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Master thesis

Influence of Wheat/Legumes Mixtures on Production Parameters and Grain Quality

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Declaration

I declare that I am the author of this graduation thesis and that I used only sources and literature displayed in the list of references in its preparation.

In České Budějovice on 14th April 2023

Signature

Abstrakt

Pšenice je celosvětově jednou z nejvýznamnějších plodin. Pěstuje se prakticky ve všech klimatických pásmech a je významným zdrojem energie a bílkovin. V Evropě postupně roste zájem o její pěstování v ekologickém zemědělství, což souvisí se zvyšující se poptávkou po biopotravinách.

V rámci diplomové práce byl proveden přesný maloparcelkový pokus ve Zvíkově u Českých Budějovic (Česká republika) za účelem vyhodnocení vlivu různých kombinací pěstování směsí luskovin a pšenice na produkci a kvalitu zrna pšenice seté. Polní experimenty byly založeny v říjnu 2021, sklizeny byly v srpnu 2022. V období vegetace byly sledovány i klimatické podmínky. Výsledky práce prezentují hodnocení produkčních parametrů, které se podíleli především na výnosu zrna a hodnotí vliv pěstování pšenice s různými luskovinami na kvalitativní parametry zrna.

Výsledky ukázaly, že odrůda ovlivňovala především délku stébla, přičemž na počet klasů měla vliv i varianta použití luskovin. Hmotnost tisíce zrn byla především ovlivněna způsobem založení porostu. Výnos zrna byl ovlivněn hlavně způsobem setí, ale efekt byl statisticky neprůkazný; nebyly zjištěny žádné statisticky významné rozdíly ve výnosových parametrech v případě pšenice pěstované samostatně nebo ve směsi. Vyhodnocením dat bylo zjištěno, že pěstování ozimého hrachu a bobu v kombinaci s pšenicí dosáhlo nejvyšších výnosů. Ze samostatného hodnocení podsevu luskovin po celou vegetaci, setí na jaře, případně jeho odstranění na jaře, byl patrný vyšší výnos u varianty s podsevem po celou dobu vegetace.

Dále diplomová práce hodnotila vliv zmíněné praxe na pekařskou jakost zrna. Obsah bílkovin v sušině zrna byl nízký a žádná z hodnocených variant nesplňovala minimální požadavek na obsah bílkovin pšenice pekárenské. Nejlepších hodnot mokrého lepku a gluten indexu dosáhla varianta s jarním přísevem luskovin. Odrůda Lorien dosáhla celkově horších výsledků ve srovnání s odrůdou Butterfly. Způsob setí neměl na kvalitativní parametry zrna zásadní vliv.

Klíčová slova: ekologické zemědělství, intercropping, pšenice, leguminóza

Abstract

Wheat is important crop worldwide, grown in a wide range of agro-climatic zones, and is a significant source of dietary energy and protein. There has been a growing interest in cultivating organic wheat in Europe.

The thesis conducted a field experiment in Zvíkov, Czech Republic, to evaluate the effect of different combinations of legume/wheat mixtures on winter wheat production and quality. The experiments were established on October 2021, and were harvested on August 2022. During the vegetation period, the climatic conditions were also monitored. The results of the thesis present the evaluation of the production parameters, which mainly contribute to grain yield, and evaluate the influence of growing wheat with various legumes on the qualitative production parameters.

The results showed that the variety mainly influenced stem length, while the number of ears was also affected by the variant of leguminous use. The thousand kernel weight was a matter of establishing the stand. The grain yield was predominantly influenced by the method of sowing, but the effect was statistically inconclusive; no statistically significant effects of wheat grown single or in a mixture with legumes on the yield parameters. The data evaluation revealed that growing winter peas and field beans in combination with wheat achieved the highest yields. From the separate evaluation of undersowing of legumes throughout the vegetation, sowing in the spring, or its remove in the spring, a higher yield was evident in the variant with undersowing throughout the entire vegetation period.

Next, the study evaluated the effect mentioned practice on baking quality. The protein content in the dry grain matter was low, and none of the evaluated variants met the minimum requirement for the protein content of bakery wheat. The best values for wet gluten and gluten index were achieved by the variant with the sowing of legumes in spring. The Lorien variety achieved overall worse results compared to the Butterfly variety. The sowing method did not have a significant effect on the quality parameters.

Keywords: organic farming, intercropping, wheat, legumes

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Content

1. Introduction

Organic farming practices for wheat production face several challenges, including low yields and lower protein content in grains. These challenges arise from several factors, including the absence of synthetic fertilizers and pesticides, limited nutrient availability, and pest and disease pressure.

Therefore, organic wheat production requires a comprehensive understanding of nutrient cycling and management, crop rotation, and pest and disease management strategies. Although the challenges of organic wheat farming, crop diversification through intercropping can help improve crop productivity and soil fertility. Furthermore, intercropping can improve crop yield stability and resilience to pest and disease pressure. Another reason for using mixed crops of wheat and legumes is the assumption that grain quality could improve in the case of cultivated wheat due to nitrogen fixation by legumes.

Intercropping legumes and wheat is a sustainable agricultural practice that can bring numerous environmental benefits. Legumes can fix atmospheric nitrogen in the soil, making it more fertile and reducing the need for synthetic fertilizers.

Intercropping promotes biodiversity by creating a more diverse habitat for insects, birds, and other animals. This is because a mixture of crops provides a more varied and balanced diet for these creatures, increasing their populations and enhancing the area's ecological balance. Another environmental benefit of intercropping is soil conservation. By planting a mixture of crops, the soil is less prone to erosion, as the roots of the different plants help to hold the soil in place.

Intercropping legumes and wheat also have economic benefits for farmers. Farmers can increase their yield by growing various crops and reducing the risk of crop failure due to disease or weather conditions. Overall, intercropping legumes and wheat is a sustainable agricultural practice that can bring multiple environmental benefits, including soil conservation, biodiversity, and reduced chemical inputs.

