0	1053	138	0.0
faba bean	Imposa	0.7	0.0	0.0	0.0	0.0	3.7	40.0	0.0	852	1.3	0.3
spring wheat		1.7	25.0	0.7	0.0	0.3	2.3	0.0	2.3	430	0.0	0.0

<u>Klazienaveen 2016 trial</u>

Natural infestation with plant-pathogenic nematodes was assessed in a second experimental field in Klazienaveen in spring 2016, by sampling the three blocks in the trial design as mixed samples. The field contained high populations of the root lesion nematodes *Pratylenchus crenatus* and *Pratylenchus penetrans* at the beginning of the growing season, and only small amounts of *Pratylenchus neglectus*, root knot nematodes (*Meloidogyne hapla*, *chitwoodi* and *fallax*) and free-living nematodes (*Trichodorus, Paratrichodorus* and *Paratylenchus* spp). At the end of the growing season (September) all plots were sampled to determine the final population (Pf) for each nematode species.

As shown by the reproduction factor (Rf), it is apparent that in the black fallow a number of nematode species have increased in abundance, especially *M. chitwoodi*, but also a number of root lesion and free living root nematodes. This might be due to the presence of weeds into the black fallow plots during the growing season, but also due to differentiation in initial population (Pi) between plots, which has been leveled by combined samples at block level at the start of the growing season. Significant differences between crops were found in nematode numbers and reproduction factors of *Meloidogyne chitwoodi* and *Pratylenchus neglectus*. *Meloidogyne fallax* showed a trend towards significance (P=0.075). Spring wheat and fallow showed an increase in *M. chitwoodi*, only in blue

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lupin a slight increase in *M. chitwoodi* was seen, while in the other crops the root knot nematode had decreased. *M.fallax* showed the same trend, with the exception that faba bean showed an even greater increase in this nematode species than both wheat and fallow. Although there were no significant differences in *M. hapla*, the same trend might be noticed, where especially faba bean and blue lupin seem to increase the population pressure. For the root lesion nematodes, the only significant differences are seen in *Pratylenchus neglectus*. The largest amounts of nematodes occur however in *Pratylenchus penetrans* and *Pratylenchus crenatus*, but no significant differences are seen between the crops. Also the amount of *Paratylenchus* species has increased a lot during the growing season, but no significant differences between crops have developed. Results are presented in Table 53 (nematode numbers) and 54 (reproduction factor).

Table 53 Nematode population (nematodes/100ml) at the end of the growing season in 2016 field trials (means of three replicates). Letters within columns denote significant differences (P<0.05 in log-transformed data) between crops. Mc = Meloidogyne chitwoodii; Mf = Meloidogyne fallax; Mh = Meloidogyne hapla; Mn = Meloidogyne naasi; Pc = Pratylenchus crenatus; Pn = Pratylenchus neglectus; Pp = Pratylenchus penetrans; Pap = Paratrichodorus pachydermus; Par = Paratlenchus sp; T2 = Trichodorides group 2

crop	**cultiv.**	**root knot nematodes**				**root lesion nematodes**			**free-living nematodes**		
		Mc	**Mf**	**Mh**	**Mn**	**Pc**	**Pn**	**Pp**	**Pap**	**Par**	**T2**
black fallow		6.5[ab]	1.5[abc]	0.0	0.0	65.5	5.5[a]	372.0	25.5	1.0	14.5
buckwheat		0.7[c]	0.3[bc]	0.0	0.0	330.3	1.0[bc]	64.3	16.0	225.3	11.3
faba bean	Fuego	0.7[c]	4.7[a]	1278.0	0.0	266.7	0.0[c]	574.7	66.3	383.7	42.3
blue lupin	Iris	1.7[bc]	0.0[c]	430.3	0.0	63.7	0.0[c]	365.3	12.7	108.3	10.0
white lupin	Boros	0.3[c]	0.3[bc]	62.3	0.0	211.3	0.0[c]	650.3	16.3	327.7	23.7
quinoa	Atlas	0.3[c]	0.3[bc]	0.0	0.0	314.7	4.7[ab]	56.0	33.7	12.3	23.0
soya bean	Viola	0.0[c]	0.0[c]	5.0	0.0	319.3	2.3[abc]	1000.0	67.7	6.3	54.0
spring wheat		18.3[a]	2.7[ab]	10.7	1.0	124.7	0.0[c]	292.0	29.7	32.0	24.0

