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White lupin (*Lupinus albus* L.) yield in Pays de la Loire and its nitrogen provisioning services

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Master thesis title: **White lupin** (*Lupinus albus* L.) **yield in Pays de la Loire and its nitrogen provisioning services**

41 pages; 11 tables; 11 figures; bibliography; 5 appendices.

Keywords: White lupin, Pays de la Loire, Nitrogen, Succeeding wheat, Provisioning services, Performances; Intercropping.

	Abstract I-Context and literature review II-Material and methods III-Results IV-Discussion V-Conclusion
Objectives	As a traditional crop for feed and now for food in Pays de la Loire, white lupin have really small production area despite attractive selling prices. Documentation of production performances of white lupin is important to identify key cropping practices to improve and stabilize grain yields. Also, assessment of nitrogen provisioning services of white lupin to succeeding wheat may bring new incentives to unlock crop democratization.
Material & Methods	Grading, biomass and soil samplings were realized in a network of 25 fields of white lupin over two years with three lupin cropping strategies: sole cropped winter white lupin, sole cropped spring white lupin and winter white lupin intercropped with triticale. Additionally, 13 fields of wheat succeeding to white lupin were studied the same way, with areas with and without nitrogen fertilizer application.
Results	Sole cropped winter white lupin had the highest grain yield potential with an average of 6,5 t/ha the first year. A mean yield of 3,27 t/ha the second year confirmed the yield variability. Sole cropped spring white lupin had a mean yield of 2,93 t/ha. Intercropped winter white lupin had stable mean performances between the first and second year (2,21 and 2,08 t/ha respectively). Sole cropped winter lupin was sown too densely, which, with unusual weather condition, led to massive disease development. Spring lupin was sown too late in some fields reducing plant development before flowering and therefore yield. Intercropped winter lupin did not develop enough before winter and suffered from important plant losses. Plus, regardless lupin cropping strategy harvest losses could reach 75%. Weed biomass production was high in spring lupin and reduced lupin plant development through competition while for both sole cropped and intercropped winter white lupin, weed development was fairly controlled. Its main impact was weed seed bank build up for next crops. In Pays de la Loire the amount of nitrogen fixed by white lupin depends directly on its biomass production. Yet, succeeding wheat response to lupin's residues' nitrogen content was not clear when cropped without N fertilization. However, some plots without N produced almost the same yield as the rest of the field.
Conclusion	White lupin have a great potential in Pays de la Loire if sowing practices are improved and weed management solutions are offered to farmers. A better understanding of lupin pre-crop effect and nitrogen provisioning services would bring more interest to the crop and may lead to substantial nitrogen fertilizers use reduction.

Notice Bibliographique

Auteur: Timothée Cheriere
Promotion 2011 Patrons de mémoire : Christophe Naudin (ESA)
Titre du mémoire: Rendement du lupin blanc (*Lupinus albus* L.) dans les Pays de la Loire et son service de fourniture d'azote
41 pages; 11 tableaux; 11 figures; bibliographie; 5 annexes.

Mots clef: Lupin blanc, Pays de la Loire, Azote, Blé suivant, services de fourniture, Performances, association.

	Résumé d'auteur
Plan indicatif	I-Contexte et étude bibliographique II-Matériels et méthodes III-Résultats IV-Discussion
But de l'étude	Cultivé dans les Pays de la Loire pour nourrir le bétail le lupin blanc trouve aujourd'hui un nouvel essor à travers l'alimentation humaine. Malgré des prix attractifs la production reste confidentielle. L'acquisition de références locales sur les performances du lupin blanc s'avère nécessaire pour identifier les leviers qui permettront de stabiliser la production. De plus, l'estimation de la fourniture d'azote à la culture suivante peut jouer en faveur de la démocratisation de la culture.
Matériels & méthodes	Des échantillonnages de biomasses et de sols et divers notations ont été réalisés sur un réseau de 25 parcelles au cours de deux années avec trois modes de culture du lupin : le lupin d'hiver pur, le lupin d'hiver associé avec du triticale et le lupin pur de printemps. A cela s'ajoute le suivi de 13 parcelles de blé succédant à un lupin blanc avec des zones traitées avec apports d'azote et d'autres sans.
Résultats	Le lupin d'hiver cultivé seul a donné le meilleur rendement avec 6.5 t/ha la première année. Une moyenne de 3.27t/ha la seconde année a confirmé l'instabilité des rendements d'une année sur l'autre. Le lupin de printemps pur a produit en moyenne 2.93t/ha, quant au lupin associé, les rendements sont restés stables de la première année à la deuxième avec 2.21 et 2.08t/ha respectivement. En deuxième année, le lupin pur d'hiver a été semé avec une densité trop élevée qui, au gré des conditions météorologiques, a engendré un très fort développement de maladies. Les semis tardifs de lupin de printemps dans certains champs n'ont pas permis un développement suffisant des lupins, limitant ainsi le rendement. Les lupins associés n'ont pas accumulé suffisamment de biomasse avant l'hiver ce qui a eu pour conséquences de nombreuses pertes de plantes pendant et après l'hiver. De plus, quel que soit le mode de culture du lupin, les pertes à la récolte représentaient 50% du rendement expérimental en moyenne. Enfin, la production de biomasse adventice a été très élevée dans les champs de lupin de printemps ce qui a affecté le rendement via la compétition pour les ressources. Dans les champs de lupin d'hiver, associé ou non, le développement des adventices semblait contrôlé. Son impact principal a été l'augmentation du stock de semences d'adventices. Dans les Pays de la Loire, la quantité d'azote fixé par le lupin dépend directement de sa biomasse. Cependant, la réponse du blé suivant est difficilement mise en relation avec les performances du lupin.
Conclusions	Le lupin blanc a donc un fort potentiel si son implantation est soignée et que des solutions de désherbage sont proposées. Une meilleure compréhension de l'effet précédent pourrait aussi permettre des économies importantes d'engrais azoté et accroitre l'intérêt pour cette culture.

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More generally I am grateful to the entire LEVA team for the welcome and nice mood.

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Table of content

Abbreviations and acronyms

ATP = Adenosine triphosphate

BNF = Biological Nitrogen Fixation

C = Carbone

CAP = Common Agricultural Policy

CICES = Common International Classification of Ecosystem Services

CV = Coefficient of Variation

DM = Dry Matter

ES = Ecosystem Services

EU = European Union

HI = Harvest Index

IC = Intercropping

IntWL = Intercropped Winter (white) Lupin

LEGITIMES = LEGumes Insertion in Territories to Induce Main Ecosytem Services

LER = Land Equivalent Ratio

Mg = Magnesium

Mn = Manganese

N = Nitrogen

Ndfa = Nitrogen derived from atmosphere

NHI = Nitrogen Harvest Index

P = Phosphorus

SC = Sole Cropped

SLSC = Sole Cropped Spring (white) Lupin

TGW = Thousand Grain Weight

UR LEVA = Unité de Recherche Légumineuse, Ecophysiologie Végétale et Agro-écologie

WLSC = Sole Cropped Winter (white) Lupin

Zn = Zinc

I. Context and study scope

World protein needs are and will continue rising in the future due to the world population increase, the living condition improvement and the evolution toward a meat based diet in developing countries (Alexandratos and Bruinsma 2012). In the long term, a reduction of consumption of animal-derived protein seems inevitable (Boland et al. 2013) but this changes will take time. In the short term however, animal-products consumption increase, in large developing countries such as China or Brazil, have already changed protein market trends. For example, between 1995 and 2013, China multiplied its imports of pig meat by 9 and its imports of soybean by 22 (FAOSTAT, 2016). European Union (EU) is relatively self-sufficient in animal-based proteins but highly dependent regarding plant proteins (de Visser et al. 2014) as about 70% of feed proteins are imported from the Americas, mainly soybean or soybean meal (FEFAC 2015; UNIP 2015).

Häusling (2011) spoke in favour of a European plan to increase protein crops production within the EU by supporting breeding and research programs and supporting farmers with direct payment for protein crops within the framework of the Common Agricultural Policy (CAP). In this framework, a French national research project, LEGITIMES (LEGume Insertion in Territories to Induce Main Ecosystem Services) started. The aim is to design, with local stakeholders, ways to introduce grain legume crops taking into account their strategy, technical and economic constraints. To do so, the project's ambition is to understand crop's behavior and performances within the focus regions' specific contexts (Pays de le Loire, Midi-Pyrénées and Bourgogne). In Pays de la Loire, Terrena, a cooperative has a growing market of food additives made from white lupin floor. Their main problematic is sourcing of raw material as product demand is higher than total production. Despite high buying prices (375€ per ton in 2015) farmers are reluctant to produce this crop and every year some of them are stopping lupin production. To better understand lupin's response to pedo-climatic conditions and farmers practices in Pays de la Loire, Terrena and UR LEVA (Unité de Recherche Légumineuses, Ecophysiologie Végétale, Agroécologie) a research unit specialized in leguminous crops, work together to identify leverages to stabilize yields, and to create technical references and incentives for farmers.

White lupin (*Lupinus albus* L.) represents a really small part of agricultural land use in spite of its high nutritive value. It contains 33 to 47% of protein (Huyghe 1997; Lucas et al. 2015) with few anti-nutritive factors in sweet varieties. Its oil (8 to 14%) is of good quality because it contains a lot of polyunsaturated fatty acids (Huyghe 1997; Lucas et al. 2015). In France the surface dedicated to white lupin production was only 4800 ha in 2014 with an average yield of 2.6 tons/ha (UNIP 2015). This is partly explained by the fact that lupin have low yield levels (Huyghe 1997) and the highest yield variability amongst European grain legumes (Cernay et al. 2015). For example, in United Kingdom, Shield et al. (1996) obtained a yield range of 0.3 to 4.5 t/ha in experimental station. In Chile, Espinoza et al. (2012) found an average yield of 2.3t/ha the first year and 5.37t/ha during the second year and 1.3 to 3.6t/ha were found by (López-Bellido et al. 1994), in southern Spain.

Understanding yield variability, creating technical references and presenting incentives to farmers is essential for the propagation of the crop. Ecological and economic assessment and quantification of legumes services to the cropping system (such as the pre-crop effect), may allow the implementation of adapted laws and economic measures leading to a wider adoption of these crops (Häyhä and Franzese 2014; Preissel et al. 2015).

I.1. Ecosystem services

As every ecosystem constituent, white lupin (*Lupinus albus* L.) produces biomass and has an impact on its surroundings. By domesticating this plant, civilizations could enjoy the products (seeds, straw…) and benefit from positive side-effects such as oxygen provision, soil structuration or biological nitrogen fixation. This benefits obtained from the plant and more largely by any existing ecosystem has been defined as Ecosystem Services (ES) by the Millennium Ecosystem Assessment of 2005 (Millennium Ecosystem Assessment 2005). In this document, Ecosystem Services have been classified into 4 groups:

- Provisioning services: "the products obtained from the ecosystem". This category includes, among others, food, any raw material sourced from an ecosystem (wood, fiber…) and fresh water.

- Regulating services: "the benefits obtained from the regulation of ecosystem processes". This group concerns water and air quality, erosion, climate or even pest and disease regulation as the services obtained from large scale processes and not a direct source of goods but improving the environment state and influencing human's well-being.

- Cultural services: "the non-material benefits people obtain from ecosystems through spiritual enrichment, cognitive development, reflection, recreation, and aesthetic experiences". Here, any landscape, landmark and more generally the diversity of and in ecosystems contribute to these services.

- Supporting services: "services which are necessary for the production of all other ES". Their impact and benefits for people are indirect, as these services are required for production (e.g.: photosynthesis or soil formation) or for regulation such as water quality improvement thanks to water cycling.

While any changes in provisioning, regulating and cultural services directly impact human life and well-being, changes in supporting services impact indirectly or in the longer term human well-being (Millennium Ecosystem Assessment 2005). To simplify the classification and the understanding of this concept, a Common International Classification of Ecosystem Services (CICES) has been elaborated through the work of the European Environment Agency (Haines-Young and Potschin 2013). In this classification supporting services is not a category as it is considered as parts of the underlying processes taking place to obtain the "final ecosystems services" which are provisioning, regulation and cultural services (Haines-Young and Potschin 2013).

Such definition and classification of ES aims at facilitating economic accounting of such services obtained from nature (Haines-Young and Potschin 2013). Indeed, ES, being benefits to human, avoids certain costs of production and their disruption or suppression would generate costs for society (Millennium Ecosystem Assessment 2005). To achieve sustainable management of ecosystems, Häyhä and Franzese (2014) suggested that economic and biophysical analysis and understanding of ES is necessary.

Agro-ecosystems managed by man benefit from ecosystems services (e.g.: climate regulation, pollination…) but may produce ecosystems dis-services which reduce productivity or increase production costs (e.g.: pest damage, natural disasters…) (Zhang et al. 2007).

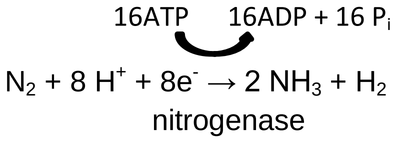
Furthermore, agriculture provides all three CICES categories of services (e.g.: food production, soil conservation, creating and maintaining landscapes…) and dis-services (e.g.: pollution of natural resources, destruction of natural ecosystems...) (Zhang et al. 2007).

Legumes crops are well known for their biological nitrogen fixation (BNF) which provides an important service to agro-ecosystems with a clear reduction of global warming potential compared to agro-ecosystems without legumes using artificial nitrogen (N) fertilizers (Crews and Peoples 2004). Other services are provided by legumes to agro-ecosystem. Faba bean services have been the center of a detailed review by Köpke and Nemecek (2010) but no such work has been done for white lupin. Provisioning services of white lupin and its components will be developed as well as regulation and maintenance services.

I.2. Legume's main ecosystem service: Biological Nitrogen Fixation (BNF)

Bacteria genus *Rhizobium* is characterized by its ability to transform inorganic atmospheric di-nitrogen (N_2) into ammonia (NH_3) when in symbiosis with legume's roots. White lupin, is able to form a symbiosis with *Bradyrhizobium lupini*. *Bradyrhizobium* genus was created to isolate a group of non-acid producing and slow growing *rhizobia* (Jordan 1982) but they have the same nitrogen fixing abilities.

When in the vicinity of root's hairs, bacteria produce the 'nod-factor' which is perceived by the root epidermis, orienting the growth of root's hair toward the bacteria. Once in contact with the root, the bacteria penetrate into the root cells and form with it a nodule, where the nitrogen fixation will occur (Moling and Bisseling 2015). The fixation process in nodule takes place in anaerobic conditions. Under the action of nitrogenase enzyme, a N_2 molecule combined with eight protons is turned into ammonia with the consumption of 16 ATPs (De Bruijn 2015). The chemical formula is:



$$\begin{array}{c} 16ATP \curvearrowright 16ADP + 16\,P_i \\ N_2 + 8\,H^+ + 8e^- \rightarrow 2\,NH_3 + H_2 \\ \text{nitrogenase} \end{array}$$

This symbiosis with *Bradyrhizobium lupini,* enables atmospheric nitrogen fixation and therefore, plant growth in low soil mineral nitrogen availability conditions. Depending on soil mineral N content and pedo-climatic conditions, white lupin has been found able to sustain 89% of its N requirements from the atmosphere (Mayer et al. 2003), or 62 to 89% of its shoot N (Espinoza et al. 2012), which corresponds to a fixation of about 93 to 311 kg of N per hectare. In their meta-analysis, Unkovich et al. (2010) found an average percentage of N derived from atmosphere (%Ndfa) in white lupin shoots of 41, with a minimum value of 14 and a maximum of 75%Ndfa. They also calculated a "root factor" of 1.71 to obtain the shoot + root N through the following formula:

$$\text{shoot N} + \text{root N} = \text{shoot total N} * 1.71$$

The 'root factor' is based on the value of the shoot:root N ratio (1.40) of white lupin obtained from previous research works with experiments made in pots. The shoot:root N ratio has been calculated for lupin around mid-flowering stage (Unkovich and Pate 2000).

$$\text{'Root factor'} = 1 + 1/(\text{Shoot : root N})$$

Nonetheless, BNF is dependent on several factors. First, the symbiosis has to occur and seed inoculation is often recommended to obtain an important nodule production and as a consequence achieve satisfactory fixation levels. Secondly, nodulation and fixation is greatly reduced by the presence of N derived compounds such as nitrate, urea or ammonium even at low concentration in the soil (Guo et al. 1992). Therefore, the less mineral N is present in the soil the more efficient BNF is. Also, compacted soils and excess or lack of water reduce significantly fixation (Sprent 1972).

I.3. Provisioning services of white lupin

I.3.A Lupin growth and yield formation

In western France, white lupin can be autumn-sown thanks to winter hardy cultivars or sown in spring. Autumn sown white lupin has a long life cycle, about 11 month whereas spring sown white lupin has a development cycle of 7 to 8 month. Winter hardy genotypes of white lupin, sown in late September, flower in April and are harvested in August. Spring genotypes should be sown between mid-February and early March for a flowering time beginning in May and a harvest occurring in September (Carrouée et al. 2003; Terres Inovia 2016).

I.3.A.a. Plant architecture and determinancy

After emergence and vegetative growth, white lupin starts its flower initiation. The main stem elongates and flowers. Once the first flowers are open, axillary buds near the inflorescence start their development into branches. These branches, called first order branches (Figure 1), flowers as well, triggering the development of second order branches and so on. The number of potential branches linearly increases with the number of nodes on the previous order branch (Julier and Huyghe 1993; Munier-Jolain et al. 1997).

When a plant is determinate, all buds become floral at a given time of the growth cycle therefore forbidding vegetative growth (Huyghe 1997). White lupin have naturally an indeterminate growth habit but determinate mutants where found and this genotype was taken into account in breeding programs (Huyghe 1997).

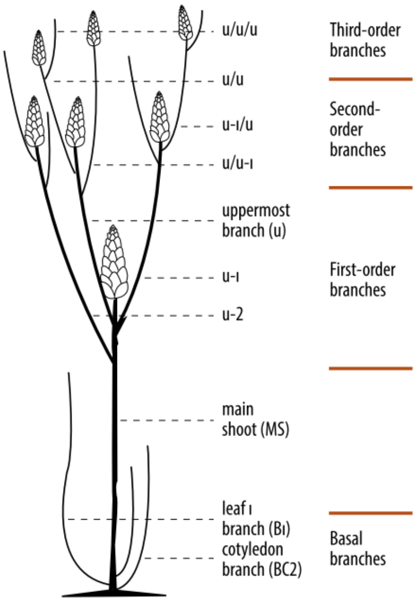


Figure 1: Lupin's plant architecture (from Walker et al. 2011)

Determinancy or indeterminancy has an impact on dry matter allocation and therefore competition between vegetative and reproductive organs of the plant. Vegetative growth and pod filling occur at the same time for indeterminate cultivars whereas pod filling becomes significant after the end of vegetative organs (stems and leaves) development (Julier et al. 1993a). This indeterminate development may induce high competition between the developing branches and the lower branches' pods.

I.3.A.b. Plant development and yield

Main yield components of French white lupin are: plant per square meter, number of pods per plant and number of seeds per pod to obtain a high seed number per square meter and seed weight (ARVALIS and UNIP 2010). Even though plant per square meter have an impact on yield when the number of plant per square meter is lower than 15 (Shield et al. 1996), above this threshold density, plant architecture changes lead to a variation in number of pods per plant but yield remains stable (Shield et al. 1996; Lopez-Bellido et al. 2000). The number of pods per square meter has been revealed an important component for yield (Lopez-Bellido et al. 2000; Noffsinger et al. 2000) and was responsible for more than 90% of yield variance in Shield et al. (1996) experiments.

According to Withers (1984 cited by Julier et al. 1993) seed number per pod is relatively constant, which is consistent with the results of Lopez-Bellido et al. (2000) who found about 3 seeds/pod, unaltered by increasing plant density nor by sowing date (López-Bellido et al. 1994). Seed weight has been found an important factor for yield by Noffsinger et al. (2000) but yield was independent from seed weight in the experiment of Julier et al. (1993). Lastly, the harvest index (HI) defined as the seed yield divided by the total above-ground dry matter at maturity was not found correlated with yield by Julier et al. (1993) and was negatively correlated with plant density (Lopez-Bellido et al. 2000). HI varied from 0.28 to 0.57 (Julier et al. 1993b). It was constant across sowing date when averaged over five years with 35% (López-Bellido et al. 1994) but not for Julier et al. (1993) where delayed sowing increased HI. It varied from 15 to 50% across years and densities with average values of 36, 30 and 27% for 20, 40 and 60 plant/m² respectively (Lopez-Bellido et al. 2000). There is no consensus on the relations of seed weight and HI to yield in scientific community.