2. Aim of the thesis and working hypotheses:

2.1 Aim of work

Evaluation of the effect of wheat/legume mixtures on production parameters and baking quality of wheat grain.

2.2 Working hypotheses

A different way of sowing, management, and removal of leguminous plants in wheat stand will affect the production parameters of the wheat.

- The presence of leguminous plants in a wheat stand may have a positive or negative impact on the yield, plant growth, and production parameters of the wheat.
- This hypothesis could be tested through experiments that compare the production parameters of wheat grown alone versus wheat grown in mixtures with different types and densities of legumes.

A different method of sowing, management, and removal of leguminous plants in wheat stand will affect the qualitative parameters of the wheat.

- The presence of leguminous plants in a wheat stand may impact the quality of the wheat grain itself, particularly its suitability for baking.
- This hypothesis could be tested through experiments that compare the baking quality of wheat grown alone versus wheat grown in mixtures with different types and densities of legumes, and through laboratory analyses of the wheat grain's nutritional and functional properties.

3. Literature review

Wheat (*Triticum aestivum* L.) is one of the most important cereal crops worldwide, with an estimated 217 million hectares of land dedicated to its cultivation (FAOSTAT, 2021). It is grown in a wide range of agro-climatic zones, from the humid tropics to the drylands of the arid and semi-arid regions. Global wheat production has steadily increased recently, with a record production of 776 million tonnes in 2021 (FAOSTAT, 2021). The major wheat-producing countries are China, India, Russia, the United States, Canada, Australia, and France, accounting for around 70% of global wheat production. Wheat is an important staple food for many people, especially in developing countries where it is a significant source of dietary energy and protein. It is also an important crop for the global food industry, with wheat flour being a key ingredient in producing bread, pasta, noodles, and many other food products and it is an essential ingredient in food products (Sapone *et al*., 2012; Tosi *et al*., 2011).

Wheat is also a significant crop in European agriculture, with an estimated 28 million hectares of land dedicated to its cultivation (Eurostat, 2021). The major wheat-growing countries in Europe are France, Germany, the United Kingdom, Italy, and Poland, accounting for around 60% of the total wheat production in the European Union. The average wheat yield in Europe is around 5 tonnes per hectare, with significant variations between countries and regions. The highest yields are typically achieved in the Western and Northern European countries, while the lowest yields are observed in the Mediterranean and Eastern European countries (Eurostat, 2021).

In recent years, there has been growing interest in cultivating organic wheat in Europe due to concerns about conventional farming practices' environmental and health impacts. Organic wheat is typically grown in smaller fields, with lower yields than conventional wheat, but with better quality and higher prices in the market (Reganold and Wachter, 2016). The main benefits of organic farming are the reduction in synthetic pesticides and fertilizers, better soil health, and a lower carbon footprint (Gomiero, Pimentel and Paoletti, 2011).

3.1. Wheat Breeding from a Global Perspective

Over the past 100 years, wheat yields in developed and developing nations have increased due to improved varietal performance and increased use of inputs such as chemical fertilizers, herbicides, and pesticides (Ceccarelli, 1996). Over the past 50 years, global wheat breeding efforts have primarily focussed on improving wheat varieties' yield potential and quality. While these have been two essential goals of breeding programs, other areas of focus have included: (1) increasing disease and lodging resistance, (2) improving the response of wheat varieties to chemical fertilizers, and (3) the development of varieties with broad adaptation to various agronomic environments (McCaig and De Pauw, 1995; Rajaram, 2001). The most significant advance in modern wheat breeding was the introduction of height-reducing (Rht) genes and the subsequent development of semidwarf varieties with a higher yielding ability under optimal conditions (Worland and Snape, 2001). Semidwarf varieties are much shorter, have greater tillering capacity, higher grain yield per spike, and are more responsive to inputs than traditional wheat varieties (Sinha *et al*., 1981). The wide-scale adoption of these new wheat varieties has resulted in higher yield potential, coupled with an increase in the use of inputs (Bramel-Cox *et al*., 1991).

Breeding wheat varieties for organic farming is crucial to developing sustainable and resilient agriculture systems. Organic farming practices rely on biological, physical, and cultural practices, which require different agronomic traits and performance characteristics from conventional wheat varieties. As a result, there is a growing need for developing wheat varieties specifically adapted to organic farming systems. Critical traits selected for organic wheat breeding programs include early vigor, disease resistance, weed competitiveness, and nutrient-use efficiency (Bingen *et al*., 2021). These traits are essential for optimizing crop performance and reducing the need for synthetic inputs, which are not allowed in organic farming systems.

In Europe, some companies are actively breeding and commercializing wheat varieties for organic farming (Bingen *et al*., 2021). These companies are developing wheat varieties adapted to organic farming systems' low-input, low-nitrogen, and weed-suppressive conditions. Generally, there is a lack of organically breeded varieties in Europe.

