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Competition at Early Growth of Kernza Intermediate Wheatgrass

Master's thesis

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Declaration

I hereby declare that I have authored this master's thesis carrying the name "Competition of early growth of Kernza Intermediate Wheatgrass" independently under the guidance of my supervisor. Furthermore, I confirm that I have used only professional literature and other information sources that have been indicated in the thesis and listed in the bibliography at the end of the thesis. As the author of the master's thesis, I further state that I have not infringed the copyrights of third parties in connection with its creation.

In Prague on 19.04.2024 Saul Kelly Roman

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Competition at early growth of Kernza intermediate wheatgrass

Summary:

Intermediate Wheatgrass (*Thinopyrum intermedium)* (Host) Barkworth and D.R. Dewey; IWG) is a perennial grain crop developed as a sustainable alternative to traditional annual grain crops. IWG and clover as companion crops have been proposed as a potential strategy for enhancing crop productivity and ecosystem services.

This greenhouse replacement study aimed to investigate the dynamics of species competition and cover rate between IWG and red clover (*Trifolium pratense*). Over 53 days, data on plant height and percent surface cover were collected approximately every week to assess these aspects and to test species competition and rate of cover. After 53 days, the final height and biomass were recorded for each plant. The central research question addressed whether red clover's presence would affect IWG growth and biomass production, and if so, to what extent.

Planting IWG with red clover in a 3:3 ratio increased ground cover by 30% compared to treatments with pots that only contained IWG. Additionally, as the proportion of red clover increased relative to IWG, there was a corresponding increase in ground cover. This suggests that red clover has the ability to establish rapid ground cover and increase overall biomass, which could positively impact various agricultural practices or ecosystem management strategies.

Regarding plant competition, in all treatments, the final weight of red clover exceeded that of IWG. Statistical regression analysis was conducted to explore the relationship between plant ratios (IWG to red clover) and the final weight of IWG plants. Although the final weight of IWG showed a significant relationship with the planting ratio, it was not negatively influenced by the presence of red clover. Notably, IWG plants with the highest final weight were observed in treatments with higher proportions of red clover, indicating a potential positive interaction between IWG and red clover, thus ruling out competitive inhibition.

These plant growth patterns warrant further investigation in field settings. The findings underscore the potential benefits of optimizing crop combinations to enhance ground cover, suppress weed emergence, and maximize resource utilization in agricultural contexts.

Keywords: perennial agriculture, Intermediate Wheatgrass (IWG), red clover, weed management, intercropping, replacement study, species competition, ground cover.

Content

1 Introduction

Annual crops are the most prominent in agricultural fields worldwide, occupying around 70% of the total cropland worldwide. These fields are commonly managed in an industrial manner and require fertilizers, energy, labour, and pesticide inputs in a significant amount (Cox et al. 2006). Although this type of agricultural systems provides benefits such as simplified cultivation practices that promote commercial scalability, high yields and economic gains (Dyer 2014) in the last century they have caused a rapid increase of harmful disservices like soil compaction and erosion, soil organic matter (SOM) loss, low water retention and infiltration, and excessive nitrogen fertilization that puts natural ecosystems at risk and the future food security worldwide (Crews 2018).

In recent years, perennial grain crops have regained the attention of farmers and researchers because they provide ecosystem services necessary for maintaining healthy, productive soils, such as the formation of SOM, the increase of the water-holding capacity of the soil, the creation of more profound and more intricate root networks that improve soils structure and reduce compaction and erosion These root systems also fix carbon into the soil, providing optimal conditions for organisms to inhabit (Asbjornsen et al. 2014). An essential advantage of perennial grain crops is that while providing these services to the soil ecosystem, they produce considerable amounts of forage and grains with lower production costs compared to more conventional annual systems (Crews 2016).

Perennial grain production refers to cultivating crops that live and produce for multiple growing seasons, different from annual crops that must be planted from seed every growing season. This type of agriculture has gained attention due to its ability to increase soil health, reduce soil erosion, and mitigate climate change (DeHaan et al. 2005, Pimentel et al. 2012).

Intermediate Wheatgrass (*Thinopyrum intermedium* (Host) Barkworth & D.R. Dewey; IWG) is a perennial grain crop that is being developed as a sustainable alternative to traditional annual grain crops (Dimitrova Mårtensson et al. 2021). IWG evolved in the shrub steppes and slopes up to lower mountain belts (Bajgain et al. 2021). Eventually it was brought to North America, where it become popularly used for erosion control, revegetation, pasture, and hay (Barkworth 2007, Plants of the World Online 2023).

The Land Institute, in Salina, Kansas, USA, has been working on breeding their trademarked IWG seed, Kernza® for over a decade, and it is now being grown on a small scale by farmers in the United States and Europe (Wayman et al. 2019). IWG populations have been bred to produce substantial biomass with relatively high nutritional value in their grain varieties. Consequently, IWG shows potential as a versatile crop capable of generating income from both grain and forage yields within a single growing season (Jungers et al. 2019).

Because IWG is a perennial crop, the early growth is slower than annual wheat. Due to this, weed management during the early establishment phase can be a practical challenge. For organic management, tine harrows are not recommended, and no herbicides are yet labelled for IWG use (Peters 2021). Furthermore, the farmers with the greatest interest in this alternative crop are not necessarily interested in chemical weed control. Intercropping IWG with other crops with different growth habits and canopy structures may help suppress weeds. For example, intercropping with legumes like clovers or vetches can provide nitrogen to the soil and help to suppress weeds through shading and competition. This thesis will explore the early growth dynamic of red clover (*Trifolium pratense* L.) and IWG as a possibility of early weed suppression without negatively affecting IWG growth.

2 Scientific hypothesis and aims of the thesis

This thesis aimed to evaluate the early growth and potential competition of IWG and red clover to determine its suitability for use as an intercrop system for suppressing weeds without significantly competing with IWG as a primary crop.

The following hypotheses were tested:

1. The inclusion of red clover will increase surface cover.
2. The inclusion of red clover will decrease the biomass of IWG.

Rejection of the first hypothesis would discourage further studies of planting red clover simultaneously with IWG for early weed management and will eliminate need to test the second hypothesis. Failing to reject the second hypothesis will prompt further research to test planting ratios and management of simultaneous intercrops in-field.

3 Literature research

3.1 Rise of annual monoculture agriculture

Annual plants are characterized by their life cycle, which is completed within a single year. From the early domestication of annual plants such as cereals and legumes to the modern era of genetically modified crops, the journey of annual plants in agriculture spans centuries and has played a role in the development of early agricultural practices and food production (Harlan 1975, Zeder et al. 2011).

The short life cycle of annual plants contributed to adaptability, allowing farmers to experiment with different crop varieties and selectively breed those with desirable traits. This facilitated the optimization of agricultural practices to suit local environmental conditions and the specific needs of a community (Harlan 1975).

Annual plants allocate a significant portion of their energy to seed production within a single growing season, allowing farmers to concentrate and harvest this energy for food, seed propagation, and, eventually, the domestication of crops (Altieri 1999). This characteristic made annual plants well-suited for the early stages of agriculture, enabling the establishment of sustainable farming practices.

The evolution of annual plants in agriculture goes beyond domestication, including the transformation of cultivation practices into adopting monocultures. Monoculture refers to the cultivation of a single crop species over a large area. The origins of monoculture date back to early agricultural societies, where the focus on specific annual crops became essential for ensuring a stable and predictable food supply (Harlan 1975).

Cultivation of annual crops in monocultures allowed farmers to optimize available resources, such as soil nutrients and water, leading to increased yields and more efficient land use (Altieri 1999). This shift was evident in the expansion of agrarian practices during the Neolithic Revolution, where populations transitioned from nomadic lifestyles to settled farming communities.

While early farmers benefited from the adaptability and efficiency of annual plants, modern agriculture has embraced monoculture practices for several compelling reasons. Monoculture has become the predominant system due to its efficiency in simplified practices, scalability, and economic gains.

Straightforward cultivation practices simplify practices such as irrigation, fertilization, and pest control. This efficiency results in higher yields per unit of land, reducing the need for extensive agricultural land to meet growing food demands (Tester and Langridge 2010). By focusing on a single crop, farmers can streamline their practices, from planting to harvesting, resulting in simplified operations and increased productivity (Dyer 2014). This efficiency is further enhanced by advancements in technology and machinery, allowing for the automation of larger-scale production and reduced labour costs. An example is Precision agriculture techniques, such as GPS-guided tractors and automated harvesting equipment, enabling farmers to optimize their operations and achieve higher yields (Javaid et al. 2022).