Finally, Noffsinger et al. (2000) found that main stem and first order branches bore the majority of the yield. And yield of white lupin was positively correlated to total above-ground dry matter (Julier et al. 1993b).

All the previously described parameters are influenced by the growing conditions and biotic and abiotic stresses may greatly influence production services of lupin.

I.3.A.c. Temperature requirements and sensitivity

White lupin is a plant which development is negligible below 3°C, therefore the base temperature used to calculate sums of temperature is 3°C (Huyghe 1991). White lupin's vernalization requirements vary across genotypes, winter white lupin Lunoble cultivar have high requirements (about 800 vernalization units (expressed in °C.d)) (Huyghe and Papineau 1990). Spring white lupin have very low vernalization needs and the cultivar 'start' has been qualified as thermo-neutral by Clapham and Willcott (1995) because it does not require any vernalization time. Huyghe (1991) showed that temperatures between 1 and 14°C allowed vernalization.

Finally, vernalization requirements decreased with the augmentation of the number of leaf primordia produced by the apex (Huyghe 1991). Autumn sown, Lunoble cultivar with high vernalization requirements (Huyghe and Papineau 1990), required 800 vernalization units when 20 leaf primordia have been initiated while it only needed 400 vernalization units with 50 leaf primordia.

During early vegetative growth and from the moment of floral initiation onward, white lupin is frost sensitive. White lupin is subjected to frost damages to roots, leaves or apex, occurring as soon as soil temperature drops to -1°C for few days, depending on the development stage and therefore root size. Seedling which produced 14 leaf primordia or less were extremely sensitive to soil freezing regardless the temperature and duration of frost. Above 25 leaf primordia produced, plants did not suffer root damages from any soil freezing treatment in Leach et al. (1997) experiment. In between those values, roots suffered various degrees of symptoms from local discoloration to severe damage causing the death of the plant.

During vegetative growth, temperature of -6°C damage cells of white lupin (Walker et al. 2011). After flower initiation, lupin is more sensitive to low temperatures as below 8°C pollen germination is delayed and tube growth is slower. Flower abortion occurs as soon as temperatures drop below 0°C.

Temperatures above 28°C during flowering can cause flower's sterility and temperatures superior to 30°C may cause abortion if it happens early in pod filling and reduces single seed size and weight therefore affecting yield. Heat stress is enhanced when combined with water stress (Walker et al. 2011).

For autumn sown white lupin, studies highlighted the importance of sowing date to avoid winter losses either by excessive or insufficient plant development before winter (Shield et al. 1996; Bateman et al. 1997; Leach et al. 1997; Shield et al. 2000; Annicchiarico and Iannucci 2007).

I.3.B. Abiotic stresses

I.3.B.a. Water stresses

Whether it is excess of water which asphyxiate roots, damaging them, and reduces nodules activity (Sprent 1972; Walker et al. 2011), or drought which reduces metabolism functioning, white lupin does not tolerate well water stresses (less than other legumes crops). Huyghe (1997) suggested that avoiding drought periods with early flowering and early pod growth cultivars was the best option. Early drought events can be tolerated by white lupin thanks to early stomatal closure during midday hours and increased rates of leaves senescence to reduce water losses (Rodrigues et al. 1995). Additionally, augmentation of fine root surface in deep soil layers enhances water uptake.

I.3.B.b. pH sensitivity

White lupin develops well on soils with pH from 4.5 to 7.5 (Huyghe 1997). Above 7.5 lime-induced chlorosis may appear in calcareous soils and below 4.5 lupin risks aluminum toxicity (Huyghe 1997). Also, in high soil pH nodulation is less likely to occur on white lupin's roots (Walker et al. 2011). Tang and Thomson (1996) showed that white lupin growth and its symbiosis with *bradyrhizobium lupini* where both affected when pH value increases. In their experiments Kerley et al. (2004) found important dry weight losses for white lupin grown in soils with a pH below 4.9 or above 7.2, and quick death of plantlets for pH below 4.4.

I.3.B.c. Sensitivity to calcareous soils

White lupin is highly sensitive to calcareous soils and, in technical literature, it is commonly said that lupin should not be grown in calcareous soils containing more than 2.5% of $CaCO_3$ (Carrouée et al. 2003; Terres Inovia 2016). A common sign of calcareous intolerance visible on lupin plant is the yellowing of the leaves with the veins remaining green. Kerley (2000) showed that lime presence in soil affects root and shoot dry weight and affects plant development potential at early stage as the leaf primordia number of the main stem decreases with the increase of soil lime concentration. The same study highlighted the lower phosphorus (P) and manganese (Mn) proportions in lupin grown in limed soil, suggesting a lower efficiency to uptake those nutrient in limed soils.

I.3.C. Biotic stresses

I.3.C.a. Diseases and insects

The main pest which may cause severe damages to white lupin is *Delia platura*. The fly's larva feeds on the roots and hypocotyls. Seed treatments and early ploughing can contribute to reduce damages do the crop (Huyghe 1997; Walker et al. 2011; Terres Inovia 2016). Slugs, rabbits, birds, *Agriotes spp*. or aphids may also damage the crops to a lesser extent (Carrouée et al. 2003; Terres Inovia 2016).

Several diseases are able to cause severe damage to white lupin: *Colletrotrichum lupini, Botrytis cinerea, Sclerotinia sclerotiorum*, *Uromyces lupinicolus* (Rust) and *Pleiochaeta setosa* (Brown spot disease). Chemicals exist to control these diseases and try to reduce damages but they have to be combined to management practices to be efficient (Huyghe 1997; Carrouée et al. 2003; Terres Inovia 2016).

I.3.C.b. Weed competition

With its long development cycle (7 months for spring-sown or 11 months for autumn-sown white lupin), low soil covering potential in early stages and wide row spacing, white lupin is quite sensitive to competition from weeds (Carrouée et al. 2003; ARVALIS and UNIP 2010; Walker et al. 2011). Several studies stressed the sensitivity of narrow-leafed lupin (*Lupinus angustifolius*) to weed competition, with yield losses of 67% compared to weed free plots (Strydhorst et al. 2008; Hashem et al. 2011). Also lupin was considered as dependent on herbicides (Perry et al. 1998 in Jensen et al. 2004). Application of non-selective herbicide in wide row fields with spray shield to avoid damage on lupin has been shown to be an efficient solution for narrow-leafed lupin weed control (Hashem et al. 2011). However with increasing environmental measures, herbicide resistance from weeds and product prices, alternative solutions are investigated. Mechanical weed control in white lupin and its consequences on plant development have been investigated and show promising results with high tolerance to soil covering by lupin (Jensen et al. 2004b) and 80% of weed population reduction in white lupin fields (Folgart et al. 2011). Nonetheless Boström (2008) reported a 5% dry matter loss when narrow-leafed lupin intercropped cereals for forage was weeded through harrowing. Folgart et al. (2011) also tested black oat as companion crop for its allelopathic effects and its high shading abilities in early growth stages. Weed control by black oat was conclusive for some weed species with up to 90% reduction but irrelevant for others.

While companion crops are not harvested, intercropping of white lupin with cereals may play an increasing role in weed control strategies. As it also allows harvest of a second crop, it may increase of overall production per hectare and provide higher resilience to climatic incidents as it has been studied for other legume species intercropping (Corre-Hellou et al. 2013).

I.3.D. Intercropping of white lupin with cereal

Intercropping is defined as growing two crops or more simultaneously on the same field in such a way that they interact agronomically (Wiley et al. 1979). It can be mixed intercropping (e.g.: intercropping with no distinct row management), row intercropping when crops are planted in alternating rows, strip intercropping when crops are planted in alternating strips of rows and/or relay intercropping when crops are simultaneously grown for only parts of their development cycle (Andrews and Kassam, 1976).

I.3.D.a. Intercropping for weed control

Mechanisms of weed suppression in intercropping of legumes and cereals have been studied. For example, in pea-barley intercropping, barley's competitiveness toward soil's inorganic N, contributes to the reduction of weeds population in comparison with sole cropped legume, even with low barley density while pea sustains its N needs through biological nitrogen fixation (Hauggaard-Nielsen et al. 2001; Corre-Hellou et al. 2011). The intercropping of narrow-leafed lupin with barley was found more competitive toward weed than sole cropped lupin (Hauggaard-Nielsen et al. 2008). Although, white lupin intercropped with triticale expressed promising results in terms of weed suppression both at flowering and maturity stages (Carton et al. 2015), really few studies have been realized regarding white lupin and cereal intercropping's effect on weed populations.

I.3.D.b. Intercropping for guaranteed production and increased total grain production

Diversity in the plant community within the same field tends to increase robustness and resilience of the system toward biotic stresses such as diseases or pests (Trenbath 1993; Boudreau 2013). Hauggaard-Nielsen et al. (2008) found a reduction of 80% of brown spot disease on narrow-leafed lupin intercropped (IC) with barley compared to its sole crop (SC). However, these phenomenon are highly variable from one study to another (Boudreau 2013).

Increased overall production in intercropping fields have been reviewed (Bedoussac et al. 2015). In intercropping of grain legumes and cereals complementary resource use (nutrient, light…) leads to a total grain yield generally superior and more stable than the same crops cultivated separately in an equivalent land surface (Hauggaard-Nielsen et al. 2008; Bedoussac et al. 2015).

For their work on maize and beans Willey and Osiru (1972) proposed to compare yields of maize and bean intercropped with their respective sole crops yields to obtain the equivalent surface of land necessary to produce the same quantity of grain of each species when maize and beans are grown in a sole cropped design. This is now expressed through the Land Equivalent Ratio (LER).

$$LER_{i/j} = (Yic_i / Ysc_i) + (Yic_j / Ysc_j)$$

For any crop i or j, intercropped, a LER can be calculated. With Yic being the yield per hectare of one specie when intercropped and Ysc the yield per hectare of the same specie cropped by itself.

For a given crop with a given production in the intercropping field, the ratio Yic/Ysc corresponds to the surface of land necessary to obtain the same production when the given crop is sole cropped. When all the ratios of individual crops of the intercropping system are added, LER can be superior to one, meaning that more than one hectare of separated combination of respective sole crops would be necessary to produce the same amount of grain. For example Jannasch and Martin (1999) found a maximum forage LER of 1.27 for wheat intercropped with white lupin meaning that 27% more surface would be necessary to produce the same amount of forage if wheat and white lupin were grown separately.

Yet, adaptation of the intercropping concept to different crops requires time and trials. As marginal crops, narrow-leafed lupin or white lupin did not undergo many trials related to intercropping.

Knudsen et al. (2004) did not find narrow-leafed lupin more or less productive in intercropping with barley than their sole crops grain yields as the LER calculated were superior to 0.9 but inferior to 1. For forage production, white lupin intercropped with cereals (oat or wheat) was more productive than white lupin sole crop as dry matter (DM) LER were ranging from 1.00 to 1.27 across year and treatment (Jannasch and Martin 1999). They also obtained crude protein LER ranging from 1.04 to 1.53. For white lupin intercropped with barley or wheat in pot experiment LER of around 1.4 were obtained for both intercropping modalities (Mariotti et al. 2009).

In the same study, white lupin shoot and root biomass was reduced by about 23% and 18% respectively (Mariotti et al. 2009). Reproductive parts of the plants were more affected by competition than vegetative parts. Below ground competition affected the dry weight per pod while above ground competition reduced the number of pods per plant.

Because of the potential weed control improvement, reduction of disease pressure and guaranteed production intercropping systems represent a part of the solution to stabilise production of white lupin seeds.

I.3.D.c. Influence of the crop cultivated before white lupin

Evidences suggest that lupin crop benefits from services of the previous crop. Lizarazo et al. (2015) demonstrated that narrow-leafed lupin was sensitive to pre-crop effect with the most positive effect attributed to barley, improving the whole plant nutrient composition. Narrow-leafed lupin seeds had higher Mg, Mn and Zn following barley. Nonetheless, the previous crop did not affect yield or protein concentration in seeds for narrow-leafed lupin (Lizarazo et al. 2015). Nitrogen fertilization of the pre-crop also has an impact on the following white lupin growth and performances due to the residual N in the soil (Wiatrak et al. 2004).

Chan and Heenan, (1996) found that soil that had been under narrow-leaf lupin wheat rotation were more porous than the ones with pea-wheat rotation or barley-wheat rotation. However this applies to Australian conditions.

White lupin is a sensitive crop and its production services may be largely affected by biotic and abiotic factors. As every single ecosystem constituent, white lupin also provides regulation and maintenance services.

I.4. Regulation and maintenance services

I.4.A. Nutrient independence

I.4.A.a. BNF in intercropping

As the management of the previous crop influences the mineral N available in the soil, a solution to maximize BNF could be intercropping as it has been shown that cereal was more competitive to uptake inorganic N from than legumes (Hauggaard-Nielsen and Jensen 2001; Mariotti et al. 2009). As cereals are more likely to uptake mineral nitrogen during growth cycle, the legume will rely more on BNF for its nutrition. Because of this, intercropping of legumes with cereals increases the proportion of N derived from the atmosphere in legumes, Hauggaard-Nielsen et al. (2008) found an increase of 10 to 15% of N derived from atmosphere in grain legumes when intercropped compared to their sole crop. Knudsen et al. (2004) found that barley's N grain concentration was improved in systems where barley was intercropped with pea or faba beans, with the legume being the dominant crop in the mixture however, this fact did not apply to barley intercropped with narrow-leafed lupin (Knudsen et al. 2004). Nonetheless, when harvested for forage, wheat was found to have a higher yield of crude protein when intercropped with white lupin than in sole crop (Jannasch and Martin 1999). These results are coherent with those of Mariotti et al. (2009) who found a higher shoot nitrogen concentration in wheat and barley intercropped with white lupin.

I.4.A.b. Phosphorus acquisition in soil

Thanks to its proteoïd roots, white lupin is able to access non-labile phosphorus from the soil that is unavailable to other plants (Braum and Helmke 1995). This is possible thanks to the production of citrate and protons which, in the vicinity of the roots, dissolve, amongst other, iron-phosphates or aluminum phosphates (Gardner et al. 1982). This ability makes white lupin tolerant to P-deficient soils, nonetheless, addition of phosphorus fertilizer increases grain yield (Mullins et al. 2001).

This ability to mobilize soil's non-labile P is a very interesting aspect in intercropping as it has been demonstrated several times. White lupin intercropping improves P nutrition of wheat through the use of different P-pools by the two plants and as well thanks to the extra-phosphorus made available by lupin to wheat through dissolution of non-labile P (Gardner and Boundy 1983; Cu et al. 2005).

I.4.B. Pre-crop effect of White lupin

I.4.B.a. Nutrient related pre-crop effect

The most expected pre-crop effect of white lupin is linked to the residual nitrogen in the field, originating from BNF. As nitrogen fixing plant some legumes leave nitrogen in the soil after harvest through BNF and residue breakdown (aboveground as well as belowground). For example, narrow-leafed lupin left 15 kg of residual N/ha more than oats in sandy soil and 18 to 27 kg residual N/ha more than oats in loamy soil (Jensen et al. 2004a) or an estimated net of 29 and 128kgN/ha in two experimental site in Florida (Doyle et al. 1988). Espinoza et al. (2012) estimated the net input of N from white lupin's crop residues of 32 kg N/ha in 2008 and of 227kgN/ha the next year. However, in literature it is more common to find yield gains due to a legume pre-crop relatively to a non-N fixing control pre-crop when wheat does not receive any N fertilization. Wheat following narrow-leafed lupin yielded 57% more than an unfertilized wheat following wheat for Doyle et al.(1988) or 77% more wheat yield after a narrow-leafed lupin compared to a oat pre-crop (Jensen et al. 2004a). Without N fertilizers, white lupin was found to increase wheat yield compared to a wheat following oat by an extra 0.78 and 1.89 t/ha in 2009 and 2010 respectively (Espinoza et al. 2012). A consensus exist on the fact that this yield advantages are reduced or suppressed with application of N fertilizers on the subsequent wheat (Doyle et al. 1988; Jensen et al. 2004a; Espinoza et al. 2012; Seymour et al. 2012). Finally, Seymour et al. (2012) found the wheat yield significantly related to narrow-leafed lupin's yield. One of the hypotheses developed to explain this relation is that the more biomass there is the more biological nitrogen fixation there is and as the harvest index 0.30 (Unkovich et al. 2010a) is low, a large amount of residues is restituted to the soil.

Furthermore, white lupin residues have a fast mineralization potential as it starts 5 days after incorporation of the residues in poor sandy soils (Carranca et al. 2009). Also, with 23 and 9.4 mgN/g of shoot and root residue respectively and a C/N ratio 18.8 for shoot and 21.2 for root residues, 1.9 and 1.6 mgP/g and a C/P ratio of 232 and 126 for shoot and root residues respectively, decomposition of white lupin residues result in net mineralization of both N and P (Mat Hassan et al. 2012). Yet, the same study highlighted reduced growth of subsequent wheat due to the presence of white lupin residues on the soil. Nonetheless, this result were obtained after six weeks of growth, therefore there are no indication about grain yield.

Regarding phosphorus nutrition, white lupin pre-crop improves following wheat P uptake (Mat Hassan et al. 2013) this may be explained by the greater P release by white lupin residues compared to faba bean or chick pea (Mat Hassan et al. 2012).

I.4.B.b. Non-nutrient related pre-crop effect

Nitrogen pre-crop effect is the easiest to quantify and the most obvious in low input systems. Other services to the subsequent crop obtained are highly related to the break-crop effect such as disease control through non-hosting mechanisms, soil structure improvement with strong root systems or weed control (Kirkegaard et al. 2008).

Weed population evolves according to the crop rotation and strategies of weed control can be based on change in plant family, plant development cycle or even competitiveness toward weeds. The long development cycle of white lupin and the limited weed control options in the late phases might result in an increase of weed seed bank which will impact the entire rotation. Strydhorst et al. (2008) found that wheat yield decreased by seven percent when weeds were not properly controlled in the legume pre-crop while the average yield decrease in the pulse crop was 42%.

I.5. Study's scope

White lupin grain is a promising alternative to soybean for animal feed and shows good technological qualities for food industry. However, its performances, highly variable, reduce its attractiveness for farmers. Subject to several biotic and abiotic stresses but tolerant regarding poor N and P soils status and density variations, white lupin may have, in Pays de la Loire (western France), preferred growing conditions and management practices. Lupin's cropping strategies might be more suited to different contexts: spring lupin for cooler regions (Terres Inovia 2016), winter lupin for region where frost is not an issue (Huyghe 1997) or intercropped white lupin with a cereal for weed control (Folgart et al. 2011). Whether it is pedo-climatic conditions, with water stresses, temperature influence, soil's condition or management practices, identification of the key cropping management is essential to improve production.

In addition to the services provided during its cultivation, white lupin may provide regulation and maintenance services to the following crop. Few studies exist to link performances of lupin to the intensity of the services it may provide to the following crop. None of them concerns white lupin.

For narrow-leafed lupin, Seymour et al. (2012) observed a strong influence of lupin's yield on subsequent wheat's yield. Their main hypothesis to explain this observation relies on a strong relationship between above ground biomass and N_2 fixation (Unkovich et al. 2010b). Thus, if high yields are obtained, a high amount of dry matter is produced and a lot of nitrogen is fixed on the field. Low harvest index would results in large quantities of lupin residues and therefore a large quantity of N released during residues break-down benefiting to the following crop.

Julier et al. (1993) found white lupin yield highly correlated to its total above ground biomass. If a relation similar to the one linking total nitrogen fixation and narrow-leafed lupin biomass (Unkovich et al. 2010b) exist for white lupin, lupin's yield would be linked to the amount of nitrogen fixed and therefore would influence the yield of the following crop through net N mineralization of nitrogen rich residues (Mat Hassan et al. 2012). However, this effect is expected to be neglected in fields where N fertilizers are applied. A direct consequence of this pre-crop effect on following wheat could be the reduction of N fertilizer applied to the field.

Performances of white lupin sole crop may be uncertain and unattractive because of its high yield variations among fields and years. Intercropping white lupin with a cereal such as triticale might be a solution to stabilize yields and enhance ecological services use. Establishment of a range of production services according to the cropping strategy, identifying key moments of the crop management and establishing a range of nitrogen fixation service which could be expected from white lupin toward the following crop may create incentive to a wider adoption of this crop.

Hypothesis:

Performances of white lupin in Pays de la Loire are highly variable:

They differ between cropping strategies (spring lupin, winter lupin and intercropped lupin).

Variation of performances is high between fields of the same cropping strategy within the same year.

High production services of white lupin's crop are linked to high biological nitrogen fixation which provides large nitrogen quantities for the following crop.

High yield implies high above ground dry matter which implies high amount of nitrogen fixed and high residue dry matter left on field.