3.2. Productivity and Quality of Organic Wheat Farming

In organic farming, wheat is vital in providing sustainable food and farming systems. Organic wheat production can improve soil health, reduce environmental impacts, and improve food quality. It also promotes biodiversity and can enhance ecosystem services, such as soil fertility, water regulation, and carbon sequestration, which can benefit crop production in the long term. On the other side, organic wheat farming has been shown to have lower yields than conventional farming. However, several studies have shown that organic wheat can produce high-quality grain with improved nutritional content compared to conventional wheat. Organic wheat can have higher levels of antioxidants, protein, and minerals, making it a more nutritious food source. Grain yields in organically managed fields are commonly lower than those of conventionally grown grain crops (Walker and Smith, 1992; Entz *et al*., 2001; Kitchen *et al*., 2003; Ryan et al., 2004). In one Australian study, organic wheat yields were 21–31 % lower than conventional yields (Kitchen et al., 2003). Another study from Australia reported that organic wheat grain yields were less than half of conventional grain yields (Ryan *et al*., 2004). In Canada, where research relating to organic grain production to date is limited, Entz *et al*. (2001) found that wheat, oat (*Avena sativa* L.), and barley yields were 23–27% lower on organic farms than conventional farms; however, maximum yields were higher on organic farms than long-term conventional averages, highlighting the potential for successful organic grain production. Competition from weeds plays a role in reducing yields. Studies have reported higher numbers of weeds and greater diversity of weed species in organic cereal crops than in conventional ones (Samuel and Guest 1990). Samuel and Guest (1990) reported that perennial weeds were more problematic in organic fields than annual weeds. On the Canadian prairies, weed populations appeared to be higher on organic farms, with wild mustard (*Sinapsis arvensis* L.) and Canada thistle (*Cirsium arvense* L.) in greater abundance in organic fields than in conventional fields (Entz *et al*., 2001).

3.3. Strategies for improving of wheat productivity and quality

The conventional efforts to boost grain yield and quality included breeding and selection. However, these take a long time and cost (Hoang *et al*., 2022). Designing cultivation practices could be a complementary strategy in grain yield and quality improvement of grain wheat in organic farming (Konvalina *et al*., 2009). The systems used should reduce input costs and improve product yields. Mixtures of wheat varieties or wheat and legumes are a viable strategy for sustainable products to help greater stability or no reduced yield, which is promoting to achieve high yields in bread quality in organic farming compared to single systems (Kaut *et al*., 2009). The use of leguminous cover crops such as astragalus and red clover is reported to be more profitable than herbaceous cover crops such as rye and wheat. This is to supply the main crop with N and reduce N requirements. Systems using legume cover crops also require less energy. Studies on this subject show that shaggy vetch is the highest yielding cover crop. Reduces nitrogen use, saves energy, and improves the effectiveness of applied nitrogen by regulating soil structure and soil water-holding capacity (Hanson *et al*., 1993). Even though mixing varieties of wheat can also improves baking quality (Aart, 2006), growing multiline cultivars and cultivar mixtures between wheat and legumes would complement properties of grain yield, grain bulk density, protein content, and also economic efficiency (Vrtilek *et al*., 2016), yield stabilization and pathogen spread in plant populations reduction (Vidal *et al*., 2020). In addition, growing mixtures of pure varieties or annual arable crop species is a promising way to improve crop productivity and complementary N accumulation, decreasing agricultural inputs, especially without chemicals use (Borg *et al*., 2018; Gaudio *et al*., 2019; Chen *et al*., 2020; Dahlin *et al*., 2020).

Table 1: Strategies to increase yields in organic arable crops that are applicable to Northern Europe. Adapted from Niggli et al. (2016)

Area of intervention	Important for	Strategies to increase yields
Soil fertility	All crops, but especially on stockless farms	• Crop rotation design and management including optimal management of legume pre-crop effects and green manure crops • Increased crop diversity • Intercropping • New technologies for reduced tillage • Increased cooperation between livestock farms and stockless farms • Adding/promoting supportive microorganisms and fungi in soil
Plant nutrients	All crops (except nitrogen for legumes)	• Optimal use of legumes in rotations • Effective use of manures • Increased recycling and use of nutrients from society • Novel treatments of organic food wastes to produce high-quality composts • Technological solutions for safe sewage sludge treatments and recycling
Crop-weed competition	All crops, but especially in stockless systems without perennial leys	• Crop rotation design and management • New physical weed control strategies and techniques including cover crop management • Use of the false seedbed technique • Precision farming and robots
Control of diseases	All crops, but especially potatoes and legumes	• Use of tolerant or resistant crop varieties • Crop rotation design and management • Preventative strategies like intercropping, deep ploughing, optimal planting date etc. • New techniques and products for preventing fungal infections, physical methods and biocontrol organisms • Replace copper that is currently used • Use of certified and dressed seeds
Control of pests	All crops, several pests in oilseed rape and potatoes	• Crop rotation design • Habitat manipulation (hedgerows, wild flower strips etc.) to strengthen functional biodiversity (e.g. natural enemies) • Physical/biological methods like nets, traps and repellents • Selective pest control products with low negative side-effects

3.4. Wheat/legume intercropping

Wheat-legume intercropping has several benefits, including improved nitrogen input due to biological nitrogen fixation by the legume, leading to increased wheat yield and reduced fertilizer application.

Contribution of legumes towards N economy in cereal-based cropping systems is well-known (Sharma and Behera, 2009). In crop rotations, grain legumes contribute to a diversification of cropping systems and as N_2-fixing plant it can reduce the mineral N fertilizer demand (Mayer *et al*., 2003). Generally in sustainable and organic farming systems, biological N_2-fixation by legumes is the main source of nitrogen for the crop rotation. Hence cropping of grain legumes is a prioritized area of research in the rain-fed areas. Grain legumes cause significant, positive yield effects on subsequent non-legumes when compared with rotations with non-legumes (Chalk, 1998; Sanginga, 2003). In addition to its beneficial factors, such as improving soil structure, breaking pest and disease cycles and the phytotoxic and allelopathic effects of crop residues, nitrogen is a key factor in the positive response of cereals following legumes (Chalk, 1998). However the improvement in N nutrition of nonfixing crops in grain legume-based cropping systems requires a more fundamental understanding of the decomposition of grain legume residue processes and the interactions with soil organic matter. Grain legume species and varieties growing at the same location differ significantly in dry matter production, N accumulation, N_2-fixation, N-balance and residue quality (Beck *et al*., 1991; Haynes *et al*., 1993; Evans *et al*., 2001). These differences may be the main factors determining the residual N contribution to subsequent crops (Hood *et al*., 1999).

