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Effects of different agronomic strategies on the short- and long-term management of weed flora in arable crops.

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Effects of different agronomic strategies on the short- and long-term management of weed flora in arable crops.

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Abstract

Since the beginning of agriculture, humans have endeavored to manage weed flora to mitigate yield losses. Post-war, the intensive use of synthetic plant protection products and mineral fertilizers significantly boosted agricultural production. Herbicides emerged as highly effective tools for weed control. However, their usage exacts a considerable toll on the environment and human health. Moreover, the rapid emergence of weed resistance to certain active ingredients poses a serious challenge to weed management strategies. Consequently, a primary challenge of the 21st century is to enhance agricultural sustainability while reducing reliance on synthetic plant protection products. The heavy reliance of farming systems on herbicides exacerbates this challenge.

The aim of this thesis is to examine the effect of different agronomic strategies on the short- and long-term management of weed flora in arable crops. Regarding short-term management, this thesis focuses on the potential reduction of herbicide usage in winter wheat crops through the utilization of mechanical weeding tools such as the harrow (annual factorial trials spanning 6 years of data between 2010 and 2022). An underlying question is whether combining direct control methods (mechanical and/or chemical) with an establishment prevention measure (delayed sowing date) could enhance weed control in winter wheat and thereby decrease herbicide applications (factorial trial, one year of data from 2022 to 2023). The second question aimed to assess the cumulative effect of different soil management measures (residue export and tillage intensity) on weed flora and yield (long-term factorial trial, data from 2021 to 2022). Finally, the last question aimed to determine the effect of different cropping systems, differentiated by their level of exogenous organic fertilization and tillage system (integrating a multitude of agronomic practices), on weed flora in organic maize crops (long-term system trial, data from 2021 to 2023).

The results showed that weed control with the harrow in winter wheat crops provided sufficient weed control in some cases. However, its effectiveness varied greatly depending on the composition of the flora, initial weed pressure, and the year. The harrow alone was not as effective as chemical weed control. The combination of mechanical and herbicide application for weed control reduced year-on-year variability and ensured highly effective weed control. Delaying the sowing date of winter wheat proved to be a very effective lever for managing weeds. Firstly, it reduced the number of emerging weeds, with a greater effect on *Alopecurus myosuroides* weeds. Secondly, delaying the sowing date improved the effectiveness of both chemical and harrow weed control. Finally, delaying the sowing date reduced weed biomass production below a threshold that did not result in any significant yield loss.

Long-term soil management proved to be a significant factor in the long-term management of weed flora and winter wheat yield. However, in this context, only ploughing demonstrated a notable impact on weeds, while the export of residues appeared to

have minimal effect. Ploughing contributed to the reduction of viable seeds in the seed bank and decreased weed abundance and biomass within the winter wheat crop. While ploughing effectively lowered pressure from *Alopecurus myosuroides*, no significant difference was observed in terms of weed diversity. Indirectly, ploughing led to higher winter wheat yields by mitigating yield losses caused by weeds, in contrast to reduced tillage practices.

Finally, the different organic farming cropping systems showed effects on the abundance and diversity of weeds present in the maize crop. Similar to the previous trial, ploughing emerged as the primary driver of community composition and weed abundance. A reduced tillage system with low organic fertilization input resulted in a much greater taxonomic diversity of weeds, including both non-harmful and problematic species such as *Cirsium arvense* and *Lolium multiflorum*. Similar to conventional farming, reduced tillage led to high weed pressure in terms of abundance and biomass. Consequently, this system did not facilitate effective weed management, resulting in yield loss due to competition. Moreover, this system was found to result in lower yields even in the complete absence of weeds. Rotation and the level of organic fertilization had no significant impact on weed composition or abundance. However, higher levels of organic fertilization tended to increase maize yields.

The coherent application of different agronomic levers can enable sustainable management of weed flora while reducing (or even avoiding) the application of herbicides.

Résumé

Depuis le début de l'agriculture, l'homme a essayé de gérer la flore adventice pour éviter les pertes de rendements. Après la guerre, l'utilisation intensive de produits phytopharmaceutiques de synthèses et d'engrais minéraux a permis d'augmenter la production agricole. Le recours aux herbicides a permis un contrôle très efficace des adventices. Cependant, l'utilisation des herbicides a un effet néfaste sur l'environnement et sur la santé humaine. De plus, très vite, des individus résistants à certaines matières actives sont apparus, mettant en péril le mode de gestion des adventices. Suite à ces conséquences négatives, l'un des grands défis du 21e siècle est d'arriver à produire tout en rendant l'agriculture plus durable et moins dépendante des produits phytopharmaceutiques de synthèses. La grande dépendance des systèmes agricoles aux herbicides rend ce défi compliqué.

Le but de cette thèse est d'étudier les effets de différents leviers agronomiques sur la gestion à court et à long terme de la flore adventice en grandes cultures. Concernant la gestion à court terme, cette thèse s'est concentrée sur la possibilité de réduire l'utilisation d'herbicides dans la culture de blé d'hiver par l'utilisation d'outils de désherbage mécanique tels que la herse étrille (essais factoriels annuels, 6 années de données entre 2010-2022). Une question sous-jacente a été de savoir si la combinaison de la lutte directe (mécanique et/ou chimique) avec une mesure de prévention de l'implantation (retard de la date de semis) pouvait améliorer le contrôle des adventices dans le blé d'hiver et ainsi diminuer l'utilisation d'herbicides (essai factoriel, une année de données 2022-2023). La deuxième question était d'évaluer l'effet cumulatif des différentes mesures de gestion du sol (exportation des résidus et intensité du travail du sol) sur la flore adventice et le rendement (essai factoriel de longue durée, données en 2021-2022). Enfin, la dernière question visait à déterminer l'effet de différents systèmes de cultures différenciés par leur niveau de fertilisation organique exogène et par le système de travail du sol (intégrant une multitude de pratiques agronomiques) sur la flore adventice dans les cultures de maïs biologiques (essai système de longue durée, données entre 2021-2023).

Les résultats ont montré que le désherbage à la herse étrille en culture de froment d'hiver permettait un contrôle des adventices qui dans certains cas était suffisant. Toutefois, son efficacité variait fort selon la composition de la flore, la pression initiale et l'année. La herse étrille n'a pas permis à elle seule d'avoir une efficacité aussi grande que le désherbage chimique. La combinaison du désherbage mécanique et chimique a permis de manière interannuelle de diminuer la variabilité et d'assurer un désherbage avec une haute efficacité. Le retard de la date de semis (semis de mi-octobre décalé à la mi-novembre) s'est avéré être un levier très efficace dans l'objectif d'une gestion de la flore adventice. Celui-ci c'est avéré agir à plusieurs niveaux, premièrement, en diminuant le nombre de levées d'adventices avec un effet accru sur les vulpins. Deuxièmement, le retard de la date de semis a permis d'améliorer à la fois

l'efficacité du désherbage à la herse étrille et chimique. Enfin, le retard de la date de semis a permis de diminuer la production de biomasse des adventices sous un seuil n'entrainant aucune perte de rendement significative.

La gestion de longue durée du sol s'est avérée être un élément important pour la gestion à long terme de la flore adventice et du rendement en blé d'hiver. Par contre, dans ce contexte, seul l'effet du labour a impacté les adventices tandis que l'exportation ou non des résidus ne semble pas avoir un effet majeur. Le labour a permis de diminuer la quantité de graines viables présentes dans le stock semencier ainsi que l'abondance (en termes d'adventices et de biomasses) dans la culture de froment d'hiver. Le labour a permis de diminuer la pression en vulpin, mais peu de différences en termes de diversité des adventices ont été observées. Le labour a permis indirectement d'obtenir un rendement supérieur en blé d'hiver grâce à la diminution des pertes de rendement engendrées par les adventices comparées à celles du non-labour.

Enfin, les différents systèmes de cultures en agriculture biologique ont montré un effet sur l'abondance et la diversité d'adventices présentes en culture de maïs. Tout comme pour l'essai précédent, le labour semble être le grand driver de la composition des communautés et l'abondance en adventices. Un système en non-labour et avec une fertilisation organique réduite permet d'avoir une diversité taxonomique d'adventices beaucoup plus importante, mais avec à la fois le développement d'adventices peu nuisibles et d'adventices problématiques, telles que le chardon et le ray gras italien. Tout comme en conventionnel le non-labour a induit une pression en adventices élevée (en termes d'abondance et de biomasse). Ce système ne permet donc pas une bonne gestion des adventices. Celles-ci ont engendré in fine une perte de rendement à cause de la compétition induite. Ce système,même en absence totale d'adventices, s'est révélé avoir des rendements plus faibles. La rotation et le niveau de fertilisation organique n'a pas eu d'impact significatif à la fois sur la composition et l'abondance des adventices. Par contre, une fertilisation organique supérieure a tendance à augmenter le rendement en maïs.

L'application cohérente de différents leviers agronomiques peut permettre une gestion durable de la flore adventice tout en diminuant (voir évitant) l'application d'herbicides.

Acknowledgments

Une thèse est un travail de longue haleine. Bien qu'il y ait qu'une unique personne qui défende la thèse, celle-ci est en réalité le fruit d'une interaction d'un ensemble d'individus dont le thésard est le point commun entre toutes ces interactions. Alors il va de soi de remercier allègrement l'ensemble des personnes ayant rendu ce travail possible.

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Acronyms

AIC Akaike's Information Criterion. 93

ALOMY *Alopecurus myosuroides* Huds.. viii, 75, 78, 79, 81–85

CFI comparative fit index. 75, 81

CS cropping system. 17, 18, 22, 23, 91, 93–95, 97, 109, 111, 112

CT conventional tillage. 20, 21, 71, 73, 78, 79, 83, 84, 89, 90, 101–103

EU European Union. 4, 29

IPM Integrated Pest Management. 4, 18, 51, 66

IWM Integrated Weed Management. 5, 18, 22, 29, 66, 107

LV latent variables. 75

MATCH *Matricaria chamomilla* L.. 75, 78, 79, 81–84

MV manifest variables. 75

NDVI normalized difference vegetation index. 112

NE newly emergent weeds. 36, 37, 40, 42, 47, 48, 50

NS new species of weeds. 36, 37, 40, 42, 47, 50

OW old weeds. vi, 36–38, 40, 42, 43, 46–50

PCoA principal coordinates analysis. 93, 95

PPP Plant Protection Product. 3, 4, 24, 25, 55, 65, 66

RMSEA Root Means Square Error Approximation. 75, 81

RT reduced tillage. 20, 21, 71, 73, 78, 79, 83–86, 89, 90, 101–103

SEM structural equation modelling. 70

SRMR Standardized Root Mean Square Residual. 75, 81

TLI Tucker-Lewis index. 75, 81

WC weed control. 37, 38, 43, 45, 49, 50

WCE Weed Control Efficacy. 57, 58, 60, 64, 66

1

Chapter I: General introduction

1. Context

Since the beginning of agriculture in the Neolithic period 10,000 years ago, humans have had to control harmful organisms (including weeds) in order to avoid yield losses. Weeds were initially managed mainly by hand and then by mechanical weeding (Oerke, 2006). After the Second World War, yields increased steadily for 50 years thanks to the intensification of agriculture based on the use of high-yielding varieties, fertilisation and the use of pesticides (Matson et al., 1997). Weed management has therefore evolved considerably with the use of chemical herbicides (Oerke, 2006). These Plant Protection Products (PPPs) were very effective in controlling weeds. Initially, the first products targeted broadleaf, and after the 1960s, the appearance of new active ingredients made it possible to control grass weeds (Chauvel et al., 2012). This greater use of mineral nitrogen and the selection of higher-yielding plants (often less competitive with weeds) were advantageous for weeds and led to greater potential losses caused by them (Figure 1.1) (Oerke, 2006; Storkey et al., 2021).

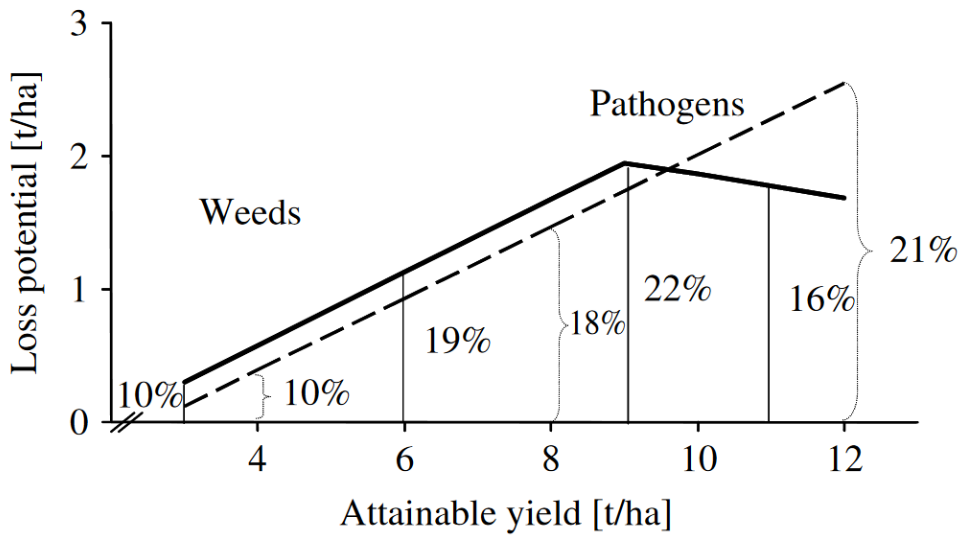

Figure 1.1: Relationship between the intensity of production (=attainable yield) and the loss potential of weeds and fungal diseases in wheat production (summary of 477 field trials on weed competition and 206 fungicide trials in Germany, 1985–90). The full line represents the loss potential caused by weeds, while the dotted line represents the loss potential caused by pathogens. Illustration from Oerke (2006)

The full line represents the loss potential caused by weeds, while the dotted line represents the loss potential caused by pathogens. The use of herbicides, the simplification of farming systems and the use of fertilizer inputs have had the impact of eroding weed diversity, homogenising it between fields and regions and selecting a more nitrophilic weed flora (Fried et al., 2009; Storkey et al., 2021, 2011, 2010).

Without control, weeds are the pests that cause the most yield losses, with potential losses estimated at 23% for wheat and 40.3% for maize (Oerke, 2006). Conversely, among pest control measures, weed control is the most effective in reducing yield losses, with an estimated reduction of 70% worldwide, leaving only an 8% loss globally (Oerke, 2006).

The intensive use of herbicides has harmful effects on the environment (Stoate et al., 2009) and public health (Waggoner et al., 2013). Furthermore, this heavy use has led to the emergence of resistance to certain active ingredients. The number of weeds resistant to certain modes of action continues to grow, jeopardising the proper management of the weed flora (Heap, 2023). By February 2023, 267 weed species had developed resistance to at least one out of the 31 known herbicide sites of action (Heap, 2023). In Europe, the resistances are sometimes clearly visible in cereal crop rotations, with, for example resistance in populations of *Alopecurus myosuroides* (Chauvel et al., 2009). These resistances compromise the management of weed flora. In addition, fewer and fewer herbicides are authorised and the number on the market is declining, which could encourage the emergence of new resistances (Chauvel et al., 2012; Qu et al., 2021). It is for all these reasons that the European Union has sought to rationalize the use of PPPs. For example, since 2009, the European Union (EU) has implemented Directive 2009/128/EC, which aims to promote the sustainable use of pesticides. This directive provides a framework for mitigating risks to human health and the environment while encouraging the adoption of Integrated Pest Management (IPM) practices. The IPM is defined by EU as follows: *«Integrated pest management means careful consideration of all available plant protection methods and subsequent integration of appropriate measures that discourage the development of populations of harmful organisms and keep the use of plant protection products and other forms of intervention to levels that are economically and ecologically justified and reduce or minimise risks to human health and the environment. 'Integrated pest management' emphasises the growth of a healthy crop with the least possible disruption to agro-ecosystems and encourages natural pest control mechanisms.»*. More recently, through the Green Deal and the Farm to Fork Strategy, the EU has set itself the ambition of reducing the use of PPPs by 50% by 2030. This ambition was temporarily suspended following farmer protests in early 2024 across Europe but remains on the European agenda and is being promoted by many worldwide organizations (of the Earth Europe, 2024). Reducing herbicide use is far from an easy task. In fact, it is considered to be the most challenging pesticide to reduce (Lamichhane et al., 2017). Our agricultural systems heavily rely on herbicides, making them the first or second (depends of the year) most consumed group of PPPs in Belgium, accounting for 40.16% of sales in 2021, which amounted to 2096 t for the same year (Corder, 2023). Despite this reliance, Belgium's PPP sales decreased by 22% between 2011 and 2020, outperforming its neighbors. For instance, the Netherlands witnessed a 14% decline between 2011 and 2021, while

Germany and France experienced increases of 11% and 13%, respectively, over the same period (2011-2021) (Eurostat, 2024). This highlights the challenge of achieving the target of a 50% reduction in PPPs. According to Triantafyllidis et al. (2023), reducing herbicide usage is possible by implementing Integrated Weed Management (IWM). IWM promotes prophylactic measures through a combination of various crop system management practices, such as tillage and residue management, shifts in sowing dates, changes in crop rotations, etc. These preventive measures aim to reduce the need for systematic herbicide use. However, when necessary, curative measures, such as mechanical weeding or chemical weeding have to be considered as well (Triantafyllidis et al., 2023). Because non-chemical weeding techniques are usually not able to compete with herbicides in terms of efficacy, some authors have suggested that various practices should be combined (Pavlović et al., 2022).

2. Objectives and structure of the thesis

This thesis is part of the Sol-Phy-Ly project titled "Evaluation of the fate of plant protection products in the field as a function of cultivation practices for the development of eco-responsible agriculture". This project is funded by the Walloon public service (Department of Agriculture, Natural Resources, and Environment, grant number "D65-1415"). The project had two main objectives: (1) to develop monitoring methods for characterizing PPPs pollution in water and soil matrices, and simultaneously to monitor lysimeters in Wallonia to better understand the leaching and degradation dynamics of PPPs, (2) to reduce herbicide use by implementing a range of agricultural practices. This thesis was conducted to support the second objective. The aim was to address weed-related issues and reduce herbicide usage using various tools studied on different scales (annual or inter-annual as illustrated in Figure 1.2). Additionally, Gembloux Agro-Bio Tech - ULiège and the Walloon Agricultural Research Center conducted long-term trials (both factorial and cropping system) of varying ages and employing different practices, which had never been previously monitored for weeds. The advantage of utilizing these diverse trials for the thesis is that they all share the same geo-pedoclimatic conditions, being located in the vicinity of the town of Gembloux. This led to the definition of the thesis subject as the "Effects of different agronomic strategies on the short- and long-term management of weed flora in arable crops". Throughout this thesis, considering the agronomic orientation given to the final document, a taxonomic approach (based upon the species name) was purposely chosen, rather than a functional trait approach.

The thesis will be structured into different chapters, as illustrated in Figure 1.2. Firstly, a state-of-the-art of the literature will be presented. The subsequent chapters will be structured based on the combination of levers studied and the aspect of short- or long-term monitoring (Figure 1.2).

Short-term management focuses on the possibility of reducing herbicide use in winter wheat crops through the use of mechanical weed control tools, such as the harrow. This objective is pursued in chapter 3 which aims to assess the combined effects of mechanical and chemical weed control, with a specific focus on the dynamic of weeds during winter wheat cropping season. An underlying question is whether combining direct control with an establishment prevention measure (delayed sowing date) could improve weed control in winter wheat; this question is developed in chapter 4.

The next two chapters (chapter 5 and chapter 6) evaluate the levers implemented on an inter-annual scale. Therefore, chapter 5 deals with the cumulative effect of different soil management (residue exportation and tillage intensity) on weed flora and winter wheat yield. The last question (chapter 6) is to determine the effect of different cropping systems differentiated by their levels of organic input and by the tillage system (integrating a multitude of agronomic practices) on weed flora in organic maize crops. Finally, a general discussion will be provided followed by a conclusion.

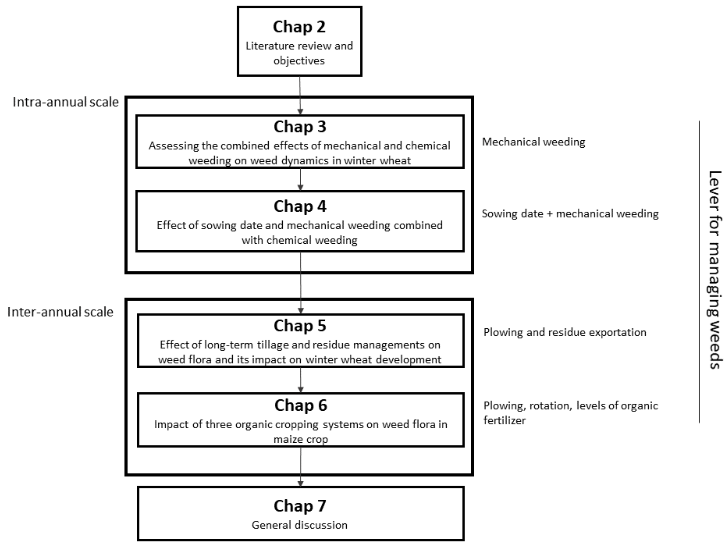

Figure 1.2: Representation of the structure of the various chapters of the thesis.

2

Chapter II: Literature review and objectives

1. Definition of weeds

To understand what we are talking about, it is important to know how to define the term "weeds". It is clear that there are a multitude of definitions, and that the same definition is not used by all scientists (Zimdahl, 2018). The European Weed Research Society defined a weed as “ any plant or vegetation, interfering with the objectives or requirements of people” (EWRS Institution, 2008 cited by Zimdahl, 2018). This very broad definition of the term weeds adds a touch of harmfulness through the "interfering with the objectives" section, but is much less so than the 2016 definition proposed by the Weed Science Society of America, which defines weeds as "a plant that causes economic losses or ecological damages, creates health problems for humans or animals, or is undesirable where it is growing" (WSSA, 2016). This is an agronomic definition of the term weed. Depending on the discipline, the definition of the term "weeds" will not be exactly the same, so ecologists have a different definition of weeds. Godinho (1984) pointed out the difficulty of finding a single definition of "weeds" because it has two meanings: an ecological sense, "plants that grow spontaneously in environments modified by humans," which corresponds to the French definition of the term “adventice”, and an agronomic, weed science sense, which would be "undesirable plants" and corresponds in French to the term “mauvaise herbe”. In this manuscript, the chosen definition of the term “weeds” corresponds to the ecological definition by Godinho (1984), meaning “plants that grow spontaneously in environments modified by humans” which therefore corresponds to the term “adventices” in French. Indeed, this broad definition allows both addressing weeds from an agronomic and harmful perspective and from an ecological and diversity perspective, which resembles more the integrated view of weeds conveyed throughout this manuscript.

2. Weed characterisation

Arable weeds thrive in environments characterised by frequent disturbances such as tillage and weeding, along with temporal variability in these disturbances. These environments also exhibit highly variable disturbances linked to crop sequences and are characterised by high nutrient availability due to fertilizers (Gaba et al., 2014; Storkey, 2020). This environment is characterised by strong competition for space and light (Perry et al., 2003). This environment, which characterises the arable field, forms an ecological niche to which weeds are adapted. Agricultural practices have exerted a selection pressure which, over the long term, has favoured weeds that mimic crop morphology and phenology (Neve et al., 2009). Some weeds are specialized in arable fields, selected by agronomic practices that create a specific ecological niche for a specialized weed flora (such as segetal weeds). However, arable weeds also encompass generalist or pre-adapted species found in open environments, like *Galium aparine*, which occupy what is known as an extended niche (Fried et al., 2010). There are several characteristics that make a weed a weed. This environment has favoured certain traits. Weeds are predominantly therophytes (annual plants that die after producing seeds), a high specific leaf area, an early and long flowering period, and a high ability to thrive in nutrient-rich soil (Bourgeois et al., 2019; Storkey, 2020). Weeds are often characterized by high growth rates (Storkey, 2004, 2020). Additionally, some weeds have developed significant shade tolerance (Bourgeois et al., 2019; Perronne et al., 2014; Storkey, 2004). Weeds tend to produce large quantities of seeds per plant, with some retaining their germination capacity for many years. Furthermore, weeds exhibit significant environmental plasticity, as a large number of species are able to grow in a wide range of edaphic and climatic conditions (Zimdahl, 2018). Finally, weeds possess trait values that vary greatly between species and within a species in response to the environment (Gaba et al., 2017; Perronne et al., 2014; Storkey, 2005).

Four main different weed strategies have been described in wheat crop by Gaba et al. (2017) (see Figure 2.1). The first are fast-growing species whose seed production starts well before harvest. These weeds are known to be less damaging. The second are species that mimic the crop. These develop more synchronously with the crop and are therefore composed of species that can cause significant yield losses (e.g. *Alopecurus myosuroides*) (Gaba et al., 2017; Gunton et al., 2011; Perronne et al., 2014). The third group are late-emerging species that tolerate shade well in their early stages of development (Bagavathiannan and Norsworthy, 2012). Finally, the last group is the fast-growing environmentally-independent emerging weeds. These independent germinating weeds can have several cycles in the crop (e.g. *Capsella bursa pastoris* L. , Aksoy et al. (1998)) (Gaba et al., 2017).

According to Grime (1977), plants can be classified into different life history strategies: competition (C), stress tolerance (S)), disturbance tolerance (R). Competition represents the interactions between plants for resources such as light, nutrients, and space. Stress tolerance refers to a plant's ability to withstand factors that limit its growth. Disturbances are events that cause partial or total loss of plant biomass. Species tolerant to disturbance are called ruderal species. Depending on the strategy employed, each plant can be positioned within the CSR life history triangle, indicating whether it is more of a ruderal species, stress tolerator, or competitor (Figure 2.2). The functional composition of the plant community therefore depends on the main characteristics of the environment, specifically disturbance and soil fertility. An environment with high fertility and low disturbance will tend to select a plant community with a "C" life history strategy. An infertile, undisturbed environment will favor a community of weeds with an "S" life history strategy, while a nutrient-rich, highly disturbed environment will favor a community of plants with an "R" life history strategy (Storkey, 2020).

Weeds tend to fall within the ruderal to competitive spectrum (Bourgeois et al., 2019; MacLaren et al., 2020; Metcalfe et al., 2019; Storkey, 2020). However, few weeds fall

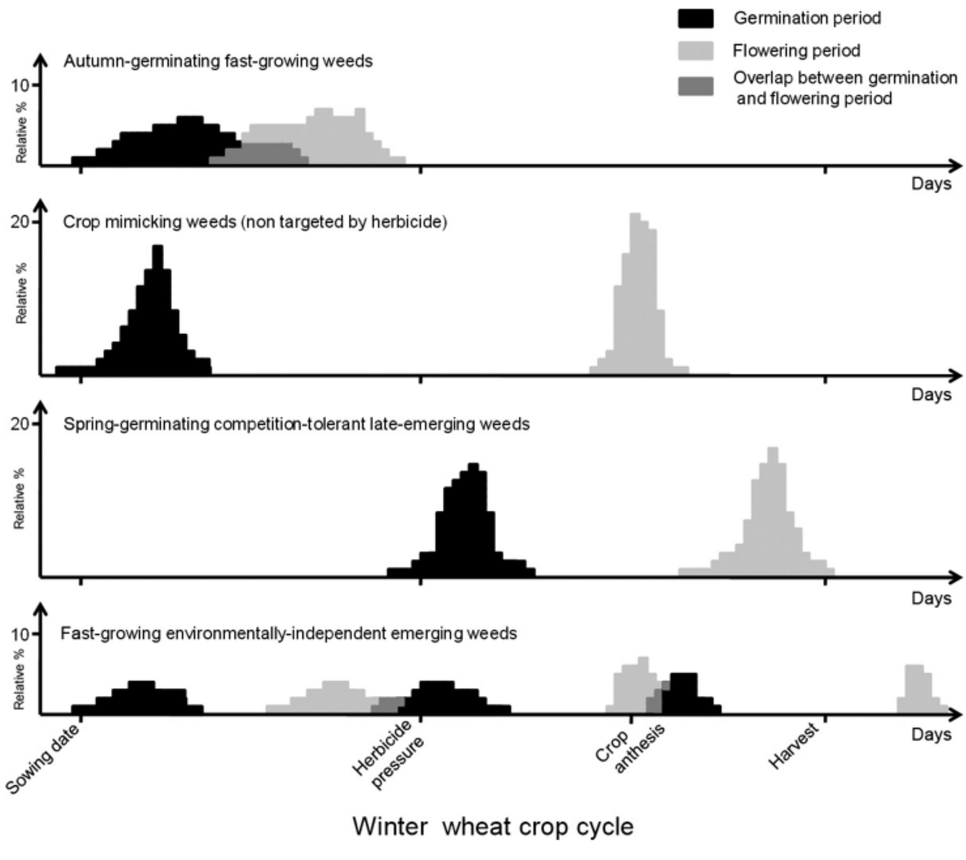

Figure 2.1: Schematic representation of the germination and flowering patterns of the four main phenological guilds observed in winter cereals.The relative percentage of individuals of a species showing a particular phenological stage at each time step along an entire cropping cycle is along the y-axis. Some species, including fast-growing environmentally independent emerging weeds, may have several cohorts during one cropping cycle. Illustration from Gaba et al. (2017)

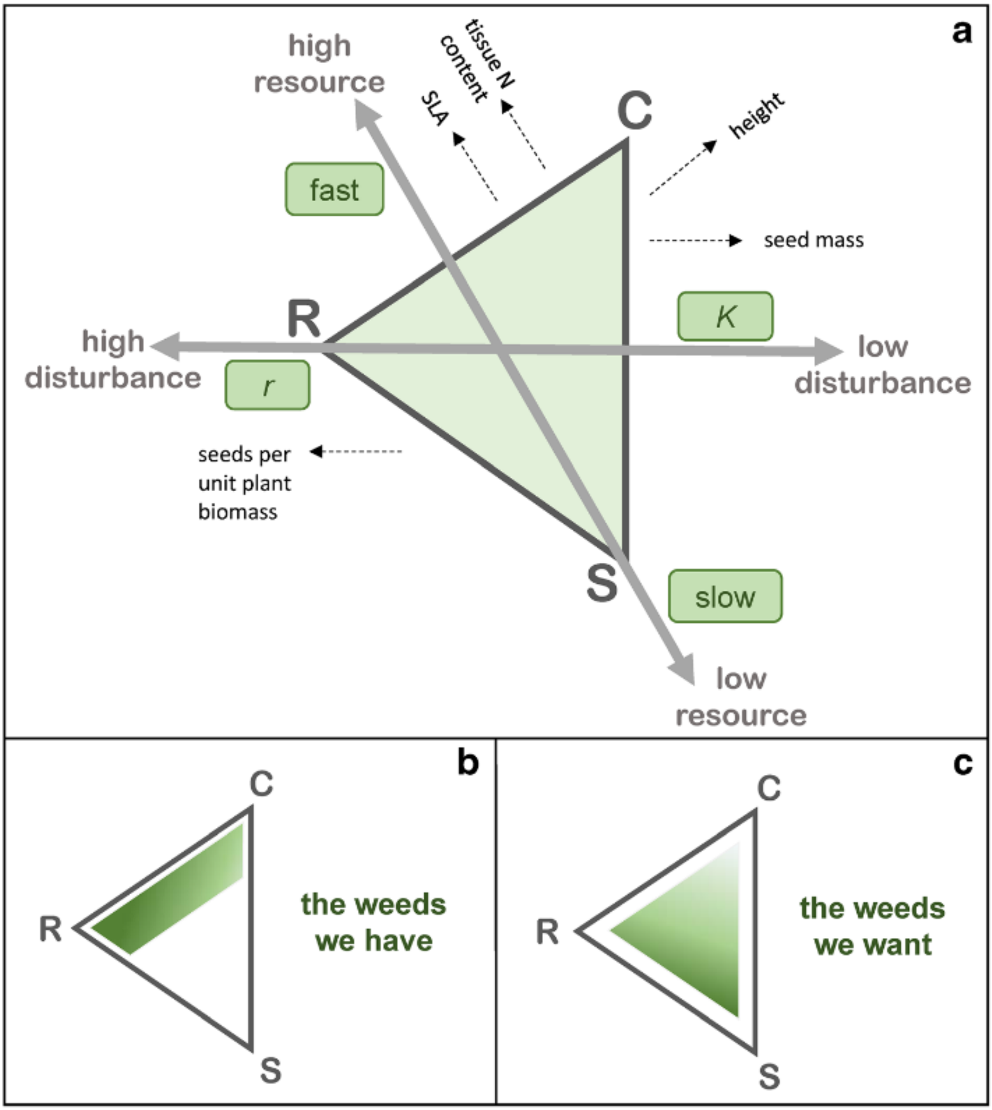

Figure 2.2: Schematic showing a) how different disturbance levels and resource availability are expected to select for weeds with different life history strategies, b) the distribution in 'CSR' space of current common agricultural weeds according to Metcalfe et al. (2019) and Bourgeois et al. (2019) and c) a more desirable distribution in 'CSR' space implying a functionally diverse community of weeds with a higher representation of species along the R-S axis. Illustration from MacLaren et al. (2020)

under the "SR" category (MacLaren et al., 2020; Metcalfe et al., 2019). Changes in crop management, with more abundant fertilisation, have led to selection pressure favouring R rather than S strategies (Storkey, 2020). According to a review by MacLaren et al. (2020), the most advantageous weeds for both ecosystem services and low competitiveness are those present along the "SR" axis of Grime's triangle. Stress-tolerant weeds are slow-growing plants that can withstand the stress caused by cultivation. In contrast, weeds along the "CR" axis, are fast-growing and tend to dominate the crop, engendering strong competition with it. According to MacLaren et al. (2020) it is possible to manage filters to select "SR" weeds. They recommend a strategy that reduces resource availability and diversifies management as much as possible at both field and landscape level. In terms of tillage intensity, the frequency of tillage should not be too high, which recommends fast-growing R weeds but not too low either, which favours perennial C weeds that have a strong impact on weed-crop competition.

3. Harmfulness of weeds

Weeds are known to have undesirable effects, which can be categorized into several aspects, as proposed by Zimdahl (2018). These include plant competition, added protection costs, reduction of farm product or animal quality, increased production and processing costs, water management issues, human health concerns, decreased land value, and limited crop choice due to aesthetic concerns. This section will focus on detailing the most significant harmful effects. Plant competition is one of the primary concerns, involving competition for water, light, and nutrients (Anderson, 1997; Zimdahl, 2018). Additionally, allelopathic interactions may contribute to competition. These competitions can ultimately lead to yield losses (Anderson, 1997; Zimdahl, 2018).

Furthermore, weeds can propagate certain crop diseases or pests, as noted in the category of Added protection costs by Zimdahl (2018), or as identified by Anderson (1997) as host plants. Weeds can serve as reservoirs for pathogens and act as sources of primary disease inoculum (Wisler and Norris, 2005). This can involve fungi such as take-all (*Gaeumannomyces graminis* var. tritici), the presence of which in annual grass weeds contributes to disease infestation (Gutteridge et al., 2006). A second well-known example is ergot disease (*Claviceps purpurea*), where certain weeds, including blackgrass (*Alopecurus myosuroides*), facilitate cross-infection and increase the risk of high infestation in wheat due to earlier flowering (Mantle et al., 1977). This contamination is particularly significant as ergot produces alkaloids that are toxic to human health and livestock (Menzies and Turkington, 2015) Weeds can also serve as reservoirs for viruses such as cucumber mosaic virus or tomato spotted wilt virus (TSWV), retaining them in the environment between cropping seasons if they are not eradicated (Laviña et al., 1996). Additionally, certain weed species can themselves be parasitic, as seen in the case of rambling broomrape (*Phelipanche ramosa*), which parasitizes plants from the Solanaceae (such as tomato and eggplant), Brassicaceae (like rapeseed), and Fabaceae (such as faba bean) families, causing significant yield losses in crops (Parker, 2013).

Another form of harmfulness, sometimes referred to as indirect, is the increase of the soil seedbank due to the seed production of weeds that have escaped weed control measures and completed their development cycle (Chauvel et al., 2018).