II. Methodology

By its participative approach, the project involves supply chain stakeholders in order to identify key elements to expend leguminous cropping areas. Therefore, experiments in farmer's fields are the most suited. Thus, regional agronomic diagnosis methodology (Doré et al. 1997; Doré et al. 2008) was chosen. This methodology directly involves farmers and may trigger changes in practices. This methodology proposes the establishment of a network of fields chosen within the area of interest and the definition of parameters to be measured in fields to establish links between the environment states influenced by climate and farmers' practices and the yield build-up.

II.1. Area's characteristics

II.1.A. Network

Located around Chateaubriant, west of France (Appendix 1), the fields' network is composed of 25 fields. 16 fields were used for the experiments of the first lupin repetition (2014 to 2016) and 9 were added to the experiment for the second repetition (2015 to 2017) (See Appendix 2). In 2015/2016 wheat crop following lupin was studied on 13 out of the 16 first year lupin fields. One field, cropped with barley, did not have repetition but was subjected to the second year protocol as barley's management and development are close to the ones of wheat. Finally spring lupin was studied only during the first year (2014/2015). Hereafter, 2015 refers to campaign 2014/2015 and 2016 refers to 2015/2016.

II.1.B. Soil characteristics

On the ploughed layer (0-30cm), soils contain between 10 and 30% clay, between 25 and 65% silt and 10 to 50% sand.

Two dominant soil types occur in the study area (see Appendices 1 and 2):

- In the southern fields, loam is the main soil type. With one exception which is silty loam.
- Silty loam soils dominate in the northern fields. However, three fields are loamy and one is composed of silty clay loam.

Over the 25 fields, soil's pH is relatively neutral, values ranging from 6.2 to 7.8 and soil organic matter varies from 1.3 to 3.7 (Appendix 2). Soil stone cover varied from 5 to 30% and soil depth, estimated from soil sampling depth, varied from 60 to 90cm.

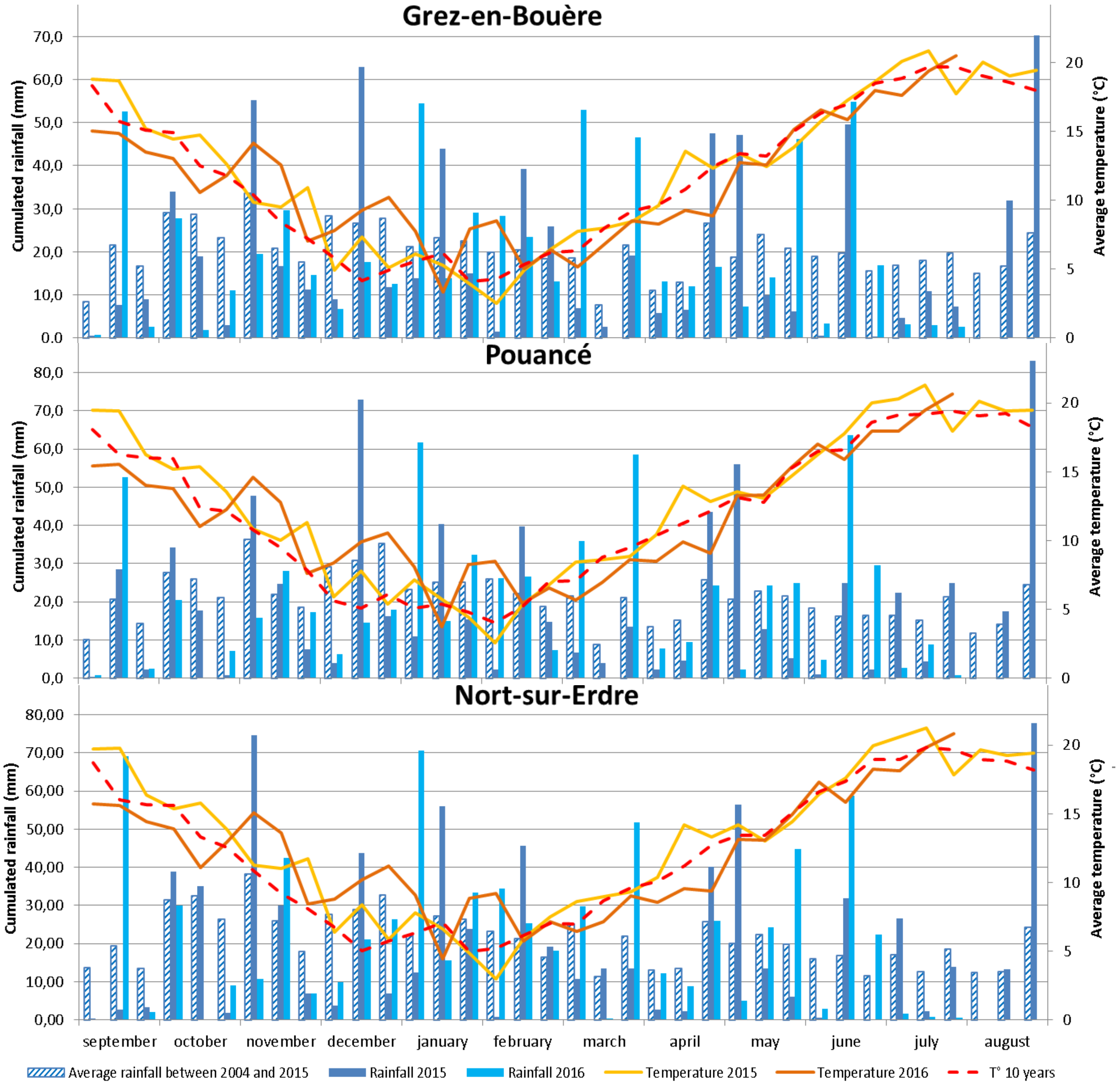


Figure 2: 10 years average, 2015 and 2016 temperatures and rainfall for the three reference meteorological stations (from Météo France, 2016)

II.1.C. Meteorological conditions

Within the same year, meteorological conditions were similar across the study territory (Figure 2 and appendix 3). Average temperatures were similar within the same year in all three stations. Grez-en-Bouère showed the highest number of days with temperatures below 0°C and above 28°C and most of the extreme values cold or hot (appendix 3). Frost was more severe in 2015 with more days with minimal values below 0°C but in 2016 frost occurred until May. High temperatures (≥ 28°C) were recorded earlier and in a higher number of days in 2015 than in 2016. In 2016 average temperatures from late October to February were above decade's temperatures and below from February to early May.

Rainfalls events occurred at the same time between all three stations within the same year with small differences in rain amount. In 2014/2015, rainfalls were higher than decade's normal in early October, early November, mid-December, mid-January and mid-February.

Then a lot of rain came between the end of April and the beginning of May, in mid-June and finally in late August. For 2016's campaign, noticeable rain events occurred in January, march late May and June. In both years, the sum of precipitation did not exceed the average sum of precipitation over the period 2004-2015. Nevertheless water shortage may have happened during the summer 2015.

II.2. Experimental setup

In the study area of Pays de la Loire, a network of fields has been created and farmers involved proceeded to their regular cropping practices during the whole season. Also exploring the impact of the lupin crop on the following crop, the experiment took place over two years. The year of lupin cropping has been named n and the year with the subsequent crop n+1.

Common terms to all experimental setups:

Plot: rectangle of 20m by 30m homogeneous in terms of soil characteristics and topography.

Subplot: small area within a plot of 1m by 3 rows of the crop, where counting and biomass sampling were done. The surface of each subplot depends on the row spacing (Appendix 2). Each of them has to be at least one meter away from the others.

II.2.A. Lupin sole crop fields

In each field cropped with white lupin, three plots are defined (see Appendix 4) at a reasonable distance from the field border (at least 10m). In each of these plots, one half is kept intact (no measurements done during year n) for the n+1 experiments (pre-crop effect) and the other half received 6 subplots (see Appendix 4). Three subplots were labeled to be harvested at flowering time and the other three were harvested at physiological maturity.

II.2.B. Lupin intercropped with triticale

Fields subjected to intercropping received the experimental design described above plus a control area with lupin sole crop to allow later comparison of performances. In the sole crop area, the same setup is applied which gives a total of 6 plots in the field (see Appendix 5).

II.2.C. Following cereal crop's setup

Fields which were in lupin (sole crop or intercropped) received, in the second year of the experiment, a cereal crop (wheat or barley). This setup aimed at quantifying pre-crop effect of the lupin. One area of the field received no fertilizer (0 N area) and received as well three plots (see appendices 4 and 5). The plots in these fields were the same as the previous year but the subplots were placed in the N+1 half of the plot to avoid sampling errors.

II.2.D. Lupin’s genetic material

The particularity of a regional agronomic diagnosis is that farmers do as they use to, therefore genetic material differ amongst fields. Table 1 presents the characteristics of the different cultivar used in farmers’ fields.

Table 1: Lupin's species characteristics (from Jouffray-Drillaud 2015 and Terres Inovia 2016).

Variety	Sowing time	Genotype	Harvest earliness	Thousand Grain Weight (gr)	% protein	number of fields
Clovis	Autumn	Dwarf determinate	Early	305	33,2	3
Lumen	Autumn	Dwarf determinate	Early	320	33,9	8
Orus	Autumn	Dwarf determinate	Early	315	34,5	8
Energy	Spring	Indeterminate	Late	324	35,8	1
Feodora	Spring	Indeterminate	Early	268	36,7	6

II.3. Measured variables

For both lupin (year n) and the following cereal crop (year n+1), similar measures were performed. Except otherwise mentioned, the following operations apply to both lupin and cereal crops.

II.3.A. Yield’s components

Plant number per square meter: in each subplot, lupin number was recorded at emergence, after winter and at maturity. As the subplots were not necessarily 1m by 1m (appendix 2), depending on the row spacing, calculation was necessary to obtain the number of plant per m². In intercropped subplots and for the following cereal, plant number was recorded at emergence only.

Crop biomass at flowering stage: in each lupin or lupin/triticale subplot, plants were cut and sorted by species if intercropped then dried during 48h at 70°C. Dry matter weight was recorded.

Crop biomass at physiological maturity: in each subplot, plants were cut, sorted by species if intercropped, grains were separated from the straw. After drying during 48h at 70°C, dry matter weights were recorded. A sample was made to have an estimate of the percentage of humidity at sampling.

N content of the crop: in each plot, the subplot PN (see Appendices 4 and 5) was dedicated to N content analysis. After being weighted for dry matter, plants from PN subplots of each plot from the same treatment (e.g.: three PN subplot of the intercropped area of the field or three PN subplots from the sole crop area of the field), were gathered in one sample. These plants were grinded, mixed and a sample was taken out to be analyzed in a laboratory for N content. At maturity stage grain and straw were processed the same way but separately. From the laboratory analysis, an estimation of the percentage of nitrogen derived from atmosphere (%Ndfa) in lupin both at flowering and maturity was obtained.

II.3.B. Quantification of the effect on soil N-pool

Soil N content: Soil N content was measured at sowing, after winter and the day after harvest. Each time, 10 soil samples were taken in the sole crop area per layer (0-30cm, 30-60cm, 60-90cm) then mixed into 1. 200 grams of soil were sent to a laboratory for KCl N mineral extraction. The same procedure was applied in the intercropped area of the field and the 0 N area when applicable.

II.3.C. Biotic pressure

Weed communities: First, at flowering and at physiological maturity, every single plot was scouted to judge the weed global repartition. The plot was then given a grade: homogeneous when weeds were spread all over the plot, intermediary when the weeds were gathered in patches spread all over the plot and heterogeneous when weeds were in one or two spots of the plot only (Fig. 3).

Then at subplot scale, weed's soil coverage was visually assessed according to the scale of Braun-Blanquet (Fig 4). After soil cover assessment, the three main weed species were identified and weighted with an estimated proportion of the total biomass.

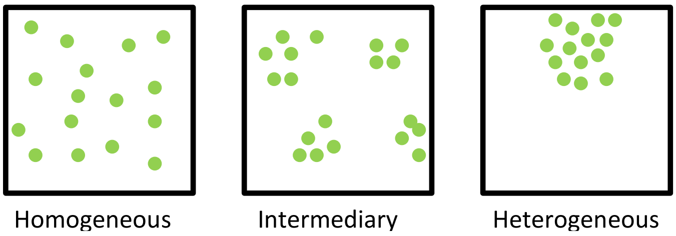


Figure 3: Sociability of weeds

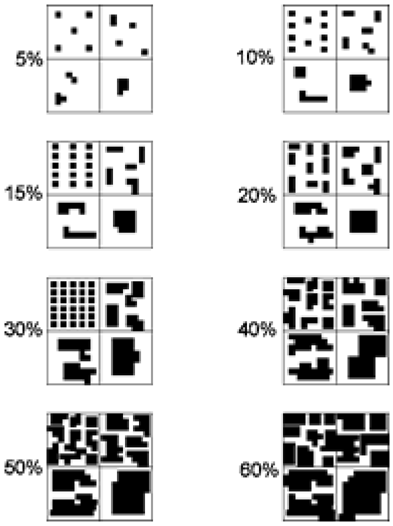


Figure 4: Braun-Blanquet scale

Following these grading, weeds were harvested at both development stages (flowering and physiological maturity) then dried during 48 hours at 70°C to obtain the weed's dry matter weight.

Diseases and pests: Detailed assessment of diseases presence took place at flowering time. In each plot, 20 plants by specie were randomly taken and examined for diseases and pest damages. Insects' damages were reported, as well as presence of *Colletrotrichum lupini, Botrytis cinerea, Sclerotinia sclerotiorum*, *Uromyces lupinicolus* and *Pleiochaeta setosa.* If signs of a disease were present on the plant, the plant was noted as infected for that disease. From these countings, a percentage of infected plants was calculated for each disease.

II.4. Calculation of theoretical nitrogen inputs from white lupin in the field

Estimation of nitrogen input to the field was calculated twice. It is worse noting that these estimations are based on above ground nitrogen content analysis because no coefficient exists to take into account root N at maturity.

Ns (nitrogen brought by seeds at sowing) = number of kg of seeds sown * average nitrogen content of seeds at maturity (spring lupin in one hand and winter lupin on the other hand).

Ndfa at maturity (quantity of nitrogen derived from atmosphere in above ground DM at maturity) = total above ground nitrogen at maturity (straw + grain) * %Ndfa at maturity

Ngrain (quantity of nitrogen contained in grains (kgN/tMS)) = obtained from the nitrogen analysis

Yt (theoretical yield is 100% of grains where harvested) = obtained from field sampling

Yr (farmer’s yield) = farmer’s feedbacks on the production

Lupin’s theoretical N balance = Ns + Ndfa - Yt * Ngrain

Lupin’s real N balance = Ns + Ndfa - Yr * Ngrain

II.5. Farmer’s interview

Field and crop management was left to the farmer. Their practices, choices and opinion were recorded and collected at the end of lupin’s cropping cycle during an interview.

II.6. Statistical analysis

After descriptive statistics, for each lupin cropping strategy, parameters were tested for correlations amongst them. Correlation significance was tested with a t-test procedure ($\alpha = 5\%$). Analysis of variance ($\alpha = 5\%$) was used when normality of distribution was assumed thanks to Shapiro-Wilkoxon test ($\alpha = 5\%$). When raw data were not normaly distributed, the following transformation formula were tested: $1/x$, x^2, x^3, log(x), ln(x), x^(1/2), x^(1/3) and asin(x). When none of them were satisfactory Kruskal-Wallis test was used for non-parametric comparisons followed by Nemenyi’s post-hoc test. Following the ANOVA and after assumption checking with Shapiro-Wilkoxon test ($\alpha = 5\%$) for normality of residuals and Levene’s test ($\alpha = 5\%$) for homogeneity of residuals’ variance, Tukey’s HSD test ($\alpha = 5\%$) was used for pairwise comparison. R software was used for statistical analysis in version: 3.3.1..

III. Results

III.1. White lupin’s performances: a comparison of yield and its component by cropping modes

III.1.A. Yield

Table 2: Yield and yield's parameters for the two experiment years

	Lupin											
	Yield ¤ (t/ha)		Thousand Grain Weight ¤ (gr)		Number of grain harvested per m^2		Number of grains per plant		Number of pods per plant		Number of branch orders bearing pods	
		CV		CV		CV		CV		CV		CV
	2015											
Intercropping winter white lupin/triticale	2,21 b	87%	309,88	10%	700,75 b	72%	37,75 b	49%	-	-	-	-
Winter Lupin sole crop	6,5 a	14%	307,91	12%	2164,4 a	13%	88,6 a	19%	-	-	-	-
Spring Lupin sole crop	2,93 b	40%	286,71	17%	1056,14 b	32%	26,14 b	32%	-	-	-	-
	2016											
Intercropping winter white lupin/triticale	2,08	52%	277,91*	7%	751,20	54%	37,25	45%	18,51	42%	2,7	24%
Winter Lupin sole crop	3,27	30%	272,09*	2%	1199,64	31%	42	48%	18,59	41%	2,81	22%

Each year was analysed separately
Numbers followed by different letters are significantly different (P < 0,05) with Tukey's HSD.
* Kruskal Wallis test was used to compare the samples distributions
¤ values normalized to 14% of humidity
- data not available
CV = Coefficient of Variation

Experimental yield obtained from the experimental design in 2015 are on average 2.21, 2.93 and 6.5 t/ha for intercropped winter white lupin (IntWL), sole cropped spring white lupin (SLSC) and sole cropped winter white lupin (WLSC) respectively (Table 2). Yield of WLSC was significantly higher than the yields of the two others and its coefficient of variation (CV) is low, meaning that the results were stable across the fields. No significant difference was found between mean yields of IntWL and SLSC. However, SLSC yield had a lower coefficient of variation (CV = 40%) than IntWL (CV = 87%).

In 2016, no difference was found between the production performances of the two crops studied. IntWL yielded 1.79 t/ha and WLSC 2.81 t/ha, both with medium variation values around the mean (CV = 52% and 30% respectively).

The accuracy of the experimental yield to farmer’s harvest was studied. In 2015, farmer’s yield on the entire field corresponded to only 34%, 49% and 41% of the experimental yield for IntWL, WLSC and SLSC respectively. For 2016, it was 77% of the experimental yield in intercropped fields and 66% for winter white lupin fields. This may have different explanations, for spring lupin one farmer reported harvest problems due to short plants and another one simply did not harvest his field. For intercropped lupin it might be explained by heterogeneity of plant density over the field.

III.1.B. Thousand Grain Weight

Thousand grain weight (TGW) was not significantly different between lupin cropping strategies in 2015 or in 2016. Furthermore, it was a stable parameter as CV does not exceed

17%. However, in 2016 the average experimental values did not reach the theoretical value proposed by the seed producer as in average the winter white lupin should reach approximately 310 grams for one thousand grains.

Yield was not found correlated to thousand grain weight in 2015 ($R^2 = 0.33$, p-value = 0.22) nor in 2016 ($R^2 = -0.2$, p-value = 0.60).

III.1.C. Grain and pod numbers

Grain number per square meter in 2015 was higher in WLSC than for IntWL and SLSC. Its variability was low for WLSC and high for IntWL. For the same year, the grain number per plant was higher for WLSC than for both IntWL or SLSC (Table 2).

In 2016, no statistical difference were found between WLSC and IntWL for grain number per square meter, grain number per plant, pods per plant or number of branch order bearing pods. Interestingly, the variability of the coefficient of variation of the last two parameters did not differ much between WLSC or IntWL. CV of IntWL grain number per square meter (54%) was higher than for WLSC (31%) (Table 2).

As shown in table 2, grain number per square meter was the main differentiating factor for yield's elaboration. 2015's regression have a $R^2=0.91$ (p-value<0.001) and 2016's regression have a $R^2=0.99$ (p-value<0.001) (Figure 5).