Table 54 Reproduction factor (Rf values) of nematode populations in 2016 field trials (means of three replicates). Rf values (Pf/Pi: final population/initial population) for each plot with the final population per plot, and the average initial population on field level. Letters within columns denote significant differences (P<0.05 in log-transformed data) between crops. Rf values of Meloidogyne naasii could not be determined, as it was not present in the initial population samples. Abbreviations: see previous table.

		root knot nematodes				**root lesion nematodes**			**free-living nematodes**		
		Mc	**Mf**	**Mh**	**Mn**	**Pc**	**Pn**	**Pp**	**Pap**	**Par**	**T2**
black fallow		9.8[ab]	0.2[ab]	0.0	*	0.2	2.4[a]	1.1	2.3	3.0	2.3
buckwheat		1.0[c]	0.0[bc]	0.0	*	1.1	0.4[bc]	0.2	1.5	676.0	1.8
faba bean	Fuego	1.0[c]	0.6[a]	639.0	*	0.9	0.0[c]	1.8	6.0	1151.0	6.7
blue lupin	Iris	2.5[bc]	0.0[c]	215.2	*	0.2	0.0[c]	1.1	1.2	325.0	1.6
white lupin	Boros	0.5[c]	0.0[bc]	31.2	*	0.7	0.0[c]	2.0	1.5	983.0	3.7
quinoa	Atlas	0.5[c]	0.0[bc]	0.0	*	1.1	2.0[ab]	0.2	3.1	37.0	3.6
soya bean	Viola	0.0[c]	0.0[c]	2.5	*	1.1	1.0[abc]	3.1	6.2	19.0	8.5
spring wheat		27.5	0.4	5.3	*	0.4	0.0[c]	0.9	2.7	96.0	3.8

Jipsingboertange 2017 trial

In 2017 nematode host status trials were carried out in naturally infected fields in Jipsingboertange. Significant differences in reproduction factor and final population of the Northern root-lesion

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nematode were measured in the different crops. Andean lupin (Rf 27.7) and soya bean (Rf 14.0) gave a very high reproduction of *P. penetrans*, followed by faba bean (Rf 7.7) and barley (Rf 6.8). Blue and white lupin gave lower reproduction (4.5 and 4.1). Sugarbeet, which is considered to be a poor host, had an average reproduction factor of 1.5.

The initial population pressure with Columbia root-knot nematode differed among the crops, with Andean lupin and barley starting with significantly higher levels of *M. chitwoodi*. In the final reproduction values, no significant differences were found between the crops. The very high reproduction in faba bean (Rf 39.9) and high reproduction in white lupin (Rf 7.5) were largely determined by the development in one of the plots. The reproduction in soya bean was remarkably low (Rf 0.17), and even lower than the very poor host sugarbeet (Rf 0.39). However, although large differences were found in Rf values, these were not significant due to the large variation between the plots. In the plots where *Paratrichodorus pachydermus* is present, no significant differences were found in nematode development between the 4 crops (Andean lupin, soya bean, sugarbeat and barley). The presence and development of saprotroph nematodes has also been monitored in the different plots. The population remained stable, starting with an average of 4277 saprotrophs at the beginning of the growing season, and 4330 individuals at the end of the cropping cycle. Results are presented in Table 55.

Table 55 Initial (Pi) and final populations (Pf) of nematode species, and reproduction factor (Rf values) in 2017 field trials in Jipsingboertange (means of three replicates). Letters within columns denote significant differences (P<0.05 in log-transformed data) between crops.