4. Ecosystem services provided by weeds

Although weeds are mainly seen through the damage they can cause (Oerke, 2006), they are an essential part of the agroecosystem, providing ecosystem services such as supporting pollinators (Bretagnolle and Gaba, 2015; Yvoz et al., 2021). The presence of pollinators can support the pollination of crop production (Bretagnolle and Gaba, 2015). The presence of weeds can also reduce nitrogen leaching in the agroecosystem (Virili and Moonen, 2024). Furthermore, weeds are at the base of the trophic chain and the seeds produced can be a food source for birds, on which farmland bird species (such as partridges) depend (Gibbons et al., 2006; Marshall et al., 2003; Storkey and Westbury, 2007; Wilson et al., 1999). Grain is also eaten by mammals such as rodents, insects (e.g. ground beetles, ants, etc.) and earthworms (Marshall et al., 2003; Petit et al., 2011; Yvoz et al., 2021; Hawes et al., 2009). According to Blaix et al. (2018) the main regulating service that can be associated with weeds is pest control. The primary mechanism is to maintain a habitat suitable for natural enemies. In fact, the food source provided by weeds can support crop auxiliaries such as omnivorous carabid beetles, which also feed on slugs. Finally, weeds can have an indirect impact on earthworms, where weed seeds are a source of food for earthworms, whose work improves the soil quality (Petit et al., 2011). However, the services provided depend very much on the species composition and abundance of the flora. Not all flora therefore provide the same level of ecosystem service (Petit et al., 2011; Yvoz et al., 2021). In addition, the various ecosystem services are rarely quantified (such as yield gain due to pest regulation, or increase in crop pollination) and there is a lack of trade-off between these services and the harmfulness of weeds at different spatial scales (Bretagnolle and Gaba, 2015).

5. Weed seedbank

The seedbank is defined as "all viable seeds present on or in the soil or associated litter" (Simpson et al., 1989). The seedbank consists of both recently deposited seed rain and viable seeds from previous years. It comprises a range of seeds with variable longevity. Seeds capable of germinating years after production constitute the persistent part of the seedbank (Simpson et al., 1989). Conversely, some seeds can only germinate within the first few months after production, forming the transient seedbank.

The dynamics of the seedbank are illustrated in Figure 2.3. The seedbank is formed through seed production by plants within the crop or from external sources through seed dispersal (via wind, rain, animals, machinery, etc.). Reduction in the seedbank occurs due to seed mortality (predation, decay caused by pathogens, and physiological death) or seed germination.

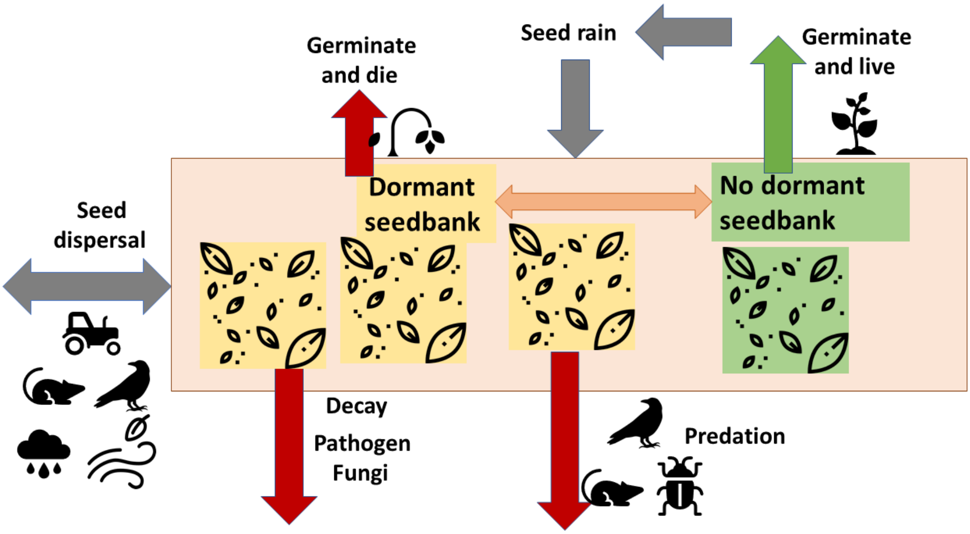

Figure 2.3: Weed seedbank dynamics. he red arrows represent seed mortality, the green arrow represents seeds that will germinate and produce seeds, the grey arrows represent new seeds that replenish the seedbank, the yellow seeds represent the dormant part of the seedbank, and the light green seeds represent the non-dormant part of the seedbank.

Weed emergence constitutes a small proportion of the total seedbank, varying between 3% and 15% depending on the source (Ball and Miller, 1989; Forcella et al., 1992; Zhang et al., 1998). The emergence percentage varies significantly among species, with some showing high annual emergence rates while others exhibit low rates. For instance, *Setaria faberi* has a first-year emergence rate of 33% (Hartzler et al., 1999), whereas *Chenopodium album* has an annual germination rate of only 0.01% (Ball and Miller, 1989). Some species possess long seed longevity in the seedbank, enabling them to persist in the environment for several years, even under unfavorable conditions, and to germinate when conditions become favorable. This characteristic is particularly crucial in highly variable environments such as agricultural fields, where environmental conditions differ annually based on crops planted and cultivation practices employed (Chauvel et al., 2018; Fried et al., 2008).

These characteristics are also associated with dormancy, defined as “the absence of germination of a viable seed under conditions favorable to germination” (Harper,1959 cited by Hilhorst (2007)). Dormancy can be classified into two types: primary dormancy and secondary dormancy (Hilhorst, 2007). Primary dormancy is "the type of dormancy that occurs prior to dispersal as part of the seed developmental program" (Hilhorst, 2007). This primary dormancy enables seeds to avoid germination while still on the mother plant, and differences in the extent of this dormancy help prevent competition among the offspring produced by the mother plant (Chauvel et al., 2018). Primary dormancy can be influenced by weather conditions during seed production by the mother plant, as observed in species such as *Alopecurus myosuroides* (Colbach and Dürr, 2003).

Secondary dormancy is defined as "the acquisition of dormancy in a mature seed after imbibition as a result of the lack of proper conditions for germination" (Hilhorst, 2007). Secondary dormancy varies greatly among species (Gardarin and Colbach, 2015) and manifests seed behavior in the seedbank through its main effect, which is dormancy cycling. Dormancy cycling involves the induction and termination of dormancy due to seasonal variations (Hilhorst, 2007; Vleeshouwers et al., 1995). These secondary dormancies ensure that plants germinate at times conducive to completing their life cycle. Dormancy can be broken by various factors, such as light exposure, cold periods, drought, or fertilization (Hilhorst, 2007; Mahé et al., 2021).

Germination is closely linked to cultivation techniques, including sowing periods and types of tillage. Consequently, the weed flora that emerges in a given year often represents only a small fraction of the weed flora present in the seedbank (Zhang et al., 1998). The variability in growing conditions, primarily influenced by crop species and climatic seasons, allows for the expression of several fractions of the seedbank (Cardina and Sparrow, 1996).

Studying the seedbank in an agro-ecosystem is an interesting tool because the seedbank is the hologram of past flora and is the reservoir of future vegetation. Consequently, it can be utilized to examine the annual weed communities of the past, present, and future (Mahé et al., 2021). Different topics studied throughout the weed seedbank in agronomy are represented by the Figure 2.4 of Mahé et al. (2021). While weed management remains the primary focus, other topics such as forecasting the emerged flora, weed diversity, and food resources for seed feeders are also addressed (Mahé et al., 2021). However, studying the seedbank can be rather complex, requiring significant time and botanical knowledge. Furthermore, comparability may be compromised by variations in methodologies, which can significantly impact the results (Mahé et al., 2021).

6. Not all weed communities have the same impact on yield

In the past, the harmfulness of weeds has often been studied by focusing on specific weed species and their relationship with cultivated crops (Cousens, 1985; Guglielmini

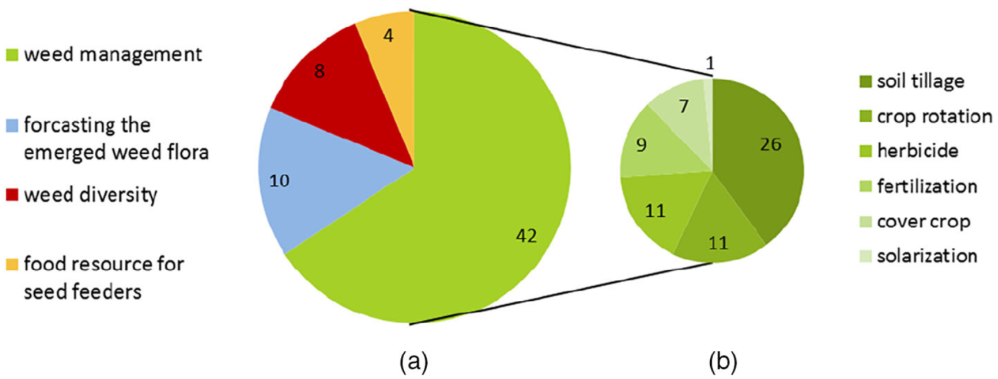

Figure 2.4: Topic studied throughout the weed seedbank (a) and details of the 'weed management' category (b). Illustration from (Mahé et al., 2021)

et al., 2017). Some models have been developed by combining the harmfulness of several different species (Florez et al., 1999; Swinton et al., 1994). However, this approach does not consider the competition between weed species (Clements et al., 1994; Garrison et al., 2014; Weigelt et al., 2007). Weed-crop interference should therefore be studied at the level of the weed community (Swinton et al., 1994). In recent years, some researchers have attempted to determine whether certain communities are less harmful than others or to investigate the idea that a more diverse flora, at equal density, could mitigate weed-crop competition (Ferrero et al., 2017; Storkey and Neve, 2018; Adeux et al., 2019b).

The hypothesis behind this theory is that the use of resources would be more diversified and less in competition with that of the crop, as well as the appearance of indirect interaction and increase asymmetric and interspecific competition within the weed community (Aschehoug and Callaway, 2015; Clements et al., 1994; Freckleton and Watkinson, 2001; Pollnac et al., 2009; Smith et al., 2010). This new research theme is one of the priorities in weed ecology according to a study by Neve et al. (2018). Three questions in the top 15 priorities are directly related to biodiversity and reducing weed:crop competition. Question 5 is "How important is weed functional diversity in maintaining ecosystem function and reducing crop yield loss from weed competition?". Question 7 "How do we increase productivity and species diversity in the arable land at the same time?". Or question 11 "How can farming systems be designed for greater resilience to weeds?".

Indeed, the simplification of cropping systems and the use of herbicides have tended to select for a less diverse flora with very high weed:crop competition (Storkey and Neve, 2018). Adeux et al. (2019b) demonstrated that certain weed communities were more damaging than others in winter wheat. In their studies, 4 out of 6 weed communities caused significant yield losses to varying degrees (ranging between 19% and

56%). These detrimental weed communities reduced the number of ears per plant and the number of grains per ear, reflecting early competition for resources (Zimdahl, 2007a; Adeux et al., 2019b).

However, a community of weeds (composed mainly of *Galium aparine*) also reduced the 1000-kernel weight, no doubt due to the climbing effect of the *Galium aparine* and its ability to overhang the crop and therefore intercept light, limiting photosynthesis and generating competition for late resources (Adeux et al., 2019b; Bauer et al., 2010; Taylor, 1999). Moreover, in this same experiment, greater diversity (in terms of richness) and greater evenness of the flora resulted in lower weed biomass and therefore a smaller impact on yield (Adeux et al., 2019b). Other studies have sometimes shown that greater diversity results in lower yield loss (see for example Storkey and Neve (2018)).

7. Various levers to reduce herbicide use

To enable more sustainable weed flora management (reducing or even eliminating herbicide use, promoting a diversified weed flora that provides ecosystem services with minimal impact on yield), diversifying the cropping system (CS) is a promising long-term management solution (Adeux et al., 2019a). The various practices associated with CS will create diverse selection pressures acting as different filters (see Booth and Swanton (2002) for the ecology theory of community assembly) that will disrupt the life cycle of weed species at different times. So depending on the crop, different sowing dates, different weeding methods (mechanical, chemical, mixed, different mode of action of herbicide), different tillage, harvesting period, quantity and management of residues, etc. are all factors leading to different filters (Fried et al., 2008; Gaba et al., 2014; MacLaren et al., 2020; Mahaut et al., 2019; Riemens et al., 2022).

As mentioned in the general introduction, an IPM approach must be applied to optimize pest management (Riemens et al., 2022). The IPM approach applied to weeds is called Integrated Weed Management (IWM). According to Riemens et al. (2022), an IWM strategy must impact weed populations at different stages of their life cycle in order to: (1) prevent weed establishment, (2) minimize the negative impact of emerged weeds on the crop, and (3) prevent the replenishment of weed seeds, rhizomes, or tubers. The various tactics of intervention, acting at different stages of the weed life cycle, were categorized into five points: (1) Diverse cropping system, (2) Cultivar choice and establishment, (3) Field/soil management, (4) Direct control, and (5) Monitoring and evaluation. Each of these sections encompasses a multitude of actions that influence weed survival at different stages of their life cycle (see Figure 2.5).

Throughout this manuscript and the various trials conducted, specific management tools within the four pillars were studied in greater detail, either individually or in

Figure 2.5: Framework for the planning and design of holistic IWM strategies that require combinations of individual management tools appropriately selected from each of the five pillars of IWM: Diverse cropping systems, cultivar choice and establishment, field and soil management, direct control and the cross-cutting pillar monitoring and evaluation. Illustration from Riemens et al. (2022).

combination. These include rotation for the diverse CS (Chapter 6), sowing date for cultivar choice and establishment (Chapter 4), tillage type for field/soil management (Chapters 5 and 6), and mechanical weeding and post-emergent herbicides for direct control (Chapters 3 and 4). In the following sections, we will delve into these management tools in more detail as studied in different chapters of the thesis.

7.1. Cultivar choice and crop establishment

It is possible to influence weed-crop interactions to mitigate the impact of weeds on crop yield. Certain management techniques employed during crop establishment can promote biotic interactions that favor the crop over weeds (Riemens et al., 2022). Additionally, selecting suitable cultivars and tolerant crops can reduce the need for direct weed control methods (Andrew et al., 2015; Riemens et al., 2022). Furthermore, delayed sowing date is an effective management tool to manage weeds in winter cereals crops (Moss, 2017; Riemens et al., 2022). In the framework of this thesis, the delayed sowing dates in winter wheat were investigated.

Delayed sowing dates can disrupt the emergence of certain weeds that prefer earlier germination periods (Moss, 2017). For autumn-sown crops like wheat, delaying the sowing date is primarily employed to prevent the emergence of *Alopecurus myosuroides*. This weed poses significant challenges in autumn-sown crops, but adjusting the sowing date helps prevent its emergence (Chauvel et al., 2009; Lutman et al., 2013; Moss, 2017). Lutman et al. (2013) demonstrated that in England, shifting the sowing date from September to the end of October reduced *Alopecurus myosuroides* emergence by 50%, and by 88% when wheat was sown in the spring. Chauvel et al. (2009) showed that in a herbicide-resistant *Alopecurus myosuroides* population, delayed the sowing date combined with other levers such as ploughing enabled a rapid reduction in the *Alopecurus myosuroides* population. A study by Rasmussen (2004) showed that delaying the sowing date had an effect on the biomass of weeds (all weeds combined) produced and helped to reduce it. However, it should not be forgotten that later sowing of winter wheat can result in lower yield potential (Bastiaans et al., 2008; Rasmussen, 2004).

7.2. Field/soil management

Field and soil management includes management tools such as tillage (primary and secondary), dead mulching (could be crop residue), nutrient placement, water management, etc. (Riemens et al., 2022). In the framework of this thesis, the levers of tillage intensity and the management of crop residue were studied.

The influence of tillage systems has been widely studied. Tillage systems can be categorized into two main types: inversion and non-inversion tillage. Inversion tillage, often referred to as conventional tillage, typically involves mouldboard ploughing followed by secondary tillage (such as cultivation or rotary harrowing). On the other hand, non-inversion tillage encompasses techniques that do not turn the soil over and are characterized by keeping at least 30% of crop residues on the surface. Depending on the intensity of tillage, this is categorized as reduced tillage (RT) or no-till (direct drilling) (Morris et al., 2010). Due to the disturbance it causes to the environment, tillage is a crucial factor in weed management and is considered one of the most impactful methods for influencing weed community within a crop (Adeux et al., 2022; Fried et al., 2008; Kouwenhoven et al., 2002). Tillage plays a significant role in the distribution of seeds within the soil horizon (Buhler, 1995; Nichols et al., 2015; Hoffman et al., 1998). Reduced tillage, and particularly no-till practices, increase the quantity of seeds on the soil surface (Buhler, 1995; Nichols et al., 2015). In no-till, the burial of seeds in the soil is a very slow process because it relies on different mechanisms such as fauna activity and the freeze-dry cycle ((Nichols et al., 2015; Hoffman et al., 1998)) Whereas conventional tillage (CT) tends to homogenize buried weed seeds at a depth that inhibits their emergence (Benvenuti et al., 2001; Buhler, 1995; Nichols et al., 2015; Hoffman et al., 1998). Mouldboard plowing tends to favor weeds with large seeds capable of germinating at greater depths, as well as species exhibiting a high level of primary dormancy (Gardarin et al., 2012, 2009). RT and no-till practices favor surface weeds, leading to an increase in germination percentage (Nichols et al., 2015). However, they also result in increased predation and seed mortality due to desiccation and harsh weather conditions (Anderson, 2005; Nichols et al., 2015; Westerman et al., 2006). RT, in particular, tends to result in higher weed density and the selection of a weed flora rich in grasses, as well as favoring perennial species (Armengot et al., 2016; Cardina et al., 2002; Schnee et al., 2023; Travlos et al., 2018; Trichard et al., 2013). This tendency to have a higher weed density makes these systems more dependent on herbicides (Melander et al., 2013; Nichols et al., 2015). Consequently, in organic farming, the adoption of RT techniques is very complicated and results in very high weed pressures (Casagrande et al., 2016; Peigné et al., 2007). However, despite the advantages of CT in terms of weed management, we must not lose sight of the other agronomic and environmental benefits that RT can bring, such as erosion control and the concentration of organic matter on the surface (Holland, 2004). If RT and conservation farming methods are adopted, all the other agronomic levers for sustainable, long-term management become all the more important (Melander et al., 2013; Riemens et al., 2022).

7.3. Diverse cropping systems

Cropping diversification can be carried out on a spatial scale (intercropping, landscape design, etc.) or a temporal scale (crop rotation). Intercropping involves growing two or more species in the same field for at least a part of their growing period. For example, cereal with legume intercropping is practiced in Europe, which reduces weed pressure compared with growing the legume alone (Bedoussac et al., 2015).

Crop rotation is an important driver of weed communities. Rotation allows for the diversification of the timing of disturbances (sowing date, harvest time, weeding operation time), the types of disturbance (weeding operation, herbicide mode of action, etc.), and the available resources (Mahaut et al., 2019). Mahaut et al. (2019) showed that diversifying sowing dates reduced weed abundance. However, in a meta-analysis by Weisberger et al. (2019), despite a lower abundance with a diversified rotation (49%), no significant impact on weed biomass was observed. It would seem that introducing a spring crop into an autumn-sown crop rotation would be more advantageous than the reverse (Buhler, 1995). Furthermore, according to Anderson (2005), growing two summer crops followed by two autumn crops results in a decrease in weed density over time, whereas alternating crops every year increases weed density. This phenomenon is all the more important in no-till systems, where seeds survive for a shorter time than when they are buried in the soil (Anderson, 2005; Nichols et al., 2015). In the case of weeds specialized in autumn crops such as *Alopecurus myosuroides*, the introduction of a spring crop in the rotation can drastically reduce this weed (Chauvel et al., 2001; Lutman et al., 2013). Crop rotation is a lever that allows for a greater reduction in weed density under a no-till system compared to a conventional tillage system (Weisberger et al., 2019). Introducing a grazed forage crop into the rotation further reduces weed abundance by increasing disturbance diversity through grazing phases (or mowing) (MacLaren et al., 2019).

Mahaut et al. (2019) showed that the variability in crop size generated by the rotation and the sowing date increased the inter-annual diversity of the weed flora, whereas at an annual level weed richness decreased with the diversification of sowing date. However, (Adeux et al., 2022) have shown that in some cases it is possible to create CSs that have both greater inter-annual and intra-annual diversity. In terms of floristic composition, the crop sowing date is the primary filter explaining the composition of the weed flora (Fried et al., 2008). A crop rotation may tend to select more generalist species, whereas a monoculture has a higher proportion of specialist species (Fried et al., 2010). Crop rotation with different traits (shape form, height, grass vs broadleaf, etc.) may also favor the coexistence of species with diverse competitive ability and different resource requirements (Mahaut et al., 2019). However, the increase in richness does not necessarily increase with crop diversification as reported in a farmer's network by Adeux et al. (2022), probably because of the intensive use of herbicides.

7.4. Direct control

When preventive measures fail to suppress weed establishment, leading to excessive weed-crop competition and subsequent yield loss or soil contamination in subsequent crops (Riemens et al., 2022), herbicides become the most commonly used and effective direct control method. However, they should only be employed as a last resort within an IWM framework (Triantafyllidis et al., 2023). Mechanical weeding, on the other hand, is a primary management tool for directly controlling weeds and serves as an alternative to herbicide use (Riemens et al., 2022). This approach was explored in more detail in this thesis.

Mechanical weeding methods such as harrowing or inter-row cultivation are widely employed today as alternatives to herbicides (Riemens et al., 2022). Inter-row cultivation, in particular, has seen a resurgence in popularity due to advancements in machine vision techniques that enable precise row detection and operation close to the crop (Fennimore et al., 2016; Riemens et al., 2022). The harrow is a versatile tool capable of weeding both within the row and between rows. However, its effectiveness depends greatly on the developmental stage of the weeds and the species composition (Kurstjens and Perdok, 2000; Rasmussen and Svenningsen, 1995; Rueda-Ayala et al., 2010). Harrowing is only effective on young weed stages and is therefore recommended during pre-emergence and early post-emergence stages (Kolb et al., 2012). For example, the harrow is not effective against perennials and is less effective against grasses and more developed weeds (Kurstjens and Perdok, 2000; Pannacci et al., 2017; Wilson et al., 1993). The effectiveness of the harrow is highly sensitive to water and soil conditions (Kurstjens and Kropff, 2001; Rueda-Ayala et al., 2010). Dry soil conditions during and after harrowing are important to allow for good desiccation of uprooted weeds and to avoid transplanting. The harrow primarily controls weeds by covering and uprooting them (Kurstjens and Kropff, 2001; Kurstjens and Perdok, 2000). Selectivity between the crop and the weeds is crucial for its adjustment to avoid yield losses (Rasmussen et al., 2009).

8. Long term experiment and cropping system

The reduction (or even non-use in organic farming) of herbicide use results, as already mentioned, from a holistic approach and a combination of management tools at the cropping system scale (Pavlović et al., 2022; Riemens et al., 2022). The aim of this management is long-term sustainability. To meet this challenge, long-term trials are needed to observe the impact of one or more strategies on weed flora over an extended period. In addition to the need for long-term factorial trials to understand one or two agronomic practices and their interaction over time (Nichols et al., 2015), reducing herbicide use requires the application of multiple coherent management tools and the design of new cropping systems that aim to achieve specific objectives within given constraints. It is therefore important to transition from a factorial approach (testing one, two, or even a maximum of three different factors) to a cropping systems approach with a larger number of factors, aiming to understand the effect of the cropping system as a whole (Drinkwater, 2002). A system, as defined by Drinkwater (2002), is "a group of interrelated elements forming a functional entity that is more than the sum of its parts." According to Lechenet et al. (2017), different perceptions of CS coexist and depend on the experiment. These authors identified three different perceptions.

The first is based on the definition of Sebillote (1974-1990) (cited by Lechenet et al. (2017)) and is defined as "CS is a sequence of technical operations implemented homogeneously on a set of plots". The CS is predefined by the set of cropping operations and by a predefined crop rotation.

The second perception of CS is a set of decision rules that define the CS implemented in a given crop sequence. These decision rules link decisions to contextual elements at the plot level, adapting to pest pressure, weather conditions and soil conditions.

The last perception listed by Lechenet et al. (2017) is a definition that provides even more flexibility and adaptability than the second. Crop sequences are not defined a priori and can be adapted to agronomic contexts (soil structure, pest pressure, etc.). Additionally, the economic aspect is taken into account in the decision-making rules. This approach allows for greater flexibility, which aligns more closely with the reality of farmers' decision-making processes.

These cropping systems are typically studied over the long term to measure the synergistic effects of different management approaches. However, they are not suitable for understanding the interaction or effect of a particular factor alone, so the two approaches (factorial and CS) are complementary (Drinkwater, 2002). On the other hand, few long-term trials have demonstrated how different agricultural practices have affected weeds and yields (Adeux et al., 2022, 2019a, 2017; Davis et al., 2012; Krauss et al., 2020).

9. How to monitor weed

Several different measurements can be carried out for weeds. Monitoring can occur at different scales of time and space which will depend on the issue being studied (Hanzlik and Gerowitt, 2016). This thesis focused solely on monitoring during the cultivation phase with a survey before weeding and at crop flowering (i.e. after all the weed control methods). The most commonly used measure in the literature is weed density (Adeux et al., 2022). Other measures, such as leaf cover, are also employed, allowing for the assessment of light and space captured by weeds (Chauvel et al., 2018). However, this variable varies greatly depending on when the measurements are taken (Chauvel et al., 2018). Weed biomass is a particularly valuable measure as it serves as the most reliable indicator of weed:crop competition compared to density or leaf cover (Adeux et al., 2022; Milberg and Hallgren, 2004). Despite these advantages, weed biomass measurements are often substituted with density or weed cover measurements due to their time-consuming nature (Armengot et al., 2015; Plaza et al., 2011; Santín-Montanyá et al., 2013). However, as noted by Adeux et al. (2022), the selection of measurement types is not always straightforward. Indicators based on density (such as richness, Shannon index, etc.) do not fully capture the capacity for weed:crop competition as biomass does, which assigns greater weight to species that have a strong impact on weed-crop competition. Therefore, indices calculated based on density are more suitable for analyses focused on species (their ability to reproduce and survive in the agroecosystem), whereas indicators based on biomass reflect an analysis centered on the agroecosystem (e.g., weed:crop competition) (Adeux et al., 2022).

The measurement of the seedbank was also conducted during this thesis. Assessing the seedbank is a highly time-consuming process that requires specific skills, and its outcomes can vary significantly depending on the method employed (such as seedling emergence or seed enumeration, and the specific protocols followed for each method) (Mahé et al., 2021). Nevertheless, despite these challenges, the study of the seedbank remains a crucial area of research, offering insights into a wide range of questions (Mahé et al., 2021) (refer to the section on the seedbank for further details (Section 5)).

3

Chapter III: Assessing the combined effects of mechanical and chemical weeding on weed dynamics in winter wheat

1. Synopsis

In this chapter, we will take an intra-annual scale approach in order to focus on the effect of different combinations of direct weed control. We will utilize 6 years of factorial trial data on mechanical weeding coupled with chemical weeding in winter wheat to observe whether mechanical weeding using the harrow could serve as a solution for reducing the use of herbicides. Additionally, we will examine which weeds are most harmful to winter wheat depending on their emergence timing and observe the dynamics of weed reduction throughout the wheat season.

This chapter relies on the following paper. Lacroix, C., Pierreux, J., Brostaux, Y., Vandenberghe, C., and Dumont, B. (2024). *Assessing the combined effects of mechanical and chemical weeding on weed dynamics in winter wheat. Weed Research, minor revision*

2. Abstract

Mechanical weeding, such as harrowing, offers a promising approach for reaching the European Union's goal of a 50% reduction in pesticide use. To assess its potential, used alone or with foliar applied herbicides without residual activity, a 6-year study was conducted on winter wheat cultivated in loamy soil under temperate conditions (Belgium). Weed density dynamics and percentage weed control (WC) were measured and compared between wheat tillering (BBCH 27-29) and wheat canopy closure (BBCH 39-75). Weeds were categorized as weeds maturing generally synchronously with wheat (OW), newly emergent (NE) weeds, or new weed species (NS) appearing in spring. The presence of OW was negatively correlated with yields (up to -0.44), while spring weeds had low or no impact on yield. Overall, one harrow pass reduced OW pressure significantly, with no statistical differences between one versus two passes (second pass performed several days after first). The percentage of weed control reached 92-94% when herbicide was used in combination with harrowing (respectively for one and two harrow passes), and proved to be more effective than harrowing alone whose efficacy reached 64% to 70% (respectively for two and one harrow passes). This study also found evidence effects of natural competition from wheat on weeds. Specifically, 54% WC was observed just after harrowing, 70% was observed when the wheat canopy was closed. These findings suggest that an integrated weed management (IWM) approach may involve an initial harrowing pass with subsequent evaluation of its effectiveness prior to commencing another intervention. In the event of poor efficacy, a full dose application of herbicide may be warranted.

3. Introduction

By implementing farm-to-fork and biodiversity strategies, the European Union (EU) aims to achieve a 50% reduction in pesticide (e.g., herbicides) (commission, 2023). Although the deadline for accomplishing this pesticide reduction has been postponed by EU, following the farmer's strikes in late 2023/early 2024, it remains at the EU agenda and is still encouraged by many organization worldwide (of the Earth Europe, 2024). Among crop protection products, herbicides may be the most difficult to reduce because weeds are amongst the most important pests impacting crop production (Triantafyllidis et al., 2023). According to Oerke (2006), without weed control, yield losses due to competition with weeds might induce up to a 23% loss in wheat yield. In contrast, when weed control measures are implemented, yield losses are reduced to approximately 7.7%. Herbicide remains an efficient method to limit weed infestations, but herbicide-resistant weeds can evolve following the repeated use of herbicides with same mode of action (Heap, 2023). Furthermore, the global availability of new herbicide molecules in the market is extremely limited (Qu et al., 2021). In addition, herbicides can have adverse effects on the natural environment, including soil and groundwater contamination (Upadhyaya and Blackshaw, 2007: cited by Rueda-Ayala et al. (2010)).

Integrated weed management (IWM) is an attractive means of reducing herbicide use (Triantafyllidis et al., 2023). To minimize weed infestation and the need to control them, IWM promotes prophylactic measures through a combination of various crop system management practices, such as tillage and residue management, shifts in sowing dates, changes in crop rotations, etc. These preventive measures aim to reduce the need for systematic herbicide use. However, when necessary, curative measures, such as mechanical weeding or chemical weeding have to be considered as well (Triantafyllidis et al., 2023). Because non-chemical weeding techniques are usually not able to compete with herbicides in terms of efficacy, some authors have suggested that various practices should be combined (Pavlović et al., 2022).

In cereal crops, spring tine harrowing can be used to control weeds (Vanhala et al., 2004). Harrowing controls weeds mainly by covering and uprooting them (Kurstjens and Kropff, 2001; Kurstjens and Perdok, 2000). Uprooting has been identified as the main mechanism responsible for weed mortality (Cirujeda et al., 2003; Kolb et al., 2012; Kurstjens and Kropff, 2001). However, harrowing efficacy is highly variable (Naruhn et al., 2021) and highly dependent on weather and local soil conditions (Kurstjens and Kropff, 2001; Rueda-Ayala et al., 2010). Efficacy is also influenced by the developmental stage of weed species and the composition of the weed population (Kurstjens and Perdok, 2000; Rasmussen and Svenningsen, 1995; Rueda-Ayala et al., 2010). For example, weeds with tap roots have extensive secondary rooting structures that are less sensitive to harrowing (Melander et al., 2003). The effectiveness of harrowing has been demonstrated to be quite limited for perennial weeds and during the late growth stages of annuals weeds. For these reasons, previous authors have recommended that harrowing be conducted in pre-emergence or early post-emergence of crop stages (Kolb et al., 2012).

Unfortunately, early harrowing is rarely possible in oceanic climates with wet autumns, such as is the case in northern Europe (Melander et al., 2003). Under such conditions, harrowing often results in injuries to winter wheat (*Triticum aestivum* L.), due to displacement of soil particles that cover plantlets, or due to mechanical damage to plantlets, potentially leading to severe reductions in yield (Melander et al., 2003). Therefore, mechanical weeding should be performed in early spring when some weeds have already reached an advanced developmental stage and are more resistant to harrowing impacts. Rasmussen et al. (2010) demonstrated that detrimental effects of harrowing on crops could be minimized by adjusting the time of harrowing to the growth stage of the crop, provided the aggressiveness of the harrow is also adjusted to the growth stage. In addition, Rueda-Ayala et al. (2011) showed that it was preferable to use a harrow in late growth stages of a crop because a crop's ability to recover is improved.

The purpose of this study was to assess the impacts of various treatments involving the use of spring tine harrowing in conjunction, or not, with chemical weeding, and to determine the potential of the treatments to enhance the efficacy of weed control. The specific research objectives of this work were: (1) to evaluate whether the effects of mechanical weeding by harrowing may be a viable alternative to herbicides in winter wheat and to what extent the combined effects of harrowing and herbicide application may enhance weed control; and (2) to examine the impacts on yield of the weeding techniques and of the weeds group by emergence time. Additionally, (3) we analyzed the dynamics of weed control throughout cropping season, from the time of wheat tillering to wheat canopy closure, to further quantify the added effects of weeding techniques over the natural competition imposed by the main crop.

4. Materials and methods

4.1. Experimental site and design

Field experiments were conducted over six growing seasons (2010, 2011, 2012, 2013, 2021, and 2022) to assess the impacts of weed harrowing combined with chemical weeding on winter wheat production. The experiments were located in Hesbaye area (Belgium) on the fields of the experimental farm of Gembloux Agro-Bio Tech-University of Liège (50.56° N; 4.71° E), but the specific locations of the studies shifted annually. The climate in this region is oceanic temperate (Climate Cfd in the Köppen-Geiger classification), with an average annual rainfall of 793.4 mm, an annual average temperature of 9.6 °C, and an average solar radiation of 825 J cm^{-2} day^{-1}. The soil type is classified as Cutanic Luvisol (IUSS Working Group WRB, 2015) with a silt loam texture (18–22% clay, 70–80% silt, and 5–10% sand). The field was plowed with a mouldboard at 25 cm depth, and the seedbed was then prepared with a rotary cultivator. The crop was sown between mid-October and mid-November. Inter-rows spacing at planting was 125 mm. Seeding density was determined by sowing date and the regional recommendation, ranging between 225 seeds m^{-2} and 375 seeds m^{-2} (Table 3.1).

Table 3.1: Year, previous crop of winter wheat, sowing date (YYYY-MM-DD) and seeding rate for all six experiments performed between 2010 and 2013 and between 2021 and 2022.

Year	Previous crop	Sowing Date	Seed Rate (Seeds m^{-2})
2010	Sugar beet	2009-10-27	225
2011	Potato	2010-11-25	375
2012	Winter wheat	2011-10-20	250
2013	Rape seed	2012-10-19	250
2021	Chicory	2020-10-28	300
2022	Rape seed	2021-10-22	300

In our experiments, a two-factorial split-plot design was employed, comprising four replicated blocks. The main plots were dedicated to weed harrowing treatment, with herbicide treatments applied as a sub-plot treatment. The sub-plot sizes were 28 m² (7 × 4 m) for experiments between 2010 and 2013 and 16 m² (2 × 8 m) for experiments from 2021 to 2022. Treatments were randomly attributed among the plots each year. Weed harrowing was performed parallel to crop rows sowing. Harrowing consisted in 0, 1, or 2 passes of the harrow (Model: Aerostar-200, manufactured by Einboeck GmbH & CokG, Austria). The various harrow passes were performed at the full tillering stage [about phenological development stage 29 of wheat (BBCH29)] for the first pass and at stem extension (BBCH 30) for the second pass (Table 3.2). The intensity and speed of harrowing (4–7 km h^{-1}) were adjusted for each pass based on winter wheat growth stage, weed size, and pedoclimatic conditions. Within the same field as the experiment, but outside the experimental plots, we conducted a visual assessment of the tine's penetration ability, its capacity to uproot weeds, and the extent of wheat cover. The tine settings were then adjusted prior to employing the harrow in the experimental plots, which then remained the same during harrowing treatment. The aggressiveness of harrowing during second passes was often set to its maximum (7 km h^{-1}, and most aggressive tine setting) because larger weeds were present and the crop was more mature and better able to withstand the more aggressive harrowing. Soil conditions were usually dry during harrowing treatments.