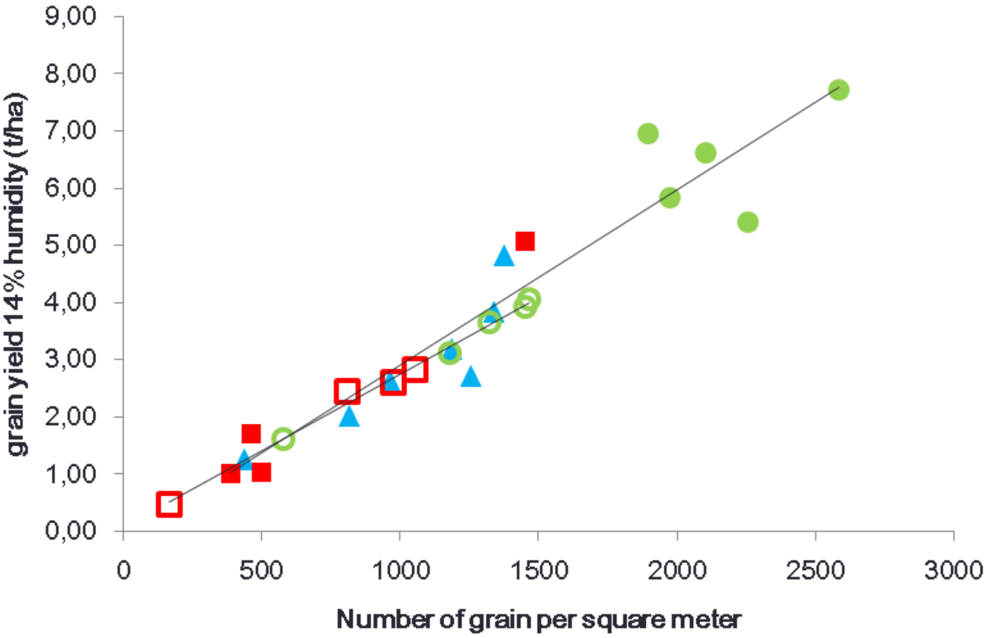


Figure 5: Relation between grain number per square meter and grain yield
Red square = Intercropped lupin, blue triangles = Spring lupin and green circles = winter lupin.
Filled symbols correspond to 2015's harvest and empty ones to 2016's harvest.

II.1.D. 2015 compared to 2016

Even though there was no proper data comparison between 2015 and 2016 in this section, it is worth noting that while IntWL expressed similar average yields, for both years, sole cropped winter lupin had, in 2016, an average yield inferior to 2015 average. Also, IntWL coefficient of variation for all parameters were similar, emphasizing the high variability of results of this crop regardless the study year, WLSC had very low CV in 2015 and high CV is pointing out that crop success may be strongly influenced by the year and more importantly that it is highly variable between years.

As grain per square meter seems to be the most important element for yield regardless the cropping strategy, its relation with field's characteristics was studied, each cropping strategy on its own.

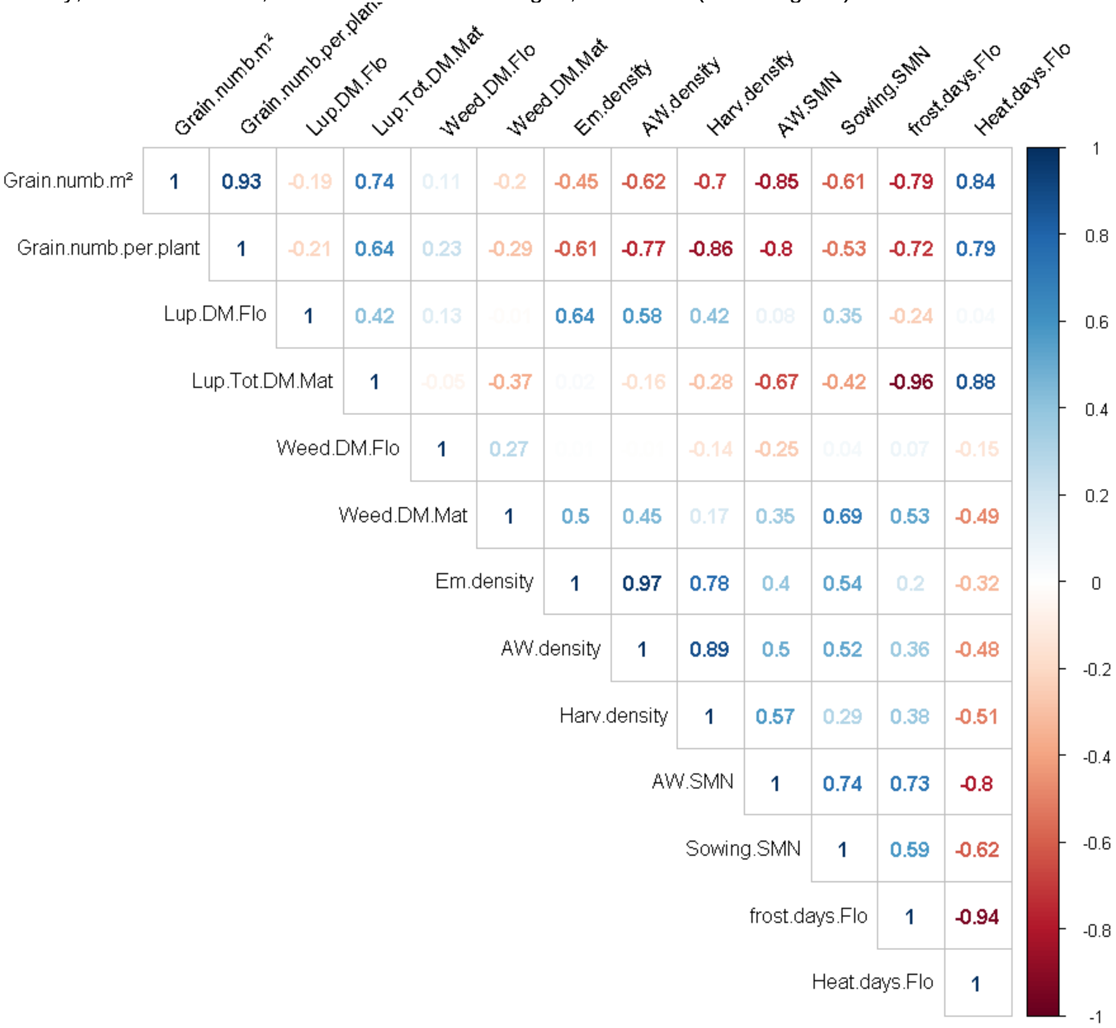
III.2. Winter lupin sole crop

For both years, grain number per square meter was the main yield component. Grain number per square meter was found correlated to grain number per plant ($R^2 = 0.93$ p-value < 0.05) and grain number per plant was negatively correlated to plant density after winter ($R^2 = -0.77$ p-value < 0.05) (table 3).

Grain number per plant and grain number per square meter were positively correlated to lupin's total above ground DM at maturity and number of hot days (when maximum temperatures reached more than 28°C). They also were negatively correlated to after winter soil mineral nitrogen quantity and number of days with frost during flowering.

Table 4 gives additional information to better identify conditions of success or failure for each situations. For example, in 2015 there was no frost registered during flowering period but there were numerous hot days while in 2016 it was the opposite. Also, fields of 2015 had overall low soil mineral nitrogen at sowing while values were high in 2016.

Table 3: Correlation table between grain number per plant and various parameters
Values superior or equal to 0.64 (or inferior or equal to -0.64) are significant correlation coefficients.
AW = after winter, DM = dry matter, Em = emergence, Flo = at flowering, Harv = harvest, Lup = lupin, Mat = at maturity, Numb = number, SMN = soil mineral nitrogen, Tot = total (straw + grain).



	Grain.numb.m²	Grain.numb.per.plant	Lup.DM.Flo	Lup.Tot.DM.Mat	Weed.DM.Flo	Weed.DM.Mat	Em.density	AW.density	Harv.density	AW.SMN	Sowing.SMN	frost.days.Flo	Heat.days.Flo
Grain.numb.m²	1	0.93	-0.19	0.74	0.11	-0.2	-0.45	-0.62	-0.7	-0.85	-0.61	-0.79	0.84
Grain.numb.per.plant		1	-0.21	0.64	0.23	-0.29	-0.61	-0.77	-0.86	-0.8	-0.53	-0.72	0.79
Lup.DM.Flo			1	0.42	0.13	-0.01	0.64	0.58	0.42	0.08	0.35	-0.24	0.04
Lup.Tot.DM.Mat				1	-0.05	-0.37	0.02	-0.16	-0.28	-0.67	-0.42	-0.96	0.88
Weed.DM.Flo					1	0.27	0.01	-0.01	-0.14	-0.25	0.04	0.07	-0.15
Weed.DM.Mat						1	0.5	0.45	0.17	0.35	0.69	0.53	-0.49
Em.density							1	0.97	0.78	0.4	0.54	0.2	-0.32
AW.density								1	0.89	0.5	0.52	0.36	-0.48
Harv.density									1	0.57	0.29	0.38	-0.51
AW.SMN										1	0.74	0.73	-0.8
Sowing.SMN											1	0.59	-0.62
frost.days.Flo												1	-0.94
Heat.days.Flo													1

For WLSC6, WLSC7 and WLSC9 row spacing was lower than recommendations and farmer's target density (sowing density) was lower than real densities (from counting in field). Flowering and maturity densities were 1.5 to 2 times higher than recommendations for WLSC6, WLSC7 and WLSC9. Densities in 2015 almost corresponded to farmer's target density (Table 4).

Table 4: Characteristics of the 10 winter white lupin fields

Colored items correspond to favorable (green), medium (yellow) or detrimental (red) conditions according to the technical institute (Terres Inovia 2016 and Walker et al. 2011).

	WLSC1	WLSC2	WLSC5	WLSC4	WLSC3	WLSC9	WLSC10	WLSC8	WLSC6	WLSC7
Production campaign	2015	2015	2015	2015	2015	2016	2016	2016	2016	2016
Soil pH	6,8	7,2	7,3	6,7	7,1	6,5	6,8	7	6,8	7,2
Soil organic matter (%)	1,6	1,4	2,4	2,7	1,9	3,2	2,8	2,3	3,7	2,8
Soil texture	Loam	Loam	Loam	Loam	Silt loam	Silt loam	Silt loam	Loam	Silt loam	Silty clay loam
Cultivar	Clovis	Clovis	Orus	Orus	Orus	Orus	Lumen	Orus	Orus	Orus
Sowing period	sept 2	sept 2	oct 1	sept 3	sept 2	sept 3	oct 1	sept 3	oct 1	sept 3
Row spacing	40	40	25	25	42	25	40	40	12,5	12,5
Sowing density (farmer's target density) (gr/m²)	32	32	28	26	25	28	29	26	25	30
Emergence density (plant/m²)	32	37	30	30	30	43	25	31	35	41
After winter density (plant/m²)	29	34	27	27	26	40	23	30	36	41
Maturity density (plant/m²)	27	27	27	24	20	33	22	25	37	42
Weeds DM at flowering (t/ha)	0,21	0,12	0,09	0,09	0,89	0,48	0,22	0,28	0,61	0,07
Weeds DM at maturity (t/ha)	0,31	0,03	0,00	0,00	0,15	3,04	0,03	1,58	0,42	0,00
Herbicide application number	3	3	2	2	3	2	2	3	1	4
Soil mineral nitrogen at sowing kgN/ha	69	121	76	142	111	218	141	152	107	163
Soil mineral nitrogen after winter kgN/ha	46	47	56	66	38	106	103	81	70	126
Lupin dry matter at flowering (t/ha)	3,64	6,60	2,17	5,70	5,00	5,80	3,07	1,78	5,35	6,01
Number of days with temperatures below 0°C at flowering (April & May)	0	0	0	0	0	3	3	5	3	3
Number of days with temperature superior to 28°C at flowering and pod filling (June and July)	11	11	13	13	11	0	0	0	0	0
Colletrotrichum lupini (observed at maturity)	-	-	-	-	-	65%	17%	0%	5%	93%
Pleiochaeta setosa (observed at maturity)	-	-	-	-	-	3%	13%	0%	0%	5%
Botrytis cinerea (observed at maturity)	-	-	-	-	-	95%	88%	60%	100%	100%
Sclerotinia sclerotiorum (observed at maturity)	-	-	-	-	-	3%	20%	63%	2%	0%
Uromyces lupinicolus (observed at maturity)	0%	0%	0%	90%	55%	0%	0%	0%	0%	0%
Fongicide aplication number	3	3	1	2	2	2	2	3	2	2
Average number of pods on main stem	-	-	-	-	-	2,95	3,52	8,52	0,81	0,95
Average number of pods on first order branches	-	-	-	-	-	10,33	8,33	9,76	6,38	1,76
Average number of pods on second order branches	-	-	-	-	-	4,95	8,9	5,05	6,52	5,95
Average number of pods on third order branches	-	-	-	-	-	0,62	7,33	0,05	0	0,24
Thousand grain weight (g/1000 grains) 14% humidity	318	357	316	291	258	272	270	275	263	282
Grain number per square meter	2585	1897	2105	1977	2258	1466	1451	1324	1180	577
Experimental yield 14% humidity (t/ha)	7,72	6,96	6,61	5,84	5,40	4,05	3,93	3,65	3,13	1,61
Farmer yield (t/ha)	3,3	3,3	2,88	3,9	2,5	2	3,1	2,5	2,2	1
Yield losses	57%	53%	56%	33%	54%	51%	21%	32%	30%	38%

In 2016, diseases were highly represented in each field with *Botrytis cinerea* in every field, *Colletotrichum lupini* in WLSC7 and WLSC9 and *Sclerotinia sclerotiorum* in WLSC7. In contrast, only *Uromyces lupinicolus* was observed in two fields (WLSC3 and WLSC4). For 2016, fields with low lupin DM at flowering also had lower *Botrytis cinerea* infection percentage.

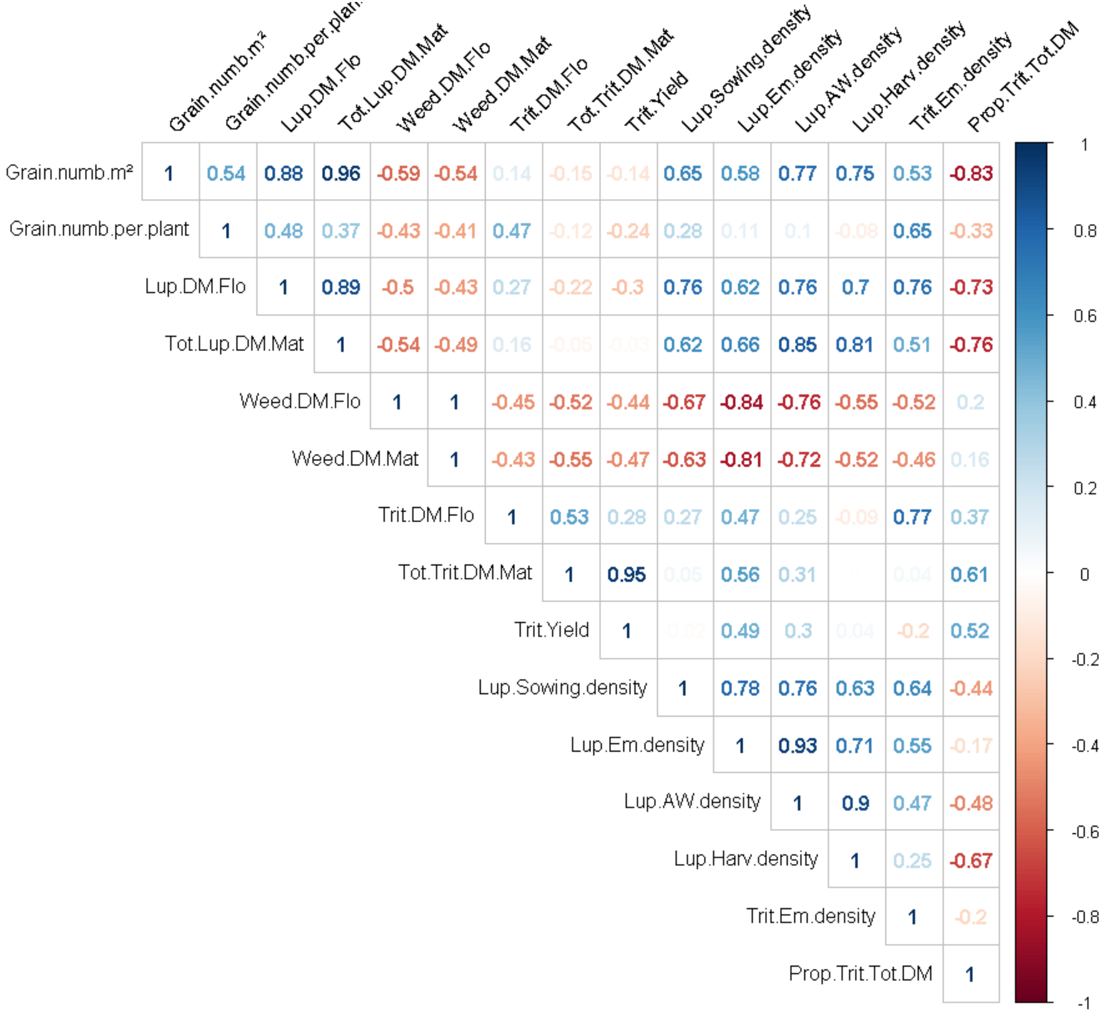
For both years, weed DM at flowering were not high and at maturity they had no real impact on grain number per square meter nor grain number per plant (no existing correlation between weed DM and other parameters) (Table 3). WLSC8 and WLSC9 had high weed DM at maturity but they were both amongst the highest yielding fields of 2016 (Table 4).

Observations on pod repartition on the plant were available only for 2016. The main stem did not bear many pods, while the first and second order branches bore the majority of pods. For the lowest yielding field (WLSC7) the first order had a low number of pods as well.

III.3. Intercropped winter white lupin

In intercropping, lupin grain number per square meter was not significantly correlated to number of grain per plant. Nonetheless, it was positively correlated to lupin above ground DM at flowering and at maturity and to lupin after winter and harvest densities (table 5).

Table 5: Correlation table between each dry matter parameters of intercropping systems.
Values superior or equal to 0.71 (or ≤ -0.71) are significant correlation coefficients (t-test).
AW = after winter, DM = dry matter, Em = at emergence, Flo = at flowering, Harv = at harvest, Lup = lupin, Mat = at maturity, numb = number, Prop = proportion, Tot = total (straw + grain), Trit = Triticale.
Grain number expressed in grain per m², dry matter and yield in t/ha.



	Grain.numb.m²	Grain.numb.per.plant	Lup.DM.Flo	Tot.Lup.DM.Mat	Weed.DM.Flo	Weed.DM.Mat	Trit.DM.Flo	Tot.Trit.DM.Mat	Trit.Yield	Lup.Sowing.density	Lup.Em.density	Lup.AW.density	Lup.Harv.density	Trit.Em.density	Prop.Trit.Tot.DM
Grain.numb.m²	1	0.54	0.88	0.96	-0.59	-0.54	0.14	-0.15	-0.14	0.65	0.58	0.77	0.75	0.53	-0.83
Grain.numb.per.plant		1	0.48	0.37	-0.43	-0.41	0.47	-0.12	-0.24	0.28	0.11	0.1	-0.08	0.65	-0.33
Lup.DM.Flo			1	0.89	-0.5	-0.43	0.27	-0.22	-0.3	0.76	0.62	0.76	0.7	0.76	-0.73
Tot.Lup.DM.Mat				1	-0.54	-0.49	0.16	-0.05	-0.03	0.62	0.66	0.85	0.81	0.51	-0.76
Weed.DM.Flo					1	1	-0.45	-0.52	-0.44	-0.67	-0.84	-0.76	-0.55	-0.52	0.2
Weed.DM.Mat						1	-0.43	-0.55	-0.47	-0.63	-0.81	-0.72	-0.52	-0.46	0.16
Trit.DM.Flo							1	0.53	0.28	0.27	0.47	0.25	-0.09	0.77	0.37
Tot.Trit.DM.Mat								1	0.95	0.05	0.56	0.31	0	0.04	0.61
Trit.Yield									1	-0.02	0.49	0.3	0.04	-0.2	0.52
Lup.Sowing.density										1	0.78	0.76	0.63	0.64	-0.44
Lup.Em.density											1	0.93	0.71	0.55	-0.17
Lup.AW.density												1	0.9	0.47	-0.48
Lup.Harv.density													1	0.25	-0.67
Trit.Em.density														1	-0.2
Prop.Trit.Tot.DM															1

It is worth noting that grain number per square meter was neither significantly correlated to triticale DM parameters nor to weed DMs. Nevertheless, grain number per square meter was negatively correlated to the proportion of triticale above ground DM matter in total crop above ground DM at maturity. Both weed DMs tended to be negatively influenced (no significant correlation) by every single crop' DM parameters (Table 5).

Table 6: Fields characteristics of intercropped winter lupin

Colored items correspond to favorable (green), medium (yellow) or detrimental (red) conditions according to the technical institute (Terres Inovia 2016 and Walker et al. 2011).