The increased biological activity of the soil caused by legume cover crops and the resulting N-cycle of the plant-soil system results in faster formation of the nitrogen needed by plants in crop production than in inorganic systems. Residual soil nitrogen from planting non-leguminous winter cover crops after summer crops is retained, reducing nitrogen loss through leaching or runoff. For example, a 65-70% reduction in nitrate levels was observed when comparing winter fallow leaching to catch. Because plant roots remove water and nitrogen. In general, wheat monocultures are more effective at recovering soil minerals than legume monocultures. For example, monocultures of rye were found to reduce soil inorganic nitrogen levels by 37-62% less than monocultures of legumes (Ryan *et al*., 2004). The most important point when growing cover crops is knowing the balance between the costs of installing a system and the benefits that come with it.

4. Material and Methods

4.1 Field experiments

According to the methodology, a precise small-plot experiment was established in all combinations of organic farming. The pre-crop was meadow clover (variety Bonus). The experiment was based on an ecologically certified area on a farm in Zvíkov near České Budějovice. Characteristics of the experimental site: grain production area, altitude 490 m, brown loam soil, loamy (medium) soil.

The experiment established stands, where mixtures of wheat (varieties Lorien and Butterfly) and several types of legumes were sown with different sowing methods. The following legumes were used: broad beans, spring and winter peas, clover incarnate, and meadow clover. Depending on the specific variant, the experiment was sown in classic grain rows (a mixture of wheat and leguminous seeds) and the row by row variant. Specifically, it was the autumn sowing of wheat and legumes in the form of under-sowing in classic grain rows (the legumes remained in the plots for the entire growing season or until frost – broad beans, peas in spring and winter forms, clover incarnate). In addition, a mechanical liquidation of legumes sown in autumn was carried out in the row by row variant (beans, spring and winter peas, incarnate). The third combination was the spring peas and meadow clover sowing in the spring in plots sown both classically and with an omitted row for spring sowing but without leguminous seeds sown in autumn. A list of all combinations is given in Table 2.

The course of the year 2021/2022 was favorable for winter wheat in some aspects and less favorable in others (details are given below). The stands were established on October 5, 2021. According to the experimental variants, leguminous crops or their mechanical disposal were planted in the spring. Within the parcels (except for the variants with clover seeding), dragging with rod gates was used to control weeding. Agrotechnical interventions are shown in Table 3. The entire experiment was harvested after reaching full maturity on 3rd August 2022 with a small-plot Wintersteiger harvester.

Table 2: Variants of setting up an experiment, Zvíkov near České Budějovice, organic farming, 2021/2022

Variant	Method of management of legume	Leguminous sowing date
Butterfly or Lorien + spring pea (undersowing)	Row-by-row, spring remove	autumn
Butterfly or Lorien + field bean (undersowing)	Row-by-row, spring remove	autumn
Butterfly or Lorien + incarnate (undersowing)	Row-by-row, spring remove	autumn
Butterfly or Lorien + winter pea (undersowing)	Row-by-row, spring remove	autumn
Butterfly or Lorien + red clover (sowing in spring)	Row-by-row	Spring
Butterfly or Lorien + spring pea (sowing in spring)	Row-by-row	Spring
Butterfly or Lorien + spring pea (undersowing)	Mixture	autumn
Butterfly or Lorien + field bean (undersowing)	Mixture	autumn
Butterfly or Lorien + incarnate (undersowing)	Mixture	autumn
Butterfly or Lorien + winter pea (undersowing)	Mixture	autumn
Butterfly or Lorien + red clover (sowing in spring)	Spring sowing	Spring
Butterfly or Lorien + spring pea (sowing in spring)	Spring sowing	Spring
Butterfly or Lorien control, standard lines (12,5 cm)	Control	-

During the growing season, the most critical parameters of the stands were evaluated, such as the number of plants after emergence and the number of ears before harvest, the number of leguminous plants in individual variants, and lodging were monitored. After harvest, the determined yield was converted to 14% grain moisture and grain weight. The incidence of diseases and pests was continuously monitored during the vegetation period. The incidence of common wheat diseases was low (as in previous years). It was instead a weak incidence of powdery mildew. We also noticed a weak occurrence of chaff and non-specific spotting in the stands. The attack of rust on plants was sporadic, and it was instead an exceptional occurrence of isolated clusters of rust on wheat leaves. In several plots, cockroach feeding was observed on wheat leaves. The level of disease and pest infestation was such that it did not cause an economically significant reduction in yield. Weeding was a bigger problem in the stands when, as a result of the heavy rains in June, weeds grew in the later developmental stages of wheat growth. None of the plots were lodged. For the reasons stated above, we present in the report statistically processed data only evaluating the length of the stalk, the number of ears before harvest, the grain yield, and the weight of a thousand seeds.

Table 3: Agrotechnology, Zvíkov u Českých Budějovic, organic farming, 2021/2022

Date	Agrotechnological operation
3. 9. 2021	Shallow plowing
25. 9. 2021	Sheep manure application (4 $t.ha^{-1}$)
26. 9. 2021	Tillage
3. 10. 2021	Pre-sowing soil preparation
5. 10. 2021	Seeding with a Hege experimental sowing machine
6. 10. 2021	Rolling
23. 3. 2022	Sowing of legumes (only some variants)
29. 3. 2022	Weeding by rod gates
11. 4. 2022	Mechanical liquidation of legumes (only some variants)
11. 4. 2022	Weeding by rod gates
3. 8. 2022	Harvest

4.2 Climatic conditions

In October 2021, there was a lack of precipitation at the location in Zvíkov, which was reflected in reduced plant emergence. The growing season for winter wheat in September 2021–August 2022 was generally average in terms of precipitation, but extreme fluctuations in the distribution of precipitation were recorded. In addition to the already mentioned dry autumn, on the other hand, an extremely wet June followed, when the monthly precipitation total was higher at 88.6 mm. This led mainly to the massive development of weeds. The remaining period was average in terms of precipitation. In terms of temperatures, the period was above average (except for April 2022). Overall, temperatures were 2.1 °C higher during the monitored period. Detailed data are in Table 4.