Herbicide treatments consisted of (1) no herbicide (zero dose or 0D) or (2) a full dose of herbicide (1D). Herbicide application was carried out using a hand-held boom sprayer, covering the width of the subplot, using a spray volume of 200l/ha. The herbicides applied were determined prior each trial, using recommended products in the region (www.cereales.be), while the dosage applied corresponded to the product-specific legal dose under Belgian legislation (all information available on www.fytoweb.be). In 2010, 30g/ha of amidosulfuron (Gratil, 750g a.i /kg (acetolactate synthase (ALS) inhibition), WG, Bayer cropscience) and 6g/ha of metsulfuron-methyl (Allie, 200g a.i. /kg (ALS inhibition), SG, FMC chemical) were used. In 2011, 2012, and 2013, respectively 60g/ha, 3g/ha,9.36g/ha and 27g/ha of diflufenican, iodosulfuron-methyl-sodium, mesosulfuron-methyl-sodium and mefenpyr-diethyl (Othello 50g/L of diflufenican (phytoene desaturase (PDS) inhibition), 2.5g/L of iodosulfuron-methyl-sodium (ALS inhibition), 7.8g/L of mesosulfuron-methyl-sodium (ALS inhibition), 22.5g/l of mefenpyr-diethyl (Safener),OD, Bayer cropscience) were applied, and respectively 300g/ha and 360g/ha of bifenox and mecoprop-P (Verigal D 250g/L of bifenox (protoporphyrinogen oxidase (PPO) inhibition) and 300g/L of mecoprop-P (auxin mimics), SC, Adama registrations B.V.) were employed. In 2021 and 2022, respectively 3.78 g/ha and 49.98g/ha of florasulam and tritosulfuron (Biathlon Duo 54g/kg of florasulam (ALS inhibition) and 714g/kg of tritosulfuron (acetyl-Coenzyme A carboxylase (ACCase) inhibition), WG, BASF Belgium Coordination Center) were applied. Table 3.2 presents dates of data collection for counting weeds in plots and for applying herbicides and the types of active ingredients in herbicides applied, by year. Although herbicide was applied before T1 (see Table 3.2), none of the herbicide applications resulted in a reduction in weeds at the T1 count because the herbicides did not have sufficient time to fully affect the treated weeds.

Table 3.2: Dates weeding operations and weed counts in plots were performed. Herbicides are foliar-applied with no residual activity. T0= before weed control, T1= after one harrowing pass, T2= after two harrowing passes, and T3= when wheat canopy was closed.

Year	Date	Operation	Active ingredients and mode of actions
2010	2010-04-12	Initial counting (T0)	
	2010-04-15	first pass of harrow	
	2010-04-19	herbicide application	amidosulfuron (ALS inhibition) metsulfuron-methyl (ALS inhibition)
	2010-04-21	counting T1	
	2010-04-27	second pass of harrow	
	2010-05-04	counting T2	
	2010-05-19	counting T3	
2011	2011-03-23	Initial counting (T0)	
	2011-03-24	first pass of harrow	
	2011-03-29	counting T1	

Continued on next page

Table 3.2 continued from previous page

Year	Date	Operation	Active ingredients and mode of actions
	2011-04-13	herbicide application	mefenpyr-diethyl (Safener), diflufenican (PDS inhibition), mesosulfuron-methyl-sodium (ALS inhibition), iodosulfuron-methyl-sodium (ALS inhibition) bifenox (PPO inhibition), mecoprop-P (auxin mimics)
	2011-04-15	second pass of harrow	
	2011-04-20	counting T2	
	2011-07-14	counting T3	
2012	2012-03-26	herbicide application	mefenpyr-diethyl (Safener), diflufenican (PDS inhibition),mesosulfuron-methyl-sodium (ALS inhibition), iodosulfuron-methyl-sodium (ALS inhibition) bifenox (PPO inhibition), mecoprop-P (auxin mimics)
	2012-03-27	Initial counting (T0)	
	2012-03-27	first pass of harrow	
	2012-04-02	counting T1	
	2012-04-06	second pass of harrow	
	2012-04-19	counting T2	
	2012-05-31	counting T3	
2013	2013-04-18	Initial counting (T0)	
	2013-04-18	first pass of harrow	
	2013-04-24	herbicide application	mefenpyr-diethyl (Safener), diflufenican (PDS inhibition),mesosulfuron-methyl-sodium (ALS inhibition), iodosulfuron-methyl-sodium (ALS inhibition) bifenox (PPO inhibition), mecoprop-P (auxin mimics)
	2013-05-02	counting T1	
	2013-05-03	second pass of harrow	
	2013-05-21	counting T2	
	2013-07-11	counting T3	
2021	2021-03-15	Initial counting (T0)	

Continued on next page

Table 3.2 continued from previous page

Year	Date	Operation	Active ingredients and mode of actions
	2021-03-30	first pass of harrow	
	2021-03-31	herbicide application	tritosulfuron (ALS inhibition), florasulam (glsACCase inhibition)
	2021-04-14	counting T1	
	2021-04-16	second pass of harrow	
	2021-05-03	counting T2	
	2021-06-21	counting T3	
2022	2022-03-03	Initial counting (T0)	
	2022-03-10	first pass of harrow	
	2022-03-22	herbicide application	tritosulfuron (ALS inhibition), florasulam (ACCase inhibition)
	2022-04-06	counting T1	
	2022-04-20	second pass of harrow	
	2022-04-28	counting T2	
	2022-06-03	counting T3	

Weather conditions 5 d before and 10 d after weed harrowing treatments were collected from the Sombreffe weather station, located 5 km from Gembloux (Pameseb, 2023). Cumulated daily total rainfall and cumulated daily mean temperature were recorded from 5 d before to 10 d after weed harrowing treatments (Table 3.3).

Table 3.3: Cumulated daily total rainfall [mm] and cumulated daily mean temperature [°C] for five days before to 10 days after weed harrowing.

Harrowing:	First pass		Second pass	
	$\sum$ rainfall (mm)	$\sum$ T (°C)	$\sum$ rainfall (mm)	$\sum$ T (°C)
Year				
2010	0.1	126.3	15.1	160.6
2011	16.2	145.5	0.6	209.4
2012	0.0	138.0	20.2	98.2
2013	7.0	177.8	31.7	182.6
2021	9.0	119.9	4.5	102.8
2022	1.9	113.0	1.7	178.8

4.2. Data collection and transformation

4.2.1. Grain yield normalization

Grain yields were characterized from a reference area of 2 × 5 m within the sub-plot between 2010 and 2013 and over the entire experimental sub-plot of 2 × 8 m between

2021 and 2022. Yields were expressed at 15% water content. To allow an inter-annual analysis, standardized relative yields were calculated. for each year, The standardized relative yields were normalized on an annual scale using Eq.3.1:

$$\text{Yield}_{\text{ni},y} = \frac{Y_{i,y} - Y_{\text{H},y}}{Y_{\text{H},y}} \tag{3.1}$$

Where, *i* is a given plot, *y* is a given year, $Yield_{ni,y}$ represents the standardized relative yield of plot i and year *y*, $Y_{i,y}$ and $Y_{H,y}$ are, respectively, the yield of a specified plot *i* and the average yield obtained across the treatments involving using only herbicide without weed harrowing (considered as the standard farming practice), for the same given year *y*.

4.2.2. Counting and classifying weeds

Weed density (from counts) was calculated before and after all weed harrowing treatments. Counts of weeds were made at temporal intervals (Tn), hereafter labelled T0, T1, T2, and T3, where T0 is the initial sampling (BBCH- 27-29), T1 is the sampling after first harrowing (BBCH 29-30), T2 is the sampling after the second harrowing (BBCH 30-31), and T3 corresponds to the last sampling performed when the wheat canopy closure has happened (data were collected between flag leaf (BBCH 39) and mid-grain filling (BBCH 75)). Between 2010 and 2013, we measured weed density in six random quadrats per sub-plot (0.44 m × 0.57 m), whereas in 2021 and 2022, measurements were made, respectively, in two and one random quadrat per sub-plot (0.50 m × 0.50 m). For each year, measurements were performed in the same location throughout the growing season.

Weeds were recorded by species and later aggregated into "broadleaf" and "grass weed" categories. Weed density was furthermore separated in three different temporal groups, specifically old weeds (OW), newly emergent weeds (NE), and new species of weeds (NS). The OW group was comprised of weeds that had already established in plots at dates T0 and T1. OWs would be expected to also be present at dates T2 or T3 if not controlled by weed harrowing or herbicide application. The growth of OW was considered synchronous with that of winter wheat. Conditions at T1 were used as reference to comparing with other weed groups. The NE group was comprised of weeds belonging to species already surveyed at date T1, but whose abundances had increased due to emergences of more individuals after time T1. Finally, NS are individuals belonging to weed species appearing only after date T1. This group was comprised of spring and summer weed species.

4.3. Data analysis

4.3.1. Analysis of variance

Homogeneity of variance and normality of distributions of data were tested first with the DHARMa package (see software section). A linear mixed-effects model was implemented for normalized yields, with weed harrowing and herbicide application defined as a fixed effect and plots (due to the split-plot layout) within a given year as a random effect.

As proposed by Vanhala et al. (2004), generalized mixed-effects models with densities of total OW, broadleaf OW and grass OW were performed. Two different generalized linear mixed models were used, each with specific objectives. The aim of the first model (qualified as an auto-regressive model and hereafter referred to as Model 1) was to assess the impact of weeding methods at each sampled date (Tn) while accounting for the previous weed infestation. To achieve this objective, herbicide application and weed harrowing passes were adjusted as fixed factors. Weed density at the previous sampling was included as a covariate (sensu Cirujeda et al. (2003); Vanhala et al. (2004)). The random effect encompassed a structured component involving the subplot (due to repeated measurements) within a plot (due to the split-plot layout), which in turn, were nested within year of sampling. Year was included as a random variable. As the data were counts, a Poisson distribution (or negative binomial in the case of overdispersion) was used with a log link function. The sampled subplots areas were included as an offset parameter (due to the different sampled areas between years) within the model (Zuur et al., 2009). A generic expression of model 1 was expressed following Eq.3.2. Specific models for each counting time and weeds categories are detailed in Table A.1.

$$\begin{aligned} \text{OWcategories}_{\text{Tn}} = \text{Herbicide} * \text{Weed harrowing} + log(\text{OWcategories}_{\text{Tn-1}} + 1) \\ +1|(\text{sub-plot/plot/year}). \\ \text{Offset} = log(\text{sampled area}), \\ \text{family distribution} = \text{poisson or negative binomial} \end{aligned} \tag{3.2}$$

The aim of the second model (qualified as global model and hereafter referred to as Model 2) is to show the overall effect of the various weed control methods, across the whole season. To pursue this objective, a model estimating weed density at date T3 was applied. The fixed effects were the same as in Model 1 (i.e., herbicide application and weed harrowing passes). Weed density at date T0 was added as a covariate. The random effects were identical to Model 1. As the data were count data, a Poisson distribution was used with a log link function. the sampled subplots areas were included as an offset parameter (due to the different sampled areas between years) within the model (Zuur et al., 2009). A generic expression of model 2 was expressed following Eq.3.3. Specific models for each weeds categories are detailed in Table A.1.

$$\begin{aligned} \text{OWcategories}_{\text{T3}} = \text{Herbicide} * \text{Weed harrowing} + log(\text{OWcategories}_{\text{T0}} + 1) \\ +1|(\text{sub-plot/plot/year}). \quad (3.3) \\ \text{Offset} = log(\text{sampled area}), \text{family distribution} = \text{poisson} \end{aligned}$$

For the grass OW category, only 2010 and 2013 data were used because these two datasets were the only years with sufficient grass weed abundances. The presence of only two years of data was deemed too low to be included as random effects. Therefore, year was included as a fixed factor. An analysis of variance (ANOVA) was performed on the statistical models followed by an estimated marginal means analysis. Details of all models can be found in supplementary material (Table A.1).

4.3.2. Correlations between weed density and crop yield

Spearman rank correlations between normalized yield and weed density (by total and by weed type: grass and broadleaved) for the various samplings were computed. Spearman tests were applied to the data because data did not always follow a normal distribution. Correlations were further computed by weed group (OW, NE, NS).

4.3.3. Dynamics of weed control

Percentage weed control (WC) can be expressed following the equation of Rasmussen et al. (2009, 2010). To our knowledge, the equation of Rasmussen has never been used to compare treatments in which techniques of control are implemented over time during a growing season. Prior analyses revealed that the original equation proposed by Rasmussen et al. (2009, 2010) exhibited a lack of fit in our situation, especially for the control treatment. Therefore, a sigmoid equation to better represent our study approach was proposed (Eq.3.4).

$$\mathrm{WC}_i = 100\left(1 - \frac{W_{ti}}{W_{t0i}}\right) = 100\left(\frac{a}{1 + e^{-b \cdot C + 10}}\right) \tag{3.4}$$

Where WC_i represents the weed control for treatment "*i*" W_{ti} is the weed density observed under treatment "*i*" at count "*t*", W_{t0i} denotes the number of weeds for treatment "*i*" at T0, before any weeding operation (intended to account for a normalization by initial weed density and the subsequent aggregation of data over years), parameters "*a*" and "*b*" control the shape of the curve, and *C* is date of sampling. Parameter "*a*" reflects the plateau of WC, whereas parameter *b* controls the slope of the curve. Therefore, parameter "*b*" influences the rate at which maximum WC is achieved over time. The date of sampling is the date at which the weed survey was conducted after each weed harrowing operation and after wheat canopy closure.

The reason for re-expressing WC as Eq.3.4 was to account for the dynamic aspects of treatments applied over time, and the corresponding changes in weed densities over time. We also believe that this formalization provides a way to represent the main crop's natural ability to compete against weeds. Thus, the WC value of the treatment without active weed control characterizes of the effects of natural (unassisted) competition by wheat, disease mortality, and natural mortality. The Eq.3.4 was tested for lack of fit and root-mean-squared errors (RMSE) were computed. Eq.3.4 was calculated on OW only. For each combination of herbicide and harrowing treatment, Eq.3.4 was fit to data from individual replicates across all years (n= 96 per sigmoid). For clarity, WC means across replicates and years are presented in the figure in the main body of the paper. Average annual WC data are presented in supplementary materials (Figure A.1).

4.4. Software

Data were analyzed with the statistical software package R (Version 4.05). GLMM and a linear Mixed Effects Model were performed with the package lme4 or the package glmmTMB. Model diagnostics were examined with the DHARMa package. Estimated marginal means analyses were achieved with the emmeans package. Parameters of the WC equation were fitted with the nls function. Lack of fit test was calculated with the qpcR package.

5. Results

5.1. Initial weed population

The lowest initial weed infestation was observed in 2012 with 6 weeds m^{-2} and the highest initial weed density was recorded for 2021 at 191 weeds m^{-2} (Table 3.4). Grass weeds were recorded in 2010 (*Poa annua* as dominant grass, 17% of total weed density) and in 2013 (*Alopecurus myosuroides* as dominant grass, 76% of total weed density). Grass weeds were also present in 2011, but did not dominate plots. *Matricaria chamomilla* was thedominant broadleaf weed in 2010, 2011, 2012, and 2021, but was only the third most abundant weed in 2022 (at 20%).

5.2. Changes in densities of weed populations over time

We found that changes in weed populations within a cropping season varied from year to year (Figure 3.1). OW tended to decrease in density over a growing season across all treatments, including in the control treatment. However, the extent of the reduction in OW density was greater in plots where weed control techniques were applied. Herbicide application tended to provide the greatest and most stable reduction in OW density. The declines in OW densities after weed harrowing varied by year as well, with noticeably lower densities occurring in 2010, 2011, and 2012, whereas OW densities remained high in 2021.

NE weeds were observed every year between 2010 and 2013. In contrast, there were almost no NE weeds in 2021, even though the initial weed density was much higher in 2021 than in other years. In 2022, despite an initially moderate weed density, no NS emerged (Figure 3.1). The highest densities of NS were recorded in 2012 after one and two passes of harrowing, but densities were even higher when harrowing was not coupled with herbicide application.

Table 3.4: Annual average initial weed density and dominant weed species before weed control, computed from measurements across the whole experimental area.

Year	Weed density [m^{-2}]	Dominant species
2010	13	*Matricaria chamomilla* L. (59%)
		Poa annua L. (17%)
		*Galium aparine*L. (12%)
2011	10	*Matricaria chamomilla* L. (45%)
		Veronica hederifolia L. (44%)
		Alopecurus myosuroides Huds. (8%)
2012	6	*Matricaria chamomilla* L. (30%)
		Veronica persica L. (14%)
		Capsella bursa-pastoris (L.) Medick. (11%)
		Viola arvensis Murray (11%)
		Veronica hederifolia L. (8%)
2013	20	*Alopecurus myosuroides* Huds (76%)
		Galium aparine L. (14%)
		Veronica hederifolia L. (8%)
2021	191	*Matricaria chamomilla* L. (94%)
		Polygonum aviculare L. (3%)
2022	35	*Papaver rhoeas* L. (36%)
		Galium aparine L. (22%)
		Matricaria chamomilla L. (20%)
		Veronica hederifolia L. (13%)

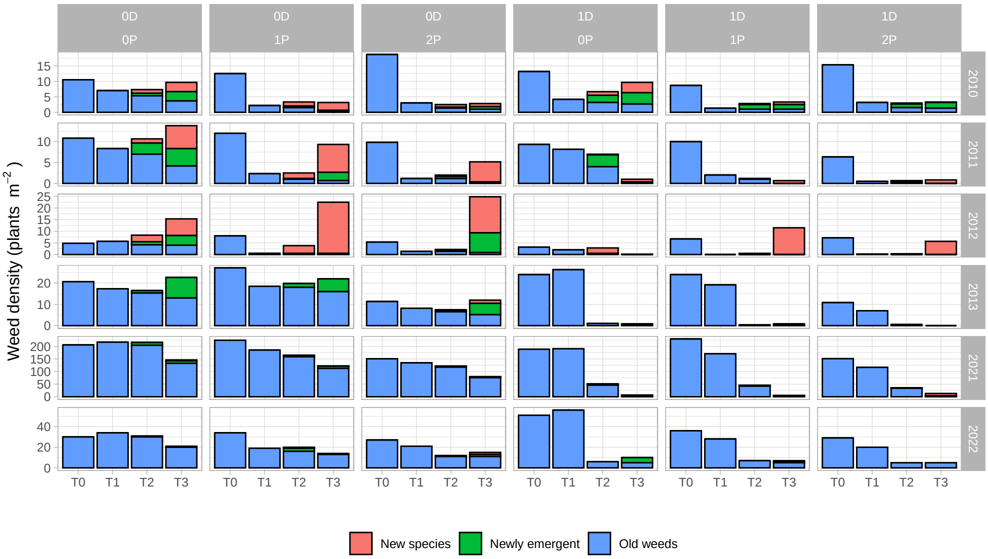

Figure 3.1: Weed density by treatment and year at different samplings (T0= before weed control, T1= after one harrowing pass, T2= after two harrowing passes, and T3= when wheat canopy was closed.) Old weeds are comprised of all weeds that were already in plots at times T0 and T1. Newly emergent is comprised of new seedling of weed species already surveyed at time T1. New species are weed species appearing only after time T1 and that species wasn't present before. 0D is no chemical weeding, 1D is full chemical weeding, 0P is no harrowing, 1P is one harrowing pass, 2P is two harrowing passes.

5.3. Effect of herbicide application and weed harrowing on yield

There was not an interactive effect between weed harrowing and herbicide treatments for normalized yield (p-value = 0.336), and weed harrowing did not affect normalized yield (p-value = 0.58). Only the use of herbicide influenced yield significantly (p-value = 0.0039), specifically within a 0.99 average normalized yield when the herbicide was used (averaged across all weed harrowing treatments) relative to an average of 0.92 without herbicide application. This herbicide treatment effect translates to an average increase in yield of 7% (herbicide applications vs. absence of herbicide treatment).

5.4. Correlation between yield and weed categories

Correlations between normalized yields and weed density are reported by weed category in Table3.5. Correlations were computed using data collected across all treatments and years and are reported separately for density at each sampling period (T0 to T3). Total weed densities exhibited negative correlations with yields, ranging from -0.28 (T0) to -0.36 (T2), depending on the time of observation (see the relationship between normalized yield and weeds density at T3 in Figure A.2). Similarly, both broadleaf and grass weeds showed significant negative correlations with yield, except at time T3 for broadleaved weeds, for which no significant correlation was found. The strength of correlations increased over time, changing from no significant correlation at time T0 to -0.24 for broadleaf weeds at time T2 and to -0.28 for grass weeds at time T3.

When looking at the three different weed groups, OW displayed the highest negative significant correlation (-0.44) with normalized yield at T3. NE exhibited a negative correlation with yield in T3 for total NE and grass NE, but no significant correlation was observed with broadleaved NE. The NS group was positive and significantly correlated with yield at time T3 for both broadleaved weeds (0.18) and total weeds (0.17).

5.5. Effect of herbicide application and weed harrowing on densities of old weeds

Considering the relatively strong correlations between OW and normalized yield, we focused subsequent analyses on the OW category. Average weed density of total OW, broadleaved OW, and grass OW are shown in Table 3.7 by treatment and reported under each assessed treatment. Model 1, used to evaluate the efficacy of weeding operations at each time step, never showed any interaction between herbicide application and weed harrowing treatments (p-value>0.05). For total OW and broadleaf OW, the weed harrowing treatment was significant at time T1. At that time, the first harrowing reduced weed density from 53 weeds m^{-2} at T0 to 37 weeds m^{-2} at T1 relative to no harrowing treatment, in which no reduction in weed density occurred. On the other hand, the second harrowing had no additional effect on OW densities because weed

Table 3.5: Correlation between normalized yield and weed density observed at each sampling period, across all treatments and years. The gradient of colour indicates the level of the correlation between the variable and yield. The more positive the correlation, the more intense the green colour, and the more negative the correlation, the more intense the red colour. Only significant correlations are reported. Time T0= before weed control, T1= after one harrowing pass, T2= after two harrowing passes, and T3= when wheat canopy was closed. Old weeds are comprised of all weeds that were already in plots at times T0 and T1. Newly emergent is comprised of new seedling of weed species already surveyed at time T1. New species are weed species appearing only after time T1 and that species wasn't present before.

	T0	T1	T2	T3		T0	T1	T2	T3
					Old weeds	-0.28	-0.31	-0.38	-0.44
Total weeds	-0.28	-0.31	-0.36	-0.32	Newly emergent				-0.27
					New species				0.17
					Old weeds		-0.18	-0.28	-0.30
Broadleaf weeds		-0.18	-0.24		Newly emergent				
					New species				0.18
					Old weeds		-0.19	-0.28	-0.27
Grass weeds		-0.19	-0.24	-0.28	Newly emergent				-0.27
					New species				

densities reported at T2 (after two passes) did not significantly differ from density after one pass. Densities for both total OW and broadleaved OW were significantly different after herbicide application at T2 and T3. After herbicide application, total OW density decreased from 48 weeds m^{-2} at T1 to 10 weeds m^{-2} at T2, and was further reduced to two weeds m^{-2} at T3. For OW grass (evaluated only for years 2010 and 2013), the first pass of harrow was effective in reducing weed density in 2013, but not effective in 2010. Herbicide application was very effective (weed density approximately zero) at time T2.

Model 2, which was used to evaluate the overall effect of weed control treatments for the entire growing season, was influenced by both herbicide and harrowing weed control techniques on total OW and broadleaf OW. No interaction between treatments occurred. Greater reductions of weed density occurred after herbicide application relative to reductions after harrowing. Weed density after one pass of harrowing decreased from 53 weeds m^{-2} at time T0 to 24 weeds m^{-2} at T3; for the same timeframe, weed density after herbicide application decreased from 48 weeds m^{-2} to 2 weeds m^{-2}. With one pass of harrowing and herbicide application (applied concurrently), weed density decreased from 52 weeds m^{-2} at T0 to one weed m^{-2} at T3. Model 2 results showed that OW grass density responded only to herbicide application (weed density was close to zero). Harrowing of weeds had no effect on the overall density of grasses, suggesting that the effect of harrowing on density observed under Model 1 (at T1 for year 2013) was probably due to the natural competitiveness of winter wheat against grasses

Table 3.6: Average for old weed density per m² and standard deviation (in parenthesis) for the various treatments, by samplings (data for six years). Old weeds are comprised of all weeds that were already present within plots at times T0 and T1 Treatment values with the same upper-case letter and lower-case letter do not differ significantly from one another, according to a Tukey adjustment at $p < 0.05$. Model 1 compared treatments at each time of data collection (counts in plots), Model 2 evaluates the overall effect of weed control treatments throughout the growing season. Average effect is the average result over the other factors (in column across herbicide application and in line across weed harrowing). Letters in upper case refer to Model 1, whereas letters in lower case refer to Model 2. Grass weed models are based solely on 2010 and 2013 data. Letters with the * symbol mean that the effect is only significant for 2013. T0= before weed control, T1= after one harrowing pass, T2= after two harrowing passes, and T3= when wheat canopy was closed. 0D is no herbicide, 1D is full herbicide application.

			Herbicide						
Timing		**Weed harrowing**	0D		1D		**Average effect**		
	Total weeds	0 Pass	47.06	(94.68)	48.19	(90.29)	**47.63**	**(91.52)**	-
		1 Pass	52.94	(105.70)	52.39	(112.18)	**52.67**	**(107.82)**	-
		2 Passes	37.11	(61.00)	36.61	(60.74)	**36.86**	**(60.22)**	-
		Average effect	**45.70**	**(88.16)**	**45.73**	**(89.20)**			
			-		-				
	Broadleaf weeds	0 Pass	43.81	(95.81)	45.11	(91.41)	**44.46**	**(92.64)**	-
T0		1 Pass	49.03	(106.97)	48.39	(113.49)	**48.71**	**(109.10)**	-
		2 Passes	35.06	(61.94)	34.31	(61.75)	**34.68**	**(61.19)**	-
		Average effect	**42.63**	**(89.20)**	**42.60**	**(90.28)**			
			-		-				
	Grass weeds	0 Pass	9.00	(7.57)	9.00	(6.80)	**9.00**	**(6.95)**	-
		1 Pass	11.17	(10.89)	11.17	(10.00)	**11.17**	**(10.10)**	-
		2 Passes	5.50	(6.53)	5.92	(6.24)	**5.71**	**(6.17)**	-
		Average effect	**8.56**	**(8.50)**	**8.69**	**(7.82)**			
			-		-				
	Total weeds	0 Pass	48.14	(93.49)	47.86	(92.04)	**48.00**	**(91.77)**	B
		1 Pass	37.92	(81.62)	36.83	(75.92)	**37.38**	**(77.98)**	A
		2 Passes	28.19	(55.09)	24.56	(47.94)	**26.38**	**(51.12)**	A
		Average effect	**38.08**	**(77.71)**	**36.42**	**(73.81)**			
			ns		ns				
	Broadleaf weeds	0 Pass	45.97	(94.33)	44.25	(93.26)	**45.11**	**(92.80)**	B
		1 Pass	35.58	(82.24)	33.94	(76.84)	**34.76**	**(78.74)**	A
T1		2 Passes	27.03	(55.54)	23.42	(48.41)	**25.22**	**(51.57)**	A
		Average effect	**36.19**	**(77.37)**	**33.87**	**(72.84)**			
			ns		ns				
	Grass weeds	0 Pass	2.06	(3.17)	3.61	(2.39)	**2.83**	**(2.66)**	-
		1 Pass	2.34	(1.53)	2.89	(1.56)	**2.61**	**(1.55)**	-
		2 Passes	2.05	(2.89)	1.14	(2.89)	**1.60**	**(2.89)**	-
		Average effect	**2.15**	**(2.53)**	**2.55**	**(2.28)**			
			ns		ns				
	Total weeds	0 Pass	44.31	(90.08)	10.08	(18.59)	**27.19**	**(66.63)**	ns
		1 Pass	32.56	(68.67)	8.56	(17.39)	**20.56**	**(51.02)**	ns
		2Passes	23.14	(49.79)	6.78	(14.10)	**14.96**	**(37.13)**	ns
		Average effect	**33.33**	**(70.96)**	**8.47**	**(16.62)**			
			B		A				
	Broadleaf weeds	0 Pass	42.33	(90.84)	9.83	(18.69)	**26.08**	**(66.92)**	ns
		1 Pass	30.42	(69.31)	8.44	(17.38)	**19.43**	**(51.20)**	ns
T2		2Passes	22.17	(50.14)	6.64	(14.16)	**14.40**	**(37.28)**	ns
		Average effect	**31.64**	**(71.51)**	**8.31**	**(16.67)**			
			B		A				
	Grass weeds	0 Pass	5.92	(5.79)	0.75	(1.26)	**3.33**	**(4.85)**	ns
		1 Pass	6.42	(6.96)	0.08	(0.24)	**3.25**	**(5.78)**	ns
		2Passes	2.92	(4.26)	0.42	(0.79)	**1.67**	**(3.23)**	ns
		Average effect	**5.08**	**(5.74)**	**0.42**	**(0.88)**			

Continued on next page

Table 3.6 – continued from previous page

				B		A					
										model1	model2
	Total weeds	0 Pass		29.56	(54.34)	2.25	(3.07)	**15.90**	**(40.50)**	ns	b
		1 Pass		23.78	(48.76)	1.56	(3.06)	**12.67**	**(35.97)**	ns	a
		2Passes		15.64	(29.86)	1.39	(4.07)	**8.51**	**(22.28)**	ns	a
		Average effect		**22.99**	**(45.26)**	**1.73**	**(3.41)**				
			model1		B		A				
			model2		b		a				
	Broadleaf weeds	0 Pass		27.58	(55.13)	2.00	(3.07)	**14.79**	**(40.73)**	ns	b
T3		1 Pass		21.64	(49.35)	1.44	(3.03)	**11.54**	**(36.06)**	ns	a
		2Passes		14.72	(30.20)	1.31	(4.08)	**8.01**	**(22.37)**	ns	a
		Average effect		**21.31**	**(45.79)**	**1.58**	**(3.39)**				
			model1		B		A				
			model2		B		A				
	Grass weeds	0 Pass		5.92	(5.79)	0.75	(1.26)	**3.33**	**(4.85)**	ns	ns
		1 Pass		6.42	(6.96)	0.08	(0.24)	**3.25**	**(5.78)**	ns	ns
		2Passes		2.75	(4.27)	0.25	(0.71)	**1.50**	**(3.22)**	ns	ns
		Average effect		**5.03**	**(5.77)**	**0.36**	**(0.86)**				
			model1		ns		ns				
			model2		b		a				

5.6. Dynamic models for density of old weeds

Tests assessing the lack of fit were applied to regressions performed with Eq.3.4 Fitted models were considered to properly describe data across all treatments (p-value > 0.05 for each regression). Average weed control parameters (a and b) and their 95% confidence intervals are reported in Table 3.7. The RMSE values when no herbicides were applied and under 0, 1, and 2 harrowing were0.31, 0.28, and 0.51 respectively, while they equaled 0.27, 0.22, and 0.19, respectively when herbicides were applied and under 0, 1, and 2 harrowing.

The dynamic representation of WC, applied to total OW and in response to the tested weed harrowing and herbicide treatments are shown in Figure 3.2. The sigmoid curves obtained showed a 38% reduction in weed density at T3 for the control treatment (without weed control). Without herbicide application, similar levels of WC efficacy were observed for one pass of harrowing (70%) and two harrowing passes (64%). Herbicide application alone was more effective (93%) than harrowing weed control alone.

Curves representing change over time in weed control showed that weed control by harrowing leads to an earlier reduction in density of weeds (i.e., at T1) relative to application of herbicide alone (Figure 3.2), as confirmed by the values of parameter "*b*" (Table3.7). This difference in weed reduction appeared to be mainly a response to the timing of the herbicide application, which usually occurred after the first harrow (Table 3.2). A combination of herbicide and weed harrowing tended to improve the efficacy of WC (i.e., increase parameter "*a*") while also improving the probability of exerting greater control (narrower confidence interval).

Table 3.7: Weed control parameters "*a*" and "*b*" reported for each treatment applied to total density of old weeds. Average values and their 95% confidence intervals (CI) are reported. Parameter "*a*" reflects the plateau of WC, whereas parameter "*b*" determines the slope of the curve. 0D is no herbicide, 1D is full herbicide application, 0P is no harrowing, 1P is one harrowing pass, and 2P is two harrowing passes. Average effect is the average result over the other factors (in column across herbicide application and in line across weed harrowing).

Weed Control parameter a and b (95% CI)						
Total weeds						
	Parameter a			Parameter b		
	Herbicide treatment			Herbicide treatment		
Weed harrowing treatment	0D	1D		0D	1D	
0P	0.40 (0.25-0.54)	0.93 (0.82-1.04)	**0.66 (0.55-0.77)**	3.32 (2.79-3.84)	3.84 (3.52-4.18)	**3.66 (3.33-3.98)**
1P	0.70 (0.62-0.78)	0.92 (0.86-0.99)	**0.81 (0.76-0.86)**	5.62 (5.09-6.16)	5.14 (4.93-5.35)	**5.33 (5.10-5.56)**
2P	0.64 (0.49-0.78)	0.94 (0.88-0.99)	**0.79 (0.70-0.87)**	5.17 (4.44-5.89)	5.26 (5.07-5.45)	**5.22 (4.89-5.56)**
	0.54(0.47-0.61)	**0.91 (0.86-0.95)**		**5.07 (4.68-5.46)**	**4.86 (4.71-5.00)**	

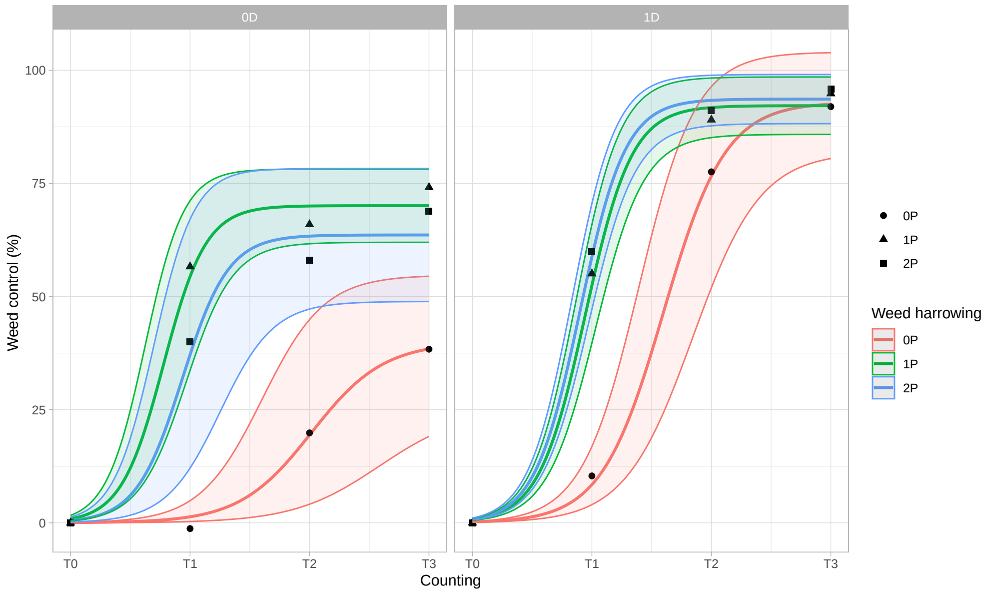

Figure 3.2: Change over time of weed control (WC) of OW for all treatments based on the Eq3.4 Sigmoid WC for each treatment were fit across all individual replicates and across all years (n= 96 per sigmoid). T0= date before weed control, T1= after one pass of harrowing, T2= after two passes of harrowing, and T3= when wheat canopy was closed. 0D is no herbicide, 1D is full herbicide application, 0P is no harrowing, 1P is one harrowing pass, 2P is two harrowing passes. The points correspond to the mean of observed data across all years and replicates, whereas the curves correspond to fitted observations. The sigmoid curves with annual observed means per year and treatment are presented in Figure A.1

6. Discussion

Within a cropping season, the fluctuation of weed density and recruitment of additional species of weeds vary from year to year depending on local weather conditions and specific crop management practices. In this study, although not consistent across years, some crop seasons were characterized by high densities of OWs and/or possibly differing densities of NE or NS seedlings. For example, in our study, OW density was very high in 2021, whereas NE and NS weeds were very abundant in 2012. In 2012, cumulative rainfall reached 20 mm (Table 3.3) around the time of second harrowing (5 d before plus 10 d after), providing well-moistened soil. Given the very shallow tilling of the upper soil associated with harrowing coupled with moist soil in 2012, conditions were probably favorable for seed germination, which likely led to the emergences of NE and NS. In contrast, in 2021 and 2022, there was not much rainfall around the times of weed harrowing and relatively few new seedlings were counted.