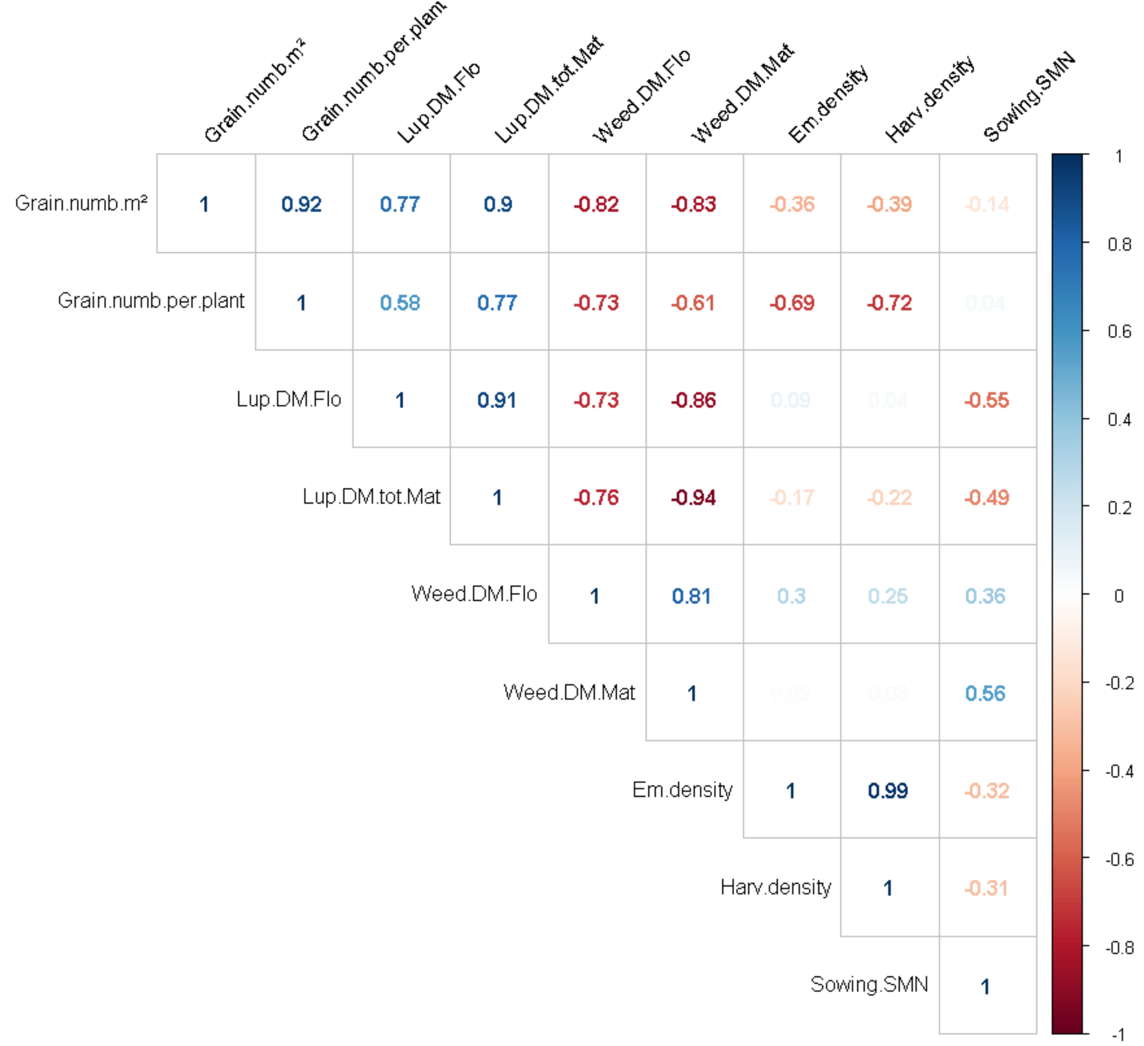
	IntWL1	IntWL5	IntWL6	IntWL7	IntWL2	IntWL4	IntWL3	IntWL8
Production campaign	2015	2016	2016	2016	2015	2015	2015	2016
Soil pH	7,2	7,1	6,6	7,1	7,2	7	6,9	6,2
Soil organic matter (%)	2,2	3	1,9	3,1	1,3	2,7	2,4	2,3
Soil texture	Silt loam	Loam	Loam	Loam	Loam	Loam	Loam	Loam
Cultivar	Lumen	Lumen	Orus	Lumen	Lumen	Clovis	Lumen	Lumen & Clovis
Row spacing (cm)	12	14	75	35	14,3	13	75	50
Triticale sowing density (grain/m²)	70	70	60	75	72	70	75	87
Triticale emergence density (plant/m²)	88	73	54	66	47	90	63	47
Lupin target sowing density (grain/m²)	31	30	30	25	27	30	28	25
Lupin emergence density (plant/m²)	45	39	29	22	36	33	37	15
Lupin after winter density (plant/m²)	38	35	26	20	27	22	28	14
Lupin harvest density (plant/m²)	29	33	23	14	16	9	23	9
Nitrogen fertilization	Yes - May	Yes - August	No	Yes - April	No	No	No	No
Number of days with temperatures below 0°C at flowering (April & May)	0	3	3	3	0	0	0	3
Number of days with temperature superior to 28°C at flowering and pod filling (June and July)	13	0	0	0	11	11	11	0
Herbicide application number	1	1	1	1	2	1	1	1
Weed DM at flowering (t/ha)	0,01	0,10	0,19	0,31	0,10	0,13	0,16	1,22
Weed DM at maturity (t/ha)	0,00	0,28	0,40	0,84	0,03	0,30	0,26	4,24
Colletrotrichum lupini (at maturity)	-	30%	5%	43%	-	-	-	2%
Pleiochaeta setosa (observed at maturity)	-	8%	7%	20%	-	-	-	0%
Botrytis cinerea (observed at maturity)	-	77%	50%	25%	-	-	-	12%
Sclerotinia sclerotiorum (at maturity)	-	13%	3%	3%	-	-	-	0%
Uromyces lupinicolus (at maturity)	0%	0%	0%	0%	0%	0%	87%	0%
Fongicide aplication number	2	2	1	2	3	0	1	2
Average number of pods on main stem	-	4,52	5,19	3	-	-	-	2,95
Average number of pods on first order branches	-	8,57	9,52	12,1	-	-	-	4,57
Average number of pods on second order branches	-	4,09	6,33	9,81	-	-	-	0,57
Average number of pods on third order branches	-	0,62	0,71	1,47	-	-	-	0
Lupin dry matter at flowering (t/ha)	3,74	3,40	1,71	1,32	0,44	1,98	0,74	0,47
Thousand grain weight (g/1000 grains) 14% humidity	313	267	267	307	351	302	273	272
Grain number per square meter	1452	1056	974	809	462	500	389	165
Triticale experimental yield 15% humidity	2,88	1,03	0,96	1,33	6,92	1,67	3,88	0,33
Lupin experimental yield 14% humidity	5,06	2,83	2,60	2,45	1,71	1,04	1,02	0,46
Farmer's triticale yield (t/ha)	2,76	0,9	0,8	-	4,75	1,5	2	0,3
Farmer's lupin yield (t/ha)	1,68	1,8	2,1	-	0,36	0,6	0,25	0,4
Triticale yield losses	4%	13%	17%	-	31%	10%	48%	9%
Lupin yield losses	67%	36%	19%	-	79%	42%	75%	14%

Total lupin dry matter was positively correlated to after winter density and harvest density. Also, sowing (farmer's target density), emergence, after winter and harvest densities were all positively correlated with each other. Over-winter losses were important in 2015 as each field lost about 10 plants yet, only three in 2016. Losses between after winter and maturity countings varied from 2 to 13 plants/m² regardless the year (Table 6).

Disease presence did not seem to be a limiting factor for yield formation and weed development was particularly high in one specific (IntWL8) field which was a fallow and was not ploughed before lupin sowing. Frost occurred during flowering in 2016 but not in 2015. Also triticale yield were different between fields, ranging from 0.33 to 6.92 t/ha over the two years. Finally, lupin DM at flowering had values from 0.44 to 3.74 t/ha. Sowing took place between late September and early October.

III.4. Spring lupin sole crop

Table 7: Correlation table for a selection of parameters of spring white lupin
Values superior or equal to 0.76 (or inferior or equal to -0.76) are significant correlation coefficients.
DM = dry matter, Em = emergence, Flo = at flowering, Harv = harvest, Lup = lupin, Mat = at maturity, numb = number, SMN = soil mineral nitrogen, tot = total (straw + grain).



	Grain.numb.m²	Grain.numb.per.plant	Lup.DM.Flo	Lup.DM.tot.Mat	Weed.DM.Flo	Weed.DM.Mat	Em.density	Harv.density	Sowing.SMN
Grain.numb.m²	1	0.92	0.77	0.9	-0.82	-0.83	-0.36	-0.39	-0.14
Grain.numb.per.plant		1	0.58	0.77	-0.73	-0.61	-0.69	-0.72	0.04
Lup.DM.Flo			1	0.91	-0.73	-0.86	0.09	0.04	-0.55
Lup.DM.tot.Mat				1	-0.76	-0.94	-0.17	-0.22	-0.49
Weed.DM.Flo					1	0.81	0.3	0.25	0.36
Weed.DM.Mat						1	0.02	0.03	0.56
Em.density							1	0.99	-0.32
Harv.density								1	-0.31
Sowing.SMN									1

Spring lupin grain number per square meter was found correlated to grain number per plant and to above ground DM at maturity and at flowering (table 7). It was also negatively correlated to weed biomasses both at flowering and maturity. Nonetheless, grain number per square meter seemed rather independent from plant density across the cycle probably because they were relatively stable across fields (Table 8). Weed DMs were also negatively correlated to both lupin dry matters.

Each field was exposed to high temperature during flowering and during pod filling, possibly leading to flower and pod abortion and may have created water shortage. Only two had drainage, which may lower waterlogging risks. Finally, diseases where observed in two fields only (without clear identification and quantification).

Tableau 8: Summary of Spring lupin fields' parameters.

Colored items correspond to favorable (green), medium (yellow) or detrimental (red) conditions according to the technical institute (Terres Inovia 2016 and Walker et al. 2011).

	SLSC3	SLSC5	SLSC7	SLSC4	SLSC6	SLSC1	SLSC2
Soil pH	7,5	6,7	7	6,2	6,9	6,7	7,8
Soilt texture	Silt loam	Silt loam	Silt loam	Silt loam	Silt loam	Loam	Silt loam
Drainage	No	No	No	No	No	Yes	Yes
Cultivar	Energy	Feodora	Feodora	Feodora	Feodora	Feodora	Feodora
Cover destruction	Ploughing	Glyphosate and shallow tillage	Glyphosate and shallow tillage	Glyphosate + Ploughing	Glyphosate and shallow tillage	Glyphosate and shallow tillage	Glyphosate and shallow tillage
Sowing date	12/02/2015	09/03/2015	10/03/2015	17/03/2015	10/03/2015	19/03/2015	19/03/2015
Sowing density (farmer's target density)	57	80	52	50	52	56	56
Sowing incident	No	Yes	Yes	No	Yes	No	Yes
Density at emergence (plant/m²)	40	53	38	31	39	51	48
Date of pre-emergence herbicide date	19/02/2015	10/03/2015	23/03/2015	01/04/2015	23/03/2015	23/03/2015	23/03/2015
Second herbicide date	-	25/04/2015	25/04/2015	29/04/2015	25/04/2015	-	-
Grass weed herbicide application date	-	30/04/2015	20/05/2015	18/05/2015	20/05/2015	-	-
Fongicide aplication number	Yes	Yes	No	No	No	Yes	Yes
Fertilization	Horse manure (50 kgN/ha) and P-K	No	No	No	No	Small amount of N-P-K-Ca-S-Mg	Small amount of N-P-K-Ca-S-Mg
Max T° at flowering (Late may + June)	34,7°C	34,5°C	34,5°C	34,7°C	34,5°C	36,9°C	36,9°C
Number of days with T° ≥ 28°C at flowering	7	8	8	7	8	4	4
Max T° at pod filling (July)	36°C	36,1°C	36,1°C	36°C	36,1°C	34,8°C	34,8°C
Number of days with T° ≥ 28°C at pod filling	6	8	8	6	8	7	7
Disease observation	No	No	No	No	No	Yes	Yes
Weed DM at flowering (t/ha)	0,02	0,84	0,93	0,88	0,59	0,81	3,29
Weed DM at maturity (t/ha)	0,02	0,45	1,86	2,70	1,41	2,71	4,13
Alopercurus myosuroides	-	F	-	-	-	-	-
Avena fatua L.	F	-	-	-	-	F	F-M
Chenopodium album L.	M	F-M	F-M	F-M	F-M	-	-
Cirsium arvense L.	-	-	M	-	-	F-M	F-M
Convolvulus arvensis L.	-	-	-	-	-	-	F-M
Daucus carota L.	-	-	M	F-M	M	M	-
Fumaria officinalis L.	F	-	-	F	-	F	-
Galium aparine L.	F-M	-	F-M	-	-	-	-
Matricaria recutita L.	-	M	-	M	F	-	-
Senecio vulgaris L.	-	F	F	-	F	-	-
Density at maturity (plant/m²)	39	49	38	33	41	49	45
Experimental yield 14% humidity (t/ha)	4,82	3,85	3,19	2,72	2,63	2,02	1,24
Farmer's yield (t/ha)	2,58	2,20	1,70	1,00	1,70	0,50	0,00
Yield losses	46%	43%	47%	63%	35%	75%	100%

A large variability of sowing dates was observed. Spring lupins were implanted between mid-February and late March. Both flowering and maturity lupin's DM were the highest with early sowing and decreased with later sowing dates (Fig 6). On the contrary weeds' DM increases with later sowing. Finally, lupin gain of DM between flowering and maturity was high when the difference between weed's maturity DM and weed's flowering DM was low and vise-versa (figure 3).

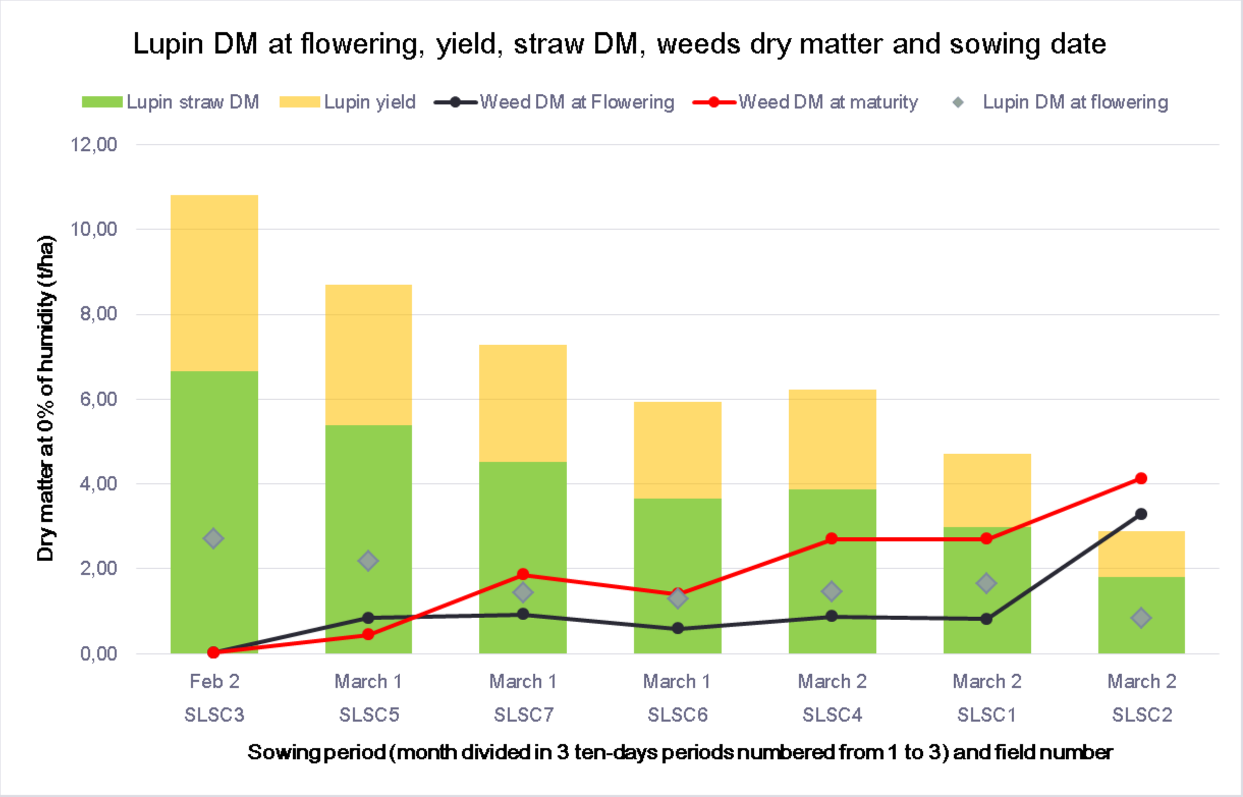


Figure 6: Lupin and weed above ground dry matter at different development stage according to sowing date

Weed dry matter was similar for all fields at flowering except for SLSC3 which had barely any weeds and the SLSC2 that had already a lot of weeds present at flowering (fig 2 and table 6). Every field went through a procedure to destroy weeds and cover crop before lupin's sowing. It was, for SLSC3 and SLSC4, ploughing and for the other, Glyphosate application and shallow soil tillage (most of the time twice). To control dicotyledonous weeds, each farmer applied a pre-emergence herbicide in their field and a second herbicide was applied before flowering in SLSC4 to SLSC7. Regarding grass weed management, a grass weed specific herbicide was applied in SLSC4 to SLSC7 also before flowering.

III.5. Lupin's regulating service: Biological Nitrogen fixation (BNF) and the effect on following wheat

III.5.A. White lupin yield and total above ground biomass at maturity

Figure 7 presents the relation between lupin's yield and total above ground (straw + grain) dry matter of white lupin.

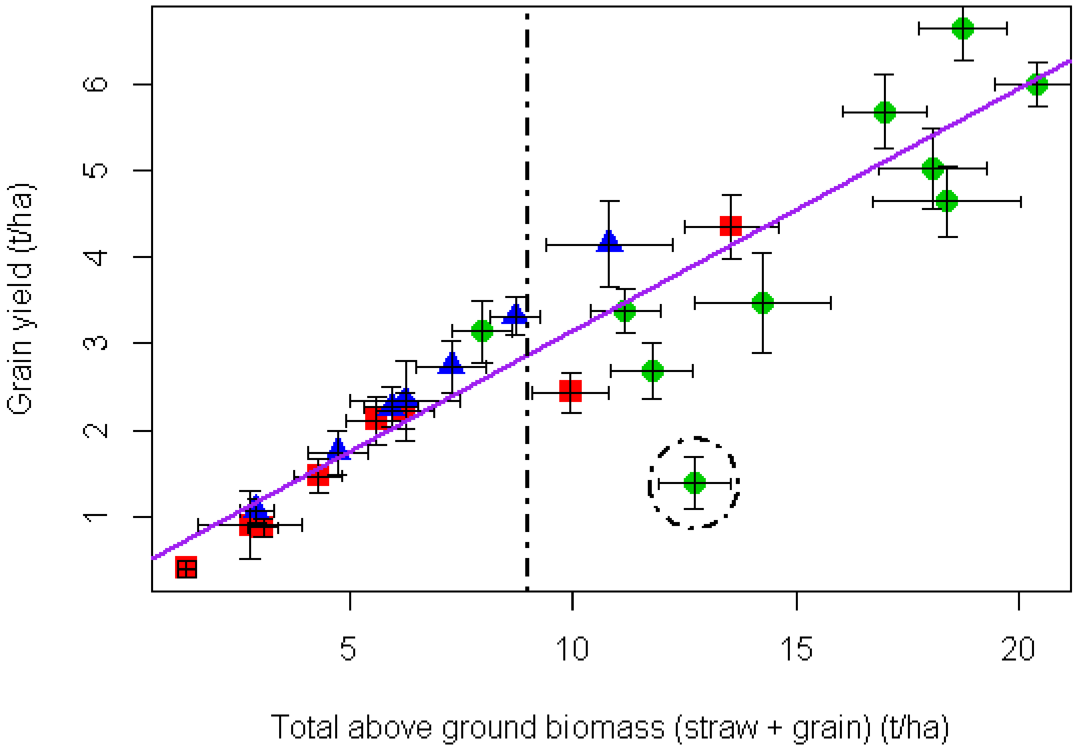


Figure 7: Linear relation between lupin's yield and total above ground dry matter at maturity
Red square = Intercropped lupin, blue triangles = Spring lupin and green circles = winter lupin.
Biomass and yield expressed at 0% humidity.
The point circled was withdrawn from the linear regression calculation because of its high leverage potential. The yield for this field was greatly affected by diseases (most of the plants damaged by botrytis and anthracnose).

A strong linear relation exists between white lupin grain yield and lupin's total above ground DM. the equation is: $Y = 0.35774 + 0.27903x$ ($R^2 = 0.90$, p-value < 0.001) with the intercept not significantly different from zero.