The large scalability of monoculture operations allows for standardized and simplified farming practices, making it easier to implement technological advancements and achieve economies of scale. This is desirable for commercial agriculture, where the production of vast quantities of a single crop aligns with market demands and facilitates the distribution of uniform products ensuring reliability in supply chains and allowing for efficient processing, packaging, and marketing, meeting consumer preferences and maximizing profitability for farmers and agribusinesses (Dyer 2014). In the economic aspect, the specialization in growing one high-demand crop allows farmers to focus on optimizing production and maximizing profits. In a globalized market, monoculture practices align with the demands of efficient supply chains and standardized agricultural products, making them economically viable and competitive (Power and Follet 1978).

While the advantages of annual monoculture in modern agriculture are evident, concerns are rapidly growing regarding the sustainability of such practices. The incorporation of annual monoculture in modern times underscores environmental issues that put the future of food production as we know it at risk, some of the main environmental issues include soil compaction, erosion, organic matter loss, low water retention and infiltration, and excessive fertilization (Crews 2018). These practices associated with annual monoculture can be reduced with the adoption of perennial agriculture systems, these systems provide an alternative source of staple foods and animal forage that can also bring benefits to the environment with more energy-effective and low-cost production lines (Glover et al. 2010).

3.1.1 Soil compaction and erosion

The transformation of land from natural ecosystems to annual crops causes the degradation of soils. When native vegetation is switched for agricultural use, the soil is vulnerable to wind, rain, and the effects of gravity for most of the year (Crews et al. 2018). This exposure is a contributor to soil erosion and compaction. The median rate of soil formation is 0.004 mm per year, and the rate of soil loss to annual tilled agriculture is 1.52 mm per year (Montgomery 2007). Loss rates, being 360 times greater than formation rates, illustrate the exploitation of soil by monoculture annual fields, which contrasts with the natural ecosystems dominated by perennials that existed before agriculture (Crews et al. 2018).

In the long-term extensive tillage can have negative impacts on soil health, including soil erosion, compaction, and loss of soil organic matter. Tillage refers to any practice that involves turning over the soil to a significant depth and disturbing soil structure. Soil tillage is needed to establish crops in the field, in annual agriculture, this must be done at least every year, and often, multiple times for proper seed bed preparation and subsequent weed control. Every time a field is ploughed it alters soil structure and functioning, it reduces microbial biomass and food-web complexity, lowering the amount of water present in the soil, and reducing soil productivity over time (Dupont et al. 2010). Tillage practices require high energy costs and high-cost machinery such as tractors and different disc attachments. About 835 L of oil equivalents per hectare are required to till the soil to produce annual crops (Pimentel et al. 1995).

3.1.2 Soil organic matter loss

Soil Organic Matter (SOM) refers to a wide range of substrates that form in the decomposition process of plants, animals, and soil microbes (Brady and Weil 2014). SOM reflects a dynamic equilibrium of inputs of organic compounds from photosynthesis and losses of organic matter to the metabolism of bacteria and microorganisms living in the soil into CO_2 (Crews et al. 2016). Russel (1997) studied SOM and its relationship with soil fertility, finding that soils with higher percentages of SOM had better stabilization of soil structure, increased water holding capacity, and increased release of elements like nitrogen, phosphorus, and sulphur into the soil profile during the growing season. Transforming natural ecosystems into croplands alters the dynamic equilibrium of organic matter inputs and losses (Davidson and Ackerman 1993).

3.1.3 Water retention and infiltration

Soils of undisturbed natural ecosystems have high water infiltration rates because aggregates and large pore spaces develop through soil fauna activities such as insects and worms and generations of root growth and decay. Soils with high infiltration capacity can store large volumes of water. The disturbance of converting natural ecosystems into monoculture fields alters the water retention and infiltration flow of the system (Wuest et al. 2006). A study by Rockstrom (2003) traced rainfall in a water-limited maize dryland crop system. Maize plants transpired 15-30% of precipitation, while 70-85% of water left the agroecosystem via surface evaporation, runoff, or leaching below the rooting zone.

3.1.4 Excessive nitrogen fertilization

Nitrogen fertilization is a common practice in annual fields to increase production, low absorption rates of fertilizers by annual monoculture fields, combined with the low water retention and infiltration, give the disservice of nutrient loss, causing overaccumulation of nutrients in runoff water (Crews et al. 2018). On average, annual crops absorb less than 50% of the N applied as fertilizer (Cassman et al. 2002). Culman et al. (2010) compared the physical and chemical properties of annual monocultured wheat fields, that have been harvested for 70 years and native perennial grasslands that have been used for harvesting hay. Annual monoculture fields were notably degraded compared to perennial grasslands, annual monocultured soil contained significantly lower amounts of SOM, soil organic carbon, readily oxidizable carbon and total soil N compared to perennial grasslands at a depth of 60 cm, showing how fertilizers deplete nutrients from soils. Heavily fertilized agricultural regions are also a threat to wildlife and water sources (United Nations Environment Programme n.d.). The water runoff from these areas ends up in water bodies where it disrupts natural cycles creating hypoxic environments where the lack of oxygen ends up being detrimental and often fatal for the organisms living in the water (Rabalais et al. 2008). This phenomenon is known water eutrophication, while it can occur naturally, since 1960 it has spread in coastal marine zones creating dead zones that remain for longer periods of time, especially during summer season (Diaz et al. 2008).

3.2 Benefits of perennial cropping systems

Perennial agriculture systems utilize crops that can be maintained and harvested over multiple growing seasons and include a range of tree, shrub, and herbaceous crops that can produce an array of products such as grains, forage, textile fibres, edible carbohydrates, protein, and oil (Scott et al. 2022). In most natural terrestrial ecosystems, perennial plants become a dominant plant species over annuals. Once they are established as seedlings, they will have an advantage over annual plants at the beginning of the next growing season. Annual plants re-start their growth cycle from seed every year, creating a disadvantage in the competition for sunlight and soil resources with perennial plants that are already established and will emerge from dormancy at the end of winter or a dry season (Tilman 1982).

Perennial vegetation aids in soil formation under certain circumstances, it allocates carbon to the root systems, contributing to the formation of organic matter, which also helps to protect the soil against erosion (Crews et al. 2018). Perennial crops that remain planted for multiple growing seasons can sequester carbon in the soil, which can help mitigate the effects of greenhouse gases that are related to climate change (Keunbae et al. 2022). Perennial crops develop deep root systems that can store carbon in the soil for extended periods, a study of perennial grasses in the United States found that perennial grassland species can sequester an average of 0.84 metric tons of carbon per hectare per year (Gelfand et al. 2011). This thesis will mainly focus on perennial grain crops and their potential, which, in the last decades have been studied and developed by scientists to provide ecosystem services while maintaining productive systems.

3.2.1. Environmental benefits

Plant communities that occur in native habitats throughout the world, are usually characterized by diverse perennial plant species that provide year-round soil coverage, protect soil from erosion, and sequester carbon in their root system, increasing the formation of SOM (Crews et al. 2018). Natural perennial grasslands are highly efficient at nutrient uptake of elements like nitrogen from shallow and deep soil depths during the growing season (Woodmansee 1978). This efficiency is related to the uptake efficiency of perennial root systems and the diversity of their plant communities in the ecosystem.

Experiments in plant diversity found that plant communities that include different and complementary functional groups will take up different forms of nitrogen from the soil profile at different times of the growing season, reducing nutrient loss, runoff, and leaching through the soil profile (Kahmen et al. 2012).

Due to their deep and abundant root systems, perennial systems reduce fertilizer runoff and more efficiently use water and nutrients (Jungers et al. 2017). In an experiment conducted by Jungers et al. (2019) three crops (maize, switchgrass, and IWG) were used to test the nitrogen leaching. Results showed that in both treatments Nitrate leaching was highest for maize followed by switchgrass and IWG, leaching was 96% lower in IWG compared to maize in the low N fertilizer treatment, and up to 99% at high N treatment.

In a study by Beniston et al. (2014), two crop systems were compared: remnant perennial tall grass prairie used for hay production and conventional croplands used to produce winter wheat. The cropland soils contained significantly less soil organic carbon than the prairie from 0 to 40 cm. At 60cm the wheat croplands contained 30% less soil organic carbon, and 25% less organic carbon. At 1 m depth root biomass was significantly greater in the tall grass prairies, there were more visible roots at 1m soil profile equating to 400-600% more root biomass carbon compared to annual cropland soil (Figure1).