Table 4: Monthly meteorological data for September 2021 – August 2022, Zvíkov u České Budějovice

Month	Air Temperature (°C)			Precipitation (mm)		
	Average	Long term average	Difference	Sum	Long term average	Difference
09/2021	14,3	12,4	+1,9	13,3	50,6	-37,3
10/2021	7,8	7,5	+0,3	14,2	33,9	-19,7
11/2021	3,2	2,4	+0,8	30,5	38,6	-8,1
12/2021	1,1	-1,0	+2,1	45,7	30,2	+15,5
01/2022	1,4	-2,7	+4,4	20,8	26,5	-5,7
02/2022	3,4	-1,1	+4,5	34,3	27,1	+7,2
03/2022	3,2	2,4	+0,8	15,5	34,4	-18,9
04/2022	6,5	6,9	-0,4	47,5	48,6	-1,1
05/2022	14,4	11,9	+2,5	39,3	76,7	-37,4
06/2022	18,6	15,2	+3,4	187,8	99,2	+88,6
07/2022	18,7	16,8	+1,9	76,7	84,6	-7,9
08/2022	18,7	16,1	+2,6	82,1	83,3	-1,2
09/2021-08/2022	9,3	7,2	+2,1	607,7	633,7	-26,0

4.3 Yield data and quality parameters evaluation

Plant height and the number of spikes/m^2 were determined before harvest. Wheat grain yield was recorded at the final harvest for each plot and was calculated at 14% moisture content. A thousand kernel weight (TKW) also was determined.

4.4 Quality and baking quality analysis

4.4.1 Milling flour

The process of milling flour involved using two machines, namely the PSY MP 20 from Mezos, Hradec Kralove, Czech Republic, and the Quadrumat Junior from Brabender, Duisburg, Germany. The resulting white flours were subjected to the Flour Mill Test, which evaluates the milling properties of small wheat samples. This data is helpful for commercial flour mills as it enables them to adjust their mill settings to optimize flour extraction (Smith and Jones, 2015).

To carry out the Flour Mill Test, small wheat samples were milled using the abovementioned machines to produce flour. The properties of the resulting flour, such as ash and protein content, were evaluated, and it was also used to test gluten strength, such as through the farinograph test (Brown et al., 2019).

4.4.2 Volume weight

Test weight is a measure of the weight of a given volume of grain, expressed in kilograms per hectolitre ($kg.hl^{-1}$) (Smith and Johnson, 2008). It is considered an essential measure of grain quality, with higher test weight indicating higher quality grain. This is because high test weight wheat usually has a higher proportion of extractable flour and less bran, making it more valuable to end-users. For wheat to be graded as No. 1, it must have a minimum test weight of 76.65 kilograms per hectolitre, and discounts are usually applied when values fall below this threshold (Jones *et al*., 2016). Kernel density, influenced by the ratio of starch to protein and the tightness of starch granules within the kernel, is a significant determinant of test weight (Jones *et al*., 2016). Several factors can impede starch deposition during grain filling, such as leaf diseases, scabs, lodging, drought, and high temperatures, which can reduce kernel density. Additionally, kernel shape plays a role in how well kernels pack, with smooth and rounded kernels packing more tightly than wrinkled ones. Shriveled or not fully filled Kernels will also have a lower test weight (Miller *et al*., 2009).

4.4.3 Protein content

The protein content percentage of the wheat samples was determined using the Kjeldahl method with the Kjeltec 1002 System (Tecator AB, Hoganas, Sweden), which is based on multiplying the nitrogen content by a factor of 5.7 (AOAC, 2016). Protein content and gluten quality are the main determinants of bread wheat quality (Shewry *et al*., 2003).

4.4.4 Wet gluten content

The wet gluten test is widely used for evaluating the quantity and estimating the gluten quality in wheat or flour samples. Gluten, which is composed of proteins, is primarily responsible for the elasticity and extensibility characteristics of flour dough (Shewry *et al*., 2003). The wet gluten content is an indicator of the protein content in the flour, and is commonly used as a specification required by end-users in the food industry (AACC International, 2012).

4.4.5 The falling number

The falling number (FN) is a widely used method for determining the sprout damage and α-amylase activity in wheat grains, critical factors in determining the flour quality for bread making (AACC International, 2000; ICC, 1995). The FN was determined using a Perten Falling Number 1310 instrument (Perten Instruments, Hagersten, Sweden) according to the AACC 56-81 B and ICC Standard 107/1 methods. The level of α-amylase activity measured by the FN test is an essential factor affecting product quality. If the falling number is too high, enzymes can be added to the flour to compensate, while a too low FN indicates excessive enzyme activity and makes the flour unsuitable for bread making (Wang *et al*., 2008).

4.5 Statistical data analysis

The data were analyzed using the Statistica 12 program (StatSoft, Inc. USA). An analysis of variance (ANOVA) was performed to evaluate the independent influence of the four factors (wheat/legume mixture, sowing time, management, and legume removal) on each quality parameter. The Tukey HSD test was used to evaluate differences between individual combinations. The significance level was set at $p<0.05$ for all analyses.

5. Results and discussion

This part of the thesis presents the results of basic production parameters, which mainly contribute to grain yield. The next part evaluates the results of the influence of growing wheat with various legumes on the qualitative production parameters.