Also, the distribution of points on figure 7 featured a threshold of 9 t/ha. When looking at the harvest index, below this threshold, HI was high and stable (HI = 0.36, CV=9%) while above 9tDM/ha, the average HI was lower (0.28) and more variable (CV=26%). This suggested two different behaviors of lupin according to the DM per unit of area of the plant population.

III.5.B. Nitrogen content of white lupin

Nitrogen composition of white lupin's above ground biomass at flowering did not differ between WLSC, IntWL or SLSC and have similar values between 2015 and 2016. Additionally, the coefficients of variation of these values are low (between 1 and 15) meaning that white lupin had a rather constant nitrogen composition at flowering.

Table 9: Lupin's nitrogen content and fixation rate (%Ndfa)

	Lupin's nitrogen composition and percentage of nitrogen dirived from atmosphere											
	in above ground DM at flowering ¤ (kg N/t DM)		%Ndfa at flowering		in straws at maturity ¤ (kg N/t DM)		in grains at maturity ¤ (kg N/t DM)		% Ndfa at maturity [2]		% proteins in grains	
		CV		CV		CV		CV		CV		CV
	2015											
Intercropping winter white lupin/triticale	32,59	4%	91,41 % a	9%	6,85	21%	48,34 b	5%	88,47%	20%	30,21 % b	5%
Winter Lupin sole crop	32,46	3%	63,18 % b	14%	10,02	35%	52,80 b	6%	86,47%	14%	33 % b	5%
Spring Lupin sole crop	31,72	15%	72,03 % b	22%	7,16	17%	57,94 a	4%	88,92%	14%	36,21 % a	4%
	2016											
Intercropping winter white lupin/triticale	32,69 *	1%	72,40%	11%	-	-	-	-	-	-	-	-
Winter Lupin sole crop	29,49 *	15%	67,80%	7%	-	-	-	-	-	-	-	-

Each year was analysed separately
Numbers followed by different letters are significantly different (P < 0,05) with Tukey's HSD.
* Kruskal Wallis test was used to compare the samples distributions
¤ DM with 0% of humidity
- data not available
CV = Coefficient of Variation; DM = Dry matter

The proportion of nitrogen derived from the atmosphere (%Ndfa) in lupin’s above ground dry matter was, on average, 91, 63 and 72% for IntWL, WLSC and SLSC respectively in 2015. In 2016 it was 72% for IntWL and 67% for WLSC. It was significantly higher in IntWL than in WLSC or SLSC in 2015 while it did not differ between IntWL and WLSC in 2016. Values were stable in 2016 (CV of 7 and 11%) and were more variable in 2015 (CV between 9 and 22%). Across the territory, at least 60% of the nitrogen contained in white lupin at flowering time originated from BNF. In 2015, %Ndfa at maturity did not show any statistical differences between cropping strategies.

At maturity, nitrogen content of straw did not differ significantly between cropping strategies but the average value for WLSC straw N was three points higher than the other values and its coefficient of variation was slightly higher than the others.

Nitrogen content of spring lupin’s grain was significantly higher than winter lupin grains N content whether it was intercropped or not and it did not differ between IntWL and WLSC. This was confirmed by the percentage of proteins in the grains. Both grain nitrogen content and percentage of protein in grain have very low coefficient of variation highlighting the stability of protein content in grains across the region.

Nitrogen fixation rate (%Ndfa) and nitrogen composition per ton of DM were high and relatively stable across cropping strategies. Therefore N_2 fixation was probably mainly correlated to crop biomass.

III.5.C. Relation between biomass and nitrogen fixation

III.5.C.a. At flowering

At flowering the quantity of nitrogen derived from atmosphere was proportional to the above ground dry matter of lupin, regardless the cropping strategy. The relations presented in the graph are highly significant (p-value < 0.001). Both intercept values are not different from zero. Below 2 tons of dry matter per hectare (12 situations) the average %Ndfa was 76% with a CV of 21% while above 2tDM/ha (13 situations), average %Ndfa was 70% with 14% of coefficient of variation.

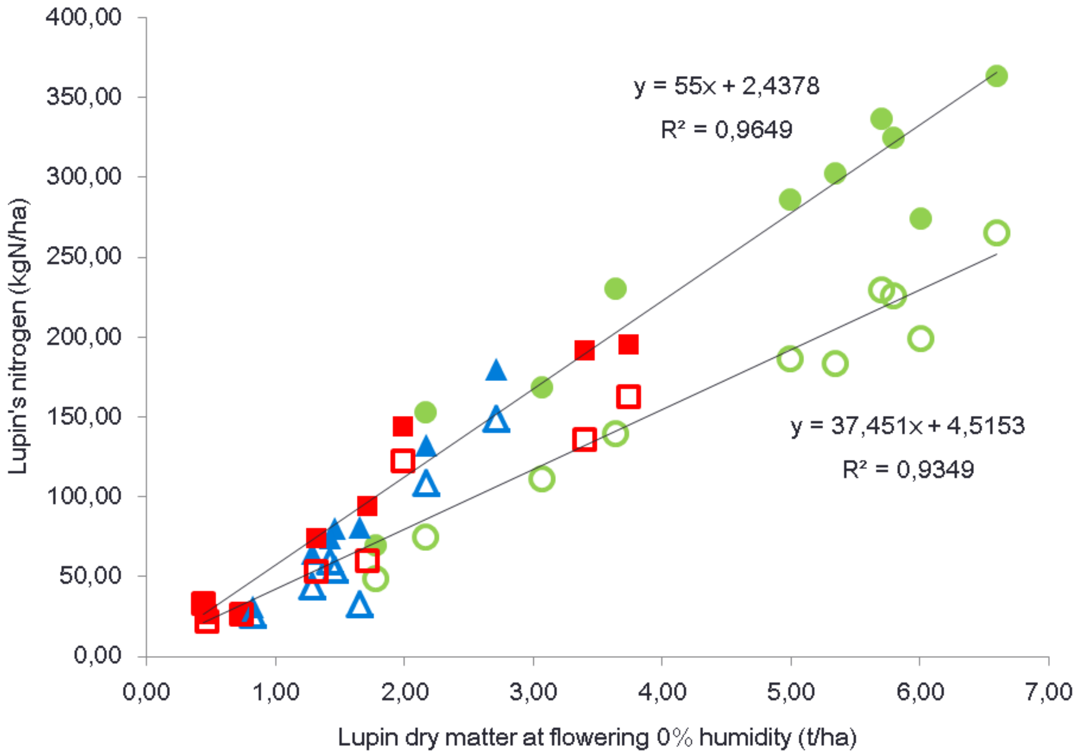


Figure 8: Relation between lupin's dry matter at flowering and nitrogen
Red square = Intercropped lupin, blue triangles = Spring lupin and green circles = winter lupin. Filled symbols are total nitrogen from lupin (shoot N * root factor). Empty symbols correspond to nitrogen derived from atmosphere. Biomass is expressed at 0% humidity.

III.5.C.b. At maturity

At maturity, a similar relation was obtained: the higher the above ground biomass the higher the quantity of N derived from atmosphere (fig 9). In this case root nitrogen was not taken into account. Some values of nitrogen derived from atmosphere in above ground DM reached 100% of total plant nitrogen.

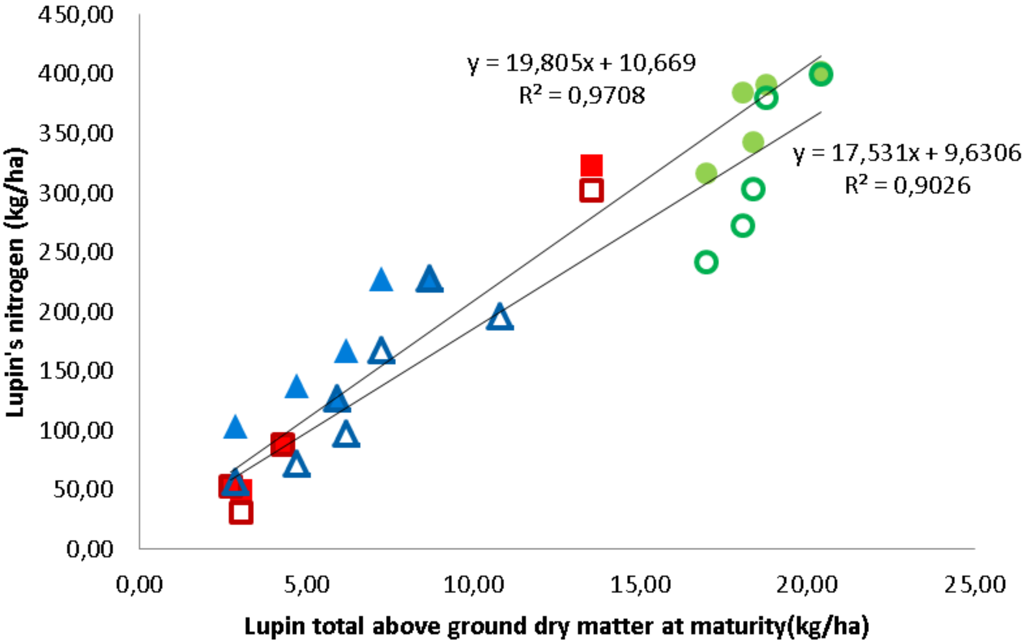


Figure 9: Nitrogen and lupin total above ground dry matter at maturity
Red square = Intercropped lupin, blue triangles = Spring lupin and green circles = winter lupin. Filled symbols are total above ground nitrogen from lupin. Empty symbols correspond to nitrogen derived from atmosphere.
Dry matter (DM) (straw + yield) expressed at 0% humidity.

III.5.D. Lupin’s experimental and real nitrogen balance

Nitrogen balance from lupin crop was first estimated with experimental yield (Lupin’s experimental N balance) and secondly with farmer’s yield (Lupin’s real N balance). Lupin’s real N balance values vary from 25.07 to 259 kgN/ha and are presented in Table 10. Plus, based on experimental yield, nitrogen harvest index (NHI) was determined. It was high (0.85) and stable (CV = 10%) across all fields and all cropping strategies, meaning that in average, 85% of the above ground nitrogen was exported with the grains.

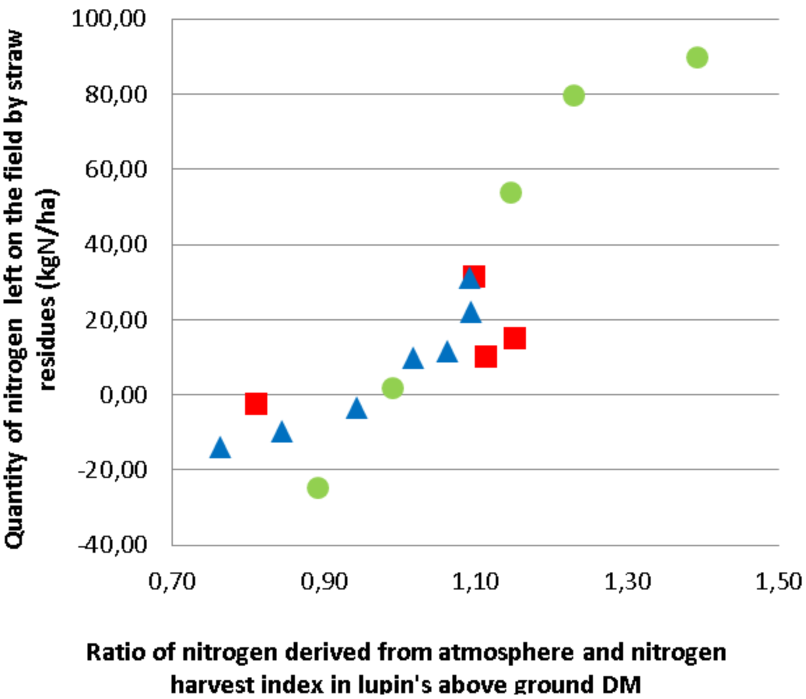


Figure 10: Lupin's experimental nitrogen balance in relation with the ratio percentage of nitrogen derived from atmosphere / Nitrogen Harvest Index
Red square = Intercropped lupin, blue triangles = spring lupin and green circles = winter lupin

In a situation where every grain produced is harvested and exported from the field and where the ratio of nitrogen derived from atmosphere on nitrogen harvest index is lower than 1, there are net exportations of nitrogen through grain exports (Fig. 10). Above this value, lupin’s straws contribute to field’s N pool because there was, in proportion, more nitrogen fixed than exported in above ground DM. Nonetheless, each lupin’s real N balance values were positive and tended to increase with the ratio value augmentation, highlighting the important contribution of grain losses (variable from one site to another) to residues’ N pool (Figure 11). This was confirmed by the positive correlation between the quantity of grain left on field and lupin’s real N balance (R^2 = 0.88 P-value < 0.001).

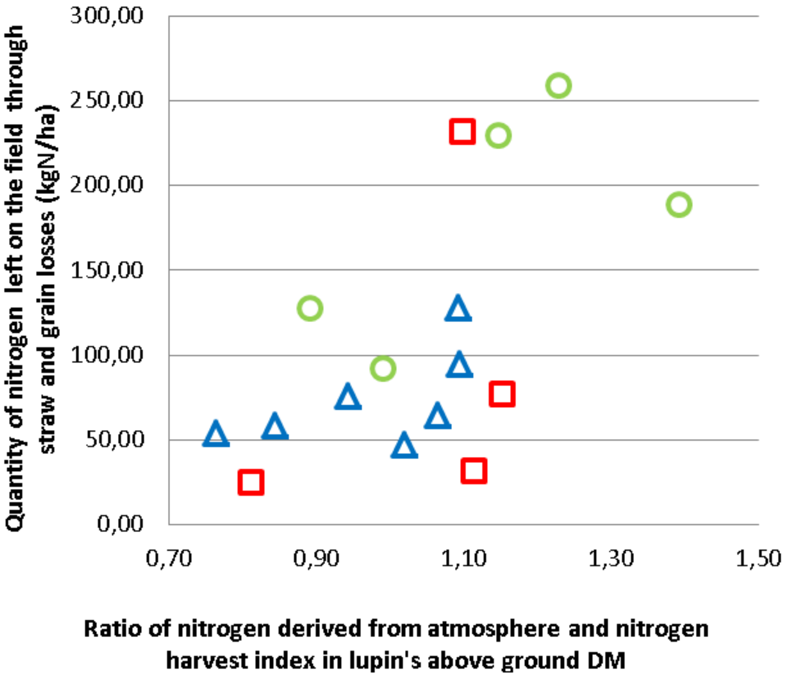


Figure 11: Lupin's real nitrogen balance in relation with the ratio percentage of nitrogen derived from atmosphere / Nitrogen Harvest Index
Red square = Intercropped lupin, blue triangles = spring lupin and green circles = winter lupin

III.5.E. Succeeding wheat response to lupin’s nitrogen input

Table 10: Wheat yield summary, soil mineral nitrogen at wheat emergence and lupin’s real N balance

	Site	Lupin's real N balance[2] (kgN/ha)	Soil mineral N in November (kgN/ha)	Wheat yield with N (t/ha) 15% humidity	Wheat yield without N (t/ha) 15% humidity	Ratio of wheat yields without N/with N	N fertilizers input in wheat areas with N (kgN/ha)
Intercropping	**IntWL1**	231,96	147,66	**8,42**	**3,95**	47%	133
	IntWL2	76,72	155,60	7,70	4,10	53%	211
	IntWL3	25,07	83,51	7,24	3,27	45%	105
	Average	111,25	128,92	7,79	3,77	41%	
	CV	97%	31%	8%	12%	9%	
Winter lupin sole crop	WLSC1	229,38	91,24	7,76	6,78	87%	190
	WLSC2	259,01	126,03	8,18	7,60	93%	190
	WLSC3	188,36	207,4 *	11,25	11,65	104%	277
	WLSC4	91,92	202,91 *	9,31	4,30	46%	270
	WLSC5	127,57	145,04	8,37	5,65	67%	121
	Average	179,25	154,52	8,98	7,19	68%	
	CV	39%	32%	16%	39%	29%	
Spring lupin sole crop	SLSC1	53,68	117,80	6,24	3,63	58%	-
	SLSC3	75,40	153,45	7,50	5,34	71%	153
	SLSC4	58,09	163,05	8,00	7,97	100%	154
	SLSC6	46,72	67,83 *	5,03	5,83	116%	214
	SLSC7	94,13	121,14 *	7,35	5,30	72%	214
	Average	65,60	124,65	6,82	5,61	71%	
	CV	29%	30%	17%	28%	28%	
	Total	**119,85**	**137,13**	**7,87**	**5,80**	**74%**	
	CV	**67%**	**31%**	**19%**	**40%**	**32%**	

Soil mineral nitrogen values follow by * are fields which received nitrogen before soil sampling for analysis

Data in bold characters (IntWL1) are yields from barley

[2] Does not take root N into account.

N fertilization values in red correspond to farmers who declared they reduced the amount of fertilizer used due to lupin effect

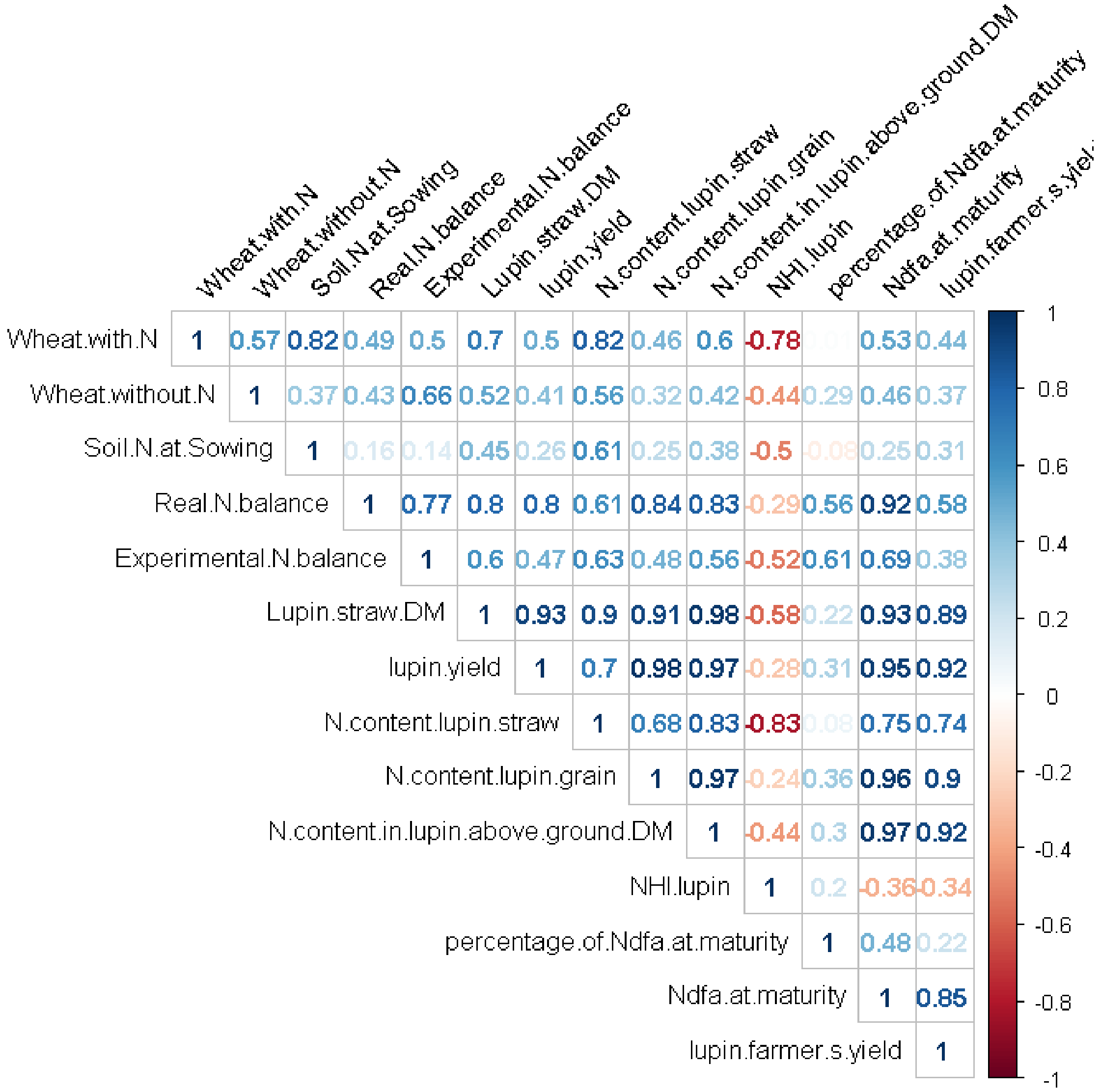
Regardless lupin’s cropping strategy, succeeding wheat yield with nitrogen ranged from 5.03 to 11.25 t/ha and average 7.87 t/ha with a CV of 19%. When cropped without nitrogen the average is 5.8 t/ha (CV = 40%) with data ranging from 3.27 to 11.65 t/ha (Table 10). However, the latter is to be considered carefully as it is amongst the 4 fields which received mineral or inorganic nitrogen fertilizers even in areas without N fertilizers application.