		Meloidogyne chitwoodi			**Pratylenchus penetrans**			**Paratrich. pachydermus**		
		Pi	**Pf**	**Rf**	**Pi**	**Pf**	**Rf**	**Pi**	**Pf**	**Rf**
faba bean	Imposa	17	428	39.9	390	2379[b]	7.7[bc]	*	*	*
Andean lupin	Branco	61	392	4.7	312	8705[a]	27.7[a]	7	8	1.3
blue lupin	Regent	17	58	2.9	431	1290[c]	4.5[cd]	*	*	*
white lupin	Feodora	36	88	7.5	317	1167[c]	4.1[bcd]	*	*	*
soya bean	Adsoy	20	3.3	0.17	531	6502[a]	14.0[ab]	10	8	1.3
barley		67	190	3.0	350	2128[bc]	6.8[bc]	10	17	1.7
sugarbeet		27	12	0.39	357	533[d]	1.5[d]	7	8	1.3

Host status of grain legumes - Bioassay

Results of the bioassay are presented in Table 56. During the *M. chitwoodi* assay, it was observed that soya bean developed many nodules, but without development of egg masses. As the nematode causes root nodule formation on soya bean, but cannot propagate, this could be an indication that soya bean could be a trap crop for *M. chitwoodi*. Faba bean and blue lupin are not sensitive for *Heterodera schachtii*, but white lupin is. Soya bean developed a small number of cysts per gram root. All lupins are sensitive for *Meloidogyne hapla*, and especially blue lupin contained a large number of egg masses per gram root. Faba bean is a little bit less sensitive, and soya bean even less.

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Table 56 Results of susceptibility assays for spring faba bean, winter faba bean, blue lupin, white lupin and soya bean for *Meloidogyne chitwoodi*, *M. hapla* and *Heterodera schachtii*.

		Meloidogyne chitwoodi		Heterodera schachtii		Meloidogyne hapla	
		# plants	eggs/g[1]	# plants	cysts/g	# plants	eggs/g
spring faba bean	Fuego	10	22.1	10	0.1	10	15.0
	Imposa	10	17.1	10	0.6	10	21.0
	Lynx	10	18.2	10	0	10	8.3
	Pyramid	10	7.2	10	0	10	12.0
	Taifun	10	24.1	10	0	10	6.9
	Tiffany	10	25.2	10	0	10	6.8
	average		**19.0**		**0.1**		**11.7**
winter faba bean	Bumble	10	14.3	10	0.4	10	10.4
	Diva	10	20.9	10	0	10	16.0
	Hiverna	10	21.7	10	0	10	16.0
	Organdy	10	22.8	10	0	10	16.0
	Tundra	10	7.4	10	0	10	17.6
	Wizard	10	5.2	10	0	10	12.3
	average		**15.4**		**0.1**		**14.7**
blue lupin	Boregine	1	50.0	10	0.3	10	75.4
	Boruta	1	7.5	10	0.1	10	86.3
	Iris	1	50.0	8	0	6	73.7
	Heros	4	19.6	10	0.1	10	103.7
	Lazur	6	19.9	10	0	10	58.9
	Regent	8	21.2	10	0.1	10	48.8
	Wars	8	26.0	10	0	10	80.0
	average		**22.4**[2]		**0.1**		**75.2**
white lupin	Boros	10	44.6	10	49.1	10	28.3
	Butan	10	42.4	10	15.6	10	35.2
	Feodora	10	19.1	10	26.1	10	29.8
	average		**35.4**		**30.3**		**31.1**
soya bean	Abelina	10	0	10	0.5	10	0.9
	Adsoy	10	0	10	5.4	10	1.0
	Alexa	10	0	10	4.1	10	1.0
	SG Anser	10	0	10	0.4	10	1.1
	average		**0**		**2.6**		**1.0**

[1]The average number of eggs (M. *hapla* and *M. chitwoodi*) or cysts (*H. schachtii*) per gram root

[2]Average of Lazur, Regent and Wars. The number of plants of other cultivars were too low: due to a technical failure the humidity in the climate chamber was too high, causing fungal disease in a number of cultivars.