5.1 Production parameters

Two wheat varieties of the winter wheat (Lorien, Butterfly) were grown in various combinations with legumes and control plots. A combination of classic rows (12.5 cm) and a variant sown by row (row by row) were used to establish the stands. In the autumn, stands were established with field beans, winter peas, spring peas, and incarnate. In classic rows, the underseeding was left throughout the vegetation period. In the row-by-row variant, the underseeding was mechanically eliminated in the spring. At the same time, in part of the plots sown in classic rows and sown in rows (row by row) without underseeding in the fall, spring peas and meadow clover were sown early in the spring. Within this part of the experiment, we had control variants without underseeding, a variant with underseeding for the entire vegetation period of sown wheat, a variant with spring seeding of legumes, and a variant with spring mechanical disposal of legumes established in autumn. All plots were relatively balanced, but as mentioned in the previous section, the wheat stands were negatively affected by the relatively dry weather during the establishment of the stands and, on the contrary, by a very wet June. A low incidence of common wheat diseases, mainly grass powdery mildew, chaff blight, and fusarium was recorded within the stands. Cockroaches have also caused isolated leaf blights. Due to the minimal attack, the detailed evaluation needs to be presented further. Table 5 shows a noticeable influence of individual factors (varieties, leguminous plants, row widths, leguminous cultivation variants) on individual parameters.

Stem length was dominantly a varietal issue. More factors influence the stem length. The most important is genetics. Different varieties of wheat have different inherent stem lengths, with some being shorter and more compact and others being taller and more elongated (Fischer and Edmeades, 2010). Significant is also environmental conditions (Richards, 2000) and also agrotechnology. Planting density, fertilization, and irrigation can impact stem length (Rebetzke *et al*., 2016). In the previous year, a more substantial influence of the method of the leguminous establishment was also noted, which has yet to be confirmed.

The number of ears before the harvest was also most influenced by the variety, partly by the variant of leguminous use. Rebetzke *et al*. (2008) confirmed the findings in their study because this parameter is strongly influenced by genetics. There are also other essential factors such as environmental conditions, plant density (Wiersma and Carter, 2010) and nutrient availability (Sadeghzadeh *et al*., 2017).

The weight of a thousand seeds (TKW) was again a matter of establishing the stand (classic rows or row by row). Thousand kernel weight is strongly influenced by genetics (Kahlown *et al*., 2011) but also by agrotechnology (Riar and Sharma, 2017). In literary sources, there are different conclusions regarding the influence on TKW. Moore *et al*. (2010) reported reduced grain weight in wider rows in their study. In contrast, McLeod *et al*. (2011) stated that no differences were found.

Table 5: Evaluation of individual factors for monitored parameters using analysis of variance (ANOVA), Zvíkov by České Budějovice, 2021/2022

Factor	**DF**	**Stem length**		**Number of ears**		**Yield**		**TKW**	
		MS	F	MS	F	MS	F	MS	F
Variety	1	157,0	74,5*	35086	39,8*	0,9	4,2*	0,02	0,01^{ns}
Legume	5	0,8	0,4^{ns}	174	0,2^{ns}	0,1	0,2^{ns}	1,22	0,41^{ns}
Row	1	2,0	1,0^{ns}	41	0,1^{ns}	6,3	13,7^{ns}	14,90	4,93*
Variant	2	3,3	1,6^{ns}	3997	4,5*	0,4	0,8*	3,54	1,17^{ns}
Error	67	2,1	-	882	-	0,5	-	3,02	-

Note: *statistically significant P ≤ 0.05; not significant; MS = mean square; F = test criterion; DF = degree of freedom

Factors: Variety = Butterfly, Lorien; Leguminosis = field bean, spring pea, winter pea, incarnate, red clover, without leguminosis; Row = 12.5 cm and 25 cm (row by row); Variant = underseeding throughout the growing season, spring seeding, liquidation of underseeding in spring, without underseeding

The grain yield in 2021/2022 was dominantly influenced by how the stand was established (classic row, row by row). However, the effect was statistically inconclusive. The variety also had some influence. In general, it is clear from the results that wheat grown alone or in a mixture with legumes had no statistically significant effect on the yield parameters. The effect of wheat growing in broad lines was published in

1994. A study conducted in Canada found that wide-row wheat (37.5 cm) had significantly higher grain yields compared to normal-row wheat (12.5 cm) under organic management (Entz and Guilford, 1994). The same results were also concluded Luna *et al*. (2018) when the study conducted in the United States found no significant difference in grain yield between normal-row wheat (12.5 cm) and wide-row wheat (25 cm) under organic management.

Table 6 provides detailed data, where monitored parameters are separately evaluated from the point of view of the sowing method (classic rows / row by row sowing; variety; specific leguminous plant; method of growing leguminous plants). The data evaluation shows differences between the two-row variants (classic rows/row by row sowing). This factor was not significant for the stalk length in the current year but affected the number of ears before harvest. In terms of yield, it was achieved when sowing in classic rows. From the separate evaluation of the varieties, a higher yield of the Lorien variety is evident, just as in the case of various mixtures and previous vintages. Evaluating individual leguminous combinations did not yield statistically significant results for any of the monitored traits. The highest yields were achieved when growing winter peas and broad beans in combination with wheat. As already mentioned, this is a statistically inconclusive result. From a separate evaluation of undersowing throughout the vegetation, sowing in the spring, or its disposal in the spring, a higher yield is evident in the variant with undersowing throughout the entire vegetation period. However, this is a lower yield than was achieved in the case of separately grown wheat (control). The positive effect of underseeding throughout the growing season can be seen from the Figure 1, where there was a significant increase in yield on some plots (see the range of yields). In previous years, the effect of spring liquidation of leguminous plants had the most significant influence.