When looking at the groups, there were no significant differences between lupin’s cropping strategies for both yields with and without N fertilizers (at alpha = 5%). However, yield stability of wheat following intercropped lupin (CV = 8 and 12%) was higher than for wheat following spring lupin or winter lupin.

The ratio between wheat yield without nitrogen and wheat with nitrogen reached three times 100% or more, revealing non respect of 0 N treatment or sampling procedure problems or that lupin N furniture may be sufficient to obtain the same yield as fertilized wheat.

Lupin’s real N balance was not significantly different between lupin cropping strategies neither was soil mineral N at sowing. Finally, N fertilizers inputs by farmers ranged from 105 to 277 kgN/ha and wheat yields in fertilized areas were not correlated to this value. Most of the farmers did not adapt their nitrogen fertilization after lupin crop destruction and those who did still applied a large amount of nitrogen on the field. Usually, it is during late flowering fertilization that farmer reduce N fertilizers quantity. This probably led to nitrogen leaching and economic losses.

Tableau 11: Correlation coefficient between wheat yield and various lupin parameters
Values superior or equal to 0.56 (or inferior or equal to -0.56) are significant correlation coefficients.
Nitrogen contents are expressed in kgN/ha.



	Wheat.with.N	Wheat.without.N	Soil.N.at.Sowing	Real.N.balance	Experimental.N.balance	Lupin.straw.DM	lupin.yield	N.content.lupin.straw	N.content.lupin.grain	N.content.in.lupin.above.ground.DM	NHI.lupin	percentage.of.Ndfa.at.maturity	Ndfa.at.maturity	lupin.farmer.s.yiel
Wheat.with.N	1	0.57	0.82	0.49	0.5	0.7	0.5	0.82	0.46	0.6	-0.78	0.01	0.53	0.44
Wheat.without.N		1	0.37	0.43	0.66	0.52	0.41	0.56	0.32	0.42	-0.44	0.29	0.46	0.37
Soil.N.at.Sowing			1	0.16	0.14	0.45	0.26	0.61	0.25	0.38	-0.5	-0.08	0.25	0.31
Real.N.balance				1	0.77	0.8	0.8	0.61	0.84	0.83	-0.29	0.56	0.92	0.58
Experimental.N.balance					1	0.6	0.47	0.63	0.48	0.56	-0.52	0.61	0.69	0.38
Lupin.straw.DM						1	0.93	0.9	0.91	0.98	-0.58	0.22	0.93	0.89
lupin.yield							1	0.7	0.98	0.97	-0.28	0.31	0.95	0.92
N.content.lupin.straw								1	0.68	0.83	-0.83	0.08	0.75	0.74
N.content.lupin.grain									1	0.97	-0.24	0.36	0.96	0.9
N.content.in.lupin.above.ground.DM										1	-0.44	0.3	0.97	0.92
NHI.lupin											1	0.2	-0.36	-0.34
percentage.of.Ndfa.at.maturity												1	0.48	0.22
Ndfa.at.maturity													1	0.85
lupin.farmer.s.yield														1

Wheat yield without nitrogen was correlated to straw nitrogen content and lupin’s experimental N balance but was not significantly correlated to lupin’s real N balance.

On the other hand, wheat yield with nitrogen positively correlated to soil mineral nitrogen at wheat sowing, lupin straw dry matter, nitrogen content in lupin straw and total lupin above ground nitrogen. It was also negatively correlated to lupin NHI.

IV. Discussion

Yield of white lupin have been described and they are variable across sites, years and cropping strategies. Results obtained from the field work are discussed to highlight key elements involved in yield build-up for each cropping strategy.

IV.1. Lupin yield in Pays de la Loire

Except form winter white lupin of 2015, white lupin grain yields were highly variable across sites, cropping strategies and years as suggested by the literature review. Whether it was sole cropped spring or winter white lupin or intercropped lupin with triticale, lupin yield was not found correlated to mean seed weight and highly correlated to number of grains per square meter as highlighted by Julier and Huyghe (1993). However, in 2016, the lower thousand grain weight of lupin (compared to the seed characteristics given by the seed producer) decreased yield. This limited TGW may be due to the late formation of pods on the second order branches which shortened the grain filling period (Walker et al. 2011). It is more probably due to the specific weather conditions of 2016, with more than 80 mm of rain in the second part of June and half of the average sun radiations over June which reduced photosynthesis and assimilates formation. Additionally, during these rainfall events, soil may have been waterlogged which penalized biological nitrogen fixation and other nutrients uptakes.

In 2015 losses between experimental yield and farmer’s yield were more than 50%. In 2016 it was less dramatic and was about 30%. Nonetheless it is high compared to the 20% report by Snowball (1986) in Unkovich et al. (2010a). This may be explained by a very low grain water content due to a dry summer which was favorable to pod rupture with any impact, for example, the combine harvester (Terres Inovia 2016). Additionally it can also highlight a certain non-representativeness of the sampling made as the experimental yield was calculated over nine sub-plots (more or less 9 m²) while farmer’s yield was an average of the entire field yield (between 3 and 24 hectares). If there were spatial inequalities in the field, it was not really represented in the collected data but had an impact on farmer’s yield.

IV.1.A. Lupin nitrogen content

Lupin seeds from winter lupin are containing significantly less nitrogen than spring lupin’s seeds. This comes partly from the genetic material as spring lupin is supposed to have a nitrogen content of about 36% while winter white lupin should have 34% according to Jouffray-Drillaud (2015) and Terres Inovia (2016). The value of 30% of protein in lupin grain in intercropping may reveal problems during pod filling as it is, even though not significantly, 3 points lower than winter white lupin sole crop and 4 points lower than the expected value.

Yields of white lupin are variable and are dependent to the grain number per square meter. For sole cropped winter white lupin, intercropped winter lupin and finally sole cropped spring lupin, key elements of yield formation are discussed and improvement possibilities presented.

IV.2. Winter lupin yield

Grain number per square meter was the main yield component. It was negatively correlated to plant density which contrasts with the results of (Lopez-Bellido et al. 2000) who found no significant plant density effect on grain yield.

2015 plant densities were good according to recommendations (Terres Inovia 2016) and yield even decreased with the lowest density value. This might be explained by the higher number of grain per plant at low density and the hot summer which did not provide good conditions for high order branches' pods development (Walker et al. 2011).

In 2016, days with minimal temperature below zero degrees Celsius occurred at least three times during flowering. In one hand these frost events probably damaged main stem flowers and lead to numerous abortions. But on the other hand, higher order branches' flowers and pods development plant led to yield compensation (Lopez-Bellido et al. 2000; Walker et al. 2011) thanks to a longer growing period allowed by moderate temperatures in July and high rainfalls in late June.

Nonetheless, on top of frost damages, humid and low temperature conditions during flowering coupled with high densities and high DM of plants led to massive *Botrytis cinerea* development on lupin plants. Indeed, closed canopies created by high above ground biomass and narrow row spacing reduced air movement within vegetation maintaining high humidity rate favorable to mold development. These humid conditions are also good for *Colletotrichum lupini* spread and development. The latter disease did not appear in every field because a spore source is necessary (e.g.: contaminated seeds) while *Botrytis cinerea* spores are hosted by many broadleaf plants and are very likely to be present in every fields.

Lopez-Bellido et al. (2000) highlighted the increasing importance of main stem in yield formation with increasing density. The field with narrow row spacing and high lupin densities dry matter at flowering did not bear any pods on the 2 first branch orders because of frost and/or *Botrytis cinerea* and *Colletotrichum lupini* development. On the contrary, the field with the lowest dry matter and wide row spacing bore pods on main stem and branches and was less affected by *Botrytis cinerea*. In the first field yield was the lowest while in the second one it was amongst the good yields of 2016.

Uromyces lupinicolus and *Sclerotinia sclerotiorum* were also observed in 2015 and 2016 respectively and probably slightly reduced grain yield but they were not directly associated with major yield losses even though they can have an important impact on yield (Walker et al. 2011).

Weed did not seem to be a major problem for crop development when present in large quantities on the field. However, in WLSC9 weeds probably led to harvest problems as the main weed represented was *Galium aparine* L. which covered entire parts of the field. Main impacts which could be expected from uncontrolled weed development in winter lupin fields would be weed seed bank build-up and harvest difficulties.

To have better chances of success with sole cropped winter white lupin, sowing should be given special attention. This implies sufficient row spacing (i.e. between 30 and 40 cm wide) and moderate plant density (i.e.: between 20 and 30 plants/m²). Investigation work should also be carried out to maximize harvest efficiency and minimize grain losses.

IV.3. Intercropped lupin yield

When intercropped with triticale, white lupin's grain number per square meter is highly related to dry matter at both flowering and maturity. As biomass per square meter is related to plant development and plant density, these two elements play a role in yield build up.

In intercropping, white lupin biomass was relatively low partly because both below ground and above ground competition with cereal reduce white lupin biomass (Mariotti et al. 2009). As there was competition between triticale and white lupin, lupin development before winter had probably been reduced. Reduction of lupin development before winter may lower the number of leaf promordia and affect root development. Yet, poor lupin development before winter increases over-winter losses (Shield et al. 1996; Bateman et al. 1997; Leach et al. 1997). Coupled with severe frost, this lack of development probably explains the losses which occurred during winter 2014/2015.

Also, after winter and harvest plant densities were correlated to grain number per square meter. In some fields lupin density decreased of ten plants between these two countings. This can also be attributed to poor plant development abilities as some dead lupin plants were found at harvest measuring less than 50 cm of height amongst triticale and other lupin plants measuring between 70 and 120 cm of height (personal observation). These plants were probably killed because they could not access light at some point of the canopy development.

In intercropped white lupin the main problem which limits lupin grain production is the plant number at harvest. Plant losses are very likely to be linked to plant development before winter, either because they are not winter hardy and will freeze or because they do not produce enough leaf primordia, diminishing their flowering and branching potential and will be suppressed by other lupin and triticale development. It is therefore important to improve sowing practices to allow good plant development before winter. This might be done by earlier sowing, by separating lupin and triticale on different rows or by delaying sowing of triticale some weeks after lupin sowing.

IV.4. Spring lupin yield

First the high correlation between dry matter and yield is confirmed by the study of Noffsinger and van Santen (1995) in which they obtained a correlation coefficient of 0.90 between biomass yield and grain yield for spring lupin. Difficulties at lupin's sowing and establishment was a source of diminution of plant density and therefore total DM. Recommended sowing density is 60 grain per square meter (gr/m^2) for Feodora cultivar and 50 for Energy (Terres Inovia 2016). Energy was sown at 57 grain per square meter. In SLSC5 Feodora was sown at 80 gr/m^2, while in the other fields sowing density was between 50 and 56 gr/m^2. Sowing occurred in good weather conditions except for SLSC1 and 2 were soils were full of water despite drainage (farmer's observations). For SLSC3 the day following sowing, rainfall created a crust on soil surface, leading to emergence difficulties (farmer's observations).

The recommendations of the technical institut Terres Inovia (2016) for sowing date for Pays de la Loire are: "as early as possible" and between mid-February and early march. This seemed to be confirmed by our results as the earliest sown field produced the highest yield and the last ones the lowest.

However, it seems that SLSC3, which was sown first, was in optimal conditions to grow, no competition between lupin and weeds, long growth cycle, a different cultivar and, in principle, no N-P-K limitations.

The high rainfalls of the end of April and early may (between 90 and 100 mm in less than 20 days), have probably weakened lupin's root system (Huyghe 1997; Walker et al. 2011) as water surely stayed in the fields (no drainage), and probably penalized growth.

Weed DM at flowering seemed to be constant across fields at flowering, except for SLC3 and SLSC2. In the first one, almost no weeds were present and the farmer chose field with low weed development potential. In the latter one, the main weeds which were present at flowering was *Avena fatua* L. which have great biomass production early in spring and *Cirsium arvense* L. which is really aggressive weed during reproductive growth in summer. This explains why weed DM was already high at flowering.

The increase between lupin flowering and maturity differ between fields. This comes from the composition of weed community. In fields were weed DM increased a lot, weeds were producing main stems with high DM content: *Chenopodium album* L.*, Daucus carota* L. *or Cirsium arvense* L.. In parallel, some weed species realized their development cycle and/or died before lupin's maturity and therefore did not contribute to weed's maturity DM. It was the case of *Senecio vulgaris* L.*, Alopercurus myosuroides* or *Fumaria officinalis* L..

Grass weed species can easily be controlled within grass weed specific herbicides (the only field were grass weed was really a problem did not receive grass weed herbicide). Therefore the main problem for weed management in lupin is broadleaf weed species presence. According to the spectrum of the herbicide used by farmers and their functioning, most of the dicotyledonous species should have been controlled (Terres Inovia 2016). This may highlight some problems of herbicide use, weed resistance or herbicide offer inadequacies. However, some perennial broadleaf weeds such as *Cirsium arvense* L. represented a common problem for most spring lupin fields and may not be affected by the herbicides uses due to their specific root system.

Finally, in June and July, during flowering and pod filling, there was several days were temperatures reach more than 30°C which is source of flower abortion and problems during pod filling (Walker et al. 2011). It was also coupled with low rainfalls which probably slowed down seed development, hence limiting final yield.

With regard to 2015 data, spring white lupin yield was highly correlated to dry matter per hectare. It is therefore necessary to obtain high population density and work to obtain high biomass per plant by early sowing and weed control to reduce competition for resources and later harvest problems (Noffsinger and van Santen 1995).

IV.5. Comparaison of the three lupin cropping strategies

Even though many more analysis could be done on data collected during the two years of experimentation, the present study highlighted the importance of the installation of a controlled plant population and sowing time. Not too many plants for sole cropped winter white lupin, early sowing for sole cropped spring white lupin with high density (between 50 and 60 grains/m² (ARVALIS and UNIP 2010)) and early sowing (or strip intercropping or delayed triticale sowing) for intercropped white lupin to improve plant population and plant development before winter.

Also weed dry matter did not seem to play a major role in yield limitation for both sole cropped and intercropped winter white lupin. Except for specific fields, weed biomass rarely exceeded half a ton per hectare. The difference between sole cropped winter white lupin and intercropped winter white lupin is that weed management involved at least 2 herbicide applications for the first while the second one received only 1. A part of the problem here comes from the weed seed bank build-up for next crops.

For sole cropped spring lupin, weeds likely played an important role in yield limitation through their competitive abilities and high dry matter amount per hectare. More investigation work will be required to fully understand the origin of such uncontrolled weed development, which could be linked to bad timing for herbicide application or field preparation before sowing.

IV.6. Lupin provisioning service and wheat response

As found by Julier and Huyghe (1993) white lupin yield was correlated to total above ground dry matter and harvest index (varying from 0.28 to 0.57 in their work) was independent from yield. A threshold (set to 9t of dry matter per hectare) of biomass was observed, dividing the yield response to the above ground biomass in two (figure 7). Below 9 tons of dry matter per hectare, the yield was closely related to above ground biomass with little deviation around mean values. When biomass per hectare was low, individual lupin plants have low biomasses (data not shown). Low DM accumulation probably imply low number of leafs on the main stem reducing branching potential (Julier and Huyghe 1993; Munier-Jolain et al. 1996). Also, because vegetative growth is concomitant to reproductive growth and pod filling, a low branching potential will reduce the overlapping of these two phases decreasing competition for resources within the plant.

Above the threshold, biomass per individual plant was higher. The hypothesis to explain the larger variability of grain yield is that high individual plant biomass implies large number of leaves produced on the main stem leading to high branching potential. High branching potential means longer flowering period as the probability of second and third branch order to develop is higher (the development of a new branch order depends on the development of the previous one (Julier and Huyghe 1993; Munier-Jolain et al. 1996)).

When second and third branch order develop, vegetative growth and flower production overlap main stem and first order pods, which were pollinated, delaying their full development. Also, flowers from upper branch order develop later in the season, exposing them to higher temperatures and water shortage risks which cause of flower abortion (Walker et al. 2011). As a consequence, with higher biomass, there is longer competition within the plant for resources and longer exposure to weather stresses. If weather conditions are favorable, the longer development will increase yield but it may reduce it because high order branches' flowers may not be pollinated.

Finally the higher variability of yield above the threshold of biomass can be explained by higher potential diseases rate caused by the fact that the canopy is closed and humidity stays in, creating favorable condition for disease development.

In 2015, nitrogen fixation rate at flowering was higher for white lupin in intercropping than in sole cropped spring or winter lupin. This can be explained by the fact that the cereal is more competitive for soil N acquisition (Hauggaard-Nielsen and Jensen 2001; Mariotti et al. 2009) which exhausts soil mineral nitrogen available for white lupin faster than in sole crop. When soil mineral nitrogen is exhausted fixation rate increases drastically (Unkovich et al. 2010b). As triticale uptakes nitrogen more efficiently than white lupin, lupin plants relies faster on biological fixation in intercropping than sole cropped explaining the higher fixation rate at flowering for intercropped white lupin than for the other in 2015.

Values of fixation rate are within the range of existing values (Mayer et al. 2003; Unkovich et al. 2010b; Espinoza et al. 2012). However, absolute values have to be considered with care as the Beta fix value originate from an experiment conducted in Switzerland with lupin grown for green manure purpose between August and November (Büchi et al. 2015). The main outcome is that total quantity of nitrogen fixed depends on white lupin total above ground biomass as it was demonstrated by Unkovich et al. (2010b) for narrow-leafed lupin. Furthermore at flowering the relation allows a precise estimation of nitrogen fixed at this stage thanks to the application of the root ratio. This relation was developed from lupin dry matter at 0% humidity. Therefore, if water content was stable across samples, a coefficient could be applied to include water content in the relation. This allow easy and precise assessment of net nitrogen input from a white lupin cover crop or a failed lupin destroyed at flowering.

At maturity an average of 88% of lupin nitrogen was derived from atmosphere. Applied to a calculation of nitrogen balance of lupin crop, this leads to N soil pool depletion in some cases under the hypotheses of a total harvest. But this might not be totally true as the calculation did not consider N contained in roots. Nonetheless, farmers never harvest 100% of their production. The calculation of a “real” nitrogen balance for the fields resulted in net nitrogen inputs for all fields and was highly dependent on the quantity of grain lost on the field. Indeed, grain’s N represents 78 to 91% of the total above ground nitrogen (Julier et al. 1993b). From our results, nitrogen provisioning services of white lupin seemed to rely on grain losses more than the quantity of straw left on the field. In some cases, grain losses led to increase tillage before wheat sowing.

According to the findings of (Mayer et al. 2003), only 12,1% of lupin residues’ nitrogen is recovered by the following crop, the rest being immobilized by microbial communities and contributing to soil organic matter. Other parameters that were not investigated play an important role in the pre-crop effect: the tap-root system or non-hosting of pests are examples amongst others (Kirkegaard et al. 2008).

Wheat response to nitrogen derived from straw dry matter was positive whether it received nitrogen fertilizers or not. Globally, the high percentage of nitrogen derived from atmosphere at maturity contributed importantly to soil nitrogen content, resulting in high soil mineral nitrogen for wheat in November. However, this availability of mineral nitrogen before winter may not be valorized by wheat and generate N leaching. Also, in some situations, results suggest that wheat yields without nitrogen are the same than with nitrogen but in most cases it is due to non-respect of 0N treatment or weed presence in sampling plots.

Moreover, farmers have different strategies to valorize nitrogen from lupin, some reduce N fertilization thanks to diagnostic tools and other just ignore it. Indeed, some farmers had one set of cropping practices for their wheat regardless the previous crop. Whether it was lupin of maize, they applied the same amount of nitrogen fertilizers. The opposite practice consisted in applying nitrogen according to chlorophyll analysis during early wheat grain filling. Thanks to this farmers estimated to have saved about 30 kgN/ha.

More work is needed to evaluate soil nitrogen content, estimate N leaching and uptake by wheat and understand farmers practices regarding lupin residues management. As well as including other beneficial pre-crop effects such as pest reduction or soil structure improvement.

IV.7. Study limits and perspective

Over 2 experiment years enormous amounts of data have been collected. This work focused on comparison between fields and cropping strategies and used only a part of available data. Another scale could be chosen to understand within field yield variations for example, taking into account more parameters (e.g.: insects, proportion of soil covered by weeds).