Figure 1 Compared with annual crops on the left side, perennial plants can create more complex root systems. Photo credit: The Land Institute, Salinas, Kansas. DOI: https://doi.org/10.1017/sus.2018.11

Another aspect of perennial crop systems is their ability to provide better habitats for native fauna compared to annual monoculture systems. Perennial crops can host natural enemies of pests, such as predatory insects, which can reduce the need for synthetic pesticides and promote natural pest control (Meehan et al. 2011). A study by Wang et al. (2021) showed that perennial crops land can sustain greater ground beetle (*Coleoptera Carabidae*) species richness than semi-natural habitats. Plant species richness positively affects the overall diversity of carabids, with the most substantial effects in perennial cropland. Carabid beetles have an essential role in controlling pest species, and they are used in studies evaluating anthropogenic disturbances in agricultural ecosystems (Knapp et al. 2015).

By contrast, annual monoculture systems have a less diverse and less stable plant community, which can lead to lower biodiversity and reduced habitat for native fauna (Letourneau et al. 2011).

3.2.2. Agronomic benefits

Incorporation of perennial grain crops into agricultural systems has the potential to reduce labour and chemical inputs while keeping productive systems. Culman et al. (2013) compared the nitrogen retention of annual wheat and perennial wheatgrass in a single species planting system. Results showed that once established, the perennial grain crop reduced total Nitrate leaching by 86% compared to annual wheat. The different uptake efficiencies of perennial wheatgrass compared to annual wheat connected to the rooting systems of these two species.

Soil fertility is crucial for agriculture because it directly influences the productivity and health of crops. Perennial grasses and forbs allocate 50-67% of fixed carbon in the root or root-like tissues belowground (Saugier et al. 2001). The roots are a substrate for soil microorganisms, fostering microbial growth and activity. Increased microbial biomass contributes to nutrient cycling, as microorganisms decompose organic matter and release nutrients in forms accessible to plants (Bardgett et al. 2014). Consequently, the carbon allocation to roots directly influences nutrient availability in the soil, a key determinant of soil fertility.

Annual grain-based agriculture systems can benefit from the potential of profound and long-lived root systems of perennial plants to manage soils. The periodic use of a short-term perennial phase in crop rotation can improve soil functioning in the long term. A study by Duchene et al. (2020) investigated the impact of introducing perennial grains into grain crop rotations, focusing on the role of rooting patterns in soil quality management. The results revealed higher root biomass under perennial grain cultivation than annual wheat at 0-10 cm depth in the first spring, with subsequent expansion during the regrowth period. Over the period between the end of the first and second spring growing seasons, perennial above and belowground biomass increased by 52% and 111%, respectively. This increase led to more than three-fold higher total root biomass under the perennial treatment compared to annual rye at the same time. The root colonization of the soil profile also showed significant differences, with the perennial grain system exhibiting thorough soil colonization until 60 cm. By contrast, the annual root system displayed lower soil colonization in the same layers.

The study also found significant differences in microbial markers, with a higher abundance of fungal markers, including arbuscular mycorrhizal fungi (AMF), under perennial treatment. In the second experimental year, the AMF N-marker ratio was significantly higher in all soil layers under IWG. These findings highlight the multifaceted effects of perennial grain introduction on biomass, rooting patterns, and soil microbial communities, providing valuable insights into the potential benefits of incorporating perennial crops into agricultural systems for soil recovery (Duchene et al. 2020).

Perennial crops production generally involves reducing the frequency and intensity of mechanical tillage. Most perennial crops are established by planting once and allowing them to establish themselves. Additionally, minimal tillage reduces the need for fossil fuel-powered equipment and labor associated with tillage, resulting in lower greenhouse gas emissions and lower costs for farmers (Crews 2016). In the production aspects, perennial crops can provide consistent yields over time, reducing the risk and variability associated with annual crop production, providing economic stability for farmers, and improving food security (Crews et al. 2016).

Switching to perennial crops may offer significant advantages in adapting to an increasingly unpredictable and extreme climate. The anticipated impacts of climate change, such as the emergence of new pests and diseases, heightened risks of flooding, prolonged droughts, and heatwaves, have the potential to negatively affect crop yields and profits (IPCC 2014). Due to climate change, essential crops like wheat, maize, and rice are projected to suffer damage (Rosenzweig et al. 2014). Current estimates indicate that with a 1°C rise in global temperature, global wheat yields may have already declined by 4.1 to 6.4% (Liu et al. 2016). Shifting to staple crops that demonstrate more efficient utilization of nutrients and water, and therefore possess greater resilience to extreme weather conditions, could be a beneficial strategy.

Researchers of the Land Institute in Salina, Kansas, USA, are creating cross breeds between existing annual crops and their wild perennial relatives, this cross breeding is successful after generations of selection and intermating. These wild hybrid crosses produce plants that maintain seed yield and quality like their annual parent while inheriting the perennial physiology of the other parent (Crews et al. 2018).

3.2.3. Economic benefits and challenges

The profitability of crops is a crucial factor in the adoption of crops and drives management decisions. Perennial crops have lower annual seeding costs, and their production requires less fertilizer than annual crops (Pimentel et al. 2012). However, grain quality challenges exist because breeding lines are still in development and grain yield of current perennial crops is lower than most annual crops (Zhang et al. 2017). An experiment by Culman et al. (2010) comparing a perennial crop of IWG with annual wheat at Kellogg Biological Station in Hickory Corners, Michigan, USA found that yields of IWG were 67% lower than those of annual wheat. Although the yields are expected to increase through breeding improvements, farmers can now deal with lower yields impact by progressively integrating perennial grain crops into their operations. Some of these integration strategies can increase yield stability, enhance biological pest control, and provide the following ecosystem services:

Rotation with annual crops: In annual agroecosystems degraded by excessive soil tillage, farmers can use perennial grains to regenerate and restore soil health before rotating back to annual crops (Ryan et al. 2018).

Grow on sloped land or as a buffer crop: Soils in perennial crop systems have increased water retention rates, decreasing runoff, and soil erosion (Glover et al. 2012). Integrating perennial grains into agroecosystems can protect surface water from sediment, agrochemicals, and nutrient pollution.

Harvest vegetation or grazing livestock: Harvesting biomass from perennial grain crops can increase profitability and help compensate for low grain yields. A study on farm profitability in the drylands of western Australia found that production of dual purpose of perennial crop systems with grain and grazing value increases its profitability and results as part of optimal farm planning, without this grazing value, perennial systems were not an optimal land management strategy for that area (Bell et al. 2008).

Regarding labour expenses, perennial crops require less labour than annual crops because they do not need to be replanted every year, therefore reducing labour costs associated with planting and harvesting. According to a study by Glover et al. (2010), perennial wheat production costs could be 20% lower than annual wheat production costs due to reduced seed, tillage, and planting costs.

Perennial crops often require fewer pesticides than annual crops. Over time perennial crops develop stronger and deeper root systems and aboveground structures, allowing them to better tolerate and recover from pest and disease attacks (DeLonge et al. 2016).

3.3 Intermediate Wheatgrass as a multipurpose crop

Intermediate wheatgrass is native shrub steppes and slopes up to lower mountain belts (Bajgain et al. 2021). IWG grows naturally in Southern Europe, Afghanistan, Albania, Austria, Bulgaria, France, Germany, Greece, Hungary, Iran, Iraq, Italy, Kazakhstan, Kyrgyzstan, Lebanon-Syria, North Caucasus, Pakistan, Poland, Romania, Russia, Spain, Switzerland, Tajikistan (Plants of the World Online 2023). IWG has been naturalized to North America, where it is a tool for erosion control, revegetation, pasture, and hay (Barkworth 2007, Plants of the World Online 2023). In North America, utilization of IWG for agricultural purposes began between 1935 and 1950 (Hitchcock, 1935). By 1956, IWG was frequently used for mixtures with alfalfa in the Northern half of the United States (Schwendiman 1956).