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0. Conclusion and next steps

Italy

According to the results obtained from the experiments during these four last years, it can be concluded that the most productive agronomic interventions for quinoa were the early spring sowing date and sowing density of 100000 plant ha^{-1}. Rotation of fava/quinoa did not have a positive impact on quinoa seed yield. Deficit irrigation and saline water (abiotic stress) treatments had no significant effect on quinoa yield and its components in the three experimental growing seasons. In 2016, the water irrigation level had an effect only on the vegetative development.

Similar to quinoa, the rotation of fava/amaranth did not have a positive impact on amaranth seeds yield. Fertilizer in the form of NH_4NO_3 with 300 kg N ha^{-1} was the best treatment for amaranth and buckwheat. Because of its ability to offset row planting density, amaranth did not have any differences in sowing density. In the 2016 growing season, early and late spring sowing dates did not affect amaranth seed yield. According to the results of the three growing seasons for abiotic stress trials, by increasing the drought and saline stress the amaranth yield decreased and the HI had the opposite trend due to a lower biomass production. Winter and spring sowing dates had no effect for fava bean and lupin when grown in clay soil. In sandy soil, winter sowing date was the best management for fava bean.

Denmark

There is potential for the following crops in Denmark: quinoa, amaranth, lupin, pea, lentils, spring and winter fava beans. For most of these crops, there has been at least a 10% increase of arable land with protein crops in Denmark.

Amaranth has potential to be consumed both as leaf vegetable and as seed under moderate defoliation. Quinoa cultivars can be targeted to optimize yields under organic production systems. With sufficient rain (>300 mm), there is potential from growing lentils under intercropping systems with oats in Denmark with the benefit of weed reduction and yield gains. The final year of trials in the Protein2Food project will provide further observations and data for all presented trials (Screening, GxE, Intercropping, Fertilisation / Density in organic quinoa and winter faba beans). Scientific publications that synthesize these results are in preparation and await for the last year of replication to be completed. Results from this report should be shared with related projects and farmers in order to obtain their feedback and recommendations, being able to validate them and put to use in the near future.

The Netherlands

White and blue lupin

Crop protection by means of fungicide use, depending on the climatic conditions, result in a slightly higher yield on average. In case of contamination of seed with anthracnosis, more often occurring in white lupins, the use of fungicides has very limited possibilities. As soon as the disease appears in the field, it is often too late to make fungicide application effective. As anthracnose is a seed-borne fungal disease, a high level of hygiene in seed propagation, and could therefore be one of the best

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ways to prevent crop losses. Insect damage seems until now to be of little problem under Dutch conditions. Although the lupin aphid has been observed on bitter lupins, little damage has been reported from sweet lupin cultivation in the Netherlands.

Based on the results of our trials, there is no need to adapt the currently applied sowing densities for indeterminate and restricted branching varieties of blue (L. angustifolius) and white (L. albus) lupin. The hypothesis that especially restricted branching varieties could profit from a higher sowing density was not confirmed in our trials. In the table below, the recommended sowing densities are summarized. Furthermore, a sowing distance of 25 cm is usually applied under Dutch conditions, as this provides sufficient opportunities for mechanical weeding.

Recommended sowing densities for different growth types of white (L. albus) and blue (L. angustifolius) lupin.

Lupin species	**Growth type**	**Sowing density (plant/m2)**
L. albus	restricted branching	90
L. albus	indeterminate	50
L. angustifolius	restricted branching	110-120
L. angustifolius	indeterminate	90

Spring and winter faba bean

Crop protection in faba bean involves usually the use of fungicides, against chocolate spot disease, faba bean rust and sclerotinia, as well as the use of insecticides, against pea leaf weevil and black aphid, which might be a vector for several virus diseases. In climatic conditions favorable for the development of fungal diseases, crop protection can result in a considerable increase in yield. Caution should be taken with the application of pre-emergence herbicides in winter faba bean, as the active ingredients may damage the growth tip and true leaves that re-appear from below the soil surface after severe frost.