Over the 6 years of this study, no statistical differences in yield were measured among any of the weed harrowing treatments and control treatments. Previous authors reporting a lack of response of yield to mechanical weeding have hypothesized that competition from weeds may be insufficient to impact yield (Gerhards et al., 2021; Rasmussen et al., 2010; Sobkowicz and Tendziagolska, 2022). Yet, in our trial weed density was around 10-fold higher during the 2021 crop season relative to other years (Table 3.4); in this specific year, harrowing showed a positive relationship with yield (data not explicitly shown). Furthermore, although an impact of harrowing on yield is frequently not observed, harrowing is probably beneficial in that it can potentially reduce the rate at which weed seeds contribute to the seedbank (Mertens and Jansen, 2002; Pannacci and Tei, 2014). Another potential explanation for the apparent lack of effect of weed harrowing on yield is the selectivity between crop and weeds. Indeed injuries to crop could reduce yield and counterbalance the decline of weed:crop competition (Rasmussen et al., 2009). Data were not collected specifically on this subject in our study. However, we obtained a standardized yield of 0.99 when herbicides were applied across all weed harrowing treatments, suggesting that there were very few, if any, problems associated with selectivity between crop and weeds.

Among the various weed categories, total density of OW exhibited the strongest negative correlation with yield (-0.44 at time T3). The OW population is composed of weeds that grow synchronously with wheat; therefore, they tend to have a greater negative impact on yield because they are directly competing for resources in early growth stages (Horvath et al., 2023; Wilson and Wright, 1990; Zimdahl, 2007b). In contrast, NS exhibited a slightly positive correlation with yield. However, this correlation does not necessarily imply causality. If NS germinate during a growing season characterized by a higher yield, both NS and yield would be expected to be similarly enhanced. Thus, the more meaningful relationship would be between a favorable growing season (in which yields tend to be higher) and the germination of new weed species, rather than between NS and yield. Even so, our results suggest that NS densities did not impact yield negatively. Because NS germinate when wheat is already well established, wheat plants can out-compete new weeds (Bagavathiannan and Norsworthy, 2012). A similar interaction may explain non-effects of NE of pre-existing broadleaf weeds on crop yield.

The negative correlation between yield and NE density of pre-existing grass weeds may be consequent to the physiological similarity of grass weeds to cereal crops like wheat (Zimdahl, 2007b). It is also possible that given the morphological similarity of some grass species with emerging wheat, some weeds may have been misclassified as "new emergent" due to not being identified as weeds at time T0 or T1. From our results, it seems clear that the significant negative correlation between yield and total density of NE weeds can be explained mostly by grass weeds. Recording the early growth stages of grass weeds during weed counts could reduce potential NE versus OW classification errors.

Because OW appear to be particularly detrimental to wheat yields, they are the most important weeds to control during the growing season. Performing an analysis similar to our study, Rueda-Ayala et al. (2011) examined the impact of weed infestation on wheat yield after excluding weeds that emerged after harrowing. They found that yield could be described as a function of weed infestation and crop tolerance to infestation, without even considering later weed emergences. Weeds emerging in spring exhibit a low biomass and are unlikely to achieve the end of their cycle or detrimentally impact grain production before harvest, particularly because well-established winter wheat is an adept competitor for resources (light, nutrients, water) (Adeux et al., 2019a; O'Donovan et al., 1985). Such spring-emergent weeds are usually present in crop fields due to cropping system diversification (i.e. including spring crops within crop rotations) and are not specific to winter wheat cultivation (Anderson, 2005). Therefore, such weeds can generally be controlled after the main crop is harvested, using various mechanical methods (e.g., stubble-breaking operations).

Our analysis of 6 years of data indicated that both weed harrowing and herbicide treatments reduced OW. Our results further showed that only one harrowing pass was needed to reduce OW to a density that was significantly less than that seen with no harrowing and not different than that seen after two harrowing passes, indicating that a second harrowing pass may not improve weed control. Such inferences are supported by the work of Pardo et al. (2008), who identified a relationship between the developmental stage of weeds and their sensitivity to harrowing. The intensity of a second harrowing may be insufficient to eradicate weeds that survived a prior early-season harrowing given that such weeds would be more firmly established later in the growing season and thus would be more likely to withstand additional harrowing passes (Kolb et al., 2012). On the other hand, harrowing had little effect on OW grasses, despite providing a small suppressive effect at T1 in 2013, revealing a lack of efficacy of harrowing on grass weeds, consistent with prior reports (Bàrberi et al., 2000; Pannacci et al., 2017; Pannacci and Tei, 2014; Wilson et al., 1993).

Weed control efficacy on OW under the sole herbicide application (93%) was found to be similar to treatments where whether one or two additional harrowing passes were applied, with absolute efficacy respectively of 92 and 94% (Figure 3.2). Yet, the temporal evolution of weed control along season provided completely different results with and without harrowing. We found that a single pass of harrowing reduced weed emergence during the tillering phase. Herbicides applied after one harrowing pass (except in 2012) require some time before they are fully effective at suppressing weeds (duration before effects are noticeable depends on weather conditions and/or mode of action of active ingredients). That is, we found that herbicides applied just before T1 did not typically have noticeable effects before T2 counting (Table 3.7 and Figure 3.2).

Competitive interaction between crops and weeds is an important factor of weed growth in wheat fields (Sardana et al., 2017), and thus should be considered concomitantly with potential impacts of mechanical weeding. Without weed management, we found that wheat naturally reduced OW by 38%. Mechanical weeding with a harrow plow does not eradicate all weeds in a field; it can uproot some weeds, damage some weeds, and not affect other weeds. Some not-uprooted weeds are likely to die in response to competition with wheat. On plots where only one harrow pass was applied, we found WC increased from 54% (based on weed density after the first harrowing) to 70% at (time of second harrowing) T2 (Figure 3.2). Similar to our findings, Cirujeda et al. (2003) found that a harrow pass achieved weeding efficiency of 21–41% after 7 d and a weeding efficiency of 74–79% after 45 d. Both our study and that of Cirujeda et al. (2003) underscore how impactful a combination of mechanical weeding with interspecific competition can be on reducing weed abundance. We hypothesize that weeds damaged during harrowing are more likely to suffer subsequent mortality in response to interspecific competition.

Because weed management by harrowing alone was observed to be highly variable, we infer that such variation is probably due to variations in weed flora composition and weather conditions across years. For example, during the 2021 cropping season, the efficacy of harrowing was much lower than that of herbicide application, which showed strong efficacy. That growing season, weed density was very high and the weed flora was largely dominated by *Matricaria chamomilla*. It is well known that tap-rooted species, such as *Matricaria chamomilla* or *Papaver rhoeas*, are poorly controlled by harrowing in spring (Wilson et al., 1993). Furthermore, weather conditions could explain the poor efficacy of harrowing for weed control that we measured in 2021, when we observed the formation of a hard soil crust by the end of winter. A hard soil crust prevents harrow tines from penetrating soil fully, thus reducing harrowing efficacy, as has been described previously (Brandsæter et al., 2012; Cirujeda et al., 2003; Rueda-Ayala et al., 2011, 2010).

The confidence intervals of the sigmoid curves indicated that combining mechanical weeding with herbicide application reduces variability in WC relative to using only herbicide or only harrowing, thus suggesting that employing both weed control methods is more likely to be successful for weed control. This complementary effect of weed harrowing and herbicide application suggests that weed management could potentially be achieved with less herbicide. Indeed, harrowing operations can generally be performed early in a growing season, and it is usually possible to perform them prior the spring herbicide treatments. Therefore, if harrowing efficacy is deemed insufficient after a pass, then an herbicide application could be used to improve weeding; conversely, if a harrowing is deemed to be effective enough, the possibility of avoiding herbicide application can be considered. Furthermore, a lack of control of grass weeds with harrowing (Bàrberi et al., 2000; Pannacci and Tei, 2014; Pannacci et al., 2017; Wilson et al., 1993) was confirmed by our results. From an IWM perspective, it appears herbicide could be applied to specifically target grasses if an initial harrowing eradicates most broadleaved weeds.

Weed control may be further improved by applying other management and complementary approaches. For example, sensor-based control systems that augment harrow selectivity can increase weed removal (Spaeth et al., 2021), especially under non-homogeneous field conditions (Rueda-Ayala et al., 2013; Spaeth et al., 2021). Combining harrowing with inter-row cultivator has also been reported to improve weed control (Naruhn et al., 2021; Rasmussen and Svenningsen, 1995), though the addition of inter-row cultivator has been limited in practice by inter-row cultivator costs and the necessity for a large inter row space.

7. Conclusion

This 6-year field trial-based study demonstrated weed management efficacy in wheat crops with mechanical weeding (harrowing) as an alternative to or in combination with herbicide application. Differences in year of OW, NE, and NS were observed across years. Weeds that germinate and develop in relative synchrony with wheat (OW) were found to reduce crop yields most substantially, whereas NE and NS that emerged later in the growing season had negligible effects on yield. Therefore, we conclude that it is important to focus management on eradicating the OW. Additionally, this study demonstrated that one harrowing pass was as effective as two, but less effective than herbicide application alone. This study reaffirmed that harrowing has very little effect on grass weeds. Furthermore, our research indicated that weed control efficacy increased over time after weeding, for example rising from 54% after one harrowing pass to 70% after canopy closure. In the present context, weed control reflected a combination of suppressing small weeds and weakening of more developed weeds such that that wheat could better out-compete weeds. Finally, variability in weed control efficacy was reduced when both harrowing and herbicide application were employed, resulting in a more frequent achievement of greater weed control efficacy. We conclude that an initial harrowing pass is suitable to IPM prior, after which herbicide can be applied in an as-needed basis.

4

Chapter IV: Effect of sowing date and mechanical weeding combined with chemical weeding

1. Synopsis

After observing the potential of the weed harrowing to reduce the systematic use of herbicides, this chapter will assess whether delayed sowing of winter wheat could improve weed management on an intra-annual scale by improving the efficacy of direct control.

2. Abstract

Combining agronomic levers to reduce the use of plant protection products is essential. Although the use of individual levers is well known, the combined effect of different levers is less understood. A study was therefore carried out in loamy soil under temperate conditions (Belgium) to observe the potential of combining delayed sowing of winter wheat with mechanical weeding, either coupled or not coupled with chemical weeding, on weed control and its impact on yield. The wheat was sown on two dates (mid-October and mid-November). In the spring, several direct weed controls were applied. Zero, one, or two passes of weed harrowing were combined with zero, half, or a full dose of herbicide. Weed density before and after direct weed control was measured, as well as weed biomass at wheat flowering. Finally, crop yield was measured. Delaying the sowing date proved to be a promising lever in winter wheat cultivation. Not only did it reduce initial weed pressure, especially for *Alopecurus myosuroides*, but it also increased the efficiency of both harrow and chemical weed control by applying direct weed control to weeds at a younger stage than when winter wheat was sown in mid-October. In addition, the delayed sowing date enabled us to achieve a low weed biomass without significant yield losses, even without direct control. Mechanical weeding did not provide effective weed control when winter wheat was sown in mid-October, whereas it did when sown later. We conclude that delayed sowing is an important lever for weed management and can reduce herbicide use. This study is based on a single year of data and must therefore be confirmed by repeating the trial over several years.

3. Introduction

In order to reduce herbicide use, it is important to adopt an IPM approach. According to Triantafyllidis et al. (2023), herbicide use is the most complicated PPP to reduce. To cut down the use of herbicides, a combination of levers must be used (Pavlović et al., 2022). Previous results based on 6 years of data from Belgium highlighted the effectiveness of mechanical weeding in winter wheat crops (Lacroix et al., 2024 (under review)). However, Lacroix et al (2024, under review) showed that weed control using the harrow was not systematically effective and was on average less effective than chemical weed control. Moreover, the harrow has often been found to be ineffective against grass weeds (Bàrberi et al., 2000; Pannacci et al., 2017; Pannacci and Tei, 2014; Wilson et al., 1993). The effectiveness of the harrow is highly dependent on the stage of the weed, with high effectiveness for young weeds, but this rapidly declines as the weeds become more developed (Kolb et al., 2012; Kurstjens and Perdok, 2000; Rasmussen and Svenningsen, 1995; Rueda-Ayala et al., 2010). Due to the wet autumn climate in Belgium, it is uncommon to perform pre-emergence or early post-emergence mechanical weed control. As a result, mechanical weeding is typically carried out during vegetation resumption after winter, at the tillering stage, as soon as weather conditions permit. However, mechanical weed control at this stage often results in more developed weeds that are less sensitive to the weed control.

Delayed sowing has been shown to be an important avoidance method for *Alopecurus myosuroides*, a very damaging weed in winter wheat (Andrew and Storkey, 2017; Chauvel et al., 2001, 2009; Lutman et al., 2013). For example, Lutman et al. (2013) demonstrated that shifting the sowing date in England from September to the end of October reduced *Alopecurus myosuroides* emergence by 50%, and by 88% when wheat was sown in the spring. Additionally, Chauvel et al. (2009) showed that in a herbicide-resistant *Alopecurus myosuroides* population, delayed sowing combined with other measures such as plowing enabled a rapid reduction in the *Alopecurus myosuroides* population.

A study by (Rasmussen, 2004) found that delayed sowing reduced the overall biomass of weeds. However, it is important to note that later sowing of winter wheat can result in lower yield potential (Bastiaans et al., 2008; Rasmussen, 2004). According to a study by (Andert et al., 2024), farmers who sow winter wheat later in the season in Germany apply less herbicide on average and reduce the use of modes of action with a high resistance risk. This offers a potential solution for reducing herbicide use in winter wheat fields. In addition, Andrew and Storkey (2017) showed that when winter wheat was sown late, its competitiveness against *Alopecurus myosuroides* improved.According to them, the relative growth rate between *Alopecurus myosuroides* and the crop is greatest at warm temperatures (so at earlier sowing date). There is a lack of studies combining different factors in the literature and it is difficult to know whether certain levers are additive, antagonistic or synergistic. To our knowledge, with the exception of a study by Rasmussen (2004), few articles have studied the combined effect of mechanical weeding with a harrow and shifting the sowing date of winter wheat.

The aim of this preliminary study, based on a single year of data from Belgium, is to investigate the impact of delayed sowing date on initial weed pressure and the combined effect of sowing date, mechanical, and chemical weed control on the weed flora. The final objective is to determine the effect of these methods and the resulting weeds on wheat yield. Our hypothesis is that delayed sowing reduces weed pressure before weed control, that it improves both post-emergence chemical weed control in winter and mechanical weed control using the harrow during vegetation resumption after winter, due to the presence of weeds at younger stages. We also hypothesize that wheat yields could be lower with late sowing due to the reduction in yield potential.

4. Materials and methods

4.1. Experimental site and design

To determine the combined effect of shifting the sowing date, harrowing, and chemical weeding in winter wheat, a trial was set up in autumn 2022 in the field at the experimental farm of the Gembloux Agro-Bio Tech-ULiège. This trial included two wheat sowing dates (early around 15 October, late around 15 November) on which mechanical weed control methods (harrow) with several intensity gradients (0, 1, 2 harrow passes carried out on the same day) crossed with the use of herbicides at different application rates (no application, half dose and full dose of the national reference dose of herbicide application (www.fytoweb.be)) were tested. This trial was conducted in microplots (2*8m) and laid out in split split plot design (main plot: sowing date, sub-plot: harrowing passes, sub: sub plot herbicide doses) with 4 replicates. The climate in this region is oceanic temperate (Climate Cfd in the Köppen-Geiger classification), with an average annual rainfall of 793.4 mm, an annual average temperature of 9.6 °C, and an average solar radiation of 825 J cm^{-2} day^{-1}. The soil type is classified as Cutanic Luvisol (WRB, 2015) with a silt loam texture (18–22% clay, 70–80% silt and 5–10% sand). The field was plowed with a mouldboard at 25 cm depth, and the seedbed was then prepared with a rotary cultivator. The crop was sown the 17th October for early sowing date and 21th November for the delayed sowing date. This delay between sowing dates corresponds to a difference of 363 growing degree days. Inter-rows spacing at planting was 125 mm. Seeding density was determined by sowing date and the regional recommendation 250 seeds m^{-2} for October sowing date and 300 seeds m^{-2} for November sowing date.

The herbicide treatment was carried out on March 28, 2023 and consisted of the simultaneous application of a grass and a broadleaf weed control, Axial and Biathlon duo, respectively. Axial (50g l^{-1} pinoxaden (ACCase inhibition) and 12.5 g l^{-1} cloquint-ocet-mexyl (safener)) and Biathlon duo (71.4% tritosulfuron (ALS inhibition) and 5.4% florasulam (ACCase inhibition)) were applied at a full dose of 1.2 l $ha^{-1}$and 70 g ha^{-1}, corresponding to the dose recommended on phytoweb (www.phytoweb.be). The half-dose of herbicide therefore corresponds to an application of 0.6 l ha^{-1} of Axial and 35 g $ha^{-1}$of Biathlon duo. Herbicides were applied using a 2m-wide portable spray bar with a spray volume of 200l ha^{-1}.

Mechanical treatment was carried out on April 21, 2023, as it could not be done earlier due to wet weather conditions in late winter and early spring 2023. The harrowing was carried out parallel to the sowing line.The intensity and speed of harrowing (4–7 km h^{-1}) were adjusted based on winter wheat growth stage, weed size, and pedoclimatic conditions. Within the same field as the experiment, but outside the experimental plots, a visual assessment of the tine's penetration ability, its capacity to uproot weeds, and the extent of wheat cover were conducted. The tine settings were then adjusted prior to employing the harrow in the experimental plots, which then remained the same during harrowing treatment for the same sowing date. The aggressiveness of harrowing for the early sowing date was set to its maximum (7 km h^{-1}, and most aggressive tine setting) to eradicate the presence of larger weeds and because the crop was more developed and better able to withstand the more aggressive harrowing compared to November sowing date. Treatments with two harrowing passes received the first in one direction and the second in the other (but always parallel to the sowing line). Soil conditions were dry during harrowing treatments.

4.2. Data collection and analysis

The weed survey for each treatment was carried out in a 0.25m² quadrat within each plot. It was conducted by weed species before and after each weeding operation, as well as during wheat flowering. In addition, measurements of weed biomass were taken at wheat flowering in these same quadrats. Grain yield were harvested from a reference area of 2 × 8 m within the sub-sub-plot and expressed at 15% water content. The various dates of weeding operations and sampling are shown in Table 4.1

Table 4.1: Sowing, weeding and sampling realized in the trial.

Date	Operations
2022-10-17	Sowing of mid-october sowing date
2022-11-21	Sowing of mid-november sowing date
2023-03-19	Initial weed density sampling
2023-03-28	Application of herbicide
2023-04-03	T1 weed desity sampling
2023-04-21	Harrowing
2023-05-05	T2 weed density sampling
2023-06-12	Weed density and weed biomass sampling at wheat flowering

The Weed Control Efficacy (WCE) was calculated based on the weed density measured at different sampling times. It is formulated as follows (Rasmussen, 1991; Spaeth et al., 2021):

$$WCE_t = \left(\frac{\text{Weed density}_{(t-1)} - \text{Weed density}_t}{\text{Weed density}_{(t-1)}} \right) \times 100 \tag{4.1}$$

Where Weed density$_{(t-1)}$ represents the number of weeds before the weeding operation, and Weed density$_{(t)}$ represents the number of weeds after the weeding operation. A value of 100% indicates that all the weeds have been controlled by the weeding operation, while a value of 0% means that the number of weeds after the operation is the same as before. A negative value indicates that new weeds have emerged between the two counting times.

A linear mixed-effect models or generalized linear model were made on response variable. Response variable (WCE, weed biomass and yield) were expressed as a function of sowing date, mechanical weeding, herbicide doses and their interactions. The block was included as random intercept. For the initial weed density (T0), this response variable was only expressed as a function of sowing date (because weed control had not yet been conducted). Details of all models can be found in supplementary material (Table B.1). The homogeneity of variance and normality of data distributions were tested. Outliers were identified using the *qqPlot* function in R, which highlights individuals with the largest residual values. The values of these points were then checked, and if they were too extreme (via boxplot and agricultural knowledge), they were removed. ANOVA was performed on these models to assess the significance of fixed effects. Finally, an estimated marginal means analysis was performed. All analyses were conducted in RStudio using the emmeans and lme4 packages. In case of non-normality, a transformation was applied (logarithmic). Additionally, a regression between weed biomass and yield was performed.

5. Results

Due to the very difficult weather conditions at the beginning of spring 2023, mechanical weeding could not be carried out early in the season. It was therefore carried out rather late, on April 21, as no suitable weather window was found before that date.

5.1. Initial weed flora

The initial weed flora in the trial was composed of both grasses and broadleaves. Grasses are predominantly represented by *Alopecurus myosuroides* Huds.. The dominant broadleaf species were *Veronica arvensis* L., *Papaver rhoeas* L., *Matricaria chamomilla* L., *Polygonum aviculare* L., and *Galium aparine* L. The average pressure of all the species mentioned above and their relative percentages in the initial composition of the flora are shown in Table 4.2.

Table 4.2: Initial weed flora composition (before any direct weed control).

Weed species	Weed density m^{-2}	Pourcentage of total weed density (%)
Veronica arvensis L.	67	43.12
Papaver rhoeas L.	29	18.39
Alopecurus myosuroides Huds.	27	17.47
Matricaria chamomilla L.	24	15.21
Polygonum aviculare L.	3	1.99
Galium aparine L.	3	1.76

The spatial variability of the weed density at the first sampling (T0) was very high, ranging from 16 to 532 weeds m^{-2}, with an average value of 157.7 weeds m^{-2}. However, this heterogeneity follows a gradient that can be explained by the different blocks.

5.2. Effect of sowing date on initial weed pressure

A highly significant effect of wheat sowing date on weed pressure at the end of winter was observed. This effect applied to both broadleaf weeds and *Alopecurus myosuroides*. Delayed sowing date resulted in a reduction of grass pressure by around 40% (from 23 grasses m^{-2} to 14 grasses m^{-2}) and broadleaf pressure by approximately 22% (from 143 to 115 broadleaves m^{-2}). The average number of weeds for the two sowing dates (mid-October, mid-November) is shown in Table 4.3.

Table 4.3: Average weed density (m^{-2}) at the initial sampling according to sowing date and weed type. Treatment with the same letters are not significantly different.

Sowing date	Weed density m^{-2}	Standard error	
	Total weeds		
Mid-October	177.27	31.94	b
Mid-November	138.13	31.94	a
	Grass weeds		
Mid-October	23.32	14.16	b
Mid-November	14.37	8.73	a
	Broadleaf weeds		
Mid-October	142.87	24.18	b
Mid-November	114.53	24.18	a

5.3. Evaluation of Weed Control Efficacy

In the spring, a herbicide was applied between time steps T0 and T1. However, preliminary analyses have shown that the herbicide took effect between T1 and T2. Specifically, one week elapsed between the herbicide application and the T1 measurement, making it impossible to observe the effects of the chemical weed control at T1. The T1 sampling, conducted on April 3, was carried out 18 days before harrowing (performed on April 21). Ideally, the T1 sampling would have been conducted earlier and closer to the harrowing, but the changeable weather did not allow for it. Unfortunately, no new sampling could be carried out closer to the harrowing due to a lack of availability. Therefore, the analysis were adapted accordingly: the T1-T2 time step, which lasted approximately one month (from April 3 to May 5), were analyzed to assess the impact of both chemical and mechanical weed control.

5.3.1. WCE of direct control weeding according to the sowing date

The WCE between T2 and T1 showed no three-way interaction between the three factors studied. However, a significant effect of the sowing date, herbicide, and mechanical weeding factors was observed on both the grass and broadleaf weed populations. Without any treatment, weed emergence was observed between the T1-T2 time step in the order of 10 and 25% for the October and November sowings respectively (Figure 4.1). As shown in Figure 4.1, the efficacy of mechanical and chemical weed control was improved by delaying the sowing date.

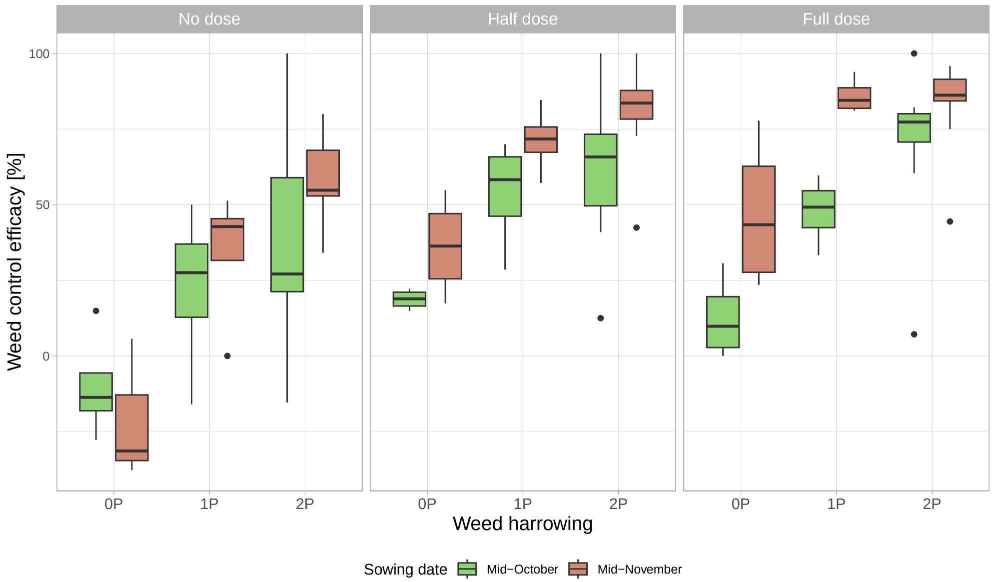

Figure 4.1: Weed control efficacy between T1 and T2 as a function of herbicide dose, weed harrowing and wheat sowing date. 0P is no harrowing, 1P is one harrowing pass, 2P is two harrowing passes. Full dose refers to the reference dose application of herbicide, half dose refers to the middle of the reference dose application of herbicide and no dose refers to no herbicide application.

The treatments of chemical weed control have in average a WCE significantly similar at half and full doses, whether on grasses or broadleaf populations. The full dose reduced the broadleaf weed population by an average of 58% and the grass population by 62% (Table 4.4). Delaying the sowing date resulted in better WCE, increasing WCE on grasses by 164%, while on broadleaf weeds it increased WCE by just over 35%. Additionally, no significant difference was observed in the reduction of the broadleaf population between one and two harrow passes (56% and 65% reduction respectively). Conversely, the reduction in the number of grasses was intermediate for one pass (38% reduction) and greatest for two passes (53% reduction) (Table 4.4).

Table 4.4: Weed control efficacy of total, broadleaf, grass weeds between T1 and T2 as a function of weed harrowing, wheat sowing date and herbicide dose. 0P is no harrowing, 1P is one harrowing pass, 2P is two harrowing passes.Full dose refers to the reference dose application of herbicide, half dose refers to the middle of the reference dose application of herbicide and no dose refers to no herbicide application.

	WCE between T2 and T1 (%)								
	Broadleaf			**Grass**			**Total**		
Herbicide application									
No dose	20.32		a	9.60	10.70	a	19.20	5.71	a
Half dose	55.05	6.36	b	39.34	9.95	b	53.90	5.64	b
Full dose	58.31	6.35	b	62.15	10.61	b	58.20	5.65	b
Sowing date									
Mid-October	37.87	6.01	a	20.31	8.72	a	34.90	4.15	a
Mid-November	51.25	6.04	b	53.75	9.78	b	52.60	4.25	b
Harrowing									
0P	12.76	6.71	a	19.70	11.26	a	13.20	5.96	a
1P	55.50	6.64	b	38.32	11.59	ab	52.60	5.90	b
2P	65.42	5.75	b	53.06	8.10	b	65.50	5.09	c

5.4. Effect on weed biomass

With regard to weed biomass at wheat flowering, no interaction was observed. However, a significant effect of the three factors separately was observed. The details for all treatments are shown in Figure 4.2. On average, the sowing date resulted in a reduction in biomass from 35.40 g m^{-2} for mid-October sowing date to 6.25 g m^{-2} for mid-November sowing date (average around the other factors). Chemical weed control reduced biomass from 42.55g m^{-2} without chemical weed control to 11.78 g m^{-2} and 6.73g m^{-2} for half and full doses respectively (with no significant difference in average). Mechanical weeding produced a higher weed biomass without harrowing (24.66 g m^{-2}), an intermediate biomass with one pass (14.62g m^{-2}), and the lowest biomass with two passes (9.73g m^{-2}).

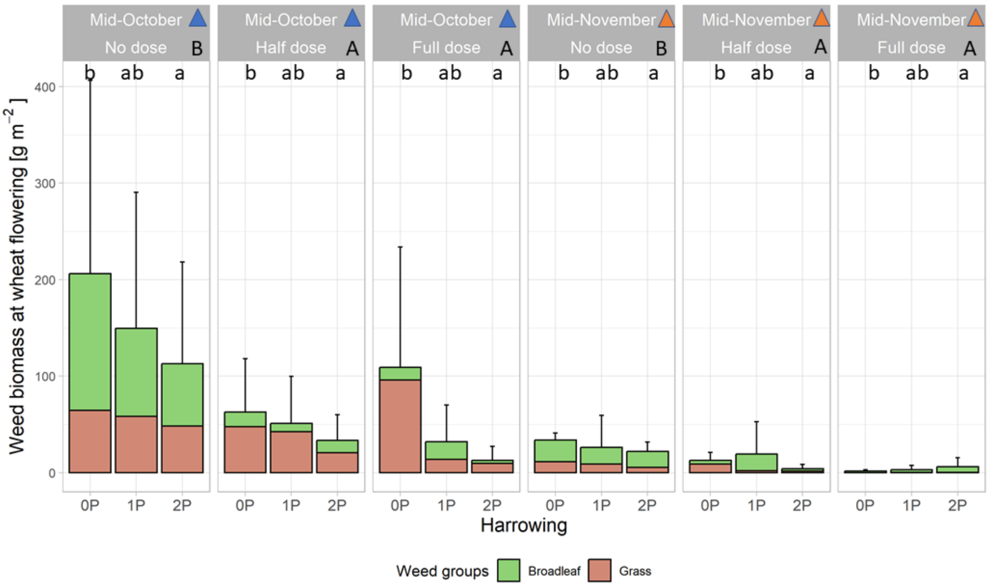

Figure 4.2: Weed biomass at wheat flowering as a function of wheat sowing date, herbicide dose and weed harrowing. Weed harrowing treatments with identical lower case letters have statistically equivalent means. Herbicide application treatments with identical capital letters have statistically equivalent means. Sowing date treatments with identical colored triangles have statistically equivalent means. 0P is no harrowing, 1P is one harrowing pass, 2P is two harrowing passes. Full dose refers to the reference dose application of herbicide, half dose refers to the middle of the reference dose application of herbicide and no dose refers to no herbicide application.

5.5. *Effect on yield*

As shown in Figure 4.3, the relationship between yield and weed biomass differed according to sowing date. No regression was observed for mid-November sowing date of winter wheat, whereas a negative regression was observed for mid-October sowing date of winter wheat (R^2=0.54).

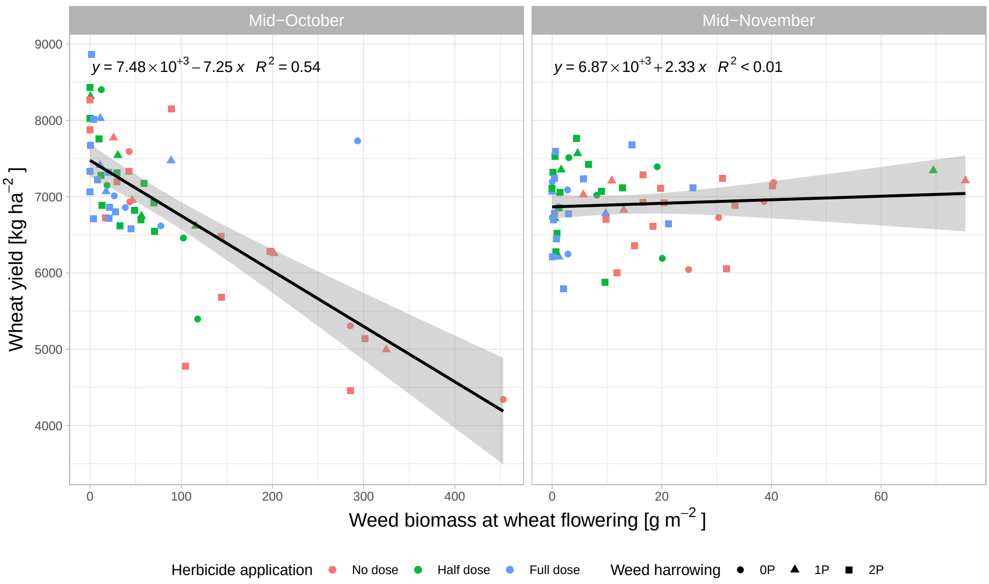

Figure 4.3: Grain yield at 15% moisture according to weed biomass in dry matter at wheat flowering according to wheat sowing date. 0P is no harrowing, 1P is one harrowing pass, 2P is two harrowing passes.Full dose refers to the reference dose application of herbicide, half dose refers to the middle of the reference dose application of herbicide and no dose refers to no herbicide application.

Upon closer examination of the results for the 2023 harvest (Figure 4.4), it was observed that there was an interaction between chemical weed control and sowing date. However, weed harrowing did not improve yield. Yields were lowest in mid-October sowing without herbicide, intermediate in mid-November sowing of wheat without herbicide and with a full dose, and highest in mid-October sowing with half and full doses of herbicide, as well as in mid-November sowing with a half-dose of herbicide.

6. Discussion

This 2023 year was marked by complicated weather conditions in the spring. The rainy weather was not conducive to early mechanical weeding, which had to be carried

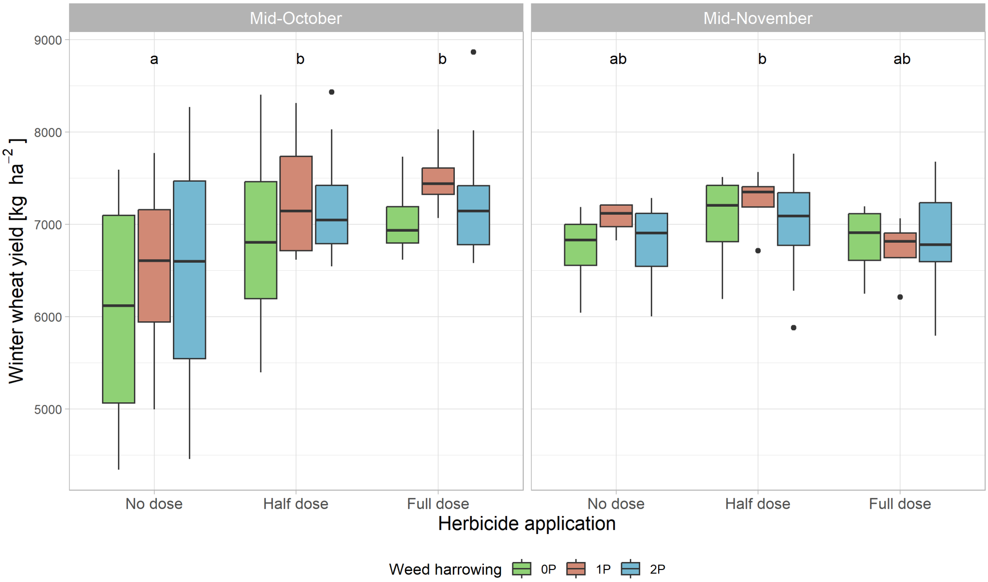

Figure 4.4: Winter wheat yield as a function of sowing date, herbicide dose and weed harrowing treatments. treatments with identical letters have statistically equivalent averages. 0P is no harrowing, 1P is one harrowing pass, 2P is two harrowing passes. Full dose refers to the reference dose application of herbicide, half dose refers to the middle of the reference dose application of herbicide and no dose refers to no herbicide application.

out very late in the season. Similarly, the very cold and windy start to spring was not conducive to chemical weed control.