The effort to develop a perennial IWG grain crop started at the Rodale Research Center (RRC) in Pennsylvania, USA (Wagoner and Schauer 1990). The purpose was to protect soil from erosion, improve soil quality, enhance wildlife habitat, reduce mechanical fuel-based operations, and reduce the costs for farmers for seeds and planting. About 100 perennial grasses were evaluated at RRC in 1983 (Wagoner and Schauer 1990). To identify potential species, they used the following criteria: vigorous perenniality, lodging resistance, seed heads above foliage for easy harvest, and potential for mechanical harvest. Based on these criteria, IWG was the chosen crop. The RRC collected 250 germplasm accessions, with half collected from the USDA Plant Introduction Office in Pullman, Washington, USA (Wagoner and Schauer 1990). The other accession samples were collected from Iran, Turkey, and countries from the former Soviet Union, and Mediterranean countries. Analysis of the collected germplasm began in 1987. Differences between accessions were minor, but seed traits such as mass per seed, fertility, and threshibility were sizeable (Wagoner and Schauer 1990). Improvement of grain yield and seed size in IWG has been major targets of selection since the initiation of IWG domestication programs (Wagoner and Schauer 1990). It is well established in recent literature that in improved IWG germplasm, significant associations exist between seed dimensions (length, width, and area) and seed weight. Multiple studies have also reported strong correlations between yield and biomass or biomass-related traits such as plant height, flag leaf area, reproductive tiller number, and spike length (Zhang et al. 2017). IWG populations developed as grain varieties still yield considerable biomass with relatively high feed values. For this reason, IWG is a promising dual-use crop that could generate revenue from both grain and forage production in the same season (Jungers et al. 2019).

Yields of IWG can vary depending on a variety of factors. However, they are generally lower than annual crops like wheat and corn; 500 to1,000 pounds per acre yield is standard for IWG (Culman et al. 2010). Grains per head may be similar and heads per plant will change by year, but the primary difference in yield is due to the difference in seed size see Figure 2.

Figure 2 IWG seed, left, it is smaller than annual wheat grains right (Derouin and Morrone 2021).

Another trait that shows the versatility of IWG as a multipurpose crop is the ability to produce forage without affecting seed production. A study by Pugliese et al. (2018) discovered that harvesting IWG forage promotes greater grain yield, seasonal forage, and root biomass in years two and three after planting. The root response of IWG to harvesting forage biomass appears to be gradual; the overall productivity of belowground biomass is influenced in the subsequent seasons.

3.4 Intercropping for environmental and agronomic benefits

Soil ground cover refers to the amount and type of vegetation, litter, and other organic and inorganic materials covering the surface of the soil. Ground cover is essential for maintaining soil health and fertility, it protects soil from erosion, helps regulate soil temperature, moisture, and provides habitat for beneficial soil organisms. The lack of ground cover reduces the ability of the soil to retain moisture, which can lead to drought and stress for crops (Blanco-Canqui et al. 2015).

Natural plant communities usually support multiple species at once without direct competition due to having different functional traits (Tilman 1982). Agriculture research into crop mixes, especially in cover crops or forage, usually sees marked benefits, particularly that total biomass of high diversity mixes can increase compared to one monoculture (Snapp et al. 2005). This can seem contradictory to primary principles of crop production regarding competition and weeds and Liebig's Law of the Minimum (Zimdahl 2004) but is very logical considering natural systems.

Legumes are an essential component of many agroecosystems due to their ability to fix atmospheric nitrogen into the soil, which when planted in crops polycultures it can be used by other crops in the mixture (Mikić et al. 2015). This nitrogen fixation process can help reduce the need for synthetic fertilizers. Fertilizers can be costly and have negative environmental impacts (Swanton et al. 2015). In addition to their ability to fix nitrogen, legumes have deep root systems that can help improve soil structure and reduce erosion (Ojiem et al. 2006). These benefits make legumes a valuable companion crop in many agricultural systems, particularly in areas with limited fertilizer inputs (Mikić et al. 2015).

IWG and red clover intercropping has been proposed as a potential strategy for enhancing crop productivity and ecosystem services. A study by Favre et al. (2019) found that intercropping red clover with IWG increased the fall forage yield by 118% and the annual forage yield by 39% despite a 50% reduction in N fertilizer. It also increased the nutritional value of the fall forage. The nutritive value of IWG harvested in the spring and fall makes it suitable for lactating beef cows, dairy cows, and growing heifers.

Law et al. (2021) conducted a field experiment to investigate the effects of intercropping red clover with IWG on weed suppression and grain yield. The treatments included monoculture stands of IWG and red clover and two intercropping treatments. The study found that both of the intercropping treatments had significantly lower weed density and biomass than IWG monoculture. The weed suppression index (WSI) was also higher in the intercropping treatments, indicating that intercropping was more effective than monoculture. The WSI measures a crops ability to suppress weed growth. The two intercrop treatments had no significant difference in WSI. Grain yield of IWG was not significantly different between the intercropping treatments and the monoculture stand. The addition of red clover increased forage yield, which was significantly higher in the intercropping treatments than in the monoculture stand of IWG (Law et al. 2021).

While legumes, specifically clover, have been used as intercrops to successfully reduce weeds without reducing yield, these crops have not been planted simultaneously since emergence. The critical time for weed suppression to prevent the excessive need for tillage early would be in the first week of growth, when IWG has a low leaf area and grows slowly relative to many weeds. The reason for this practice is because an intercrop planted simultaneously with IWG would provide too much competition. Still, this early competition is not specifically recorded in the literature. We proposed a simple pot experiment to test these two hypotheses. Hypothesis 1) ground cover could be achieved faster by including red clover and 2) early competition by red clover reduces the biomass of IWG.

4. Methodology

4.1 Experimental Design

One commonly used experimental design for studying plant competition is the replacement series design, in which two or more species are grown together at different ratios, with the total plant density constant. In this design, the relative yield of each species is measured and used to estimate the competitive effects of one species on another (Swanton et al. 2015). The general methodology for evaluating plant competition involves comparing the growth and development of plants grown in monoculture with those grown in mixed-species communities (Swanton et al. 2015). Statistical analysis of variance and regression are used to tests for significant differences between treatments and assess the relationship between biomass production at different planting ratios (Swanton et al. 2015).

Based on this, a replacement study was done to measure competition of early growth of IWG and red clover. The experiment took place under controlled conditions in a heated greenhouse with supplemental lighting. Seeds were initially established in a Sanyo growth chamber (Sanyo MLR-352 with FL40SS-W37 lamps (Sanyo Electric Co Ltd)) for 3 days to ensure that the healthiest germinated seedlings were grown in the pot experiment. Seedlings were transplanted in (10 cm x 10 cm x 11 cm) volume pots using a sandy loamy sand soil from Czech University of Life Sciences Prague demonstration field. The setup of experiment consisted of 7 different ratios of IWG to red clover, each with 6 plants per pot, 6:0, 5:1, 4:2, 3:3, 2:4, 1:5, 0:6. Three replicates of each treatment were planted in two experimental runs starting on November 18 and November 28, 2022. The greenhouse is maintained at 25 ^{o}C at 16 hours per day using Lumatek ATS200W lights set to 40% dimmability. Each experimental run used a completely randomized design, and the pots were re-randomized, to eliminate edge effects, every 7 to 10 days.

4.2 Measurements

Each plant height was measured every 7 days as well a picture of the soil cover at 40 cm above soil level. Pictures were used to visually estimate the vegetative soil coverage from the same angle of each pot and removing personal biases towards the pots. At 53 days after planting, plants were clipped at soil level, and the fresh weight of each plant was taken using a desktop scale with precision to 0.01 g.

4.3 Statistical Analysis

Final height and weight were analysed using procedure AOV (ANOVA) in R studio (R core team. 2023), on each run separately and combined if the variance was similar. The ANOVA was run as a factorial with main effects being species and ratio. The relationship between the increasing planting ratio and the final weight was also tested using regression. The plant growth over time was analysed using SAS statistical software 9.4 (SAS Institute Inc. 2023). Non-linear regression (proc nonlin) was used to fit a sigmoidal model to the height data of each crop in the treatments 6:0 and 0:6. This fitted model was tested for fit with the growth of the corresponding species in each other ratio using regression (proc reg) with that species in each planting ratio treatment.

5. Results

Emergence and early growth of both species began at the same time after germinated seedlings were transplanted. Plants were all healthy and vigorous and no complicating diseases or pest problems were apparent through the duration of the experiment (Figure 3). The growth habit of clover did not seem affected by planting ratio, but IWG in lower densities planted with clover changed leaf orientation (Figure 4).