Based on the results of a limited number of trials, we would recommend a maximum sowing depth for winter faba bean of 10 cm under Dutch conditions. Deeper sowing will lead to later emergence, which may lower the possibilities of hardening before the first frost appears. A limited number of results from sowing density trials in winter faba bean did not result in significant differences in yield. While under favorable circumstances, winter faba bean may obtain higher average yields than spring-sown faba bean under Dutch circumstances, in case of harsh winter conditions and low plant survival, re-sowing fields in spring might be a viable alternative for farmers.

Soya bean

Only one trial has been carried out with crop protection in soya bean, in which little fungal diseases developed.

Crop protection

Lupins as green manure are thought to increase root-knot and root-lesion nematodes in general, and are therefore in The Netherlands negatively advised in susceptible rotations. Detailed information on nematode species-level was however lacking until this study was performed. The various host plant

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studies performed for this deliverable, show some tendencies concerning the development of nematodes during the growth of grain legumes. However, the performed trials provide preliminary results, and follow-up studies would be necessary to confirm the trends in our trials. It should also be taken in mind, that varieties of crops themselves may vary in sensitivity as host plants.

Pratylenchus penetrans

In the Jipsingboertange trials, all tested grain legumes were hosts of *Pratylenchus penetrans*, but to a different extent. Andean lupin and soya bean gave a very high reproduction of *P. penetrans*, followed by faba bean. Blue and white lupin gave a somewhat lower reproduction. In Western Australia, lupins are known as hosts of *Pratylenchus penetrans*. Also the sensitivity of faba bean for the genus Pratylenchus has been reported in the literature (Stoddard et al, 2010). Generally, faba bean is considered a susceptible host for *Pratylenchus penetrans*, but this susceptibility is reported to be cultivar-dependent as well.

Pratylenchus neglectus

In the Klazienaveen 2016 trial, faba bean, white and blue lupin (Rf=0) and buckwheat (Rf<1) suppressed California meadow nematode (*Pratylenchus neglectus*). Soya bean suppressed *P. neglectus* compared to fallow (Rf=1). Quinoa stimulated population development (Rf=2), but population development was less compared to black fallow. This is partially confirmed by findings from Western Australia, where lupins are known to be tolerant or resistant to the root-lesion nematode *Pratylenchus neglectus* and faba bean is considered to be resistant as well. On the other hand, susceptibility for *P. neglectus* has also been reported from Mediterranean cultivars of faba bean (Di Vito et al, 2002).

Meloidogyne chitwoodi

In the Klazienaveen 2016 trials, soya bean (Rf=0), white lupin and quinoa (Rf=0.5) suppressed Columbia root-knot nematode (*Meloidogyne chitwoodi*), while faba bean and buckwheat suppressed *M. chitwoodi* compared to black fallow (Rf=1). Blue lupin slightly increased the nematode population, but less compared to black fallow. Although the results from the Jipsingboertange trial was not significant, it confirmed the suppressive effect of soya bean on *M. chitwoodi*. However for lupins and faba bean results in Jipsingboertange were different, and the *M. chitwoodi* population increased during the growing season. The bio-assay finally gave a second confirmation of these results, as egg masses did develop in faba bean and lupins, but no egg masses were seen in soya been, although nodules had been formed. It is hypothesized that the roots of soya bean actively attract juveniles, but that the nematode is not able to complete its lifecycle inside the roots. Therefore we presume that soya bean could be actually incorporated in crop rotations to lower the pressure of *M. chitwoodi*.