6.1. Delayed sowing date: an impactful lever for managing weeds

Delaying the sowing date from 17 October to 21 November has proved to be a promising lever. As reported in the literature, delaying the sowing date is an effective lever for reducing initial *Alopecurus myosuroides* Huds. pressure (Chauvel et al., 2009, 2001; Lutman et al., 2013). It also reduced the initial pressure of broadleaf weeds, but to a lesser extent (around 40% for *Alopecurus myosuroides* and 20% for broadleaf). Fewer studies have focused on the effect of delaying the sowing date on broadleaf, although the same effect of a reduction in certain broadleaf weeds has already been observed (Cosser et al., 1997). In addition to avoiding significant weed pressure, delayed sowing improved the effect of both weed harrowing and chemical weed control. This effect was greater for *Alopecurus myosuroides* (164% improvement) than for broadleaf weeds (35% improvement). In our opinion, this improvement in WCE is linked to the younger weed stages when weed control techniques are applied. Weed harrowing is very sensitive to weed stages and is very effective on weeds that are not very developed (Kolb et al., 2012). In difficult conditions for the active ingredients of herbicides to act, younger weeds are also more sensitive to herbicides and would explain this gain in efficacy. On the other hand, without any weed control, late sowing led to weed emergence throughout the spring. This can be explained by the fact that there is less soil cover in spring than with mid-October sowing, allowing light to reach the soil and promote the growth of new weeds. However, Lacroix et al. (2024, under review) showed that this spring emergence has no impact on wheat yield. These weeds are the most synchronous with the crop and tend to have a greater negative impact on yield because they compete directly for resources during early growth stages (Wilson and Wright, 1990; Zimdahl, 2007b).

Weed biomass at wheat flowering demonstrated the extent to which delayed sowing date, even without weeding, can prevent significant biomass production. In fact, the weed biomass in the mid-November sowing was on average more than four times lower than the weed biomass in the mid-October sowing. This reduction in weed biomass has been previously reported by Rasmussen (2004), who observed a 50% reduction in weed biomass, but only under intermediate weed pressure.

The large drop in weed biomass, even without direct control, seems to be the result of several phenomena. The reduction in initial pressure automatically leads to a reduction in the weed biomass produced. However, the pressure (in term on weed density) at the end of the winter is only 22% lower and could therefore not by itself explain such a large drop. We assume, as Andrew and Storkey (2017) have shown for *Alopecurus myosuroides*, that late sowing increases the competitiveness of wheat relative to weeds due to the better relative growth rate of winter wheat compared to weeds at low temperatures. We hypothesise that this higher relative growth rate for wheat during the winter phase would allow the wheat in early spring to be much more developed than the weeds, giving it a major advantage in terms of competitiveness when the weather conditions improve, by intercepting more light and taking more nutrients from the soil. This improvement in competitiveness, which results in a much weaker development of the weeds, means that there is no yield loss due to the weed presence (see Figure 4.3). Delaying the sowing date would therefore reduce weed-crop competition. Additionally, weed biomass serves as a reliable indicator of weed seed production (Andrew and Storkey, 2017; Lutman, 2002; Rasmussen, 1993). Thus, delaying the sowing date helps prevent a significant seed rain that could replenish the seedbank. Furthermore, delaying the sowing date did not lead to statistically lower yields compared to weed control at mid-October sowing. This effect was also observed by Rasmussen (2004) under conditions of moderate or high weed pressure.

6.2. Effect of weed harrowing

Despite being applied late in the spring, the harrow achieved around 50% efficacy, thanks to a very aggressive tool setting. However, earlier application, weather permitting, is still advisable, as the harrow is more effective on young weeds (Kolb et al., 2012). It can be assumed that the effectiveness of weed control in late sowing would have been much greater if the harrowing could have taken place in March when weeds were less established. Long-term data analysis spanning several years could provide insights into whether an even greater effect could be observed between mechanical weed control and shifting the sowing date. The harrow also contributed to reducing grass pressure, with reductions of 38% and 53% for 1 and 2 harrow passes, respectively, averaged over all other factors.

Numerous studies show that grasses are not well controlled by the harrow in most cases (Bàrberi et al., 2000; Pannacci and Tei, 2014; Pannacci et al., 2017; Wilson et al., 1993). However, managing grasses using the harrow alone is complicated by the fact that their morphology is very similar to that of wheat, making it difficult to achieve good selectivity with the harrow. On the other hand, combining two different strategies, such as sowing dates and weed harrowing, seems to be a promising solution for reducing the use of PPP. This first year of trials demonstrated that delaying the sowing date increased the effectiveness of the harrow and prevented yield losses associated with it. In the case of early sowing, weed harrowing did not sufficiently reduce weed biomass (more than 100g m^{-2} after two harrow passes) without compromising future plot contamination and cereal yield loss.

6.3. Effect of herbicide application

Herbicide application was not optimally effective, with an efficacy of 55 to 58% for broadleaf weeds and 39 to 62% for grass weeds, respectively, for the half and full dose of herbicide (averaged around the other factors). This lack of efficacy can be explained by the cold weather, which was not conducive to good herbicide efficacy. The herbicide often slowed or even stopped the growth of the weed but did not necessarily kill it (visual observation). Mechanical weeding has therefore improved weed control. This is in line with the results of Lacroix et al (2024-under review), who showed that combining mechanical and chemical weed control reduced the variability of effectiveness compared with herbicide weed control alone and ensured good weed control in all circumstances when a combination of herbicide and harrowing weed control was applied.In the case of early sowing, applying a full dose of herbicide seemed unavoidable to prevent yield losses. However, this was not necessary with late sowing of winter wheat due to the lower biomass produced by the weeds, as illustrated above.

6.4. General considerations

This trial demonstrated the extent to which the integration of several management tools could have an impact on weed management. These initial results are very promising from the point of view of reducing the use of PPPs and are fully in line with the IWM approach. These results prove that it is possible to do without herbicides when sowing late, thanks to the lower initial weed pressure and, above all, thanks to the weed:crop competition, which is reduced to a level that does not impact on yield. In late sowing, the WCE with the harrow is improved and seems sufficient even if it is done late (as in our only year of data). In addition, this method did not have a significant effect on yield compared with mid-October sowing with weed control. However, this result needs to be confirmed after several growing seasons and therefore several weather conditions. On the other hand, if a farmer opts for early sowing, it appears challenging to forgo chemical weed control without incurring yield losses or jeopardizing the long-term productivity of the plot due to an important seed rain. Mechanical weed control, even with well-developed weeds, does not provide sufficient reduction, even after two passes.

7. Conclusion

These preliminary results obtained from one year of trials have highlighted the importance of IPM and the significance of testing the combination of multiple strategies in experimentation. Indeed, if we aim to reduce the use of PPP, it is important to combine a range of strategies and understand how they interact with each other. This preliminary study is very promising and has demonstrated the additive effect of delaying the sowing date, weed harrowing, and herbicide application on weed control. Firstly, this study reaffirmed that delaying the sowing date decreased the pressure from *Alopecurus myosuroides*, as well as, to a lesser extent, from broadleaf weeds. Furthermore, it showed that delaying the sowing date improved the WCE of both weed harrowing and herbicide application by targeting weeds at younger stages. Delaying the sowing date drastically reduced weed biomass (reduced by 4) due to, in our opinion, a relative competitiveness between the weed and the crop, favoring the crop with greater relative growth in cold wheat conditions. This resulted in insufficient weed pressure to cause yield losses. We conclude that it is possible to forego the use of PPP with late sowing (mid November instead of mid-October), combined if necessary with mechanical weeding, without compromising yield. However, these results are based on a single year, and additional data over multiple years are needed to confirm these initial findings.

5

Chapter V: Effect of long-term tillage and residue managements on weed flora and its impact on winter wheat development

1. Synopsis

After analysing the effect of direct weed control and delayed sowing on an annual scale in the previous two chapters, a long-term weed community management approach is discussed. The management of crop residues and tillage are essential elements in the management of the agroecosystem. In addition, crop residues can compete for several uses (straw, biomethanisation, soil fertility, etc.).

In this chapter we will discuss the effect of long-term soil management through crop residue management on weed community and on the development of winter wheat in a rotation based on winter wheat.

This chapter is based on the article published (Lacroix et al., 2024)

Lacroix, C., Vandenberghe, C., Monty, A., and Dumont, B. (2024). *Effect of long-term tillage and residue managements on weed flora and its impact on winter wheat development*. *Agriculture, Ecosystems & Environment*, 366:108937.

2. Abstract

In Hesbaye region (Belgium) with a loamy soil and under temperate climatic condition, winter wheat is a key component of agricultural rotations. As part of these rotations, soil management is a known driver of soil fertility and carbon storage. However, it could also influence the weed flora. In this study, the long-term effect of four soil management on the expressed and potential weed flora was examined. Soil management levers were (1) the export (OUT) or restitution (IN) of crop residues and (2) the burial of residues by conventional tillage (mouldboard ploughing 25 cm depth, CT) or reduced tillage (cultivator ploughing 10cm depth, RT). The weed seedbank and expressed flora in winter wheat were characterized. Weed diversity was assessed using the Richness and the Shannon diversity index. Then, the impact of flora and management on yield were investigated. Tillage management showed little impact on weed diversity with only a slight increase in diversity in reduced tillage. However, reduced tillage resulted in a higher weed seedling density and a higher weed density than conventional tillage, which indirectly led to yield losses. Exporting residues had no clear effect on weeds. In conclusion, within cropping systems based on the cultivation of wheat, reduced tillage can pose problems for the long-term management of the weed flora, and great attention has to be paid to its management.

3. Introduction

Agricultural soils management is known to have an impact on carbon storage and potentially could help mitigate the rise in atmospheric CO_2 concentration (Martin et al., 2021). The management of crop residues, which can be exported (e.g. for animal fodder or bioenergy production) or incorporated into the field using reduced or conventional tillage, can therefore play a role in carbon storage (Autret et al., 2016; Hiel et al., 2018). Beside impacting the soil carbon content, soil management can have impacts on soil geochemical dynamics (Blanco-Canqui and Lal, 2009; Hiel et al., 2018) and on soil microbial communities (Degrune et al., 2017, 2016; Spedding et al., 2004). Furthermore, soil management can also have an impact on weed flora (Nichols et al., 2015).

The effect of tillage alone (without residue incorporation or exportation) on weeds is widely documented, although different trends are sometimes observed between studies on both the flora expressed and the seedbank (Nichols et al., 2015; Plaza et al., 2011; Santín-Montanyá et al., 2016). These differences are mainly explained by complex interactions with other factors such as: differences in the duration of the experiment, the history of the field, and the species present (Nichols et al., 2015). However, it is commonly reported that reducing tillage increases weed density and favours grass populations (Nichols et al., 2015; Schnee et al., 2023; Travlos et al., 2018; Trichard et al., 2013). On the other hand, residue restitution can influence weed dynamics by changing nutrient dynamics, soil temperature or soil moisture (Nichols et al., 2015; Liebman and Mohler, 2001). Yet, it is not very clear whether the burial of retained crop residues by tillage favours weed development or not (Nichols et al., 2015). Furthermore, the resulting composition and harmfulness of the weed flora in the long term are poorly documented (Nichols et al., 2015). However, the mulch effect of residues has a proven effect on reducing weed germination if the quantity is sufficient. If the quantity is insufficient, the effect may be the opposite (Nichols et al., 2015; Chauhan et al., 2012). Plaza et al. (2011) highlighted the importance of long-term trials to shed light on the effect of agricultural practices on weed diversity. Furthermore, long-term of tillage and residue management could directly impact crop yield while also exerting an indirect influence on weed flora. To highlight the direct and indirect relationships between different variables, structural equation modelling (SEM) has gained traction within ecological studies (Majdi et al., 2014; Puech et al., 2015). Moreover, recent research, such as the case study conducted by Quinio et al. (2017), has successfully employed path analysis to investigate the impact of farming practices on weeds and winter wheat production.

The aim of this paper was therefore to characterize the long-term effect of residue and tillage management on weed pressure and crop productivity after 14 years of cultivation. The focus was put on a winter wheat cropping season, as this crop exhibit an important phenotypic plasticity and as it occupies ~45% of the Walloon arable lands. Monitoring of (1) the weed seedbank and (2) the in-season expressed weed flora were performed. Finally, (3) it was determined whether differences in flora composition and levels of infestation could impact winter wheat yield potential.

4. Materials and methods

4.1. Site description and experimental design

The long-term trial is established since 2008 on the experimental farm of Gembloux Agro-Bio Tech, University of Liège, in Belgium (50°33’49.6”N, 4°42’45.0”E). The climate in this region is oceanic temperate (Climate Cfd in the Köppen-Geiger classification) with an average annual rainfall of 793.4 mm, an annual average temperature of 9.6 °C and an average solar radiation of 825J cm^{-2} day^{-1}. The soil type is classified as Cutanic Luvisol (WRB, 2015) with a silt loam texture (18-22% of clay, 70-80% of silt, and 5-10% of sand). The experiment was designed as a Latin square disposal with four replications (see Figure C.1). Each plot measured 15 m wide and 40 m long. Crop rotation since the beginning of experimentation in 2008 is present in the Table C.1. Since 2015, the rotation has remained the same, with a winter wheat crop present every other year (maize, winter wheat, sugar beet, winter wheat).

The trial compared two different factors for managing soil and crop residues: (1) the restitution (IN) or the exportation (OUT) of crop residues, and (2) the intensity of tillage: conventional tillage (CT) or reduced tillage (RT). The combination of these two factors results in four different crop residue managements: CT-IN, CT-OUT, RT-IN and RT-OUT.

Regarding the exportation of crop residue, stubble and chaff were always kept on site, but the rest of residue (straw and what’s left of leaves) were exported (OUT) or maintained (IN). Tillage is carried out to a depth of 25cm in CT and 7-10cm in RT. RT and CT treatments were both breaked with a stubble breaker after the harvest. In CT, ploughing was carried out a few days before sowing winter wheat. Finally, seedbed preparation was identical in RT and CT (using a stubble cultivator). For more information on the trial see the article of Hiel et al. (2018). Details of all winter wheat cultivation operations in 2021 and 2022 (the year in which the measurements were taken for this paper) are shown in Table 5.1.

The history of the various herbicide applications since 2008 is presented in Table C.1. Cover crops were generally terminated by applying glyphosate. Herbicides were applied at spring during within winter wheat cropping seasons and applied between one and three times, depending upon the success of the weed control. Maize crop was managed with a single post-emergence application of herbicide. Lastly, the FAR weed control itinerary (usually applied in Belgium) was applied during the sugar beet seasons, which consists of repeated low-dose passes of a mixture of foliar herbicide (phenmedipham), an activator (ethofumesate) and a residual herbicide (e.g. metamitron). Rapeseed and faba bean were cultivated only once since establishment of the experiment and herbicides were applied following business-as-usual management. More details about ingredients, modes of action and HRAC groups (HRAC, 2024) are presented in Table C.1.

Table 5.1: Winter wheat cultivation operations in 2021-2022.

Date	Operation	Depth (cm)	Additional information	CT-IN	CT-OUT	RT-IN	RT-OUT
10-25-2021	ploughing	25	with mouldboard plough	x	x		
10-26-2021	seedbed preparation	10	with stubble cultivator (Lemken Smaragd 9/300)	x	x	x	x
10-27-2021	sowing	7	wheat variety is Camesino (275 grain.m^{-2}), the tractor was equipped with a dual cultivator (Jadin) in front and rotary harrow and wedge ring roller combined with seed drill (Amazone)	x	x	x	x
03-10-2022	weeding		application of Sigma Star (0.33 kg.ha^{-1}) and Actirob B (1 l.ha^{-1})	x	x	x	x
03-29-2022	nitrogen fertilisation		liquid nitrogen (39%), 60 kg.ha^{-1} of nitrogen	x	x	x	x
04-26-2022	nitrogen fertilisation		liquid nitrogen (39%), 50 kg.ha^{-1} of nitrogen	x	x	x	x
04-27-2022	weeding		application of Axial (1.2 l.ha^{-1}), Biathlon Duo (0.060kg.ha^{-1}) and Actirob B (0.8 l.ha^{-1})	x	x	x	x
04-28-2022	growth regulator		application of Cycofix (1l.ha^{-1})	x	x	x	x
05-14-2022	fungicide		application of Balaya (1.5l.ha^{-1})	x	x	x	x
05-18-2022	nitrogen fertilisation		solid nitrogen calcium ammonium nitrate (27% N), 60 kg.ha^{-1} of nitrogen, 20kg.ha^{-1} CaO	x	x	x	x
07-26-2022	harvest		harvest of winter wheat	x	x	x	x
Aug. 2022	residue exportation		exportation of straw bale out of the field		x		x

4.2. Field data collection

4.2.1. Weed seedbank

To determine the impact of residue management on weed density and diversity, weed seedbank samples were systematically collected on the17th January, 2022. A 'W' sampling pattern was employed, with five composite samples derived from four soil cores each (diameter=2cm) per plot. The 4 soil sub-samples were collected at each corner of a 50 x 50 cm quadrat. Sampling was conducted at two different depths: 0-10cm (maximum working depth in RT) and 10-25cm (maximum working depth in CT). In total, 160 samples (4 treatments*4 replications* 5 samples/plot * 2 depths) underwent analysis using the emergence method. The composite samples were stored for 15 days in a cold room at 5°C in order to break the dormancy of some specific seed species (Mahé et al., 2021). The composite soil samples were sieved and then spread on trays, over potting soil (1cm) and argex balls (2cm). The samples were themselves spread with a maximum depth of 2cm to allow germination of all seeds (Mahé et al., 2021). A PVC tube was inserted at the corner of the tray for regular irrigation. In addition, micro-sprinkler irrigation was carried out every week to prevent the surface layer of soil samples from drying out. Weed seedlings were identified and counted every 2-3 weeks. Once identified at the species levels (or genus when it was not possible to identify at species level), the weeds were removed. Species are named using both the latin name and the EPPO code (https://gd.eppo.int/). The emergence was monitored between 02 February 2022 and 30 November 2022. The first phase of monitoring (until 11 September) was carried out in a germination room with 574 lux light and a temperature between 17 and 20°C. Between 08/04/2022 and 22/04/2022 the samples were not irrigated to force drought. On 22/04 the samples were crumbled by hand before irrigation was applied again. This period of dryness followed by crumbled is intended to stimulate germination (Mahé et al., 2021). From 12 September to the end of November, the weed seedbank was installed in an unheated greenhouse to enhance autumnal germination.

4.2.2. In-season crop and weed sampling

In order to characterise the weed flora expressed during winter wheat cropping season (sowing in autumn 2021) and its impact on yield, samples were taken during the 2022 winter wheat growing season. Weed density by species was measured at the time of wheat tillering and at flowering stages within 5 quadrats of 50 cm * 50 cm per plot. In addition, at wheat flowering, weed biomass by species and crop biomass were measured within the same quadrat as weed density.

Finally, at wheat maturity, the yield was measured in 5 quadrats of 50 cm * 50 cm per plot. Each quadrat was sampled within a 2 m radius of the quadrat within which data were collected at wheat tillering and flowering. At maturity, components of yield (stem biomass, spike biomass and number of spikes per m^2) were measured directly from samples. The average grain biomass per spike was derived as follows:

$$\text{Grain biomass per spike} = \left(\frac{\text{spike biomass.m}^{-2}}{\text{number of spikes.m}^{-2}}\right)$$

All the biomass samples were dried at 60°C in an oven until the biomass remained unchanged. Biomass were measured at the nearest 0.01g.

4.3. Field data collection

Species richness (number of species per quadrat) and Shannon-Weiner index were computed from weed-related data. Indices were computed on weed seedbank observations and were calculated for in-season field data, at tillering and flowering of winter wheat. Shannon-Weiner index, which measure the α-diversity was calculated by samples (seedbank) or quadrats (in-season) as follow:

$$H = 1 - \sum_{i=1}^{S} p_i \cdot \ln(p_i)$$

Where p_i is the relative proportion of individuals of species i in a community of S species and S is the total number of species.

4.4. Statistical analysis

Statistical analysis were perform using R statistical Software (V4.3.1; R Core Team, 2021). Response variables (weed density, weed biomass, species richness, Shannon index, yield...) were modelled with the *glmmTMB* package. Model diagnostics were verified with the *DHARMa* package. Response variable were expressed as a function of crop residue exportation, tillage intensity and their interaction. For seedbank related-data, depth of sampling was also studied. In this case response variable were expressed as a function of crop residue exportation, tillage intensity, depth of sampling and their integrated triple interaction. Rows and column of the Latin square design were always included as random intercept. Distribution was selected to meet the conditions. In addition, the model with the lowest Akaike's Information Criterion (AIC) was chosen. All selected models are presented in supplementary Table C.2. ANOVA were performed on these models to assess the significance of fixed effects. Finally, an estimated marginal means analysis was performed using the emmeans package.

Correlation between weed density, density of the two most prevalent weed species at flowering and yield components were calculated with Spearman correlation due to violation of parametric assumptions.

A path analysis (covariance structural analysis) was performed with the *lavaan* package in order to illustrate the relationships of direct and indirect effects between the variables impacting yield. The model was constructed based on standardised variables (i.e., centred mean and scaled by standard deviation). Path models are built upon both latent variables (LV) and manifest variables (MV). The first LV, "Weed pressure", initially used the same MVs as Quinio et al. (2017), i.e. richness, Shannon index and abundance (except that abundance is expressed here in terms of biomass rather than individuals). The second LV, "Soil management", comprises MV “Ploughing” (conventional or reduced tillage) and MV “Residue exportation” (residue exported or maintained). The third latent variable refers to the productivity; as proposed by Quinio et al. (2017) it was composed solely of the yield. Two MVs related to yield components (number of spikes per m² and average biomass of grains per spike) were added to the model (Figure 5.1).

The quality of the model was assessed using five indicators. First, the chi-square test (χ^2) was calculated. A p-value >0.05 indicates an acceptable model fit. Secondly the comparative fit index (CFI) and the Tucker-Lewis index (TLI) should respectively have a value above 0.90 and 0.95. Finally, the Root Means Square Error Approximation (RMSEA) and the Standardized Root Mean Square Residual (SRMR) with value below 0.08 generally indicate a well-fitting model.

Based upon preliminary results, a second model was built. Only the MV related to weed abundance indicator (expressed in biomass) was eventually kept to feed the LV related to weed pressure. The other two indicators were proven to not contribute to build a quality model. Additionally, the MV related to residue fate was removed from the LV soil management. This variable was not providing any additional insight to the model. In fact, in this trial, the lack of significant impacts of residue exportation on yield had been demonstrated in earlier studies

5. Results

5.1. Weed seedbank

The seedbank revealed a total of 18 different species (Table 5.2). The dominant species in the seedbank were *Matricaria chamomilla* L. (MATCH) and *Alopecurus myosuroides* Huds. (ALOMY), and represented respectively 73.6% and 18.7% of the seedling density. *Polygunum aviculare* L. ranked third and represented only 4.4% of all seedling density.

ANOVA revealed a significant interaction of sampling depth, tillage and residue exportation on seedling density. ANOVA results are provided in Table C.3 in the supplementary material. Results were separately analysed by sampling depth. In the 10-25 horizon, no significant difference in seedling density was observed between the different residue management methods. However, on the 0-10 horizon, weed density was lowest in CT-IN and highest in RT-IN.

Concerning the seedling density of the two most abundant species in the seedbank

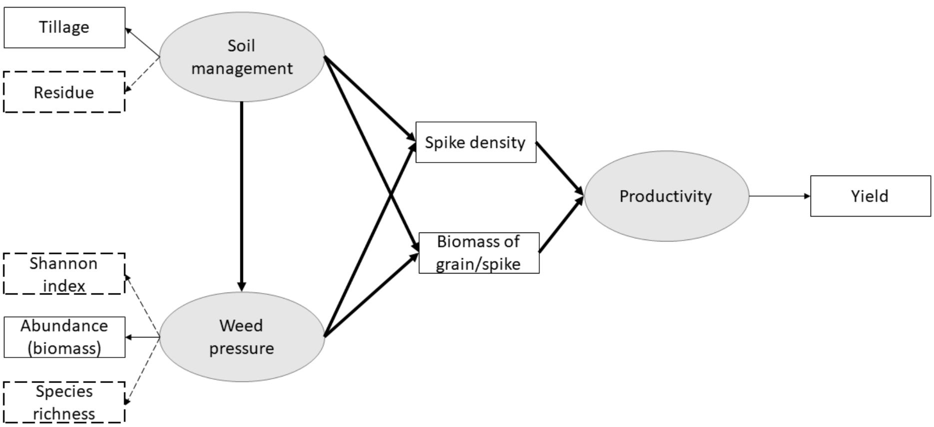

Figure 5.1: structural equation model for the relationship between productivity, yield components, weed pressure and soil management. Latent variables are in an oval colored in gray and manifest variable are in rectangle. A direct path is represented by a single arrow that directly connects two traits (e.g., residue management and weed pressure). The dotted rectangles correspond to the variables which were tested in the initial path analysis but which were not kept in order to respect the conditions of the path analysis.

Table 5.2: Number of species present in the seedbank trial and there weed seedling density proportion

Species EPPO code	Species Latin names	Number of individuals counted	% of total seedling counted
MATCH	*Matricaria chamomilla* L.	556	73.6
ALOMY	*Alopecurus myosuroides* Huds.	141	18.7
POLAV	*Polygonum aviculare* L.	33	4.4
CHEAL	*Chenopodium album* L.	5	0.7
SONAS	*Sonchus asper* (L.) Hill	5	0.7
CAPBP	*Capsella bursa-pastoris* (L.) Medick	2	0.3
CIRAR	*Cirsium arvense* (L.) Scop.	2	0.3
AETCY	*Aethusa cynapium* L.	2	0.3
ATXHA	*Atriplex prostrata* Boucher ex DC.	1	0.1
BROMO	*Bromus hordeaceus* L.	1	0.1
PAPRH	*Papaver rhoeas* L.	1	0.1
EPIAD	*Epilobium tetragonum* L.	1	0.1
GALAP	*Galium aparine* L.	1	0.1
STEME	*Stellaria media* (L.) Vill.	1	0.1
VIOAR	*Viola arvensis* Murray	1	0.1
TARSS	*Taraxacum* sp.	1	0.1
VERHE	*Veronica hederifolia* L.	1	0.1
ERICA	*Erigeron canadensis* L.	1	0.1

(MATCH and ALOMY), they both showed a significant interaction between sampling depth and tillage (see Table C.3). At depths of 10-25 no significant difference in seedling density was observed, whereas at 0-10 the weed seedling density was higher in RT than in CT (Figure 5.2).

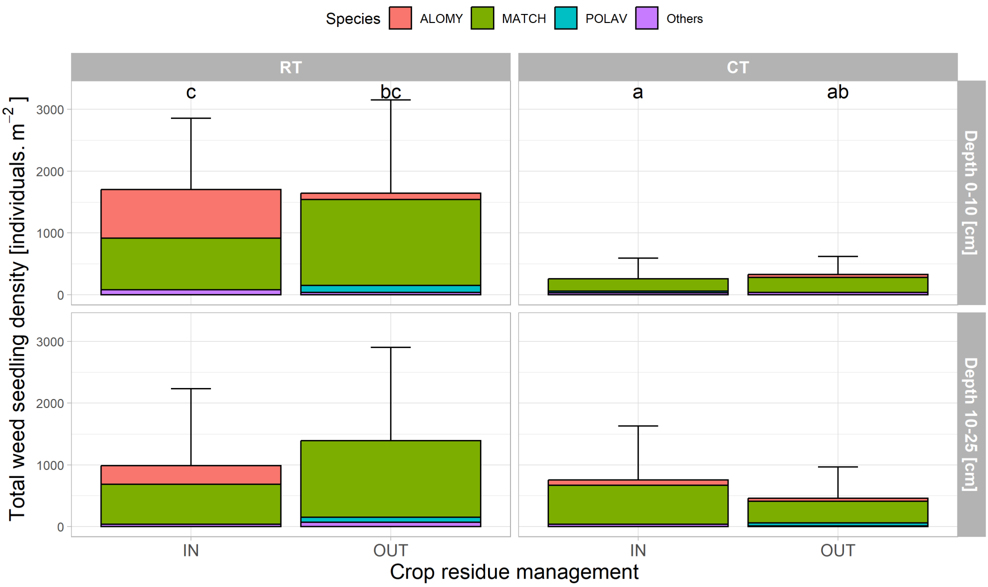

Figure 5.2: Total weed seedling density m^{-2} as a function of sampling depth and soil management. Treatments with the same coloured letters are not significantly different. Letters correspond to the interaction effect of total weed seedling density between the different soil management. "0-10" and "10-25" are respectively the sampled soil depths of 0-10cm and 10-25cm. The "others" group includes species present at less than 5%. RT=reduced tillage, CT= conventional tillage, IN = residue restitution, OUT= residue exportation.

The average species richness (sample scale) was significantly higher in RT compared to CT, with an average of one species more in favour of RT (3 and 2 respectively). The trend was identical for the Shannon index, with an average value of 0.55 in RT and 0.27 in CT (Figure 5.3). No significant difference was observed with the factor related to the exportation of residues.

5.2. In-season weed community expression

The weed flora at the end of winter was mainly composed of *Alopecurus myosuroides* Huds. (ALOMY) and *Matricaria chamomilla* L. (MATCH) (see in supplementary Table C.4 for all species present). The timing of the weed survey (at wheat tillering - before herbicide application - and at wheat flowering - after herbicide) had no impact on total weed density or on ALOMY density. However, a 56% reduction was observed in MATCH between the two surveys (pvalue=0.01213). CT reduced weed abundance

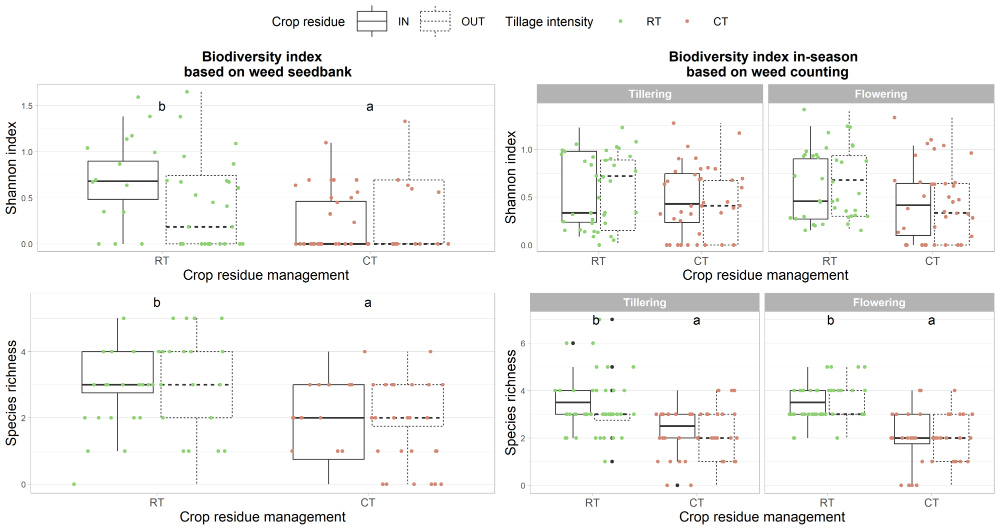

Figure 5.3: Biodiversity index (Shannon index above and species richness below) based on Weed Seedbank on the left and on weed counting in-season (in winter wheat) on the right as a function of crop residue management. Treatments with the same letters are not significantly different. RT= reduced tillage, CT= conventional tillage. IN= restitution of residues, OUT= exportation of residues.

(pvalue <0.0001) measured at tillering by 78% compared to RT (see Figure C.2 for spatial weed density). Similar trend was found for the two main weeds (ALOMY and MATCH), with an average reduction of 69% and 87% respectively (Figure 5.4). However, no significant effect of residue exportation (IN vs OUT) was observed.

At wheat flowering, weed biomass was significantly higher in RT than in CT, with an average biomass of 24 g m^{-2} and 12.2 g m^{-2} respectively (Figure 5.4). While the trend was identical for MATCH (pvalue=0.001475), there was no significant effect of tillage on ALOMY biomass (pvalue= 1.8074).

No effect of weed survey, tillage and residue exportation on the Shannon index was observed (Figure 5.3). However, tillage had an effect on the average number of species (sample scale), with an average of 3 species in RT and 2 species in CT.

5.3. *Impacts of weeds on crop growth and yield components*

Total weeds biomass exhibited a negative correlation with yield with a value of -0.58. When comparing the yield of quadrats with the highest (50g m^{-2}) and lowest (0g m^{-2}) weed biomass, 28% loss of yield was recorded (see in supplementary Figure C.3). Regarding the compartments of the plant, the greatest correlation with total weed biomass was found with spike biomass (-0.57), then total biomass (-0.55) and finally stem biomass (-0.47). At flowering, the impact of weeds on total crop biomass was

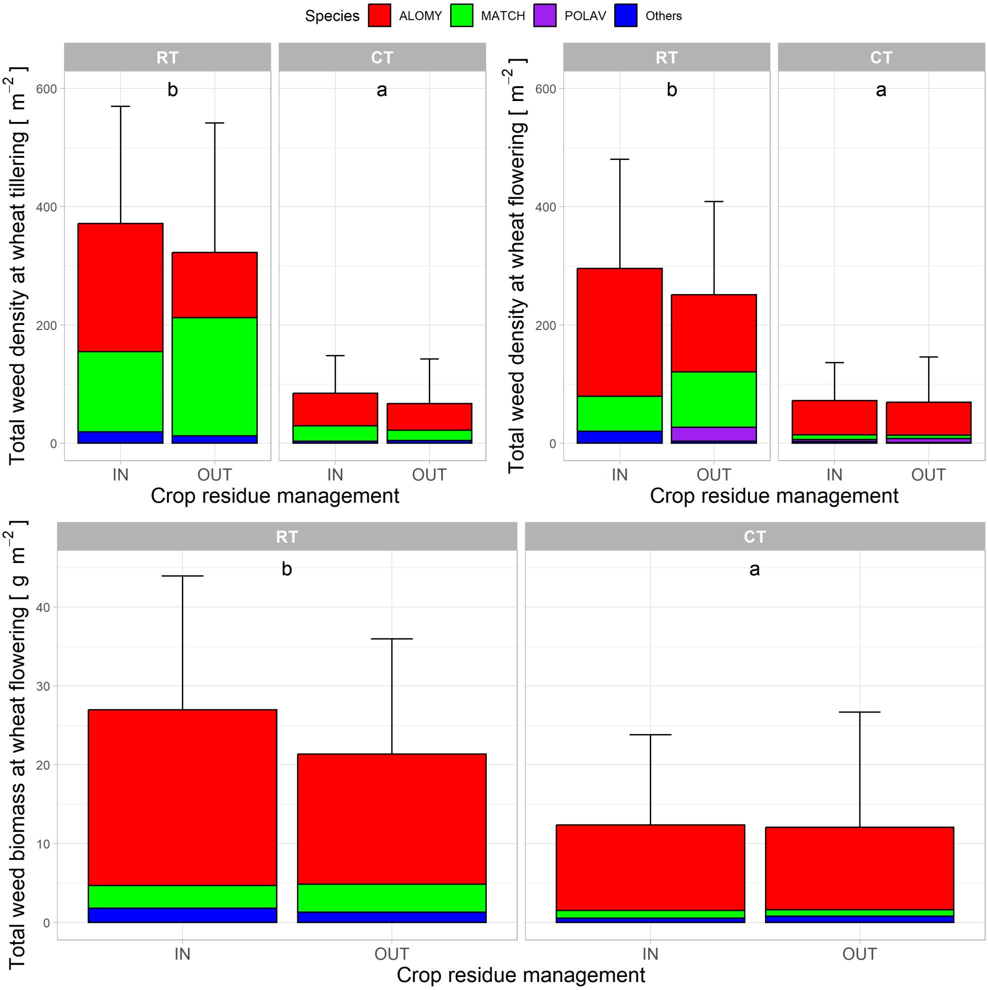

Figure 5.4: Weed density at wheat tillering and at wheat flowering (top graph) and biomass of weeds at wheat flowering (bottom graph). Treatments with the same letters are not significantly different. Letters correspond to the effect of tillage on weed density or biomass. The "others" group includes species present at less than 5%.