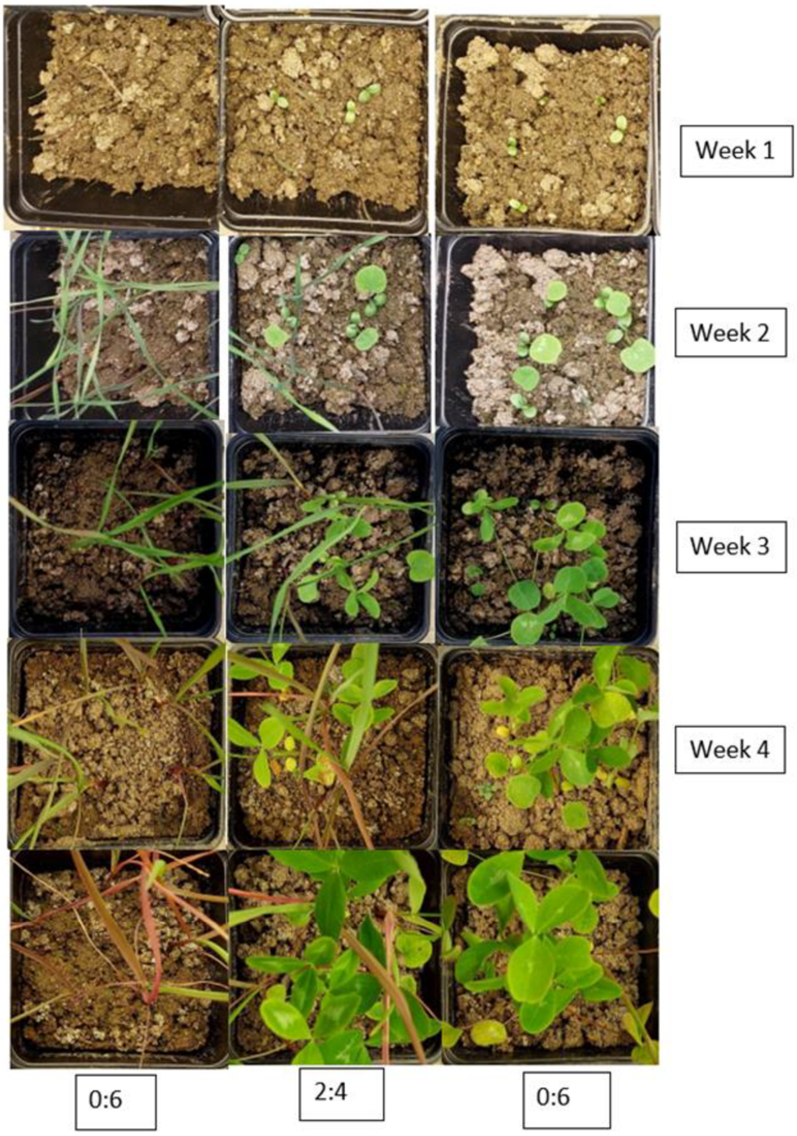

Figure 3 Example of vegetation cover progression in the IWG to red clover ratios 6:0, 3:3, and 0:6.

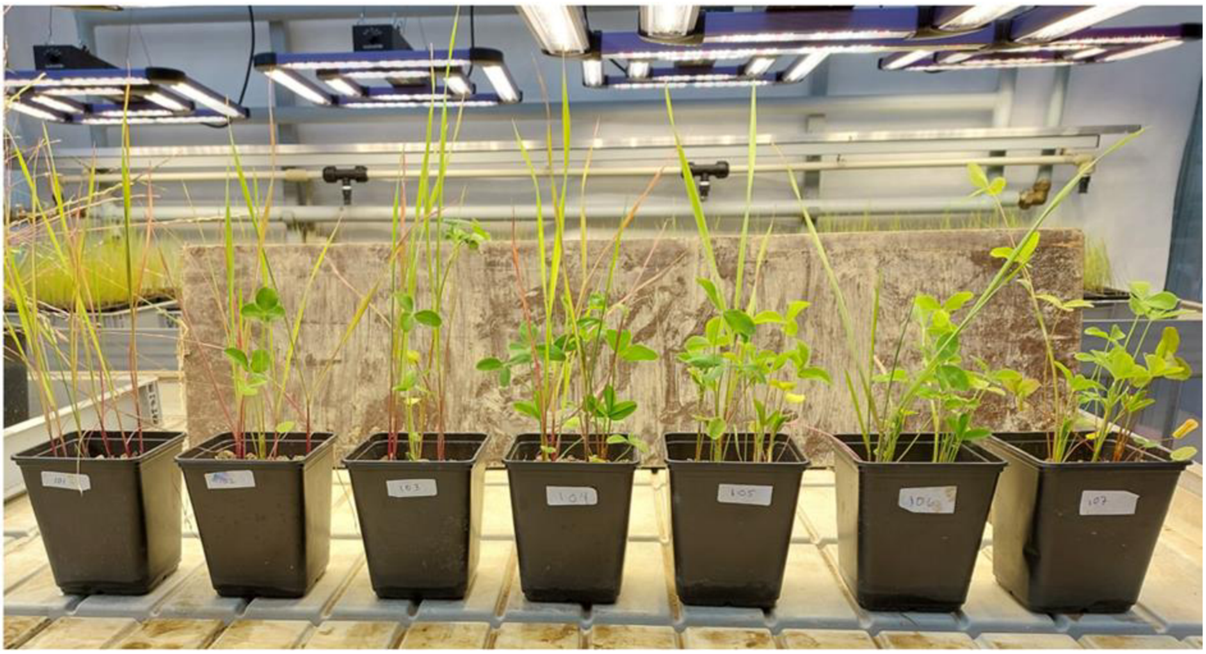

Figure 4 Visual appearance of different planting ratios of IWG and red clover with one IWG plant replaced with red clover in each pot progressing to the right. Picture taken 12/12/2022.

Pictures were taken from a height of 40 cm above the soil surface at each data collection timing. Clover mixes had more soil cover than IWG alone (Figure 5). Visual assessment of percentage vegetative cover at 30 days after planting showed a significant effect of planting ratio (Figure 6). We fail to reject the first hypothesis that adding clover would increase vegetative cover.

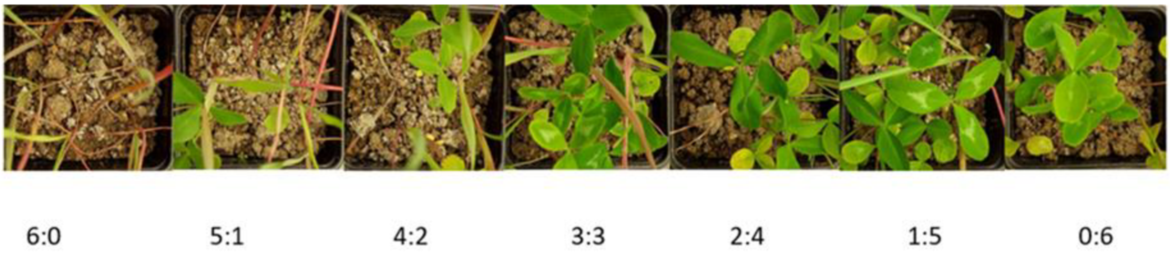

Figure 5 Comparison of aboveground green cover from different IWG: clover ratios after 4 weeks from emergence. Picture taken 16/12/2022.

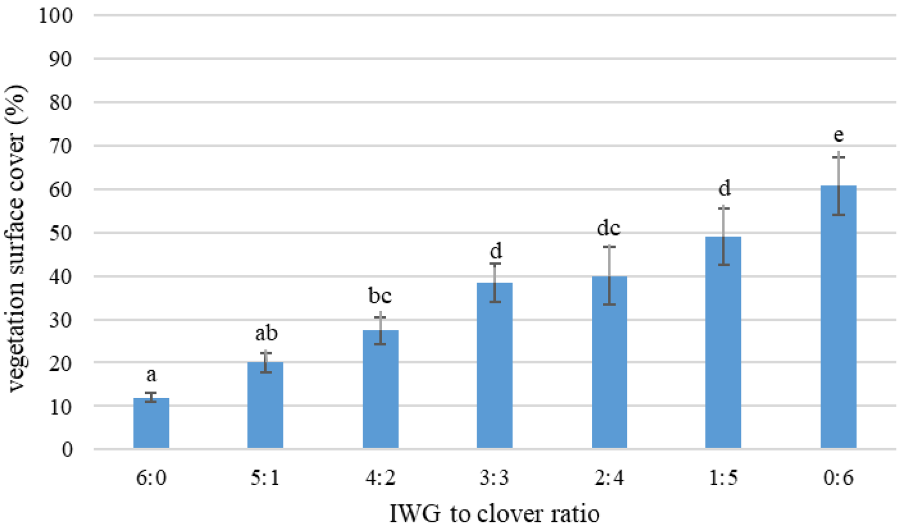

Figure 6 Visual assessment of vegetative cover at 30 days after planting. Blue bars show average cover of each ratio combined over replications and both runs. Bars with the same letter are not statistically different ($p<0.05$). Error bars represent standard error of all observations within one treatment.