Table 6: Evaluation of experimental variants on monitored parameters, Zvíkov by České Budějovice, 2021/2022

Variant	Stem lenght (cm)	Number of ears (m^2)	Yield ($t.ha^{-1}$)	TKW (g)
Lines design				
Row by row	89,2a	303,0a	4,209a	43,9a
Lines (12,5 cm)	89,3a	332,9b	5,433b	44,7b
Variety				
Butterfly	87,8a	297,9a	4,711a	44,4a
Lorien	90,7b	340,3b	5,025b	44,3a
Legume				
No legume	91,3a	340,7a	5,127a	45,1a
Field bean	89,3a	313,6a	4,872a	44,4a
Spring pea	88,3a	327,7a	5,611a	45,3a
Winter pea	88,7a	337,7a	5,322a	44,2a
Incarnate	88,8a	311,0a	4,797a	44,2a
Red clover	89,5a	322,4a	4,825a	43,7a
Method of management of legume				
Undersowing	88,6a	334,1a	4,883a	44,7a
Sowing in spring	89,4ab	325,4a	3,96a	43,9a
Spring remove	89,3ab	292,4b	4,129c	44,3a
No legume	91,3b	340,7a	5,127ab	45,1a
Note: Values marked with the same letter do not show a statistically significant difference at the level of significance $P \leq 0.05$ (Tukey HSD test).				

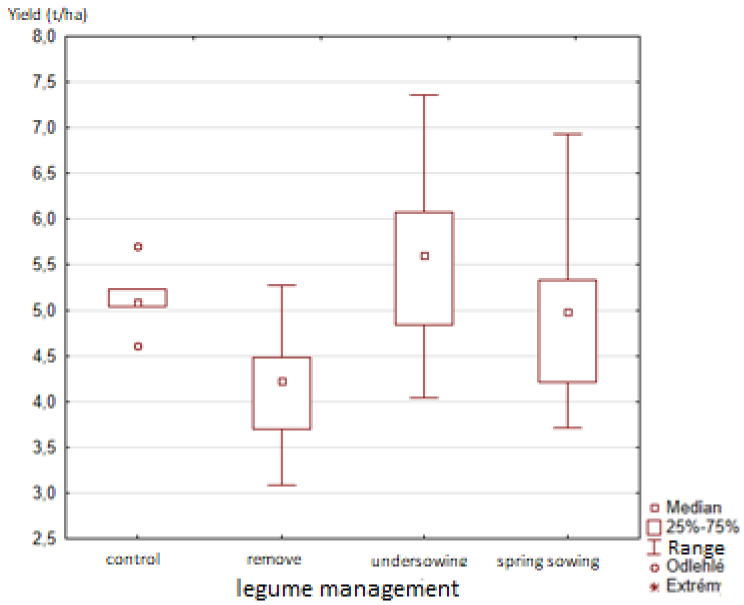

Figure 1: Graphic representation of grain yield within individual variants of the use of legumes, Zvíkov by České Budějovice, 2021/2022

5.2 Quality parameters

After the experimental harvest, samples were taken. Then fundamental analyzes of the baking quality of the wheat grain were carried out using standard methodologies in the laboratories of the Department of Agroecosystems of the Faculty of Agriculture and Technology of the University of South Bohemia in České Budějovice.

In the case of both varieties of winter wheat, relatively low contents of protein in the grain dry matter were recorded in the year 2021/2022. In the case of the Butterfly variety, the values of the content of protein in the dry matter of the grain ranged from 9.29% (Butterfly + incarnate, sowing in autumn in a mixture) to 9.99% (Butterfly + red clover, sowing in spring). The content of protein was relatively balanced. The minimum requirement for the content of N-substances in the dry matter of food grain - bakery wheat (11.5%) would not be met by any of the evaluated variants. The protein content in grain was very low in comparison with other studies. Eq., in a study conducted in Germany, the average protein content of wheat grain grown under organic farming was reported to be 11.6% (Zikeli *et al*., 2015). Another study in Hungary reported that the average protein content of wheat grain grown under organic farming was 12.5% (Szilágyi *et al*., 2017). A study in Austria reported that the average protein content of wheat grain grown under organic farming was 11.9% (Schwarz *et al*., 2016).

The reasons why protein content in grain of organically produced wheat is low is, according to many authors, caused by insufficient nitrogen fertilization Lampkin *et al*., 2002, Mäder *et al*., 2007, Taube *et al*., 2013.

The values of the protein content in the dry matter of the grain corresponded to the values of the content of wet gluten in the dry matter of the grain, which ranged from 15.81% (Butterfly + winter pea, sowing in autumn) to 19.93% (Butterfly + pea sown, sowing in spring). In both cases (content of N-substances and content of wet gluten), the best values were achieved by the variant with the addition legume in spring (Table 7).

In the Lorien variety, lower values of the content of protein in the grain dry matter were recorded compared to the Butterfly variety by 0.7%. They ranged from 8.49% (Lorien + spring peas, mixed sowing in autumn) to 9.38% (Lorien + winter peas, row-by-row sowing in autumn). None of the options would meet the min. requirement for the content of protein content in the dry matter of food grain - bakery wheat.

We recorded gluten index values in the range 66 (Butterfly, control) – 84 (Butterfly + bean, sowing in autumn in a mixture). According to a study by Smith *et al*. (2018), the optimal value of the gluten index for wheat used in bread baking is between 90-120. From our results, we can state that growing the Butterfly variety with field beans increased baking quality compared to other variants (Table 8).

The results of the evaluation of the content of wet gluten in the dry matter of the grain of Lorien variety also corresponded to the stated values, which ranged between 15.03% (Lorien + incarnate sown in the mixture in the fall) and 18.48% (Lorien + winter peas, sown in the fall with the row- by-row). We recorded gluten index values in the range of 50 (Lorien + winter pea, sown in autumn).

The falling number ranged in optimal values between 214-216 s. (Butterfly + field bean, incarnate, winter pea, sowing in autumn) - 260-263 s. (Butterfly + red clover with seeding into the stand in the spring both directly into the stand and row by row system). The falling number reached values between 229 (Lorien, control) - 293 s. The optimal value of the falling number in seconds for baking wheat is around 250-300 seconds. This value ensures that the wheat has a sufficient alpha-amylase activity to produce a good quality dough with good baking characteristics (Kweon *at al*., 2011).