RT= reduced tillage, CT= conventional tillage. IN= restitution of residues, OUT= exportation of residues.

already noticeable (correlation of -0.36).

Upon examining the yield components, total weed biomass and ALOMY biomass exhibit negative correlations with spike density (resp. -0.5 and -044), and the biomass of grains per spike (resp. -0.33 and -0.34) (Table 5.3). The correlation with weed biomass was furthermore a bit higher for the spike biomass than with the spike density. The weakest correlations were reported with the biomass per spike and with the biomass of grains per spike. The same trends were observed for ALOMY, but no significant correlations were observed with MATCH.

Table 5.3: Significant correlation between yield components and total weed biomass and ALOMY. The gradient of the red colour indicates the level of the negative correlation between the variable and yield. The more negative the correlation, the more intense the red colour.

Yield component	Weed biomass	ALOMY biomass
Yield	-0.58	-0.54
Spike biomass	-0.57	-0.52
Total biomass	-0.55	-0.52
Spike density	-0.5	-0.44
Stem Biomass	-0.47	-0.46
Biomass at flowering	-0.36	-0.35
Grain biomass per spike	-0.33	-0.34
Biomass per spike	-0.32	-0.35

5.4. *Path analysis*

When an ANOVA was carried out on yield as a function of tillage and export residue the effect of tillage is significant (pvalue =0.04326). However, when weed biomass was added as an explanatory variable to predict yield, no effect of tillage was observed (pvalue= 0.306) whereas the effect of weed biomass was significant (pvalue=0.00525). This led us to consider an integreated approach trougth the path analysis. The final model (Figure 5.1 without the dotted rectangles) met all the statistical conditions to perform a relevant path analysis (pvalue>0.05, CFI=0.997, TLI=0.990, RMSEA= 0.081, SRMR= 0.006). Soil management (here only represented by the tillage practice) had a path coefficient σ_{direct} that is exclusively significant with weed pressure ($\sigma_{\text{direct}} = -0.38$). Soil management did not exhibit any significant direct coefficient with yield components. On the contrary, weed pressure (expressed here through the manifest variable of the abundance measured in terms of biomass) was the only significant factor impacting yield components. A grater path coefficient was found for spike density ($\sigma_{\text{direct}} = -0.44$) compared to the biomass of grains per spike ($-\sigma_{\text{direct}} = -0.33$). Spike density was the most impactful component on productivity with a $\sigma_{\text{direct}} = 0.84$, while the biomass of grain per spike has a σ_{direct} equalling 0.39 (Figure 5.5).

The indirect effect of weed pressure on productivity were mainly expressed by the effect on the number of spikes ($\sigma_{\text{indirect}} = -0.37$) and to a lesser extent through the biomass of grains per spike ($\sigma_{\text{indirect}} = -0.13$). The global indirect effect on weed pressure productivity is -0.50. Finally, and consequently to those results, the indirect significant influence of tillage was expressed through weed suppression. The indirect path coefficient on spike density equalled 0.17 and the σ_{indirect} on grain biomass per spike equalled 0.13, for a global indirect path coefficient on productivity equalling 0.29.

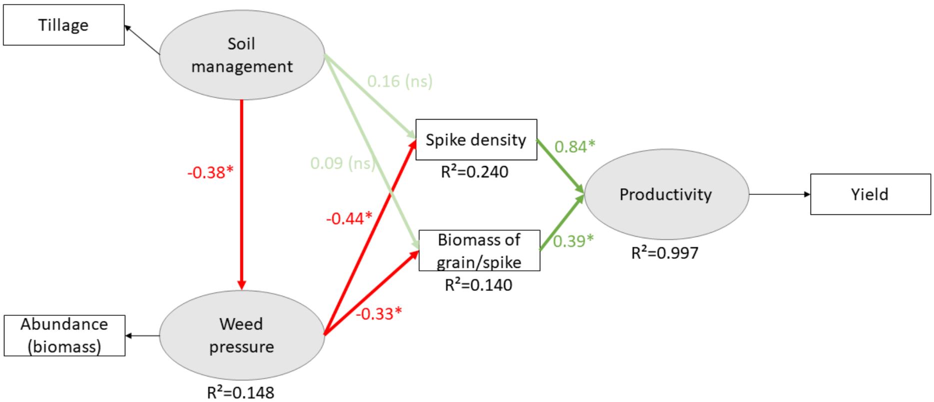

Figure 5.5: Path coefficients of the final model for the relationship between productivity, yield component, weed pressure and soil management (only tillage practices). Latent variables are in an oval colored in gray and manifest variable are in rectangle. Path coefficients (σ) were computed from regressions (red arrow = negative and green arrow = positive). A direct path is represented by a single arrow that directly connects two traits (e.g., soil management and weed pressure) whereas an indirect path occurs when the path between two variables is separated by other(s) variable(s) (e.g., productivity and weed pressure). Insignificant paths (pvalue>0.05) are indicated by "ns", statistical significance of the path coefficient at p-value ≤ 0.05 is indicated by "*".

6. Discussion

6.1. Impact of long-term soil management in weed diversity

Weed diversity was relatively low in all treatments. Only two species (ALOMY and MATCH) dominated both the seedbank and the expressed weed flora. A slight increase in both Shannon diversity and species richness (on average one more species) was observed in the seedbank in RT compared to CT. The same trend was found in the flora expressed during the winter wheat cropping season, despite no clear pattern in Shannon diversity was found. The results are in line with those of long-term trial documented in the literature. Within the seedbank, Sosnoskie et al. (2006) showed a slightly higher specie richness in RT compared to CT, with ca. 2 species more. However, they reported no difference in the Shannon index between RT and CT, while in the present study, a significant, yet low, difference was reported. The results regarding the expressed flora were in line with those of the long-term trial by Plaza et al. (2011), where no differences in terms of Shannon diversity were observed and the same trend of a slight increase in species richness (+1 species on average) in RT. It was hypothesized by the authors that RT could allow a slight increase in the number of species due to a greater diversity of ecological niches and germination opportunities. Complementary, results gain in the present study suggest that residue exportation had no reported long-term impact on weed diversity.

The prevalence of two dominant weed species, particularly associated with cereal crops, can be attributed to the rotational strategy employed. The rotation emphasizes the recurrent cultivation of winter crops, initiated in early autumn during the trial, contributing to the establishment of a distinctive flora (Nichols et al., 2015; Storkey and Neve, 2018). The effect of crop rotation is indeed known to be a much more powerful driver of weed flora composition than tillage (Fried et al., 2008).

6.2. Impact of soil management on weed density

As previously observed in the literature, RT increases the seedling density of seedbank on the upper soil layer compared with CT. However, the quantity of weed seedling within soil depth 10-25 cm was found to not be statistically different between CT and RT, which result is in line with other studies (Cardina et al., 2002; Schnee et al., 2023). This effect was confirmed independently for the two dominant weeds (ALOMY and MATCH).

The expressed weed density measured before the first weeding operation was higher in RT, in agreement with several long-term studies (Plaza et al., 2011; Santín-Montanyá et al., 2013, 2016). However, in the current study, no major effect of residue exportation was observed on the weed seedbank and the expressed weed density during the wheat cropping season. A potential explanation could be associated to the dilution of surface residue, which only occurs within the 0-10 cm soil profile in reduced tillage and would contribute to explain such results. Indeed, it is likely that the mulch effect impacting the density of germinating weeds, as observed by Anderson (1999) under no-tillage system, was not expressed in this case. Furthermore, this might be reinforced by the fact that the preceding crop (sugar beet) returns only a small quantity of residue on the field. The actual effect of residues on the expressed weed flora is more likely to be observed after a crop leaving a larger quantity of residue (such as wheat or maize).

Chemical weed control did not result in a reduction of total weed density during the season. Moreover, when examining the species individually, it became evident that the herbicide exhibited no discernible impact on the ALOMY population. Following complementary laboratory analysis (data not shown), it was determined that this ALOMY population demonstrated resistance to the spring herbicides used during winter wheat cultivation (resistance to Acetolactate Synthase and Acetyl CoA Carboxylase). The emergence of this resistance may be attributed to the recurrent use of identical active ingredients in winter wheat (Zeller et al., 2021). Indeed, since 2008 the mode of action of Inhibition of Acetolactate Synthase has always been applied in winter wheat and Acetyl CoA Carboxylase was applied in 2014, 2020 and 2022 (Table 1). Conventional tillage (CT) proved to be an efficient method for managing the ALOMY population in comparison to reduced tillage (RT). Zeller et al. (2021) demonstrated that ALOMY was reduced by 70 to 80% when rotational ploughing was implemented. Weed biomass, on average, was higher in RT than in CT, indicating that the greater number of weeds at tillering led to an increased total weed biomass. However, there was no discernible significant impact on ALOMY biomass among the tillage and residue exportation methods. One might have thought that the non-significant effect was due to a higher biomass per ALOMY in CT than in RT, but no significant effect of biomass per ALOMY was observed (see Table C.3).

6.3. Impact of tillage, residue exportation and weeds development on yield.

When performing ANOVA between yield and soil management (tillage and residue exportation), significant impact was reported for soil tillage. A higher yield were reported under CT and was in line with the European literature (Van den Putte et al., 2010). On the other hand, the ANCOVA between the yield, soil component and the biomass of weed as an explanatory variable (see Table C.3), the analysis revealed no effect of tillage. Instead the sole influential factor was the weed biomass. In this trial, the effect of tillage appears to be indirect, as highlighted by the path analysis.

Weed pressure was found to be also significantly linked with yield (using both ANOVA and path analysis). This highlights the importance of regulating weed flora in the event of weed infestations. Looking more in depth to the impact of the different species, MATCH, although present, was not significantly correlated with yield losses. However, ALOMY was found to explain the majority of the loss (-28% between an area without naturally ALOMY and an area with 50g of ALOMY m^{-2} s, Figure C.4). ALOMY is a species that is phylogenetically close to wheat and shares similarities in its development, with the same germination period and a slightly shorter cycle, which means there is a great deal of competition for resources (Adeux et al., 2019b). No direct effect between crop residue management and yield was reported by the path analysis. However, results gained in this study suggest that tillage expressed its impacts mostly through the control it puts on weeds which themselves had direct significant impacts on yield components.

Among the yield components studied, the one that explained yield the best was spike density, in agreement with the literature (Lenoir et al., 2023; Slafer et al., 2014)(Lenoir et al., 2023; Slafer et al., 2014). This component exhibited the highest path analysis coefficient with weed pressure. It was confirmed in this study that the competition induced by weeds leads to a loss of yield, mainly by reducing the wheat's capacity to produce spikes, as suggested in previous studies (Adeux et al., 2019b; Welsh et al., 1999). This confirms that competition can act early in the season (Welsh et al., 1999; Zimdahl, 2007b) and can lead to a greater tillers recession when wheat competes with weeds for light and nutrients in the environment. It would therefore be interesting to monitor tillers dynamic earlier in the season to confirm this hypothesis. Finally, weed-induced competition was found to cause yield losses, to a lesser extent, by affecting grain filling (monitored here through the grain biomass per spike). Adeux et al. (2019b) showed in their experiment that a weed community composed almost exclusively of ALOMY had no effect on the 1000-kernel weight but did have an effect on the number of grains per spike, suggesting that the competition generated by ALOMY takes place until wheat flowering. The indirect effect of tillage management on yield through weed competition could explain the earlier observation reported by Hiel et al. (2018) on the same experimental site, who did not systematically observed an impact of soil management over the year but who reported a -3.4% cumulative yield decrease between 2010 and 2015.

7. Conclusion

The long-term effect of tillage and residue management, by exporting or maintaining residues on site and incorporating them or not through tillage, showed no effect of residues exportation on yield and weeds. The lack of link between weed flora (diversity and abundance) monitored through the seedbank or during the cropping season of winter wheat showed that, in a rotation based on wheat, residue exportation was of little importance in the context of this study. The lack of effect of maintaining residues could be explained by a dilution of crop residues in the upper soil profile that still occurs in some reduced tillage (RT) systems (such as the one implemented here), preventing the mulch effect to occur. Reduced tillage was found to have no major impact on weed diversity (richness was a little bit higher compared to conventional tillage) but resulted mostly in an increase of weed density. While this increase is in line with results reported in other long-term trials, it was most likely exacerbated in this case by the frequent return of autumn crops to the rotation. In a system based on wheat, RT might facilitate the development of ALOMY, a very competitive species that is detrimental to yield. This management technique might favour the appearance of resistance

-as observed in this trial, especially in winter wheat-based cropping systems. Above all, it highlights the problem of long-term sustainable management of the weed flora. Reduced tillage management technique might indirectly lead to higher yield losses through poor control of the weed flora in systems based on wheat cultivation. In this regard, while RT is promoted for its potential to maintain or enhance soil health over the long term, it would be interesting to compare the sustainability of weed management within different soils and cropping systems management, including systems with a higher proportion of spring crops.

6

Chapter VI: Assessing the impact of three organic cropping systems management on weed flora during maize cropping season

1. Synopsis

After observing the long-term effect of tillage and residue management on weed flora in conventional farming, we move on to a second long-term trial. Using a trial system set up in 2019, this chapter looks at the effect of three different CS in organic farming. These CS are characterised by variations in the intensity of fertilisation and tillage, with rotations and cropping operations adapted to meet these objectives.

This chapter relies on the following paper: Lacroix, C., Huyghebaert, B., Sail, S., Abras, M., and Dumont, B. (2024). *Assessing the impact of three organic cropping systems management on weed flora during grain maize cropping season. European Journal of Agronomy, under review.*

2. Abstract

In organic farming, fertility and weed management are major issues. On another hand, reduced tillage is a practice widely used in conventional farming because of its benefits for the soil. In organic farming, however, reduced tillage is more challenging due to the ban on the use of herbicides for weed management. In the Hesbaye region of Belgium, characterized by loamy soil and temperate climatic conditions, a long-term trial was set up with three organic farming rotations without ley. A characterization of the weed flora in maize crops was conducted over 3 years. Weed diversity was assessed using the Shannon diversity index, species richness, and evenness. Subsequently, the impact of flora and management on yield was investigated. Weed-free quadrats were installed to differentiate the effect of weeds from the effect linked to the systems. Organic fertility showed no effect on weed flora composition, weed diversity, or weed pressure (density or biomass). However, it did result in a yield that tended to be higher. Reduced tillage resulted in greater diversity in terms of species richness and Shannon diversity index. However, evenness was lower. Species composition was significantly different, with the presence of problematic perennial weeds such as *Cirsium arvense*. Weed pressure was higher in reduced tillage, with a 190% increase in weed biomass compared to plowing. This resulted in significant impacts on the growth of maize, which had a biomass 64% higher when weeds where manually removed. Maize yield was also impacted, with a loss of 45% compared to plowing. This article concludes that reduced tillage is difficult to recommend in this context due to poor weed management, which can cause major yield losses in maize crops.

3. Introduction

The two main challenges that organic agriculture is facing are the management of crop nutrition and the management of weeds, which can lead to important yield losses (Zikeli and Gruber, 2017). Effective fertility management is paramount. Due to restrictions on the use of mineral fertilizers, fertility primarily relies on the application of exogenous organic matter (livestock effluents, compost, etc.), the use of rich and varied green manures, the intercropping with legumes and the lengthening and diversification of rotations (Watson et al., 2002). Integrated livestock systems offer the benefit of incorporating ley in the rotation, which plays a major role in organic farming in terms of soil fertility (Watson et al., 2002). Moreover, the ley is one of the major levers for the long-term management of weed flora (Grosse et al., 2021), reducing the weed seedbank (Albrecht, 2005; Melander et al., 2020; Sjursen, 2001). Additionally, ley can serve as a crucial tool for controlling *Cirsium arvense* (Grosse et al., 2021). On the other hand, with the specialisation of agriculture, the number of farms without livestock is significant in certain agricultural regions, such as the Hesbaye in Belgium (Statbel, 2024). For organic stockless farming, the integration of ley in crop rotation is less advantageous as it has low commercial value. To better control the release of nutrients and meet the needs of the crops, the use of commercial organic fertilizer is therefore a common solution that ensure quicker nitrogen availability during the season, in comparison to e.g. manure. These commercial organic fertilizers are easily mineralized within the year of application ensuring additional nitrogen supply for the crop (Hartz and Johnstone, 2006). However, these commercial organic fertilizers are quite expensive and can be an obstacle for farmers who don't have in their rotation high added-value crops -e.g. no vegetables in the rotation- and have therefore to carefully assess the cost-to-benefit ratio of using such fertilizers (Brust, 2019; Hartz and Johnstone, 2006).

Conservation tillage is mostly used in conventional agriculture for its beneficial effects on the soil. Conservation tillage, which includes reduced tillage (RT), prevents soil erosion, builds up organic matter in the upper part of the soil and improves water infiltration (Holland, 2004). While, these advantages are also confirmed in organic farming (Krauss et al., 2020; Mäder and Berner, 2012; Peigné et al., 2007), RT techniques are rarely used in organic farming because of the complexity to manage weeds, leading to higher weed pressure (Krauss et al., 2020). This is amplified by the ban on applying herbicides in organic farming and the delay in nutrient mineralization (Cooper et al., 2016; Mäder and Berner, 2012). Hence ploughing is one of the major tools for managing weed community in organic farming. In a meta-analysis by Cooper et al. (2016) RT in organic farming resulted in yield losses of around 7.6% compared to deep inversion tillage. Yield stability under RT compared to conventional tillage (CT) can be reduced due to water, air, N supply and weed pressure (Krauss et al., 2020). After 15 years of reduced tillage trials in Switzerland, Krauss et al. (2020) showed that, on average, weed infestation under RT was 173% higher than under CT. This infestation was even more marked in spring crops such as maize and sunflower, with weed infestation 265% higher than in CT. This trend was also observed in the seedbank, with a seedbank slightly more than twice as large in RT compared with CT. In addition, RT tends to favour grasses and perennials such as thistle (*Cirsium arvense*

(L.) Scop.) (Gruber and Claupein, 2009; Krauss et al., 2020; Peigné et al., 2007; Sans et al., 2011). However, higher weed pressure does not necessarily result in yield losses compared to conventional farming, as shown by the meta-analysis by Cooper et al. (2016) and by other studies (Armengot et al., 2015; Hofmeijer et al., 2019). The effect of RT on mineralisation, the risk of compaction and weed pressure on yield is often confused (Mäder and Berner, 2012). Hofmeijer et al. (2019), in their studies of winter wheat in a long-term trial in Switzerland, showed that in this crop there was no difference in yield between RT and CT, but that RT exhibited 15-18% higher yield when weeds were removed. Highlighting a yield loss caused by weed competition. A study to a group of European organic farmers practising conservation agriculture revealed that weed management was the primary concern hindering the adoption of RT practices (Casagrande et al., 2016).

To assess the associated risk and try to address these challenges, a long-term trial was initiated in Belgium in autumn 2018, examining three organic rotation systems. Globally the assessed cropping systems exclude the use of ley (as the area is mostly dedicated to crop production, with few livestock). The systems differ in the intensity of soil tillage and organic fertilizer use. To compensate for the absence of commercial organic inputs in some systems, leguminous-cereal combinations were used as levers to sustain fertility, but are also known to help in managing more effectively weeds compared to non-associated cultivated legumes crops in pure (Bedoussac et al., 2015). In addition, the composition of the cover crops in such systems were more complex than in the business-as-usual organic rotation, with a greater proportion of legumes to increase biological nitrogen fixation (Robačer et al., 2016; Tonitto et al., 2006).

This study focused on the characterization of the weed community during maize cropping season (a crop common to all three cropping systems during the three years of monitoring). The objective of this study were (1) to evaluate weed pressure in three organic cropping systems (2) to study the impact of the three systems on weed diversity and (3) to evaluate the effect of weed communities development on yield.

4. Material and Methods

4.1. Site description and experimental setup and management

The long-term trial is established since autumn 2018 on the experimental farm of Walloon Agricultural Research Centre (CRA-W), in Belgium (50°33’36.8"N 4°43’ 09.9"E). The climate in this region is oceanic temperate (Climate Cfd in the Köppen-Geiger classification) with an average annual rainfall of 793.4 mm, an annual average temperature of 9.6 °C and an average solar radiation of 825J cm^{-2} day^{-1}. The soil type is classified as Cutanic Luvisol (FAO_WRB classification) with a silt loam texture (18-22% of clay, 70-80% of silt, and 5-10% of sand).

Three organic cropping systems (CS) are being evaluated in the trial. One system represents business-as-usual conventional organic practices (inspired by the usual management of organic farms in the study area) and includes the use of soil inversion techniques (ploughing) and the use of commercial organic fertilizer (conventional tillage-commercial organic fertilizer imput (CT-COF)). The second system omitted the use of commercial organic fertilizer (conventional tillage-low fertilizer imput (CT-LF)), while the third system involved reduced tillage concomitantly with the absence of commercial organic fertilizer (reduced tillage-low fertilizer imput (RT-LF)). To sustain the soil fertility, the two systems managed without commercial organic fertilizer make use of a greater and more diverse range of intercropping as well as more complex cover crop compositions, including legumes.

The duration of the rotations for all three cropping systems is 7 years (see Table 6.1). The trial was conducted with "temporal" replication over three years (Lechenet et al., 2017), i.e. with crop rotations starting in cropping seasons n, n+1, and n+2. Three successive crops within the sequence of each cropping system are grown each year. The trial therefore comprises nine plots; one experimental single plot being 12 m wide and 225 m long (see Figure D.1).

Table 6.1: Management summary of the three cropping systems in terms of fertilisation, tillage, rotation and weed management

	CT-COF	CT-LF	RT-LF
Tillage	Conventional tillage (CT)	Conventional tillage (CT)	Reduced tillage (RT)
Fertilisation	Exogenous manure (2X rotations) + commercial organic fertilizer	Exogenous manure (1X rotation)	Exogenous manure (1X rotation)
Rotation	Winter wheat, cover crop, potatoes, spelt, cover crop, malting barley, cover crop, maize, faba beans, and rapeseed association	Wheat-pea, cover crop, malting barley, cover crop, lentil-camelina, spelt-pea, cover crop, grain maize, wheat-faba bean, and rapeseed association	Wheat-pea, cover crop, malting barley, cover crop, lentil-camelina, spelt-pea, cover crop, grain maize, wheat-faba bean, and rapeseed association
Cover crop	White mustard,phacelia	Complex cover crop (cereals + legumes + phacelia + mustard or sunflower) like: spring oats, spring vetch, berseem clover, spring faba bean, sunflower, phacelia	Complex cover crop (cereals + legumes + phacelia + mustard or sunflower) like: spring oats, spring vetch, berseem clover, spring faba bean, sunflower, phacelia
Seedbed	A dual cultivator (Jadin) in front and integreated seed drill (Kuhn)		
Manure application	Before maize and rapesseed	Before rapeseed	Before rapeseed
Comercial organic fertilizer	In all crop except faba beans	No commercial organic fertilizer	No commercial organic fertilizer
Weed control	2-3 inter-row cultivator in maize and rapeseed. 2-3 weeder (harrowing or rotary hoe or rotative weeder) for other crops		
False seedbed			If good weater condition before maize
Perennial weed	Hand weeding and stubble breaking in intercropping phase		

The organic business-as-usual CS (CT-COF) consists in a sequence of crops including winter wheat, potatoes, spelt, malting barley, grain maize, faba beans, and rapeseed association (with companion plants). In contrast, the two CS excluding the use of commercial organic fertilizer inputs follow a rotation of crops made of wheat-pea (intercropping), malting barley, lentil-camelina (intercropping), spelt-pea (intercropping), grain maize, wheat-faba bean (intercropping), and rapeseed (association with companion plants). The management itineraries for these three CS are summarized in Table 6.1, with additional details available in Table D.1 for maize crop.

4.2. Field data collection

4.2.1. Crop and weed sampling

To compare and characterise the weed community present in the three different cropping systems and its impact on yield, samples were collected during the grain maize cropping season, as grain maize was the common crop across all systems. Samples were collected annually for three years (between 2021 and 2023). Weed density by species was measured before the first maize weeding operation and at maize flowering, using six quadrats of 0.75m x 1m arranged following a 'W' sampling pattern within each plot in 2021. In 2022 and 2023, weed density was assessed using 10 quadrats of 0.75m x 1m along the 'W' sampling patterns. Additionally, weed biomass and crop biomass were measured in the same quadrats at maize flowering. Furthermore, in 2022 and 2023, maize biomass at flowering was assessed in 10 weed-free quadrats of 0.75m x 1m (hand-weeded) per plot, arranged in a 'W' sampling pattern, to evaluate maize biomass without weed competition. All biomass samples were oven-dried at 60°C to constant weight.

Finally, at maize maturity, yield was measured with combine harvester in three different zones of the field in 2021 and 2022 and in four different zones of the field in 2023. Samplings collected during the season (weed density, as well as weed and maize biomass at maize flowering) were matched to yield zones. Samplings that were too close to two yield zones were removed, while samplings that were present within a given yield zone were averaged (see Figure D.2).

4.3. Weed diversity index

Species richness (number of species present per quadrat), Shannon-Weiner index and evenness index were calculated at flowering of maize. Shannon-Weiner index (H – eq.1) and evenness (E – eq. 2) were calculated at quadrat level and express as follow:

$$H = 1 - \sum_{i=1}^{S} p_i \ln(p_i)$$

$$E = \frac{H}{\log_2 S}$$

Where p_i is the relative proportion of individuals of species *i* in a community of *S* species, *H* the Shannon diversity index and *E* the evenness. The evenness is comprised between 0 and 1. It evaluates the similarity of abundances of each species. A value of 1 indicates identical abundances across all species, while a value closer to zero suggests dominance by a few species.

4.4. Statistical analysis

Statistical analysis were perform using R statistical Software (V4.3.1; R Core Team, 2021). Response variables (weed density, weed biomass, species richness, Shannon index, eveness, yield) were modelled with the *glmmTMB* package. Model diagnostics were verified with the *DHARMa* package. Firstly, response variables were expressed as a function of CS, year and their interaction. Concerning the weed density, crop development stages were also studied. In this case response variable were expressed as a function of CS, year, crop stage and their triple interaction. The sample nested within the plot was included as a random effect due to the consistent location of the samples across both stages. Finally, the crop biomass was also expressed as a function of CS, Hand weeding, year and their interaction. Distribution was selected to meet the conditions. In addition, the model with the lowest AIC was chosen. All the selected models are detailed in Table D.2. ANOVA were performed on these models to assess the significance of fixed effects. Finally, an estimated marginal means analysis was performed using the *emmeans* package.

In a second step, regressions were conducted to analyze the relationships between weed biomass and diversity indices (Shannon diversity index, species richness, evenness) across different years. Additionally, regressions were performed to examine the relationships between crop biomass, diversity indices, and weed biomass, with year included as a fixed factor along with crop density as covariate. Finally, two regressions were conducted to assess the relationship between yield and crop (or weed) biomass, with the year included as a fixed factor. Similar to previous steps, appropriate distributions were selected to meet model conditions, and the model with the lowest AIC was chosen. When certain AICs were close, the model with the best agronomic significance was chosen (e.g. the model with no negative estimate of biomass). ANCOVA tests were then applied to these models to evaluate the significance of variables. All selected models are presented in supplementary Table D.2

To assess whether the weed composition at maize flowering differed among the three CS, a principal coordinates analysis (PCoA) was conducted using the Bray-Curtis dissimilarity matrix with the vegan package. Subsequently, a permutational multivariate analysis of variance (PERMANOVA) was performed on the Bray-Curtis dissimilarity to evaluate the effect of CS. Post-hoc analysis was conducted using the *pairwise.adonis* function to compare the weed communities between pairs of CS. Additionally, an analysis of similarities (ANOSIM) was conducted using the *vegan* package to determine if the dissimilarities between the CS were significantly greater than within the CS.

5. Results

5.1. Weed community characterisation

The most prevalent species at emergence across all CS and across three years were *Chenopodium album* L., *Persicaria lapathifolia* (L.) Delabre, *Matricaria chamomilla* L. and *Stellaria media* (L.) Vill., with a total of 33 different species observed. Weed density exhibited a significant interaction between CS and year, as well as between year and growth stage (see Table D.3 for all ANOVA results).

Before the first mechanical weeding operation, in 2021, no significant differences were observed among the three CS, and the average weed density equaled 123 weeds m^{-2}. However, in 2022 and 2023, RT-LF showed significantly higher weed pressure compared to CT-COF and CT-LF, with respective weed density before weeding of 83, 7 and 4 weeds m−2 in 2022, and 132, 12, and 12 weeds m−2 in 2023 (Table 6.2).

At maize flowering, weed density reached 130 weeds m^{-2} in RT-LF in 2022, while CT-COF and CT-LF had density of 18 and 11 weeds m^{-2} respectively. Similarly, in 2023, weed density was 116 weeds m^{-2} in RT-LF, while it equaled 14 and 11 weeds m^{-2} in CT-LF and CT-COF respectively. Weed density at maize flowering are illustrated in Figure 6.1.

Weed biomass at maize flowering was influenced by both the year and CS. RT-LF exhibited a higher weed biomass compared to CT-COF and CT-LF, as reported in Table 6.2. The weed biomass of RT-LF ranged on average from 101.55 g m^{-2} in 2023 to 260.90 g m^{-2} in 2021, while CT-COF and CT-LF ranged from 11.49 g m^{-2}in 2023 to 145.21 g m^{-2} in 2021.

The average species richness at maize flowering exhibited an interaction between the year and CS. RT-LF consistently demonstrated the highest average species richness, ranging from 9.6 species in 2022 to 11 species in 2023. In contrast, CT-LF displayed an intermediate number of species compared to RT-LF and CT-COF. Specifically, in 2021, the number of species in CT-LF was not significantly different from either RT-LF or CT-COF, with an average value of 7.6 species per quadrat. However, in 2022 and 2023, the number of species in CT-LF significantly differed from RT-LF, with approximately half the number of species observed in RT-LF (3.7 species in 2022 and 5.0 species in 2023). CT-COF, on the other hand, showed significant differences from RT-LF (but not from CT-LF) in both years, with an average number of species per quadrat ranging between 4.5 and 6.7 species.

The CS and the year influenced the Shannon diversity index. RT-LF exhibited greater diversity, with values ranging from 1.47 in 2022 to 1.90 in 2021, compared to CT-LF and CT-COF, which had Shannon diversity indices ranging from 1.21 for CT-COF in 2023 to 1.68 for CT-LF in 2021. Regarding evenness, an interaction between CS and year was observed. In 2021, no differences among CS were detected. However, in 2022 and 2023, the evenness was significantly lower for RT-LF (0.65 in 2022 and 0.71

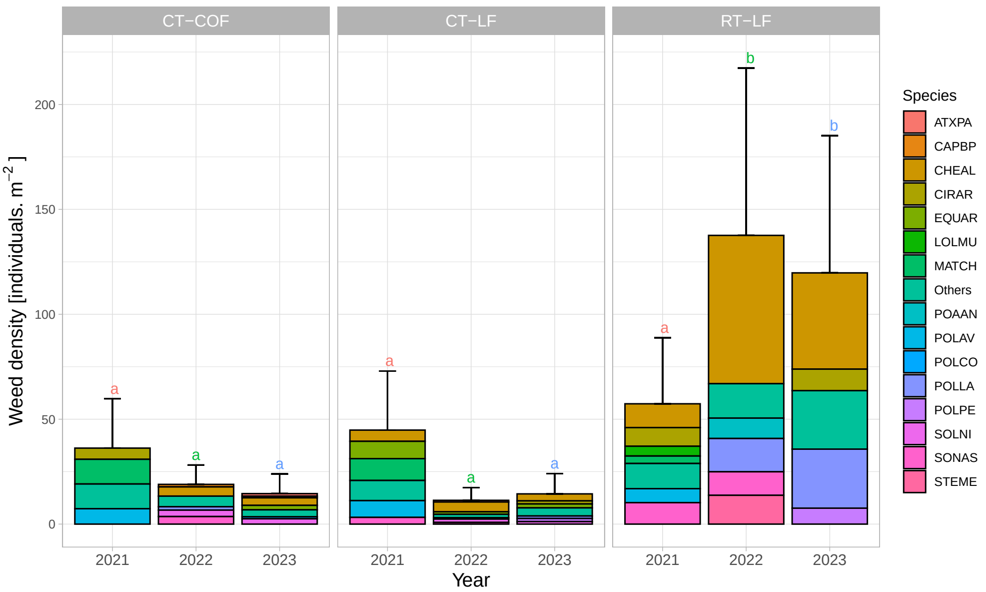

Figure 6.1: Weed density per species (named by to their EPPO code, https://gd.eppo.int/) at maize flowering according to different Cropping systems in 2021, 2022 and 2023. Treatments with the same color and letters are not significantly different. The letters correspond to the effect of the cropping system on weed density per year. The "others" group includes species present at less than 5%. CT-COF refers to the cropping system under conventional tillage with commercial organic fertilizer input. CT-LF indicates the system under conventional tillage with low fertilizer input, while RT-LF refers the cropping system with reduced tillage and low fertilizer input.

in 2023) compared to CT-LF and CT-COF (ranging between 0.85 and 0.92).