Clover's final height was more significant than IWG, having the greatest growth rate between days 40 and 54 without reaching a plateau. IWG to clover ratios of 2:4 and 1:5 had a greater IWG height on the last 20 days, resonating with the data we got for IWG height. Clover showed higher average heights in the last 20 days when planted with IWG like in the case of treatments 2:4 and 1:5. Treatment did not significantly affect the clover height during the timelapse of the experiment (Figure 8). Height over time was modelled in the treatment of each species and compared to the growth pattern of that species in the other planting ratios (Figure 7 and 8). When evaluating height of the clover and IWG, both exhibit a different pattern. IWG accumulated height quickly before stalling (Figure 7), likely due to the limitations of growing in a small pot, while clover accumulated height slowly and then quickly began to produce tall stalks and flower (Figure 8) (picture at 6 or 8 weeks). The model was fit to all observations of that species in each planting ratio, and all produced a good fit, with an RMSE less than 7 and an R2 value over 0.75, indicating that height growth rate was not significantly altered by the competition in other planting ratios.

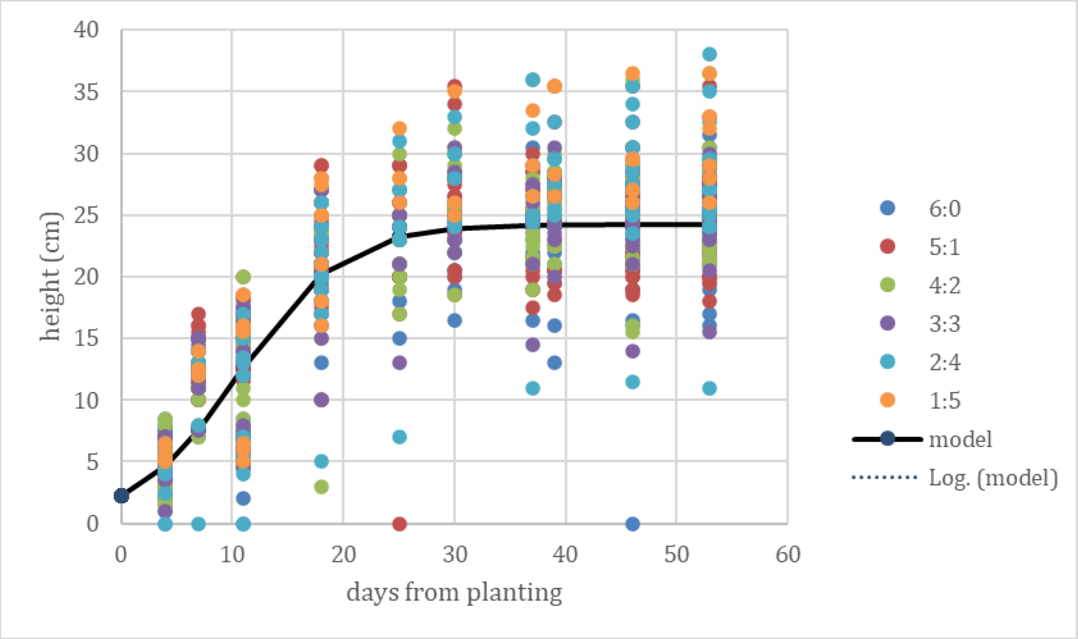

Figure 7 IWG height over time in days from planting. Each line of data point represents a data collection day of a single IWG plant across different planting ratios of IWG to clover 6:0, 5:1, 4:2, 3:3, 2:4, 1:5, 6:0.

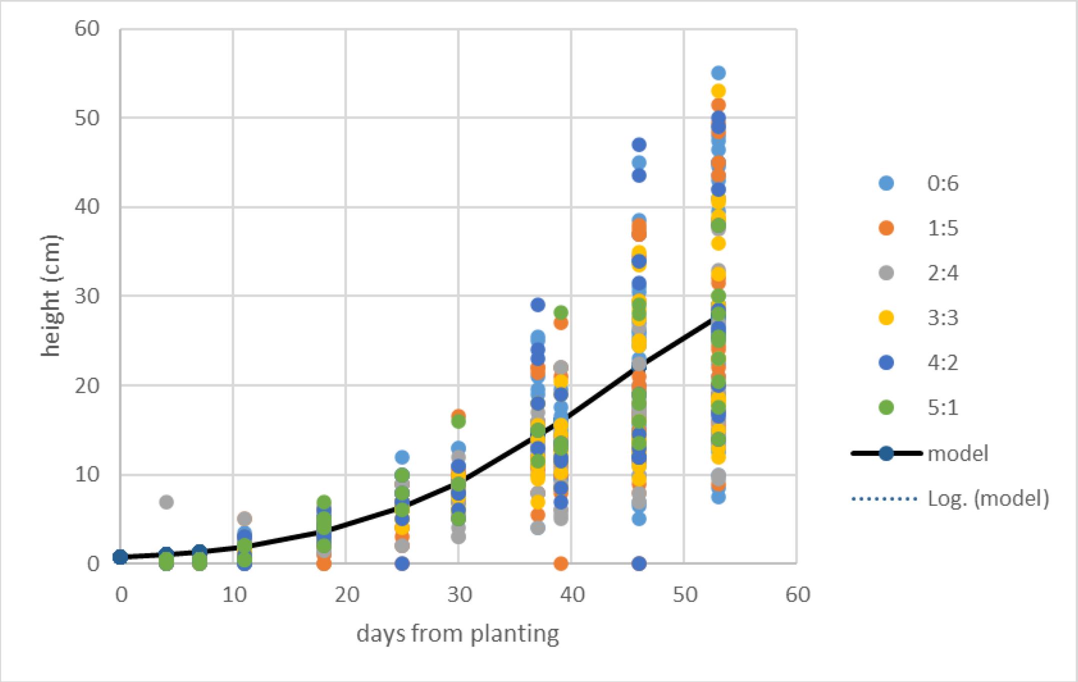

Figure 8 Clover heigh over time in days from planting. Each line of data point represents a data collection day of a single clover across different planting ratios of IWG to clover 6:0, 5:1, 4:2, 3:3, 2:4, 1:5, 6:0.

Final height and biomass were subject to analysis of variance and regression after fitting criteria for normality. Weight data needed to be normalized by log transformation and height data by a natural log transformation. The Coefficient of Variation in both runs was similar and therefore runs were combined. Main effects of ratio and species were not statistically significant for final height. Height means were all similar among different planting ratios and species. The variability of height within species is visible: with IWG heights ranging from 15 to 38 cm and clover heights of clover ranging from 13 to 59 cm (Figure 8).

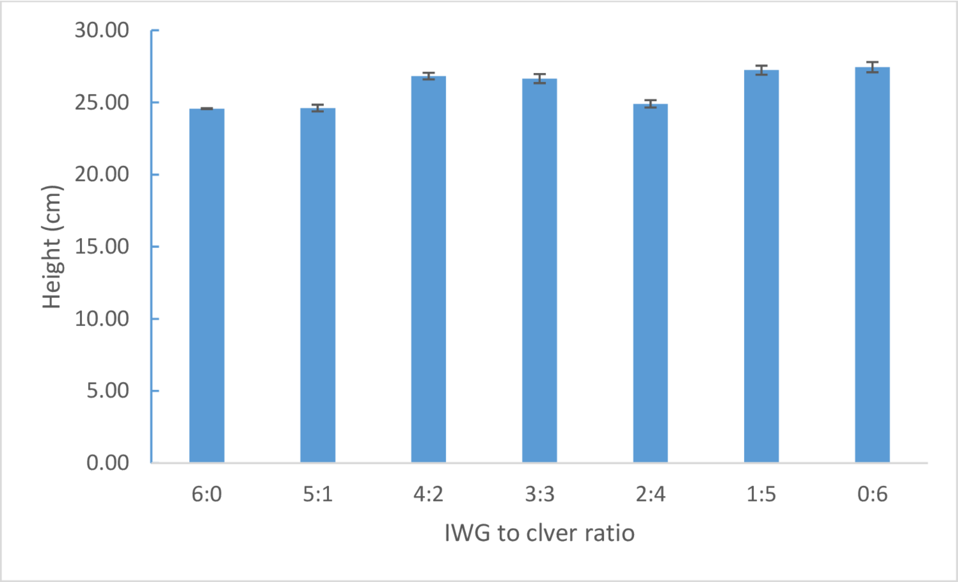

Figure 9 Main effect of planting ratio on the average final plant height per pot, 53 days after planting. Error bars show the height variation across all plants (clover and IWG) in each pot over all replications in each run.