Table 7: Quality parameters of wheat (organic farming, variety Butterfly), Zvíkov by České Budějovice, 2021/2022

Variant	Seeding	Legume management	Volume weight (kg/hl)	Protein content (%)	Wet gluten content (%)	Gluten index	Falling number (s)
Butterfly + spring pea (undersowing)	Row-by-row	autumn	76,10	9,50	17,77	75	244
Butterfly + field bean (undersowing)	Row-by-row	autumn	74,97	9,50	16,08	84	254
Butterfly + incarnate (undersowing)	Row-by-row	autumn	75,77	9,49	16,58	83	229
Butterfly + winter pea (undersowing)	Row-by-row	autumn	75,97	9,58	17,78	76	249
Butterfly + red clover (sowing in spring)	Row-by-row	spring	75,60	9,99	19,72	66	263
Butterfly + spring pea (sowing in spring)	Row-by-row	spring	75,53	9,80	19,10	69	258
Butterfly + spring pea (undersowing)	Standard lines	autumn	77,07	9,55	18,03	78	216
Butterfly + field bean (undersowing)	Standard lines	autumn	76,57	9,53	17,59	74	214
Butterfly + incarnate (undersowing)	Standard lines	autumn	76,67	9,29	18,46	77	215
Butterfly + winter pea (undersowing)	Standard lines	autumn	75,67	9,85	15,81	70	231
Butterfly + red clover (sowing in spring)	Spring sowing	spring	76,90	9,76	19,29	69	260
Butterfly + spring pea (sowing in spring)	Spring sowing	spring	76,37	9,86	19,93	69	258
Butterfly control, standard lines (12,5 cm)	Control		77,10	9,72	19,27	66	227

Table 8: Quality parameters of wheat (organic farming, variety Lorien), Zvíkov by České Budějovice, 2021/2022

Variant	Seeding	Legume management	Volume weight (kg/hl)	Protein content (%)	Wet gluten content (%)	Gluten index	Falling number (s)
Butterfly + spring pea (undersowing)	Row-by-row	autumn	70,70	9,00	17,30	63	246
Butterfly + field bean (undersowing)	Row-by-row	autumn	70,30	9,24	18,15	62	269
Butterfly + incarnate (undersowing)	Row-by-row	autumn	69,77	8,92	16,60	65	245
Butterfly + winter pea (undersowing)	Row-by-row	autumn	70,93	9,38	18,48	50	268
Butterfly + red clover (sowing in spring)	Row-by-row	spring	69,53	9,17	17,64	70	292
Butterfly + spring pea (sowing in spring)	Row-by-row	spring	70,57	9,34	18,42	59	276
Butterfly + spring pea (undersowing)	Standard lines	autumn	70,13	8,49	15,12	75	254
Butterfly + field bean (undersowing)	Standard lines	autumn	69,10	8,58	15,10	73	278
Butterfly + incarnate (undersowing)	Standard lines	autumn	68,93	8,56	15,03	77	272
Butterfly + winter pea (undersowing)	Standard lines	autumn	53,00	8,58	15,82	74	259
Butterfly + red clover (sowing in spring)	Spring sowing	spring	69,07	9,04	16,41	76	275
Butterfly + spring pea (sowing in spring)	Spring sowing	spring	68,97	9,14	17,16	72	293
Butterfly control, standard lines (12,5 cm)	Control		70,60	8,84	16,77	64	229

From the comparison of the results of both varieties and sowing methods, it is clear that the Lorien variety (B) achieved overall worse results compared to the Butterfly variety (E) - this applies mainly in the case of the Zeleny test, partially also in the case of the content of protein content and wet gluten in the grain dry matter and the falling number. The influence of the sowing method (row-by-row and mixture) was not clearly manifested on the quality parameters. If we evaluate the wheat varieties (controls) and different variants, the content of protein content in the dry matter of the grain was practically at the same level.

6. Conclusion and evaluation of the contribution of the thesis

In this thesis, two wheat varieties were grown with various combinations of legumes and control plots. Classic rows and a variant sown by row were used to establish the stands. Underseeding was left throughout the vegetation period in classic rows, while in the row-by-row variant, it was mechanically eliminated in the spring.

The stem length was dominantly a varietal issue, while the number of ears before the harvest was most influenced by the variety and partly by the variant of leguminous use. The weight of a thousand seeds was a matter of establishing the stand. The grain yield was dominantly influenced by how the stand was established. Wheat is grown alone or in a mixture with legumes had no statistically significant effect on yield parameters. From the separate evaluation of undersowing throughout the vegetation, sowing in the spring, or its disposal in the spring, a higher yield is evident in the variant with undersowing throughout the entire vegetation period. The positive effect of undersowing throughout the growing season can be seen from the significant increase in yield on some plots. The highest yields were achieved when growing winter peas and broad beans in combination with wheat. However, the results are statistically inconclusive.

In conclusion, the experiment showed relatively low protein content in the dry grain matter of both winter wheat varieties in 2021/2022, which did not meet the minimum requirement for bakery wheat. The wet gluten content in the grain's dry matter corresponded to the protein content, with the best values achieved by the variant with legume addition in spring. The gluten index values ranged from 66 to 84, lower than the optimal values for bread baking, while the falling number ranged from 214 to 263 seconds, meeting the optimal range for baking wheat. The Lorien variety achieved overall worse results compared to the Butterfly variety. The influence of the sowing method on the quality parameters was not manifested. The low protein content in organically produced wheat is caused by insufficient nitrogen fertilization. Unfortunately, the effect of tested leguminous growing in different variants on wheat grain quality was insignificant.

The diploma thesis brought exciting results regarding applying wheat and legume intercropping. However, these are one-year results from which it is possible to trace specific trends, but they are statistically inconclusive. In order to evaluate the effect of wheat legume intercropping, it would be necessary to repeat the experiments in at least three years, which, however, exceeds the time frame of the thesis.

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