Table 6.2: Least square means and their standard errors for weed density (before the first weeding and at maize flowering), weed biomass, diversity indices (species richness, Shannon diversity index and evenness, based on density at maize flowering), and crop performances (maize biomass at flowering and yield). Treatments with the same capital or lowercase letters are not significantly different. Capital letters are used when no interaction between the cropping system and the year is observed. CT-COF refers to the cropping system under conventional tillage with commercial organic fertilizer input. CT-LF indicates the system under conventional tillage with low fertilizer input, while RT-LF refers the cropping system with reduced tillage and low fertilizer input. See supp. Table.A.2 for all models used and Table D.3 for all ANOVA results

			Year						
		CS	2021		2022		2023		
Initial	Weed density (individuals $m^{-}2$)	CT-COF	93 (25)	a	7 (2)	a	12 (3)	a	
		CT-LF	89 (24)	a	4 (1)	a	12 (3)	a	
		RT-LF	128 (34)	a	83 (18)	b	132 (27)	b	
Flowering	Weed density (individuals $m^{-}2$)	CT-COF	32 (9)	a	18 (4)	a	14 (3)	a	
		CT-LF	31 (9)	a	11 (3)	a	13 (3)	a	
		RT-LF	51 (14)	a	130 (27)	b	116 (24)	b	
	Weed biomass (g m^{-2})	CT-COF	145.21 (48.13)		33.70 (10.26)		11.49 (3.96)		A
		CT-LF	142.20 (51.75)		47.76 (13.96)		26.96 (8.42)		A
		RT-LF	260.90 (80.76)		147.86 (37.88)		101.55 (27.18)		B
	Species richness	CT-COF	6.7 (0.8)	a	5.2 (0.6)	a	4.5 (0.6)	a	
		CT-LF	7.6 (0.9)	ab	3.7 (0.6)	a	5 (0.6)	a	
		RT-LF	9.8 (0.8)	b	9.6 (0.6)	b	11 (0.6)	b	
	Shannon diversity index	CT-COF	1.48 (0.16)		1.44 (0.12)		1.21 (0.12)		A
		CT-LF	1.68 (0.17)		1.06 (0.12)		1.34 (0.12)		A
		RT-LF	1.90 (0.16)		1.47 (0.12)		1.65 (0.12)		B
	Evenness	CT-COF	0.79 (0.04)	a	0.88 (0.03)	b	0.87 (0.04)	b	
		CT-LF	0.84 (0.05)	a	0.85 (0.03)	b	0.92 (0.04)	b	

Continued on next page

			Year					
		CS	2021		2022		2023	
		RT-LF	0.84 (0.04)	a	0.65 (0.03)	a	0.71 (0.03)	a
	Crop biomass ($g\ m^{-2}$)	CT-COF	534.24 (59.26)	b	869.77 (42.60)	c	633.31 (42.99)	b
		CT-LF	412.15 (60.44)	ab	642.08 (42.84)	b	542.34 (46.17)	b
		RT-LF	279.68 (64.15)	a	397.35 (43.13)	a	240.25 (43.34)	a
Maturity	Yield ($T\ ha^{-1}$)	CT-COF	3.38 (0.26)	b	7.04 (0.26)	c	7.62 (0.23)	c
		CT-LF	2.38 (0.26)	a	5.01 (0.26)	b	6.36 (0.23)	b
		RT-LF	1.62 (0.26)	a	2.22 (0.26)	a	3.33 (0.23)	a

The weed composition of RT-LF was significantly different from that of CT-LF and CT-COF (pval<0.05), as illustrated in the PCoA plot in Figure 6.2, and was characterized by a higher presence of several species, including *Lolium multiflorum* (LOLMU), *Poa annua* (POAAN), *Cirsium arvense* (CIRAR), *Trifolium repens* (TRFRE), *Trifolium pratense* (TRFPR), and *Taraxacum* spp (TARSS).

5.2. Effect of cropping system on crop biomass and yield

The biomass of grain maize at flowering exhibited an interaction between CS and year. In 2021, maize biomass at flowering was lowest for RT-LF at 279.68 g m^{-2} and highest for CT-COF at 534.24 g m^{-2}. CT-LF displayed intermediate biomass and was not significantly different from either RT-LF or CT-COF, with an average biomass of 412.15 g m^{-2}. In 2022, the trend remained consistent with that of 2021, except that CT-LF was this time significantly different from both CT-COF and RT-LF. In 2023, however, the biomass of RT-LF remained the lowest at 240.25 g m^{-2}, while CT-LF and CT-COF exhibited statistically similar biomass levels, with respective values of 542.34 g m^{-2} and 633.31 g m^{-2}.

Grain maize yields for the different CS exhibited an interaction between CS and year. The year 2021 was characterized by an exceptionally low yield. The yield in CT-COF was the highest at 3.38 t ha^{-1}, while yields in CT-LF and RT-LF were statistically identical at 2.38 and 1.62 t ha^{-1} respectively.

In 2022 and 2023, yields were highest in CT-COF (7.04 t ha^{-1} in 2022 and 7.62 t ha^{-1} in 2023), intermediate in CT-LF, and lowest in RT-LF, with the average yield in RT-LF being more than three times lower in 2022 than in CT-COF.

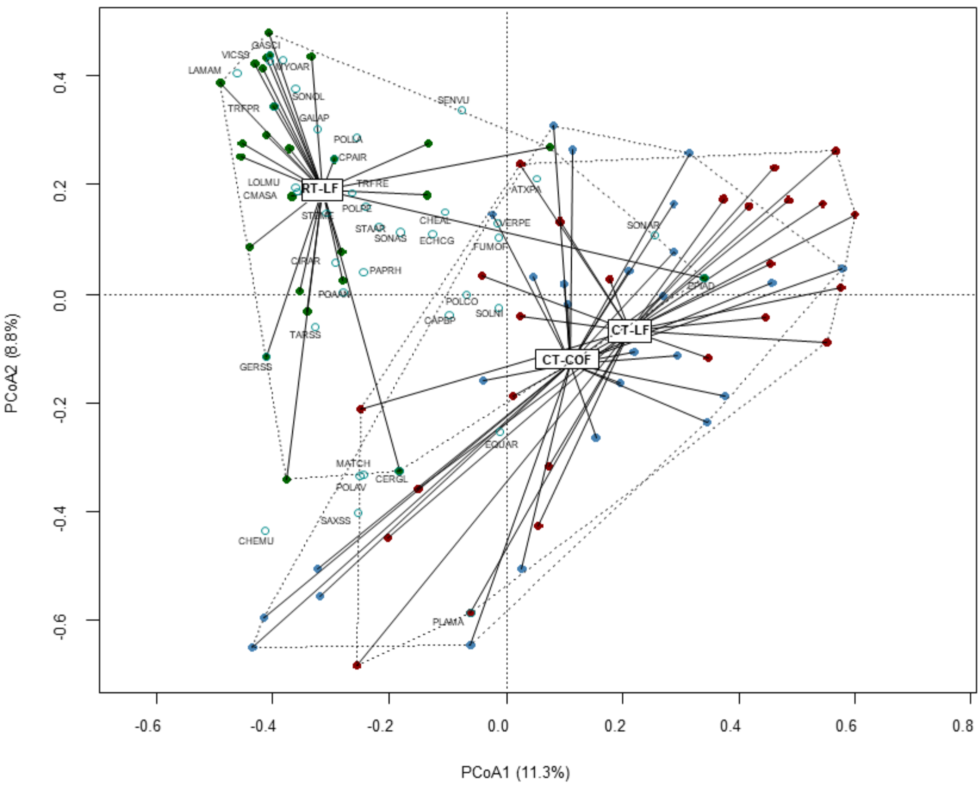

Figure 6.2: Principal Coordinate Analysis (PCoA) based on Bray-Curtis dissimilarity of weed species at maize flowering (named by to their EPPO code, https://gd.eppo.int/). RT-LF is significantly different of CT-LF and CT-COF based on PERMANOVA analysis (pvalue <0.05). CT-COF refers to the cropping system under conventional tillage with commercial organic fertilizer input. The green, blue and red dots correspond to the RT-LF, CT-COF and CT-LF quadrats respectively. CT-LF indicates the system under conventional tillage with low fertilizer input, while RT-LF refers the cropping system with reduced tillage and low fertilizer input.

5.3. Relationship between weed pressure, weed diversity and crop production

Figure 6.3 presents different relationships between maize biomass, weed biomass, maize yield and/or various diversity index, whose trends and behaviors across CS are presented below.

5.3.1. Relationship between weed diversity and weed or crop biomass

The Shannon diversity index had no effect on weed biomass and crop biomass. Weed biomass was negatively correlated with weed evenness (Figure 6.3A). In 2021, transitioning from an evenness of 0.4 to an evenness of 1 resulted in a 42% decrease in weed biomass, while in 2023, transitioning from an evenness of 0.6 to an evenness of 1 led to an 88% reduction in weed biomass. Conversely, crop biomass was positively correlated with weed evenness, with a 92% increase observed between an evenness of 0.6 and an evenness of 1 (Figure 6.3B).

The relationship between weed biomass and species richness indicated that higher species diversity was associated with increased weed biomass (Figure 6.3C). Transitioning from 4 to 12 species per sampling resulted in weed biomass more than tripling. Conversely, for maize biomass, the relationship was opposite, with maize biomass halved when the number of species increased from 4 to 12 (Figure 6.3D).

5.3.2. Relationship between maize biomass and weed biomass

Maize biomass was negatively correlated with weed biomass (Figure 6.3E). An interaction between weed biomass and year was observed (see supp. Table D.3). When weed biomass increased from 80 to 240 g m^{-2}, maize biomass decreased by 44%, 32%, and 65% for the years 2021, 2022, and 2023 respectively.

5.3.3. Relationship between yield and crop or weed biomass

A negative correlation was observed between weed biomass and yield (Fig. 3G). When weed biomass increased from 80 to 240 g m^{-2}, maize grain yield decreased by 38%, 44%, and 45% in 2021, 2022, and 2023 respectively. Maize biomass serves as a good proxy for yield, as illustrated in Figure 6.3F.

The potential correlation between yield and maize biomass is illustrated in the Figure 6.3F. With maize biomass increasing from 500 g m^{-2} to 1000 g m^{-2}, yield rose by 139%, 106%, and 126% for the years 2021, 2022, and 2023 respectively.

5.4. Effect of weed-free quadrats on maize biomass

The maize biomass exhibited an interaction between year and CS, as well as an interaction between CS and hand weeding (see supp. Table D.3 for ANOVA value). Only RT-LF showed a significant difference between the weed-free quadrat and the standard weed control quadrat (p-value < 0.001), with a difference of 274 g m^{-2}.

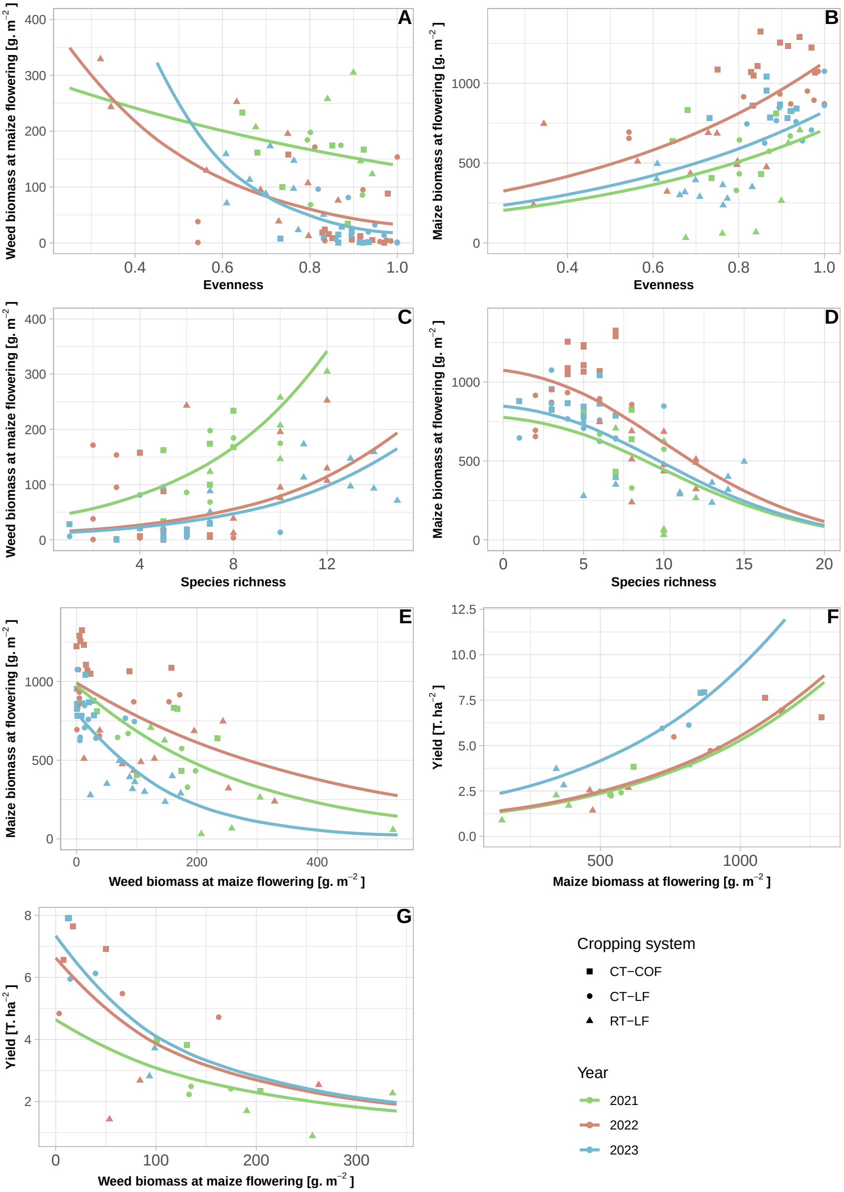

Figure 6.3: Different plots of relation between maize biomass, weed biomass, yield and weed diversity (evenness and species richness). A: Relation between weed biomass at maize flowering and evenness (based on weed density at maize flowering). B: Relation between maize biomass at flowering and evenness (based on weed density at maize flowering). C: Relation between weed biomass at maize flowering and species richness (at maize flowering). D: Relation between maize biomass at flowering and species richness (at maize flowering). E: Relation between maize biomass and weed biomass at maize flowering. F: Relation between maize yield and maize biomass at flowering (aggregated by yield zone). G: Relation between maize yield and weed biomass (aggregated by yield zone) at maize flowering. Predictions were based on linear mixed-effect models or generalized mixed-effect models. See the supplementary Table D.2 for more information about these different models. CT-COF refers to the cropping system under conventional tillage with commercial organic fertilizer input. CT-LF indicates the system under conventional tillage with low fertilizer input, while RT-LF refers the cropping system with reduced tillage and low fertilizer input.

Table 6.3: Least square means of maize biomass (g m^{-2}) and their standard errors for the different cropping systems under standard weed control and weed-free quadrats, along with their relative differences (%). P-value indicates the significance of the difference between standard weed control and weed-free quadrats. Treatments with the same letters are not significantly different. CT-COF refers to the cropping system under conventional tillage with commercial organic fertilizer input. CT-LF indicates the system under conventional tillage with low fertilizer input, while RT-LF refers the cropping system with reduced tillage and low fertilizer input.

CS	Hand weeding	Maize biomass		%differences	P value
CT-COF	Weedy	1016.47 (27.56)	e		
	Weed-free	979.95 (29.23)	de	-3.59	0.3654
CT-LF	Weedy	819.4 (27.56)	c		
	Weed-free	877.45 (27.56)	cd	7.08	0.1394
RT-LF	Weedy	426.31 (27.56)	a		
	Weed-free	700.34 (28.32)	b	64.28	<0.0001

Despite a 64% increase in maize biomass in the weed-free quadrat in RT-LF compared to the standard weed control quadrat (Table 6.3), it remained significantly lower than in CT-LF and CT-COF (standard weed control or weed-free quadrat).

6. Discussion

6.1. Weed pressure is higher in Reduced tillage

Tillage practices influence weed density that tended to be higher in RT system even before the first weeding operation and at maize flowering stage. The higher weed density reported within RT-LF system resulted in a greater weed biomass, averaging a 190% increase over the three years compared to CT-LF system. Other studies have shown similar findings, indicating higher weed density under RT (Armengot et al., 2015; Krauss et al., 2020; Peigné et al., 2015). This confirms concerns regarding long-term weed community management in reduced tillage systems in organic farming (Casagrande et al., 2016).

On the other hand, the intensity of organic fertilization and the adaptations made within the rotation did not significantly influence weed density and weed biomass. The long-term trial at Frick in Switzerland, which compared the application of slurry alone with farmyard manure containing a reduced quantity of slurry, also reported the low effect of fertilization on weed density and weed biomass (Armengot et al., 2015; Hofmeijer et al., 2019). The application of organic amendments releases nitrogen much more gradually over the season than mineral fertilizers. Such a gradual release may synchronize more effectively with crop development and consequently enhance nutrient uptake by the crop relative to weeds (Little et al., 2021).

6.2. Weed diversity and weed composition are more driven by tillage than rotation or organic fertilization.

Species composition is known to be primarily influenced by tillage practices, fertilization levels, and crop rotation, as supported by existing literature referring to both organic and conventional systems (Armengot et al., 2015, 2016; Fried et al., 2008; Mahaut et al., 2019; Sans et al., 2011; Santín-Montanyá et al., 2013). In this study, among all three factors, tillage intensity was the only one found to be effectively a driver for different species composition. A greater number of species and a higher Shannon diversity index were found in the RT system (RT-LF) compared to the other two systems. Such a higher diversity was not observed by Armengot et al. (2015), and little differences were found in comparison to conventional systems (Lacroix et al., 2024; Plaza et al., 2011; Santín-Montanyá et al., 2013). The higher species richness and higher Shannon index could be explained by the transition phase towards RT system which is in place since 2019. Reduction in tillage tends indeed to select for different functional traits and, consequently, different weed types (Armengot et al., 2016; Derrouch et al., 2022). Longer monitoring may confirm this hypothesis. On the other hand, despite having a higher number of species and a greater Shannon index, the RT-LF (reduced tillage system) exhibited lower evenness compared to the two ploughed systems. Consequently, there was a greater tendency for a few species to dominate the weed community in RT-LF in comparison to the other two systems. Weed species composition included broadleaf species such as *Chenopodium album*, *Persicaria lapathifolia*, *Sonchus asper*, and *Stellaria media*, but also problematic grasses weeds such as *Lolium multiflorum*, or even perennials weeds like *Cirsium arvense*,. The presence of perennials like *Cirsium arvense* raises concerns about the system's long-term sustainability, as noted by Armengot et al. (2015). Additionally, this present study may underestimate the presence of *Cirsium arvense*, as interrow cultivators provided effective control, and some manual removal had been performed before maize flowering during the trial. The substantial time required for manual removal (monitored in 2023) in RT-LF (8 times longer compared to CT-COF) reflects the higher abundance of *Cirsium arvense* in this system (see Table D.4). It is furthermore worth noting that RT-LF system also harbors less problematic weeds such as clover or *Myosotis arvensis*. Armengot et al. (2016) demonstrated that reduced tillage may favor less competitive species with lower nutrient requirements. In contrast, differences in rotation and organic fertilization between CT-COF and CT-LF appear to have minimal impact on the species composition in maize crops, suggesting that tillage system is a more influential factor.

6.3. Evenness can mitigate the harmfulness of weeds.

The more equitable the weed community (computed upon weed density records), the lower the weed biomass and the higher the maize biomass at flowering. This relationship is consistent with the results of Adeux et al. (2019b), who studied the effect of several weed populations on wheat yield components. However, the authors observed a similar relationship with evenness and the Shannon diversity index, whereas in the present study, no significant relationship for Shannon diversity index was observed. Furthermore Adeux et al. (2019b) did not find a relationship between species richness and weed biomass (or crop biomass). These differences suggest that the distribution of the weed community limits the impact on the crop rather than diversity. Yet, it is important to note that maize crop is characterized by low density and important inter-row, whereas it is the opposite for winter wheat. These differences could affect the behavior of the weed community, as in maize cropping system, a large number of new weed individuals may continue to emerge after the last weeding operation.

6.4. Weeds impact the productivity of maize only in reduced tillage.

When comparing hand weeded quadrat (weed-free) to others, weeds remaining after mechanical weeding operations in CT-COF and CT-LF did not cause any significant loss of maize biomass at flowering (only carried out in 2022 and 2023), which can serve as a proxy for crop yield estimates (Figure 6.3). This suggests that the weeds in these two systems would not have had a significant impact on yield. On the other hand, the weed flora in RT-LF under RT resulted in a significant reduction in maize biomass (compared to weed-free quadrats), therefore potentially also affecting the yield level within RT-LF, which were lower than CT-COF and CT-LF. This finding contrasts with Armengot et al. (2015), who did not observe any yield loss in RT system (wheat, sunflower, and spelt) despite a weed density 2.3 times higher than in conventional tillage. In the same experiment under wheat crop, Hofmeijer et al. (2019) demonstrated that, despite a higher weed biomass in RT than in CT, yields were comparable. However, in weed-free areas, yields in RT were 15-18% higher than in CT. This indicates that the yield potential in RT was affected by weeds. These findings suggest that in studies where no yield loss was observed in RT compared to CT despite higher infestation, this does not necessarily indicate the absence of harmful effects of the weed community on yield.

RT-LF was characterized, on average, by lower evenness in 2022 and 2023 at the quadrat scale, consistent with the relationship presented in Figure 6.3, indicating exacerbated weed pressure in terms of biomass when evenness is lower. Additionally, RT-LF is characterized by higher weed density, as observed in other organic reduced tillage trials (see here above). With, on average, more species than the other systems, RT-LF is marked by the presence of highly harmful weeds such as *Cirsium arvense*, *Lolium multiflorum*, and *Chenopodium album*, which could explain the substantial difference in biomass, in addition to the higher weed density.

However, even in the absence of weeds (hand weeding), RT-LF still exhibited lower maize biomass than CT-LF and CT-COF. This finding is consistent with a study conducted by Adeux et al. (2017) in a trial on conventional maize cultivation. In their study, the conservation tillage maize monoculture showed higher weed biomass, and conservation tillage resulted in a lower yield on average compared to other cropping systems, even in weed-free quadrats (compared with the weed-free quadrat of the other cropping systems). This suggests that there are other effects of RT on maize yield, such as increases water availability, soil compaction and delayed mineralization (Adeux et al., 2017; Cooper et al., 2016; Peigné et al., 2015, 2007)

In this study, the yield loss under reduced tillage was significantly higher than the average value of 7.6% reported by the meta-analysis of Cooper et al. (2016). This larger difference can be explained by the substantial variability in yield loss among different reduced tillage crops (Cooper et al., 2016; Krauss et al., 2020; Légère et al., 2013; Van den Putte et al., 2010). In a meta-analysis conducted on European crop under conservation agriculture, Van den Putte et al. (2010), demonstrated that maize grain yield was reduced by 17% under RT compared to CT. Lacroix et al. (2024) demonstrated in a long-term trial carried out under similar soil and climatic conditions that RT caused indirect yield losses due to higher weed pressure. The reductions reported here indicate a much greater reduction in yield, approximately 45%, compared to the same system using ploughing (CT-LF). This difference could be attributed to the high weed pressure in these organic farming trials, as evidenced by the significantly higher maize biomass in the weed-free quadrat. These yield results contrast with those of a long-term trial in Germany, which found no difference in silage maize yield between RT and CT (Zikeli et al., 2013). However, a long-term trial in France using organic farming methods showed that maize yields using direct seeding experienced a yield loss of around 75% (Peigné et al., 2015).

Despite a similar composition and weed pressure (in terms of weed biomass and density) between CT-COF and CT-LF, CT-COF tended to have a higher maize biomass (albeit sometimes not significantly different), resulting in a higher yield. This difference could be explained by the intrinsic aspect of the system, which receives more organic fertilization, thereby allowing for better fertilization of the plants.

7. Conclusion

The monitoring of weed flora over three years within grain maize grown under different organic cropping systems (CS) showed that the level of organic fertilization had no impact on weed flora composition, diversity, or weed pressure (density and biomass) but did result in higher maize yields. Reduced tillage (RT) with low fertilization (LF), on the other hand, showed a tendency for weed pressure to be higher than with conventional tillage (CT), both in terms of weed density and biomass (a 190% increase of weed biomass compared to CT-LF). RT system resulted in higher diversity (species richness and Shannon diversity index) but lower evenness. Under the conditions of this study, a negative relationship between evenness and weed biomass was observed, but no relationship was observed for the Shannon index. The higher the species richness, the greater the weed biomass (and vice versa for maize biomass). Selecting a more balanced weed community (but not necessarily one that is richer in species) seems to limit the harmfulness of the weed community. This study showed that weeds impacting maize productivity only under RT system with an increase in crop biomass of around 64% when weeds where manually removed compared to standard weed control but still have a lower biomass than the two CT systems. Other effects, such as water retention capacity, soil compaction or delay in mineralization, could be involved. Consequently, yields under RT were on average 45% lower than under CT. Globally, a long-term solution to weed management remains essential to avoid yield losses and promote reduced tillage systems in organic farming.

7

Chapter VII: General discussion and perspectives

Reducing or even replacing the use of herbicides requires a multitude of management tools. But the tricky question is how to combine them, and can they be transposed to other agricultural regions?

1. Intra- and interannual scale

1.1. Delaying sowing date to enhance weed control?

In Chapter 4, we explored the promising potential of delayed sowing dates in Integrated Weed Management (IWM). Delaying sowing dates reduces initial weed pressure, enhances wheat competitiveness against weeds, and improves the effectiveness of direct weeding (both mechanical and chemical). Although promising, several questions arise regarding the long-term viability of this technique. Does delaying the sowing date only provide short-term reduction in weed emergence? In fact, this practice imposes a selection pressure similar to other methods. Sowing cereals later in mid-November may favor late-emerging individuals within weed species, potentially diminishing the beneficial effects of delayed sowing dates on initial weed pressure. Furthermore, delaying the sowing date could simply filter out non-preferentially emerging weeds, just as a diversified crop rotation with crops sown in autumn and spring can favor generalist species (Fried et al., 2008, 2010).

Finally, concerning the delayed sowing date, global warming could potentially reduce the effectiveness of this approach. Warmer days later in autumn and winter may allow for new weed emergence, but they may also reduce the advantage observed in this trial with relative growth rate in favor of wheat at low temperatures. Indeed, with global warming, weed-crop competition may shift in favor of weeds in some cases (Korres et al., 2016; Peters et al., 2014). Additionally, the delayed sowing date was accompanied by an increase in wheat sowing density in accordance with regional recommendations (www.livre-blanc-cereales.be). This increase aimed to compensate for the reduction in emergence percentage and, particularly, the decrease in the number of tillers per plant. However, the factor of delayed sowing dates in the chapter 4 actually focuses on the combined effect of increasing sowing density and delaying the sowing date simultaneously. The effect of delayed sowing date and increased sowing density cannot be disentangled. Denser sowing at the same date leads to greater weed competition than less dense sowing (Andrew and Storkey, 2017). In order to maintain the yield potential, which is lower with late sowing, the sowing density of the wheat is increased. However, we can consider that these factors are linked since they are always used in pairs.

1.2. Systemic approach to weed management

In the long-term trials (chapters 5 and 6), ploughing had a major impact on weed pressure (both individual and biomass). In the organic trials, the absence of chemical weed control (resulting in a stringent filter on weed flora) led to a much greater diversity and more pronounced differences in composition. Despite ploughing being shown as an important lever for limiting weed pressure, this technique may not always be feasible due to its potential negative effects on soil health. Indeed, many farmers opt for reduced tillage or no-till primarily for the benefits to soil health, such as reduced erosion risk and increased organic carbon in the top layer, rather than for weed management purposes. A trade-off could be occasional ploughing (Peixoto et al., 2020). Ploughing every 5 to 10 years has been shown to have benefits not only for weed control but also for other aspects, such as reducing soil compaction (Peixoto et al., 2020). However, Cordeau et al. (2020) showed that after 17 years of no-till, ploughing had a significant impact on the weed community (density, richness, composition), but slightly increased weed density compared with no-till. According to the authors, this phenomenon can be attributed to the presence of species with persistent weed seeds (e.g., *Fumaria officinalis* or *Sinappis spp.*). Hence, tillage for weed management should only be considered in cases of problematic weeds in no-till systems. Nevertheless, the same study found that ploughing increased winter wheat yields by 31%.

The inclusion of a ley in the organic trials, although a constraint of the system, is one of the important levers that could be used to manage both fertility and weed management in CS in RT. In fact, the introduction of ley with successive mowing or grazing periods results in different pressures compared to an annual crop (MacLaren et al., 2019). Ley reduces the seedbank (Albrecht, 2005; Melander et al., 2020; Sjursen, 2001). Additionally, ley can serve as a crucial tool for controlling *Cirsium arvense* (Grosse et al., 2021).

As the presence of species is the result of the filters generated, it would have been interesting to position the species from the different long-term trials in Grime's triangle. In fact, classifying weeds according to the different systems in the triangle could have shown whether certain strategies were favored over others. However, without having positioned them, we can already say that the presence of grasses (*Lolium multiflorum*) and perennials (*Cirsium arvense*) in RT organic system (chapter 6) are species that are closer to C strategy along the "CR" axis. It would be interesting to observe whether, in the long-term organic trials, the CT-LF or the RT-LF will see a modification of its flora with a tendency for species to shift from the "CR" zone along the "SR" axis as suggested by MacLaren et al. (2020), since some of the recommended strategies to achieve this are being implemented (reduction in nutrient levels, crop association, etc.).

2. Fonctionnal approach

The presence of *Alopecurus myosuroides* in the soil management trials (chapter 5), especially in RT, is explained by the fact that this weed has a capacity to germinate on the surface. It has a low seed longevity (Barralis et al., 1988), which means that it can be effectively managed by burying the seed. Additionally, *Alopecurus myosuroides* has a preferential emergence in autumn, coinciding with cereal sowing. Consequently, a rotation with an autumn-sown crop every two years favors its maintenance. Finally, in this trial, *Alopecurus myosuroides* is resistant to certain active ingredients used for chemical weed control, rendering this method ineffective and exacerbating the problem. *Alopecurus myosuroides* stands out as the predominant herbicide-resistant weed in Europe (Moss et al., 2007). Belgium is not exempt from this phenomenon, as early as 1996; Eelen et al. (1996) reported populations of *Alopecurus myosuroides* resistant to several active ingredients.

The sensitivity of *Matricaria chamomilla* (the second most prevalent species in chapter 5) to ploughing is less significant than that of *Alopecurus myosuroides*. In fact, the seed longevity of *Matricaria chamomilla* is longer than *Alopecurus myosuroides*. However, this species primarily germinates in the upper part of the soil, so ploughing can only moderately control *Matricaria chamomilla* by diluting the seeds in the soil horizon. Rotation has little effect on it, as it is capable of germinating all year round.

Understanding why a weed is present or not and how it will react to agronomic levers is often based on weed traits. When the number of species is limited, it is possible to analyze why a weed is favored by the system. However, once the number of species increases (as in chapter 6), this exercise becomes more complicated, and a functional approach becomes more interesting. In fact, the thesis solely focused on a taxonomic approach to weeds, whereas a functional trait approach, as studied in other papers with topics closely related to the thesis (Armengot et al., 2016; Fried et al., 2012; Adeux et al., 2022; Mahaut et al., 2020), would have been interesting. In essence, a functional trait approach does not center on a particular species but rather on the traits associated with it (Violle et al., 2007). This information provides a more generalizable response by characterizing the traits of the weed community favored by specific environmental conditions (response traits). Indeed, the presence of a species in a given location is influenced by historical and stochastic effects. It's possible that another species could have been present, but simply lacked individuals in the seedbank initially. For instance, in the crop residue and tillage management trials, reduced tillage favored the presence of *Alopecurus myosuroides*, while on the organic platform, it was *Lolium multiflorum*. Both are grasses with relatively short seed life. Some functional traits of these two species are similar and are favored by no-tillage (Fried et al., 2012). Originally, a functional trait approach was planned for Chapter 5. However, due to the limited number of species, this approach seemed less meaningful, and a taxonomic approach was preferred. Nevertheless, a functional trait approach for organic trials (Chapter 6), which exhibit higher species richness, would be an interesting avenue to explore. In addition, the functional trait approach allows for the characterization of how the selected weed community influences the agroecosystem in terms of the (dis)services provided (effect traits). This aspect is developed in the next section.

3. Harmfullness and ecosystemic services

The thesis solely focused on a small aspect of weed harmfulness, specifically weed-crop competition. However, weeds have other negative effects, such as crop quality loss or causing harvest difficulties due to green weeds. It would be interesting to consider these additional forms of harmfulness when monitoring cropping systems, as they are significant factors that can result in financial losses but are unfortunately often overlooked in studies. Furthermore, according to a survey conducted by Cordeau and Schwartz (2019), in addition to yield losses, the increase in the seedbank and the decline in crop quality are concerns for a large number of farmers.

On the other hand, besides the sheer diversity of the weed community (at quadrat scale for a perspective on level of competition), no consideration was given to the value of ecosystem services. However, as mentioned in the introduction, weeds play a crucial role in the agro-ecosystem as they represent the primary link in the food chain. Information regarding a weed community that may be slightly more competitive due to its greater abundance, but also provides important ecosystem services such as support for pollinators, is crucial but often overlooked. It would be interesting, based on the species identified in the surveys, to assign them ecological values (e.g., pollinator support, seed source for birds and insects). However, Yvoz et al. (2021) have developed indicators to estimate the contribution of weeds to disservices and services. This indicator-based approach encompasses various types of harmfulness (potential weed competition against crops, potential weed contribution to harvest difficulties, potential weed contribution to future infestations) and various types of ecosystem services (resources for pollinators, resources for natural enemies of pests). Therefore, this approach would enable the assessment of weed communities associated with certain agricultural practices not only in terms of composition and yield losses, but also in terms of other forms of harmfulness and services. It raises the question of trade-offs between diversity, environmental support, and harmfulness that need to be identified and quantified (positive-negative balance). It appears that much remains to be explored in this area (Bretagnolle and Gaba, 2015), and a collaborative approach between weed ecologists and weed agronomists should be promoted in future research.

4. Experimental limitations and opportunities

In addition to fostering a more integrated approach among weed scientists, implementing a multidisciplinary research approach (taking into account the various components comprising CS) could prove beneficial. Despite the drawbacks in terms of weed management, reduced tillage and no-till are often adopted for other reasons. Therefore, it is crucial to bring together different disciplines in long-term CS trials, including physical, biological and chemical soil fertility, yield, weed management, economic aspects, environmental aspects, etc. (Lechenet et al., 2017). While researchers from different fields may conduct various studies within the same experimental platform, it does not necessarily imply that the link between these different disciplines has been established. For instance, on the soil managements trial (Chapter 5), numerous studies have been conducted or are currently underway on a range of topics. These include investigations on soil microbial flora (Degrune et al., 2016, 2017), yield and carbon storage (Hiel et al., 2018), long-term modeling of yield and carbon storage in the context of global warming (Delandmeter et al., 2024-inprep), N_2O emission studies, and so forth.

An approach with indicators that showcases the positive and negative effects resulting from these different studies would be a significant asset in understanding the adoption of one technique over another and could provide valuable insights for policymakers at the European level. Additionally, the competition generated by weeds can vary greatly from one season to another or from one location to another, and could be explained using information from other disciplines (e.g., a lower availability of certain nutrients caused by a less aerated soil structure in no-till could increase weed competition with crops if the weeds have a better absorption capacity than the crop).

In this manuscript, we followed the effects of levers on two different crops: winter wheat and maize. Winter wheat is the leading crop produced in Belgium in arable crop (Statbel, 2024). Maize, on the other hand, is the most widely produced cereal in the world (FAO, 2024) and is therefore of great importance. In addition, through these two crops we have been able to study weed communities in winter and spring crops but never in the same trial. However, in the long-term trials (chapters 5 and 6), assessing a cropping system based on a single crop (wheat 5 maize 6) raises questions about its effects on other crops. Weeds that pose problems for one crop (such as specialized flora on wheat, for example) may not necessarily pose problems for other crops in the rotation. In addition, in the crop residue and tillage management trial (chapter 5), the impact of ploughing appears to be highly influenced by rotation, as according to the literature (Nichols et al., 2015). This long-term trial was not originally designed to monitor weeds, and due to the need for early data collection, several consecutive years of wheat were planted. Given the interaction between rotation and ploughing, different results could have been observed with a more balanced rotation. As the long-term effect results from the accumulation of the impacts of various practices (Lechenet et al., 2017), the effect of the studied lever on a single plot with a single rotation implies that the results are difficulties to be extrapolated (Colbach et al., 2020). The major challenges encountered with cropping systems and their long-term effects lie in demonstrating local feasibility without necessarily explaining the causes of reduced crop losses (Colbach et al., 2020).

Some agronomic levers were not explicitly addressed in this thesis. For example, the effect of false seedbed in reducing the seedbank and emergence during cultivation was not examined. Its effectiveness is variable and depends on environmental conditions such as humidity and soil temperature (Travlos et al., 2020). The cover crop is another element that has not been studied. Although it may reduce biomass during the growth of the cover crop, little effect is observed in the subsequent crop (Adeux et al., 2021) and the next autumn crop (Rouge et al., 2023). The cover crop destruction method seemed to have the greatest effect on weed suppression (Rouge et al., 2023). A long-term trial showed that regular use of cover crops tended to favor the presence of generalist short-cycle weed species (capable of producing seeds during the short cover crop period) such as *Poa annua* and *Capsella bursa-pastoris* (Adeux et al., 2023). This two lever was used in Chapter 6 on organic farming. However, it was not possible to isolate its effect in this CS trial. This highlights the importance of a complementary approach between CS and factorial trials to observe both the effect of the system as a whole and isolate the different drivers of the weed community and its management. A factorial approach on different cropping systems (reduced tillage, conventional tillage, levels of fertilizers, etc.) would be beneficial (Lechenet et al., 2017). This would make it possible to highlight the advantages or disadvantages of this lever within the system (e.g., varietal trials, weeding itineraries, false seeding, etc.) (Lechenet et al., 2017).