Meloidogyne fallax

In the Klazienaveen 2016 trials, soya bean, blue lupin, white lupin, buckwheat and quinoa (Rf=0) suppressed *Meloidogyne fallax*. Faba bean suppressed *Meloidogyne fallax* as well (Rf=0.6), but less than black fallow (Rf=0.2). The 2015 Holten trials are more difficult to interpret, as no initial

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population levels were measured. Black fallow and lupins did not contain M. fallax at the end of the season, but faba bean did. More research will be necessary to confirm these results. In comparison: in Australia, the most widely used blue lupin cultivar Mandelup is reported to be slightly susceptible to *Meloidogyne fallax* (Duke, 1981).

Meloidogyne hapla

Meloidogyne hapla was present in the 2016 trials in Klazienaveen, but results among the different plots were not significantly different. However, very high reproduction of *M. hapla* took place in faba bean, blue lupin, and to a lesser extent in white lupin. In 2017, bioassay trials were performed with *Meloidogyne hapla* as well. In this trial, the high sensitivity of blue lupin was confirmed, faba bean being somewhat less sensitive. Soya bean was the least sensitive to *Meloidogyne hapla* in the bioassay, with on average only 1 egg/g root. In the literature it has been reported that both white and blue lupins are susceptible for *Meloidogyne hapla* (Duke, 1981), but little information is available on differences between cultivars. According to Stoddard et al. (2010) also faba bean is affected by the genus *Meloidogyne*. In (sub)tropical regions it is known to be susceptible for *Meloidogyne javanica* and *M. incognita*.

Heterodera schachtii

In the bioassay, we found several faba bean varieties and blue lupin varieties, that completely lacked any development of cysts. In contrast, all white lupin varieties did reproduce *H. schachtii* to a considerable extent. Soya bean slightly reproduced *H. schachtii*. In crop rotations that include beets, blue lupin or faba bean may be more adequate to cultivate compared to white lupin.

5. Delays and difficulties

The most difficult factors to control in field trials are the environmental conditions (weather, pests and diseases, soil conditions). High variations of environmental factors reduces the consistency of results. It is in the nature of field experiments that not all factors are controlled; therefore the level of variation is to take in account by using multiple replicates for the same experiment and the trials are repeated for at least three years (five years for the screening).

For instance, during these trials weather extremes were detected in Denmark in 2017 (humid year) and 2018 (the driest year in a century). In terms of pests and diseases, hares were a problem for some legumes in the first years, which were subsequently controlled using electric fences. In Italy some problems occurred due to birds that destroyed cotyledons of lupin plants during emergency, this problem was controlled using soil coverings after sowing.Diseases in the seed were present in crops such as quinoa, which have reduced the crop's potential and seeds germinability. Furthermore the results are from experimental trials with limited plot sizes, so extrapolations to kg ha^{-1} may give overestimation of results. However, trials in open fields are be made just to replicate farm conditions as close as possible during growing season.

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6. Impact and outreach

All the information produced in D1.10 will be collected and added to specific recommendations resulting from all trials implemented with the different species tested in Denmark, Netherlands and Italy. After validation, these results can be arranged in recommendations in order to increase availability and quality of proteins by dissemination to farmers. The data collection of all trials and other relevant information from all work packages of the project could be used to help gain optimal selection of species and cultivars to increase the agrobiodiversity and sustainability in Europe and diversify production systems.

Among the species tested, the majority are legumes, which encourages the reduction of chemical fertilizer use and increase of arable land destined to protein-crop production, using also marginal soils, promote transition from animal-based protein to plant based protein with clear impact on reduction of carbon footprint, of fertilisers and of water supply. All these effects bring positive contributions to the society for its sustainable and environmentally friendly growth and development. All the trials have been implemented in close communication with seed companies to test their materials, SMEs to share experiences in the species of interest. During all growing seasons we had visits from different stakeholders (farmers, researchers, breeders, students, other projects, SMEs and media). These interactions strengthen future collaborations to upscale production of protein crops and build new market opportunities.

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