Treatment showed statistical significance in the ANOVA test for the weight of final biomass (Figure 10). The final average biomass was higher for the IWG to clover ratio 0:6 than 6:0 and increased as the amount of clover per pot increased. Results show that clover produced more biomass per pot than IWG in the first 50 days since emergence (Figure 11).

While no ratio by species interaction was found, regression analysis was done to test if there was a significant relationship between the final weight of each species across planting ratio. The average weight of IWG per pot did have a significant relationship with planting ratio. While there are limitations to this analysis because of the imbalance of the sample size, the weight of IWG individuals was not reduced by increasing clover ratios (Figure 12). We reject the second hypothesis.

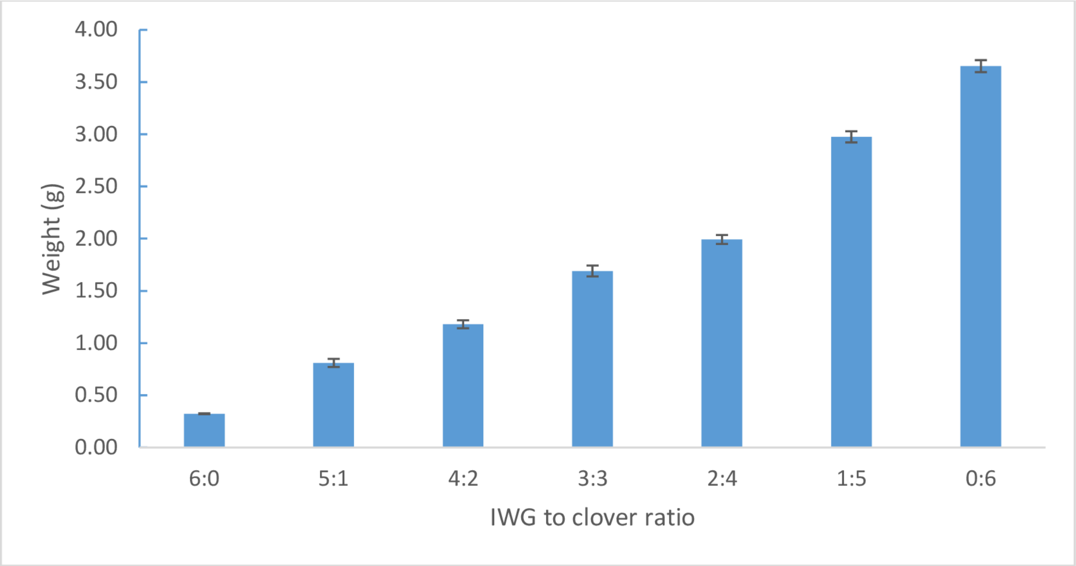

Figure 10 Main effect of planting ratio on the average plant weight per pot. show the height variation across all plants (IWG and clover) in each pot over all replications in each run.

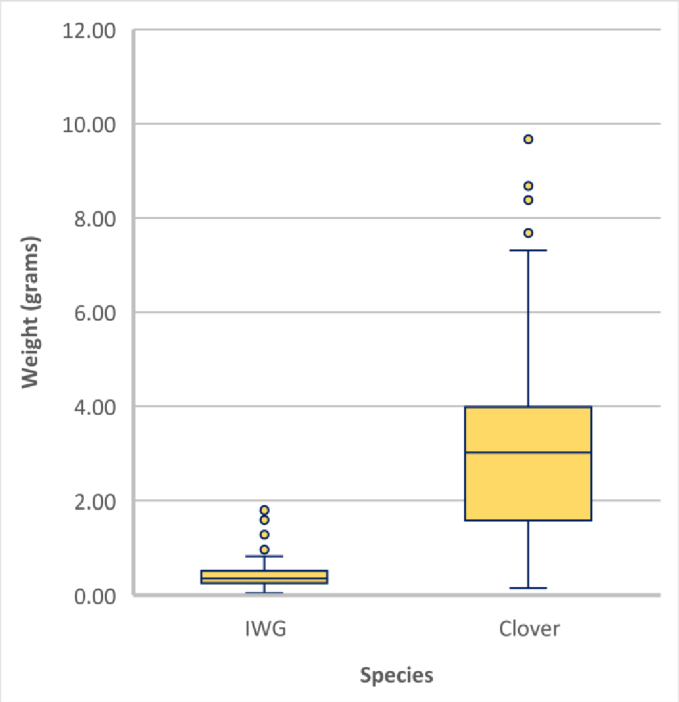

Figure 11 Distribution of final weight across species

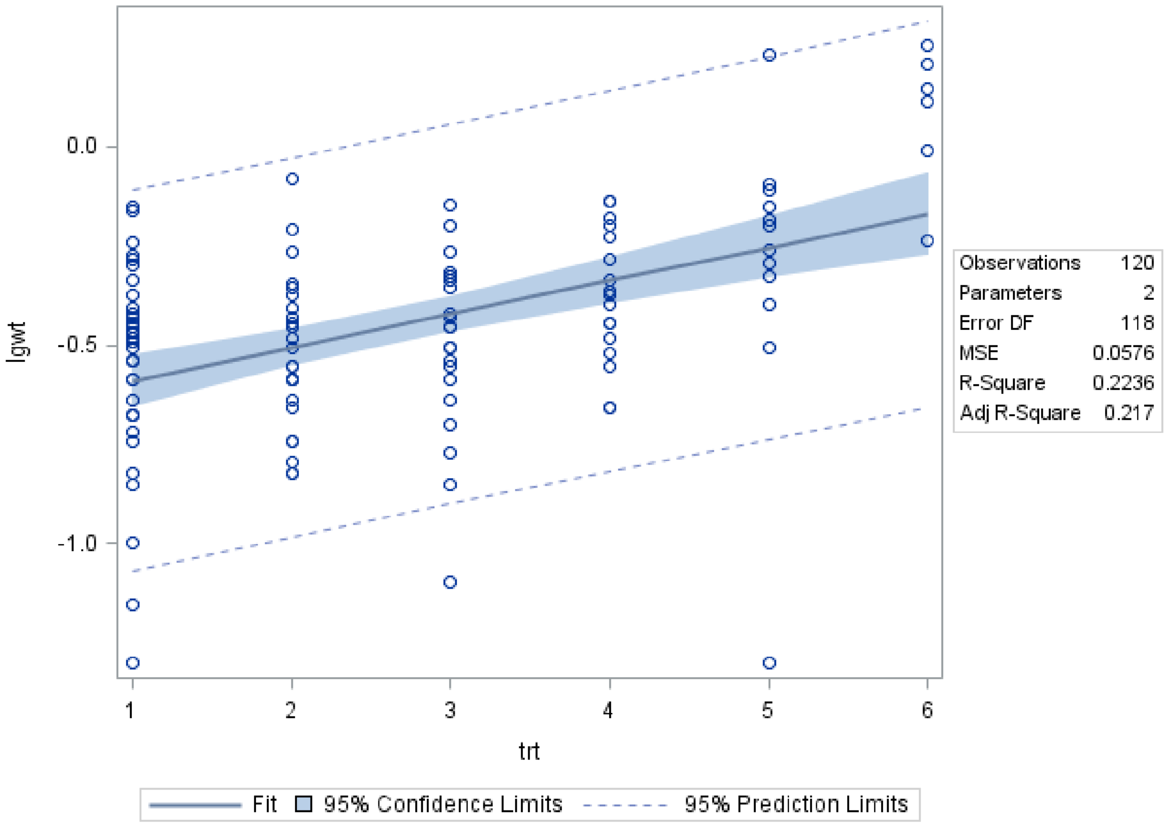

Figure 12 Regression model of IWG final height across treatments

6. Discussion

This experiment aimed to test the following hypothesis:

1. Ground cover could be achieved faster by including red clover.
2. Early competition by red clover reduces the biomass of intermediate wheatgrass IWG.