Secondly, due to technical constraints, some data, mainly related to soil and lupin root development, were lacking. For succeeding wheat study, there was no control possible to compare wheat development after lupin to wheat after another cereal for example.

This study highlighted the importance of improvement of sowing practices for all lupin cropping strategies, established a robust relation to estimate easily nitrogen amount derived from atmosphere at lupin flowering from simple biomass sampling and revealed lupin harvest difficulties. For succeeding wheat, the relations with lupin development remain unclear and more repetition and analysis are required.

V. Conclusion

As traditional crop for cattle protein supply in Pays de la Loire, white lupin found revival in food ingredients. However, production area remain really small and numerous farmers give up this crop after few years of trial despite the attractive contracts proposed by Terrena.

This work had two purposes. In one hand it aimed at documenting production performances of white lupin in Pays de la Loire and providing insights on which cropping practices were to be improved to stabilize grain yield and increase crop success rate. And on the other hand, to assess nitrogen provisioning services of white lupin to quantify the potential effect on succeeding wheat.

Regional agronomic diagnosis was performed thanks to a network of 25 fields of white lupin studied over two year. Three lupin cropping strategies were studied: sole cropped winter white lupin, sole cropped spring white lupin and winter white lupin intercropped with triticale. 13 of those fields with wheat succeeding to white lupin were studied with and without nitrogen fertilizer application for pre-crop effect.

Sole cropped winter white lupin had the highest grain yield potential in 2015 with an average of 6,5 t/ha but it decreased to 3,27 t/ha in 2015 confirming the yield instability and sensitivity to weather conditions. Sole cropped spring white lupin had an average yield of 2,93 t/ha. Intercropped winter white lupin did produce significantly less than sole cropped winter lupin but its mean performances remained stable from 2015 to 2016 (2,21 t/ha and 2,08 t/ha respectively). For the three cropping strategies, the existing variability in grain yields have been partly attributed to one common factor: sowing practices. Indeed, sole cropped winter lupin was found sown too densely in 2016, which, with unusual weather condition, led to massive disease development, spring lupin was sown too late in some fields reducing plant development before flowering. Intercropped winter lupin did not have enough development time before winter and suffered from important plant losses. Three propositions were made to solve this problem: earlier sowing of the plant mix, strip intercropping where each row is dedicated to one specie or delayed sowing to let the lupin grow before triticale implantation. A second common problem for farmers, regardless lupin cropping strategy, was harvest losses which could reach 75% of grain. It has to be investigated as only few elements arose to explain it: short plants, over dried grains at harvest or non-representativeness of the sampling plots. The last element which impacted only spring lupin yield formation but played a major role in farmers' discouragement is weed development. Weed biomass production was high in spring lupin and reduced lupin plant development through competition while for both winter lupin sole cropped and intercropped, weed development was fairly controlled and its main impact was weed seed bank build up for next crops.

The present work confirmed that in Pays de la Loire the amount of nitrogen fixed by white lupin depends directly on its biomass production. Yet, succeeding wheat response to lupin's residues nitrogen content was unclear when cropped without N fertilization. Despite this results, white lupin pre-crop represents a real opportunity to reduce nitrogen fertilizers input on succeeding wheat as some spots without N produced almost the same yield as the rest of the field and because it is already done by some farmers. Many other pre-crop effects played a role in wheat development and have not been taken into account in this work.

White lupin have a great potential in Pays de la Loire if sowing practices are improved, weed management solutions are offered to farmers. A better understanding and quantification of pre-crop benefits is necessary to unlock white lupin production in Pays de la Loire.

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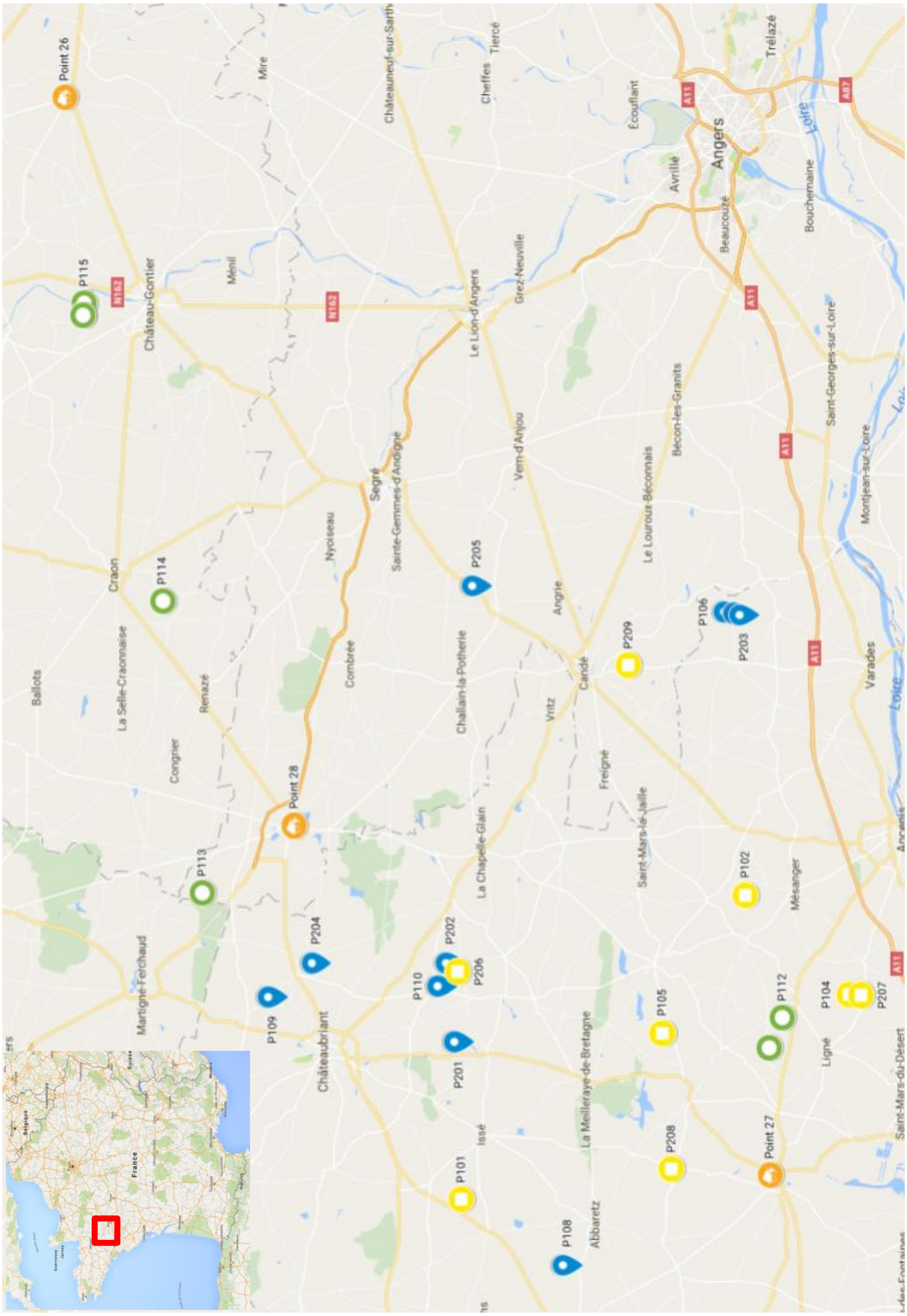
Appendix 1: Fields' network for 2015/2016 LEGITIMES experiments
Blue points are sole cropped winter white lupin fields
Yellow points are intercropped winter white lupin with triticale fields
Green points are sole cropped spring white lupin fields
Orange points correspond to meteorological stations.



Appendix 2: Fields and soil characteristics

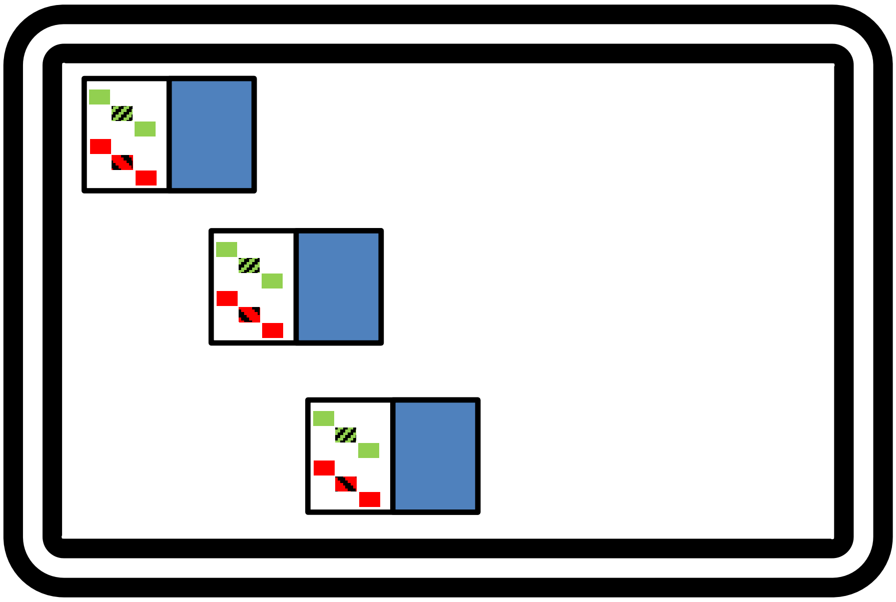
Field Id	Crop		pH	OM (%)	Soil type	sub plot size (m²)	soil sampling depth (cm)	Portion of soil covered by stones	Nearest meteorological station
	2014/2015	2015/2016							
IntWL1	Lupin /Triticale	Barley	7,2	2,2	Silt loam	0,53	80	15%	Pouancé
IntWL2	Lupin /Triticale	Wheat	7,2	1,3	Loam	0,58	60	7-10%	Nort-sur-Erdre
IntWL3	Lupin /Triticale	Wheat	6,9	2,4	Loam	1	90	25%	Nort-sur-Erdre
IntWL4	Lupin /Triticale	-	7	2,7	Loam	0,37	90	<15%	Nort-sur-Erdre
WLSC1	Winter Lupin	Wheat	6,8	1,6	Loam	1	80	<15%	Nort-sur-Erdre
WLSC2	Winter Lupin	Wheat	7,2	1,4	Loam	1	70	<15%	Nort-sur-Erdre
WLSC3	Winter Lupin	Wheat	7,1	1,9	Silt loam	1,15	80	15<x<50%	Nort-sur-Erdre
WLSC4	Winter Lupin	Wheat	6,7	2,7	Loam	0,75	70	<15%	Pouancé
WLSC5	Winter Lupin	Wheat	7,3	2,4	Loam	0,75	80	<15%	Pouancé
SLSC1	Spring Lupin	Wheat	6,7	1,6	Loam	0,32	90	10%	Nort-sur-Erdre
SLSC2	Spring Lupin	-	7,8	2,4	Silt loam	0,32	90	NA	Nort-sur-Erdre
SLSC3	Spring Lupin	Wheat	7,5	1,7	Silt loam	0,45	90	50%	Pouancé
SLSC4	Spring Lupin	Wheat	6,2	2,2	Silt loam	0,45	90	<15%	Pouancé
SLSC5	Spring Lupin	-	6,7	2,7	Silt loam	0,36	60	5-10%	Grez-en-Bouère
SLSC6	Spring Lupin	Wheat	6,9	2,6	Silt loam	0,6	90	5%	Grez-en-Bouère
SLSC7	Spring Lupin	Wheat	7	2,5	Silt loam	0,6	90	5%	Grez-en-Bouère
WLSC6	-	Winter Lupin	6,8	3,7	Silt loam	0,45	90	25%	Pouancé
WLSC7	-	Winter Lupin	7,2	2,8	Silty clay loam	0,4	90	15%	Pouancé
WLSC8	-	Winter Lupin	7	2,3	Loam	1,2	90	20-25%	Nort-sur-Erdre
WLSC9	-	Winter Lupin	6,5	3,2	Silt loam	0,6	90	1-5%	Pouancé
WLSC10	-	Winter Lupin	6,8	2,8	Silt loam	1,2	90	5%	Pouancé
IntWL5	-	Lupin/Triticale	7,1	3	Loam	0,45	60	10-15%	Pouancé
IntWL6	-	Lupin/Triticale	6,6	1,9	Loam	1,4	90	5%	Nort-sur-Erdre
IntWL7	-	Lupin/Triticale	7,1	3,1	Loam	0,9	60	15%	Nort-sur-Erdre
IntWL8	-	Lupin/Triticale	6,2	2,3	Loam	1,5	60	30%	Pouancé

Appendix 3: Table of the number of days with extreme temperatures and the maximal and minimal values

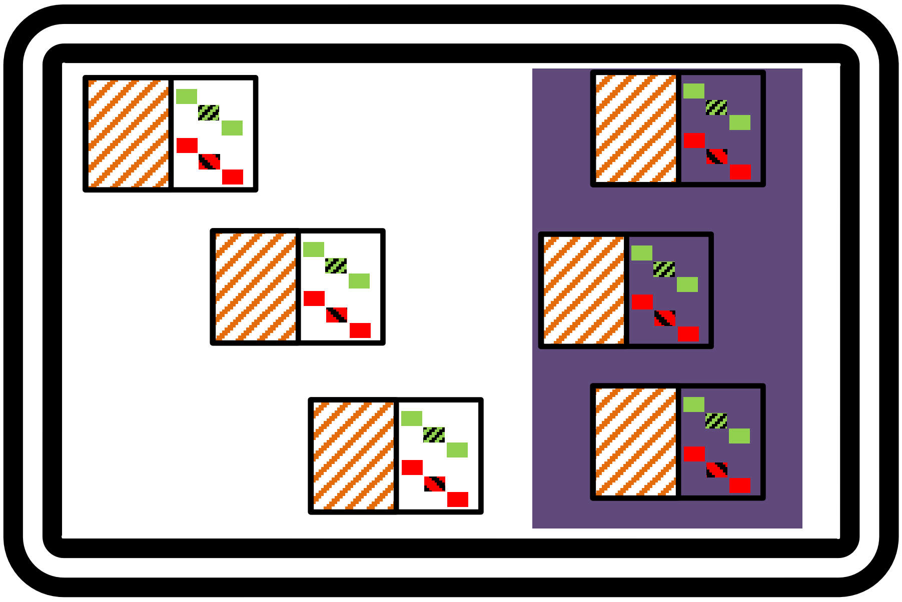
		Station	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	March	Apr.	May	June	July	Aug.
Year 2014/2015	Number of days with minimal T° < 0°C	Grez-en-Bouère	0	0	1 (-0,6°C)	9 (-5,9°C)	11 (-6,6°C)	14 (-4,9)	6 (-2,3°C)	0	0	0	0	0
		Pouancé	0	0	1 (-0,3°C)	7 (-6,1°C)	10 (-5,7°C)	12 (-2,7°C)	2 (-0,3°C)	0	0	0	0	0
		Nort-sur-Erdre	0	0	0	7 (-5,5°C)	7 (-5,2°C)	11 (-2,7°C)	4 (-1,4°C)	0	0	0	0	0
	Number of days with maximal T° ≥ 28°C	Grez-en-Bouère	0	0	0	0	0	0	0	0	0	8 (34,5°C)	8 (36,1°C)	12 (32,8°C)
		Pouancé	1 (28°C)	0	0	0	0	0	0	0	0	7 (34,7°C)	6 (36°C)	11 (32,4°C)
		Nort-sur-Erdre	6 (29°C)	0	0	0	0	0	0	0	0	4 (36,9°C)	7 (34,8°C)	9 (33°C)
Year 2015/2016	Number of days with minimal T° < 0°C	Grez-en-Bouère	0	0	3 (-1°C)	3 (-1,7°C)	6 (-3,5°C)	6 (-5,8°C)	6 (-4°C)	5 (-1,8°C)	1 (-0,2°C)	0	0	NA
		Pouancé	0	0	0	1 (-1°C)	4 (-1,5°C)	5 (-4,2°C)	3 (-3,4°C)	3 (-1°C)	0	0	0	NA
		Nort-sur-Erdre	0	0	0	2 (-1°C)	3 (-2,6°C)	4 (-5,2°C)	3 (-1°C)	5 (-1°C)	1 (-0,3°C)	0	0	NA
	Number of days with maximal T° ≥ 28°C	Grez-en-Bouère	0	0	0	0	0	0	0	0	0	1 (28,6°C)	9 (36,4°C)	NA
		Pouancé	0	0	0	0	0	0	0	0	0	0	8 (35,6°C)	NA
		Nort-sur-Erdre	0	0	0	0	0	0	0	0	0	0	7 (37,8°C)	NA

Appendix 4: Experimental design for sole cropped lupin

Lupin sole crop year n :



Following cereal year n+1 :



Legend :

▣ Field border with 10m margin to avoid border effect

🟦 Plots' half kept for n+1 subplots

▨ Plots' half where subplots were at year n

🟩 Subplots sampled at flowering stage

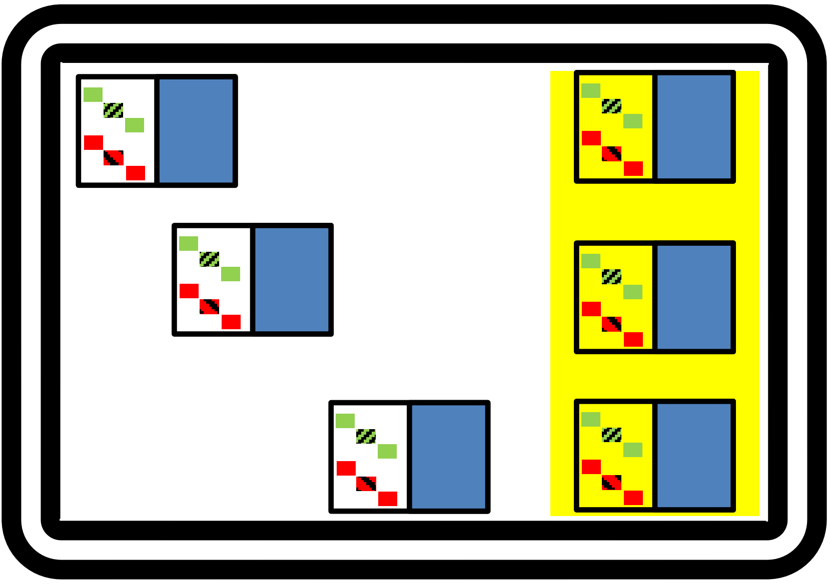
🟥 Subplot sampled at physiological maturity

▨ ▨ Subplots (PN) sampled for N content analysis at flowering and maturity.

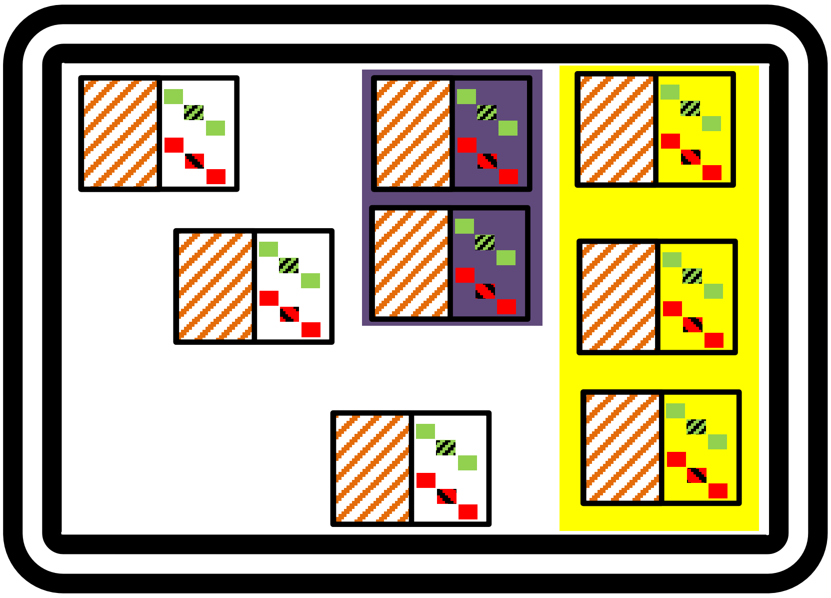
🟪 Control area: 0 N applications

Appendix 5: Experimental design for intercropped lupin

Lupin intercropped with triticale year n:



Following cereal year n+1:



Legend :

▣	Field border with 10m margin to avoid border effect
🟨	Lupin sole crop area for intra-field comparison
🟦	Plots' half kept for n+1 subplots
▨	Plots' half where subplots were at year n
🟩	Subplots sampled at flowering stage
🟥	Subplots sampled at physiological maturity
▨ ▨	Subplots (PN) sampled for N content analysis at flowering and maturity.
🟪	Control area: 0 N application