The experiment results provide insights into the dynamics of mixing red clover with IWG and its implications for ground cover and biomass production. Firstly, the overall health and growth of red clover and IWG, without any complicating diseases or pest issues, underscore the suitability of the experimental conditions for studying their interactions. Both species' similar rate of emergence and early growth timing highlights their adaptability to the experimental setup.

6.1 Ground cover

The significance of achieving faster ground cover lies in its potential to suppress weed growth, enhance soil health, and optimize resource utilization in agricultural systems. Faster ground cover can effectively shade the soil, reducing light availability for weed germination, growth and minimizing competition for water and nutrients. For example, mature alfalfa stands exhibit competitiveness against weeds. However, when an alfalfa seedling emerges it is more vulnerable for weeds invading the space, there will be more competition for territory and resources because the crop has not yet been stablished (Dillehay et al. 2017). During early establishment of crops, competition with emerging weeds can limit favourable results for the crop and lead to weeds gaining territory and resources (Zimdahl 2004). Ott et al. (1989) found when the weed (*Triticum aestivum L.*) emerged in newly seeded alfalfa during fall season, if uncontrolled, after 30 days it could reduce the yields by over 80%.

The Critical Period for Weed Control (CPWC) further supports this idea (Knezevic and Datta 2015). Crops that achieve canopy closure more rapidly tend to have a shorter CPWC, as they can outcompete weeds for resources sooner. Promoting rapid ground cover establishment in agricultural systems can effectively suppress weeds and improve crop productivity (Knezevic and Datta 2015).

Percentage of ground cover is a widely used method for quantifying the extent of vegetation cover over a given area (Afolayan 1979). This approach involves determining the proportion of the ground surface covered by vegetation, typically expressed as a percentage. For this experiment visual evaluation of green cover was rated from 0 to 100 %. Here we demonstrated that treatments with red clover and IWG were the ones that showed the most vegetation coverage in the first 30 days since planting (Figure 4 and Figure 5). Red clover showed a faster cover development than IWG; pots that only contained IWG had over 30% less ground cover, increasing chances for potential weeds to develop (Figure 5). This observation supports the hypothesis that including red clover can accelerate ground cover establishment and aligns with the evidence from Law et al. (2021). Their study revealed that intercropping reduced weed density and biomass compared to IWG, indicating enhanced weed suppression.

Furthermore, the higher weed suppression index in the intercropping treatments emphasize the effectiveness of intercropping in managing weed growth. The correspondence between our findings and the results of Law et al. (2021) supports that integrating red clover into intercropping systems can enhance ground cover and contribute to effective weed management strategies.

6.2 Biomass

The inclusion of red clover did increase the visual cover, but this will only be useful in the field if it does not significantly reduce the biomass of IWG. Most crop plants are bred to be very tolerant of intraspecific competition (able to thrive at high densities of their own species) but not necessarily to be tolerant of interspecific competition (competition with weeds for example or in a mixture of crops) (Worthington and Reberg-Horton 2013). While other researchers have looked at intercropping clover in the field (Law et al. 2021), the measured effect was on final crop yield impact, and not on early growth; often, the intercrop was also planted after IWG was already established. With the goal to increase ground cover for weed control without stunting the IWG, this study needed to also evaluate how clover competed with IWG. Early competition can be classically measured by weighing and partitioning final biomass through a replacement study (Swanton et al. 2015).

Early competition did not reduce the biomass of IWG; the results of the final weight showed that the treatments that contained IWG and clover intercropped had the most biomass, mainly because red clover showed a faster growth rate starting 20 days after emergence. To corroborate that the final weight of IWG was higher, not only because of the red clover, we used a regression analysis of final weight data by species, which showed that IWG plants present in the treatments intercropped with higher red clover ratios had a slightly higher mean final weight than the ones that contained more IWG than red clover or just IWG. Even though the sample size of IWG was lower than the ones that contained just IWG or a higher IWG ratio, it provides some evidence that red clover does not inhibit IWG biomass formation, therefore ruling out competition. The obtained results match the ones of Law et al. (2021), where lower grain yields, biomass, and fodder production of IWG were not observed when intercropped with red clover. Additionally, the significant difference in final height between red clover and IWG, with red clover exhibiting greater height overall, suggests distinct growth patterns between the two species. The observation that red clover showed higher average heights when planted with IWG in specific ratios indicates potential facilitative interactions between the species.

Based on observation, there is more competition between IWG plants between each other in this confined space since the treatments that only contained IWG achieved the lowest values for plant height and biomass. Since grain plants generally compete for soil resources, they experience intra-specific competition under non-fertilized conditions if cereal plants are intercropped with legume plants, like in this case, red clover, which competes less for soil N than cereals (Mariotti et al. 2009) and can fix atmospheric N. Crop mixes of grains and legumes decrease the competition experienced by the cereal plant due to functional complementarity in N acquisition strategy (Duchene et al. 2017).

In addition, while cereals and legumes do not exhibit significant competition when planted together, it is essential to recognize the importance of implementing effective management strategies. Factors such as planting ratios, spacing, and the timing of planting play crucial roles in optimizing the performance of intercropped systems (Mtei and Massawe 2016). Both cereals and legumes require essential resources such as water, nutrients, and sunlight, emphasizing the necessity of strategic management practices. An example is the study by Peoples et al. (2009), which highlights the competition for soil mineral nitrogen between legumes and cereals in intercropping systems, indicating that practices such as intercropping can impact nitrogen fixation by limiting the presence of effective rhizobia in the soil. By considering these factors, farmers can mitigate potential competition and maximize the benefits of intercropping for enhanced agricultural sustainability and productivity. An example of an agricultural practice that can reduce competition is strip cropping and planting alternating strips of cereals and legumes (Mtei and Massawe 2016). Strip cropping avoids some of the disadvantages of intercropping: managing the single crop within the strip is easy, and competition between the crops is reduced (Ojiem et al. 2007).

7. Conclusion

Based on the results and our initial hypotheses, the following conclusions were reached:

- The combination of IWG and red clover provided a greater ground cover at 40 days from emergence. Higher ratios of red clover relative to IWG resulted in more soil cover. More soil cover provides shade for the ground thereby blocking sunlight and reducing weed emergence.
- By day 53, when the final weight was measured, red clover produced significantly more biomass than IWG, treatments with greater red clover ratios had a higher final weight than than treatment that only contained IWG, suggesting that combining IWG with red clover can optimize space and create more biomass with the same resources.
- The addition of red clover did not reduce the weight per plant of IWG in any planting ratio. Pots that contained a higher number of red clover relative to IWG contained the tallest and heaviest IWG plants in the data set. Pots that contained only IWG plants showed the most competition with each other since they had the lowest height and weight.

This research emphasizes the significance of incorporating legumes, particularly red clover, as a companion crop for IWG. Through the study, we observed that within 40 days of planting, this combined cultivation reduced the available space for weeds to establish themselves. This natural weed management approach prevents the necessity for herbicides or mechanical removal, offering farmers a sustainable alternative that minimizes environmental harm and protects natural nutrient cycles in soils.

Additionally, these crops collectively generate more plant biomass, optimizing the utilization of essential resources such as water, light, and soil nutrients. The resulting biomass holds potential as a nutrient-rich forage mix, enriching the diet of livestock This dual-purpose cultivation enhances farm productivity and promotes agricultural sustainability by harnessing the multifunctionality of crops within the ecosystem.

Future research into this topic could investigate further into the dynamics of IWG biomass production when mixed with red clover, particularly by ensuring equal IWG plant numbers per treatment. By comprehensively understanding the interactions between red clover as a weed suppressant and its role as a beneficial companion to IWG, we can optimize cultivation practices for enhanced efficacy and productivity in weed management systems.

Conducting further investigations of this topic in field settings offers the potential to translate research findings into practical applications. By implementing more efficient organic weed management strategies, farmers can reduce reliance on harmful herbicides while ensuring the production of high-quality grain crops from IWG.

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9. Abbreviations

AMF : Arbuscular mycorrhizal fungi
AOV : Analysis of variance
CO_2 : Carbon dioxide
CPWC : Critical Period for Weed Control
GPS : Global Positioning System
IPCC : Intergovernmental Panel on Climate Change
IWG : Intermediate wheatgrass
N : Nitrogen
RRC : Rodale Research Center
SAS : Statistical Analysis System
SOM : Soil organic matter
USA : United States of America
USDA : United States Department of Agriculture
WSI : Weed suppression index