The size of plots in long-term trials can also influence the results (Lechenet et al., 2017). In the soil management trial (Chapter 5), the plots measured 40m by 15m, with a 3m-wide cultivation area between modalities and a 16m-long cultivation area between modalities. In the organic trials (Chapter 6), the plots were 12m wide by 225m long, with 3m of grass between them. Small plots can result in contamination from the edges. It is well known that the diversity of weeds at the edge of the field is greater than inside the field. The edge is often considered a refuge for biodiversity (see for example (Fried et al., 2009; Bourgeois et al., 2020)). Additionally, cultivation operations and farming tools can serve as sources of seed dispersal and thus contribute to contamination (Petit et al., 2013). According to Petit et al. (2013), this spread can extend several meters in the direction of the tractor's travel and more than one meter perpendicular to the travel direction. Regarding soil management trial, the distance between plots should therefore mitigate contamination. However, over several seasons, individuals from the buffer strip could propagate and contaminate adjacent plots. Grass strips, as utilized in the organic cropping system trials, serve to reduce contamination between plots (Cordeau et al., 2012). The harvester can be a major source of dispersal for species that do not set seed at harvest (Petit et al., 2013). Blanco-Moreno et al. (2004) showed that the dispersal of *Lolium rigidum* could exceed 18 meters by the harvester. This may therefore lead to slightly different results than if they had been carried out in much larger agricultural plots. The presence of *Lolium multiflorum* in the plots (mainly ploughed) in the CS organic trials is very probably due to contamination from the grass strips sown with this grass despite regular mowing. Invasion from the edge towards the centre is very rapid due to the narrow width of the plot (12 meters). On the other hand, despite the fact that the contamination originally came from the grass strips, potential management lack of this grass proves that there is a problem with the management of the system and could therefore have occurred with this species or another grass species with similar functional traits in larger plots. Anemochory species (species whose seeds are spread by the wind) are known to disperse seeds over great distances (Benvenuti, 2007). Although thistle contamination is primarily through root buds, it does not preclude the possibility of primary contamination from nearby plots via wind dispersal (Tiley, 2010). While the majority of thistle seeds fall near the mother plant, a small percentage is carried by the wind, contributing to establishment in new environments (Tiley, 2010). Thistles could even contaminate neighboring plots through the grassed area. Tiley (2010) mentions that thistles can be dispersed by horizontal root growth over long distances (between 3.4m and 13m).

Furthermore, the fragmentation into such small plots, with diverse crops, increases the diversity of the site and can therefore be a source of management of pests in a general sense (Lechenet et al., 2017; Rusch et al., 2016; Chaplin-Kramer et al., 1 09). This could enhance the effect of biological regulation with reinforced seed predation (Perrot et al., 2023). Petit et al. (2023) showed that a grass strip promotes seed predation early in the spring, extending up to 32m from the field edge.

Monitoring weed communities in quadrats is not suitable for monitoring all types of weeds. Perennials, because of the way they multiply and are dispersed by tillage tools, tend to grow in patches. Monitoring in small quadrats therefore does not necessarily give a good idea of its development and, above all, its spread over several years. Unmanned aerial vehicle (UAV) imagery to map these patches or GPS point mapping (Weigel et al., 2023; Rasmussen et al., 2019) could have focused on these weeds with dispersed propagules. In addition, new pre-harvest satellite imagery methods to detect patches of *Cirsium arvense* based on normalized difference vegetation index (NDVI) measurements are also being developed (Rasmussen et al., 2021). However, perennial management is often a problem in no-till, reduced tillage or organic farming, and deserves to be monitored in long-term trials.

It is not easy to disentangle weed competition from the effects of cultivation practices (such as tillage and fertilization) and direct weed control in systems trials. (Colbach et al., 2020). The presence of weed-free areas, as set up in Chapter 6, seems to be a good solution for highlighting the level of weed-crop competition and yield differences linked to other CS factors (estimation of actual crop yield losses). It would have been interesting to use larger areas to obtain data at crop flowering and maturity rather than just at flowering to know the actual crop yield loss and not only the crop biomass loss. In addition, to better understand weed-crop competition and the effects of different levers, having unweeded areas (such as lifting the tool in mechanical weeding or not spraying in conventional farming) is interesting (Colbach et al., 2020). Indeed, it is important to have both unweeded and weed-free areas in order to estimate the real yield losses of the system (Colbach et al., 2020; Adeux et al., 2019b). Chemical weed control can have phytotoxic effects on plants, and treated areas are not necessarily free of weeds from the start of the crop. Mechanical weed control can modify the nitrogen dynamics by tillage or impact the crop by uprooting (Colbach et al., 2020). The difference between weed free and unweeded gives us the yield loss potential of the system. The difference between weed free and the pressure actually observed gives us the actual crop yield losses. However, the difficulty of implementation and above all the already small size of the experimental plots makes it difficult to collect this data. In addition, the question arises of when to destroy the unweeded areas so as not to contaminate the plot in another area and ensure the sustainability of the cropping system experiment.

Another point of concern is the duration of the CS implementation (Lechenet et al., 2017). In Chapter 6, the trials have been ongoing since 2018-2019. Given that some of the levers may not be fully implemented until later phases, it is possible that the trial is currently in a transitional phase (Lechenet et al., 2017). In the organic trials, only the effect of ploughing was observed, but it is worth noting that less direct effects may take time to manifest (Lechenet et al., 2017). For instance, rotations that have not yet completed their first crop cycle or levels of organic fertilization could influence the outcomes later. Derrouch et al. (2021) monitored weed communities in conventional agriculture across an adoption gradient spanning 1 to 20 years, covering 100 fields of winter wheat in France. Their study revealed an increase in species richness and total weed abundance over time, but no significant change in species diversity or evenness. Additionally, they observed a homogenization of weed communities across fields over time.The beginning of the CS is therefore important.With only temporal repetition of the rotation (shifted by one or two years), there is an interaction between the crop and meteorological conditions. However, until the first rotation is completed, a year effect since the beginning of the experiment may be conflated with other factors. For instance, in 2021, with the first temporal iteration, there were only two different crops since the adoption of the CS, whereas the temporal repetition (n+2) of 2023 had witnessed four crops since the CS implementation. If there's an increase in weed pressure over time or a shift in weed communities, highly contrasting results may emerge among our three temporal replicates.

In addition to having data from experimental trials, it would have been interesting to monitor a farm-field network (with, for example, a gradient of agro-ecological practices). Researching innovative systems that work for farmers and comparing different techniques and rotations adapted by several farmers on weed communitites is very important information that allows us to draw more robust conclusions (Lechenet et al., 2017). On the other hand, in a farm network, fewer parameters are managed and the logistics between researchers and farmers are sometimes very complicated. This type of research, although very interesting, is still very time-consuming, with a loss of data that can be very significant due to a lack of communication or a last-minute change of plan.

5. Further considerations

5.1. Barriers to the adoption of IWM

The adoption of agricultural practices that enable sustainable weed management by farmers is a key point that has not been developed in this manuscript. Indeed, many methods and combinations of techniques have been shown to be effective by research but have not been widely practised (Riemens et al., 2022; Deguine et al., 2021). Farmers have to continually trade off a multitude of diverse factors. They tend to minimise risk in order to secure income and avoid excessive yield losses (Deguine et al., 2021; Doohan et al., 2010). Moss (2019) highlights some reasons why farmers have difficulty adapting non-chemical methods to replace herbicide use. These include the greater complexity of non-chemical methods and their often lower and more variable efficacy (See Moss (2019) for all reasons). One of the major obstacles to adopting agronomic practices and reducing herbicide use mentioned by Moss (2019) is the fact that indirect and long-term control methods are not directly visible to farmers. In fact, unlike herbicide application, where weed mortality is observed, the effectiveness of levers such as ploughing, delaying the sowing date and a diversified rotation are difficult for farmers to perceive. To overcome these obstacles, trials can be a good means of communication to facilitate the spread of practices.

The potential obstacles to the adoption of the agronomic levers studied in this manuscript are discussed in greater depth below.

Diversifying the rotation can be a problem for the farmer, for example by replacing high-value crops with lower-value crops. In addition, crop diversification requires increased knowledge of different crops and sometimes specific equipment that is not available on the farm. Lastly, crop diversification can also be hampered by the lack of market channels and outlets in the country, as in the case of hemp growing in Belgium.

The feasibility in the field of combining mechanical and chemical weed control in winter wheat is an issue. Apart from land with resistance problems, weed control using a harrow is less effective than application of herbicide and generates additional costs if, in any case, a subsequent pass has to be made (even if it's just for grass control). The use of a half-dose herbicide combined with mechanical weeding, even though in many cases it was just as effective as chemical weeding (data not shown, see Solphyly report (Vandenberghe et al., 2022)), raises the question of long-term sustainability, with the risk of resistance developing and therefore a loss of total long-term effectiveness (Manalil et al., 2011).

Delayed sowing date in cereals is a well-known way of reducing pest pressure in general, but it is still not widely used. This can be explained by the notion of risk associated with the technique. The later the farmer wants to sow, the smaller the weather window available for sowing, with the risk of planting the crop in poorer conditions that could have an impact on yield potential (which is already reduced by delaying the sowing date). In some cases, there may even be a risk of not having an all-weather window. The autumn of 2023 is a good example, where many farmers were unable to sow all their land with winter wheat (or very late) due to an exceptionally wet autumn and winter, and were therefore forced to buy seed from another crop in order to be able to cultivate their fields. The staggering of sowing dates for different plots of wheat over the autumn is therefore normal, but it would be interesting, given the advantage of delayed sowing in terms of weed pressure and the resulting weed-crop competition for farmers to have the reflex of delayed wheat sowing dates only on land where weeds are a problem (e.g. problem with *Alopecurus myosuroides*) and not on the whole of their fields.

5.2. Perception of weeds

The perception of weeds by farmers must be taken into account as it can serve as a barrier to the adoption of certain weed management practices (Doohan et al., 2010). According to a study by Doohan et al. (2010), conventional farmers tend to prefer controlling weeds through herbicide application, a choice largely influenced by their strong aversion to weeds. Additionally, the study revealed that few farmers perceive preventive measures as offering significant benefits relative to the perceived risks (Doohan et al., 2010). Moreover, the perception of risk associated with weed management varies not only among different stakeholders (such as farmers, technicians, and researchers) but also within these groups (Cordeau and Schwartz, 2019).

We believe that enhancing farmers' knowledge of weeds (encompassing species identification, their harmfulness, and dynamics) is crucial for dispelling weed-related misconceptions. Extension programs and coaching initiatives targeting farmers seeking to improve their weed management practices are essential for addressing the challenges of integrated weed management.

5.3. Artificial intelligence in weeding tools and its integration for future responsible weed management

An important point in terms of reducing the use of herbicides is the integration of artificial intelligence into weeding tools. Weed detection using imagery means that only weeds can be targeted, so a very small area can be sprayed (see ecorobotix for example https://ecorobotix.com/en/). These techniques make it possible to reduce the quantity of herbicide applied per hectare by up to 95%, thereby reducing the environmental impact (water, soil, human health) and meeting the European Union's demand for fewer plant protection products. However, it's important to note that while these methods are effective in reducing herbicide use, they do not address the risk of herbicide resistance development, which remains a significant challenge. Therefore, the adoption of AI-based weed control technologies should not lead to the abandonment of other agronomic practices crucial for effective weed management. Furthermore, laser weeding robots are currently being developed that would further reduce the environmental impact of weed control (Andreasen et al., 2022). In addition, as the weed is recognised by imagery, weeds that are morphologically close to the crop could be selected (Coleman et al., 2023). Crop mimicry is actually an adaptive strategy employed by weeds. As with manual weeding, which is known to select the mimicry of plants at the vegetative stage, the phenomenon is the same for image recognition and could therefore eventually reduce the precision and recognition of certain weeds, reducing the effectiveness of weed control in the long term. Consequently, there is a potential for weed phenotypes mimicking the crop to emerge, posing long-term challenges in weed management (Coleman et al., 2023).

The risk of these new technologies, which could exert very strong selection and eradication pressures once perfected, is that they might indirectly lead to the simplification and homogenisation of agricultural systems, similar to what occurred post-war with the use of herbicides and mineral fertilizers. In contrast, agricultural systems should focus on diversity and become more agro-ecological by adopting a systems approach based on ecological principles, as proposed by (MacLaren et al., 2020). However, the technological approach should not be seen as a dualism with the agro-ecological approach. In fact, rather than focusing solely on eradication, weed recognition could serve as a tool for managing weed communities. Indeed, only a limited number of weed species are truly problematic and result in significant yield losses, such as *Gallium aparine*, *Chenopodium album* and *Alopecurus myosuroides* (Storkey and Neve, 2018; Adeux et al., 2019b). Targeted spraying of these problematic weeds, along with selectively controlling other species to maintain low weed pressure, could be an effective strategy. Furthermore, incorporating recognition algorithms that identify rare and protected weed species can ensure they are never inadvertently sprayed. This approach offers a potential trade-off between the negative impacts of problematic weeds and the positive contributions of other species within the ecosystem. This opportunity to use weeding robots to find a trade-off between yield and weed diversity has been proposed by Zingsheim and Döring (2024). However, to realize this concept, weeding robots would require additional capabilities, including the ability to recognize individual weed species or genus, estimate weed cover, and process this information in real-time to precisely target and destroy weeds at the individual level (Zingsheim and Döring, 2024). While the idea of achieving weed-free fields may seem ideal for some farmers, we believe that it is undesirable, considering the significant role weeds play in the agro-ecosystem, such as providing seed production for birds and supporting pollinators. One potential risk associated with precision weeding robots, whether mechanical or chemical, is that they become so efficient that no weeds are allowed to complete their life cycles.

8

Chapter VIII: Conclusion

The use of agronomic levers has demonstrated their importance and impact on weed communities.

The application of the harrow in winter wheat proved to be sufficiently effective under certain conditions but less so than herbicide application. This underscores the necessity of substituting herbicide application with a combination of levers. Effectiveness varied depending on initial weed pressure, composition and the year. Combining harrow weeding with herbicide application provided effective control in all circumstances, with a lower level of variability in effectiveness compared to herbicide application alone. Furthermore, the emergence of new weed flushes after weeding in the spring had no impact on yield.

Delaying the sowing date has proven to be an effective lever for integrated weed management. In addition to reducing the abundance of weeds before weeding (mainly within the *Alopecurus myosuroides* population), it improved direct weed control (harrowing and herbicide application) by targeting younger weeds compared to early sowing. Late sowing prevented yield losses caused by weeds, as weed pressure was too low to affect yields. Weed biomass at crop flowering was indeed low, possibly due to an enhancement in the competitiveness of winter wheat against weeds, resulting from improved relative growth in cold conditions during the establishment phase. However, these results are the fruit of a single year and should therefore be confirmed.

The long-term effect of soil management, with or without the export of crop residues and with or without the incorporation of residues by ploughing, showed no effect of crop residue management on weeds and wheat yield. The lack of effect of crop residues on weeds could be explained by the dilution of crop residues in the upper soil horizon due to reduced tillage, preventing the mulch effect from taking place. Ploughing, on the other hand, proved to be an important long-term lever for reducing the seedbank and the initial abundance of weeds compared to reduced tillage in winter wheat, although it had little impact on weed diversity. Reduced tillage increased the abundance of *Alopecurus myosuroides*, a highly competitive species that negatively affects yield. The high pressure of *Alopecurus myosuroides* could have been favored by the high presence of autumn crops in this trial. This higher weed pressure in winter wheat crops under reduced tillage ultimately resulted in greater yield loss compared to winter wheat under ploughing. Above all, it highlights the challenge of long-term sustainable management of weed flora. It would be interesting to compare the sustainability of weed management across different soil and cropping systems, including systems with a higher proportion of spring crops.

Different organic farming systems characterized by their levels of organic fertilization (rotation adapted to fertilization levels) and tillage (ploughing vs. reduced tillage) have shown an effect on the abundance, composition, and diversity of weed communities in maize crops. However, at this time, only the ploughing lever seems to have had a significant impact on the weed flora. Reduced tillage has resulted in higher weed pressure (weed density and biomass), ultimately leading to yield losses. However, cropping systems with reduced tillage and lower organic fertilizer inputs showed lower yields than the other systems, even without weeds. Other factors such as soil compaction and delayed mineralization also impacted maize yields. Unlike the conventional farming trials, reduced tillage increased weed diversity (higher species richness and Shannon diversity index) and resulted in a weed community significantly different from the other two cropping systems. This system fostered the development of both less harmful weeds and problematic ones, such as *Cirsium arvense* and *Lolium multiflorum*. Rotation and the level of organic fertilization had no significant impact on either weed composition or abundance. On the other hand, higher levels of organic fertilization tended to increase maize yields. Longer-term monitoring of this trial could reveal less direct effects than ploughing, such as rotations that have not yet completed their first crop cycle or levels of organic fertilization. Under these conditions, omitting ploughing in organic farming seems to lead to weed management issues, raising questions about long-term sustainability.

Finally, despite monitoring various agronomic levers through multiple experimental trials, it would be valuable to continue this monitoring in long-term trials. Additionally, conducting monitoring on a network of farms with gradients in the use of certain agronomic levers (and gradients in herbicide dependency) would allow for exploration in a more diverse context. Furthermore, as the goal is to achieve sustainable agricultural management, it is crucial for these management methods to be adopted by farmers. Convincing them to implement these agronomic levers and establish virtuous cropping systems poses a significant challenge. Extension work and coaching for farmers seeking to improve their weed flora management are essential to meet the challenges of integrated weed flora management.

List of Achievements

Published papers as first author

Lacroix, C., Pierreux, J., Brostaux, Y., Vandenberghe, C., and Dumont, B. (2024). *Assessing the combined effects of mechanical and chemical weeding on weed dynamics in winter wheat*. *Weed Research*. (Under review)

Lacroix, C., Vandenberghe, C., Monty, A., and Dumont, B. (2024). *Effect of long-term tillage and residue managements on weed flora and its impact on winter wheat development*. *Agriculture, Ecosystems & Environment*, 366:108937.

Lacroix, C., Huyghebaert, B., Sail, S., Abras, M., and Dumont, B. (2024). *Assessing the impact of three organic cropping systems management on weed flora during grain maize cropping season*. *European Journal of Agronomy*. (Under review)

Report contributions

Vandenberghe, C., Blondel, A., Lacroix, C., Lefébure, K., Colinet, G., Dumont, B., and Pigeon, O. (2022). Evaluation du devenir des produits phytopharmaceutiques en plein champ en fonction des pratiques culturales pour le développement d'une agriculture éco responsable : Sol-phy-ly. Technical report.

Vandenberghe, C., Blondel, A., Pierreux, J., Lacroix, C., Colinet, G., Dumont, B., and Pigeon, O. (2021). Evaluation du devenir des produits phytopharmaceutiques en plein champ en fonction des pratiques culturales pour le développement d'une agriculture éco responsable (sol-phy-ly). Rapport d'activités final 2018-2021. Technical report.

Scientific oral presentations and posters

Lacroix, C., Vandenberghe, C., Pierreux, J., Géruzet, G., and Dumont, B. (23 June 2022). Combining chemical and mechanical weeding in wheat. Oral presentation, 19th European Weed Research Society Symposium.

Lacroix, C., Abras, M., and Dumont, B. (30 August 2022). Characterisation and

impacts of weed flora on maize crop in different organic cropping systems.Oral presentation, 17 ESA Congress.

Lacroix, C., Van Den Abeele, M., Monty, A., De Clerck, C., and Dumont, B. (23 May 2023). Effect of crop rotation on weed community: The case of an innovative long-term cropping system in Belgium. Oral presentation, 74th International Symposium on Crop Protection.

Lacroix, C., Pierreux, J., Géruzet, G., Vandenberghe, C., and Dumont, B. (03 November 2022). Contrôle des adventices en céréales tout en réduisant l'utilisation de produits phytopharmaceutiques. Poster, Phloème 2022.

Other Communications

Lacroix, C., Pierreux, J., Vandenberghe, C., Dumont, B., and Henriet, F. (2024). Intégration du désherbage mécanique en froment d'hiver. Oral presentation, Livre Blanc Céréales février 2024.

A

Appendix of chapter 3

1. Supplementary material

Table A.1: List of all models for the different response variables in R syntax ("*" indicates main and interaction effects). Number with" *" indicates that the model was perform only with 2010 and 2013 data set. Models 1 and 2 are GLMM models while the model 3 is a linear mixed effects model.

Number	Response	Explanatory variable	Random effects	Offset	Zero inflation	Family
1.1	Total weed density T1	herbicide application*weed harrowing +log(Total weed density T0 +1)	1|sub-plot/plot/year	log(sampled area)	No	poisson
1.2	Total weed density T2	herbicide application*weed harrowing +log(Total weed density T1+1)	1|sub-plot/plot/year	log(sampled area)	No	poisson
1.3	Total weed density T3	herbicide application*weed harrowing +log(Total weed density T2+1)	1|sub-plot/plot/year	log(sampled area)	No	nbinomial1
1.4	Broadleaf weed density T1	herbicide application*weed harrowing +log(broadleaf weed density T0 +1)	1|sub-plot/plot/year	log(sampled area)	No	poisson
1.5	Broadleaf weed density T2	herbicide application* weed harrowing+ log(broadleaf weed desnity T1+1)	1|sub-plot/plot/year	log(sampled area)	No	poisson
1.6	Broadleaf weed density T3	herbicide application*weed harrowing+ log(broadleaf weed density T2 +1)	1|sub-plot/plot/year	log(sampled area)	No	nbinomial1
1.7*	Grass weed density T1	herbicide application*weed harrowing*year+ log(grass weed density T0 +1)	1|sub-plot/plot		No	poisson
1.8*	Grass weed density T2	herbicide application*weed harrowing+year+log (grass weed density T1 +1)	1|sub-plot/plot		No	poisson
1.9*	Grass weed density T3	herbicide application*weed harrowing+year+log(grass weed density T2+1)	1|sub-plot/plot		No	poisson
2.1	Total weed density T3	herbicide application*weed harrowing +log(Total weed density T0+1)	1|sub-plot/plot/year	log(sampled area)	No	poisson
2.2	Broadleaf weed density T3	herbicide application*weed harrowing+ log(broadleaf weed density T0 +1)	1|sub-plot/plot/year	log(sampled area)	No	poisson
2.3*	Grass weed density T3	herbicide application*weed harrowing+year+log(grass weed density T0+1)	1|sub-plot/plot		No	poisson
3	Yield (normalized)	herbicide application*weed harrowing	1|plot/year		No	gaussian

2. Supplementary results

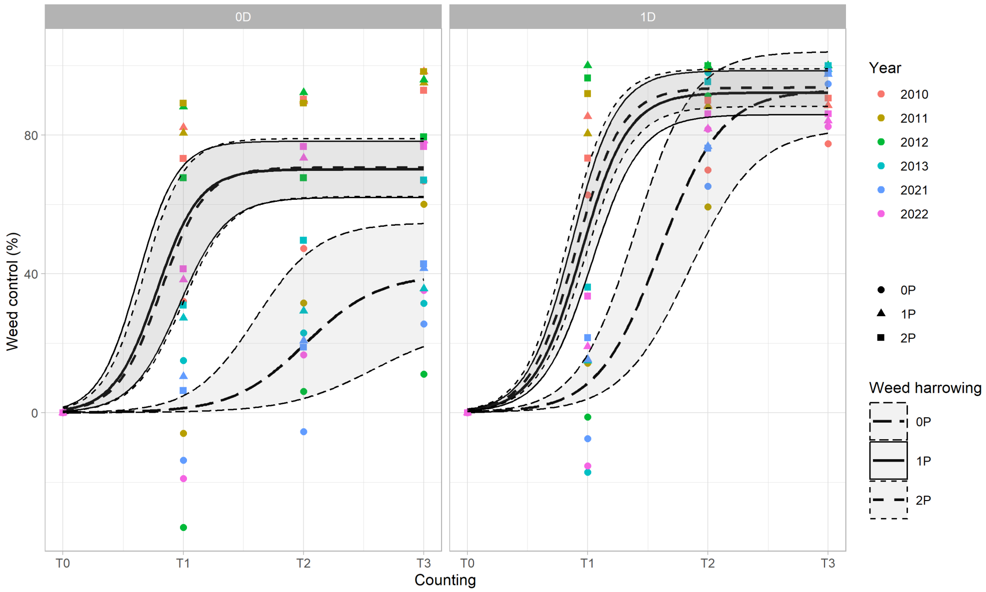

Figure A.1: Change over time of weed control (WC) of OW for all treatments based on the Eq.3.4. Sigmoid WC for each treatment were fit across all individual replicates and across all years (n= 96 per sigmoid). T0= date before weed control, T1= after one pass of harrowing, T2= after two passes of harrowing, and T3= when wheat canopy was closed. 0D is no herbicide, 1D is full herbicide application, 0P is no harrowing, 1P is one harrowing pass, 2P is two harrowing passes. The color points correspond to the annual observed means per treatment, whereas the curves correspond to fitted observations.

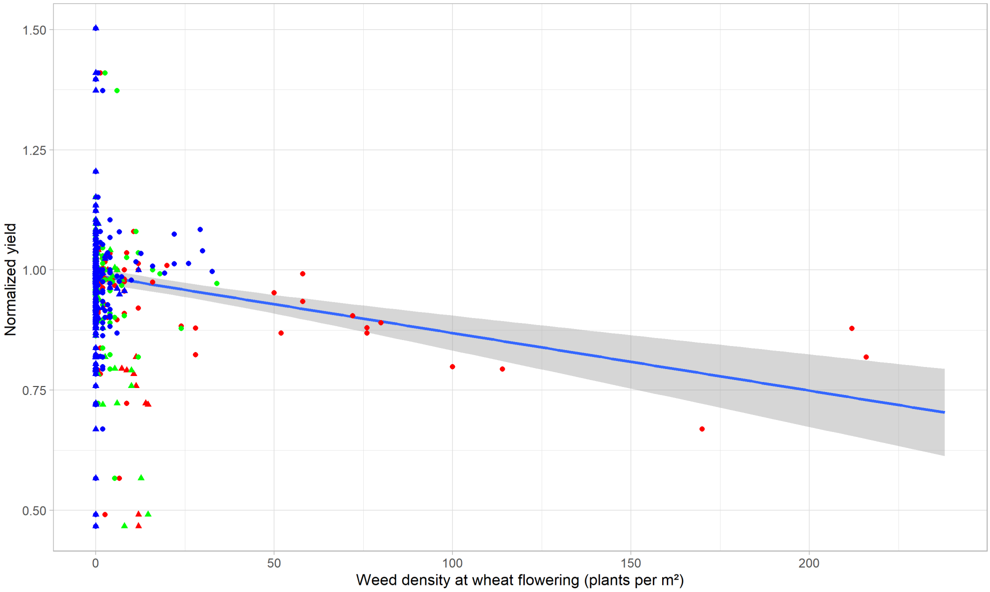

Figure A.2: Normalised yield as a function of weed density at wheat flowering. The red colour corresponds to the density of old weeds, the green colour corresponds to the density of newly emergent weeds and the blue colour corresponds to the density of new species weeds. The triangle represents the grass weeds categories while the circle represents the broadleaf weeds.

B

Appendix of chapter 4

Table B.1: List of all models for the different response variables in R syntax.

<table>
<tr><th>Number</th><th>Response</th><th>Explanatory variable</th><th>Random effects</th><th>Family</th></tr>
<tr><td>1.1</td><td>Total weed density T0</td><td>Sowing date</td><td>1|Block</td><td>gaussian</td></tr>
<tr><td>1.2</td><td>Grass weed density T0</td><td>Sowing date</td><td>1|Block</td><td>nbinomial2</td></tr>
<tr><td>1.3</td><td>Broadleaf weed density T0</td><td>Sowing date</td><td>1|Block</td><td>gaussian</td></tr>
<tr><td>2.1</td><td>Total WCE T2-T1</td><td>Herbicide application*weed harrowing*sowing date</td><td>1|Block</td><td>gaussian</td></tr>
<tr><td>2.2</td><td>Broadleaf WCE T2-T1</td><td>Herbicide application*weed harrowing*sowing date</td><td>1|Block</td><td>gaussian</td></tr>
<tr><td>2.3</td><td>Grass WCE T2-T1</td><td>Herbicide application*weed harrowing*sowing date</td><td>1|Block</td><td>gaussian</td></tr>
<tr><td>3.1</td><td>Log(total weed biomass+1)</td><td>Herbicide application*weed harrowing*sowing date</td><td>1|Block</td><td>gaussian</td></tr>
<tr><td>3.2</td><td>Log(broadleaf weed biomass+1)</td><td>Herbicide application*weed harrowing*sowing date</td><td>1|Block</td><td>gaussian</td></tr>
<tr><td>3.3</td><td>Log(grass weed biomass+1)</td><td>Herbicide application*weed harrowing*sowing date</td><td>1|Block</td><td>gaussian</td></tr>
<tr><td>4</td><td>Yield</td><td>Herbicide application*weed harrowing*sowing date</td><td>1|Block</td><td>gaussian</td></tr>
</table>

C

Appendix of chapter 5

1. Supplementary materials

Table C.1: Crop rotation between 2008 and 2022 and weeding history applied to trial between 2008 and 2022. HRAC group are the herbicide mode of action group made by Herbicide Resistance Action Committee

Year	Crop	Date	Weeding	Ingredients	Modes of Action	HRAC Group
2008-2009	Rapeseed	09-03-08	Application of Roundup [6.43 L.ha^{-1}] prior sowing	Glyphosate, potassium salt	Inhibition of enolpyruvyl shikimate phosphate synthase	G
		10-13-08	Application of Butisan [1.7 L.ha^{-1}]	Metazachlor	Inhibition of very long-chain fatty acid synthesis	K3
2009-2010	Winter wheat	04-14-10	Application of Atlantis WG [0.30 kg.ha^{-1}], Milan [1.25 L.ha^{-1}], Primus [0.05 L.ha^{-1}] and Vegetop [1 L.ha^{-1}]	Mesosulfuron-methyl-sodium, iodosulfuron-methyl-sodium, mefenpyr-diethyl, bifenox, pyraflufen-ethyl, florasulam, esterified rapeseed oil	Inhibition of acetolactate synthase, inhibition of protoporphyrinogen oxidase	B, E
2010-2011	Winter wheat	04-13-11	Application of Othello [1.2 L.ha^{-1}] and Legacy [0.2 L.ha^{-1}]	Diflufenican, iodosulfuron-methyl-sodium, mesosulfuron-methyl-sodium, mefenpyr-diethyl, MCPA	Inhibition of phytoene desaturase, inhibition of acetolactate synthase, auxin mimics	F1, B, O

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Table C.1 continued from previous page

Year	Crop	Date	Weeding	Ingredients	Modes of Action	HRAC Group
2011-2012	Winter wheat	03-28-12	Application of Othello [1.2 L.ha^{-1}] and Legacy [0.4 L.ha^{-1}]	Diflufenican, iodosulfuron-methyl-sodium, mesosulfuron-methyl-sodium, mefenpyr-diethyl, MCPA	Inhibition of phytoene desaturase, inhibition of acetolactate synthase, auxin mimics	F1, B, O
2012-2013	Cover crop (mustard)	03-18-13	Application of TAIFUN 360 [2.59 L.ha^{-1}]	Glyphosate, isopropylamine salt	Inhibition of enolpyruvyl shikimate phosphate synthase	G
2013	Faba bean	04-08-13	Pre-emergence weeding with application of Lingo [1.4 L.ha^{-1}] and Stomp 400 SC [1.8 L.ha^{-1}]	Clomazone, linuron, pendimethaline	Inhibition of deoxy-D-xylulose phosphate synthase, inhibition of photosynthesis at PS II, inhibition of microtubule assembly	F4, C1 C2, K1
		06-10-13	Manual weeding on thistle with application of GLYFOS [5.67 L.ha^{-1}]	Glyphosate	Inhibition of enolpyruvyl shikimate phosphate synthase	G
		08-28-13	Application of Diquanet SL	Diquat dibromide	PS II electron diversion	D
2013-2014	Winter wheat	04-01-14	Application of Atlantis [0.3 kg.ha^{-1}], Hussar Ultra [0.1 L.ha^{-1}], and Actirob B [1 L.ha^{-1}]	Mesosulfuron-methyl-sodium, iodosulfuron-methyl-sodium, mefenpyr-diethyl, esterified rapeseed oil	Inhibition of acetolactate synthase	B

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Table C.1 continued from previous page

Year	Crop	Date	Weeding	Ingredients	Modes of Action	HRAC Group
		04-25-14	Application of Axial [1.47 $L.ha^{-1}$]	Pinoxaden, cloquintocet-mexyl	Inhibition of acetyl-CoA carboxylase	A
		05-16-14	Application of Allie [30.55 $g.ha^{-1}$]	Metsulfuron-methyl	Inhibition of acetolactate synthase	B
2021	Sugar beet	05-20-21	Application of DIANAL 160 [0.99 $L.ha^{-1}$], ETHOMAT 500 [0.40 $L.ha^{-1}$], Allitron 700 Sc [0.79 $L.ha^{-1}$], SAFARI [14.90 $g.ha^{-1}$], and VEGETOP [0.70 $L.ha^{-1}$]	Phenmedipham, ethofumesate, metamitron, triflusulfuron-methyl, esterified rapeseed oil	Inhibition of photosynthesis at PS II, inhibition of very long-chain fatty acid synthesis, inhibition of acetolactate synthase	C1 C2, K3, B
		05-30-21	Application of DIANAL 160 [1.74 $L.ha^{-1}$], ETHOMAT 500 [0.40 $L.ha^{-1}$], Goltix Queen [0.99 $L.ha^{-1}$], SAFARI [19.87 $g.ha^{-1}$], and VEGETOP [0.50 $L.ha^{-1}$]	Phenmedipham, ethofumesate, metamitron, quinmerac, triflusulfuron-methyl, esterified rapeseed oil	Inhibition of photosynthesis at PS II, inhibition of very long-chain fatty acid synthesis, inhibition of acetolactate synthase, auxin mimics	C1 C2, K3, B, O
		06-05-21	Application of DIANAL 160 [0.99 $L.ha^{-1}$], ETHOMAT 500 [0.30 $L.ha^{-1}$], Goltix Queen [0.99 $L.ha^{-1}$], SAFARI [19.87 $g.ha^{-1}$], and VEGETOP [0.70 $L.ha^{-1}$]	Phenmedipham, ethofumesate, metamitron, quinmerac, triflusulfuron-methyl, esterified rapeseed oil	Inhibition of photosynthesis at PS II, inhibition of very long-chain fatty acid synthesis, inhibition of acetolactate synthase, auxin mimics	C1 C2, K3, B, O

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Table C.1 continued from previous page

Year	Crop	Date	Weeding	Ingredients	Modes of Action	HRAC Group
		06-15-21	Application of MATRIGON [1.50 $L.ha^{-1}$] and VEGETOP [1 $L.ha^{-1}$]	Clopyralid, monoethanolamine salt, esterified rapeseed oil	Auxin mimics	O
		06-19-21	Application of CENTIUM 360 CS [0.07 $L.ha^{-1}$] and FRONTIER ELITE [0.79 $L.ha^{-1}$]	Clomazone, dimethenamid-p	Inhibition of deoxy-D-xylulose phosphate synthase, inhibition of very long-chain fatty acid synthesis	F4, K3
2021-2022	Winter wheat	03-10-22	Application of Sigma Star [0.33 $kg.ha^{-1}$] and Actirob B [1 $L.ha^{-1}$]	Iodosulfuron-methyl-sodium, mesosulfuron-methyl-sodium, thiencarbazone-methyl-sodium, mefenpyr-diethyl, esterified rapeseed oil	Inhibition of acetolactate synthase	B
		04-27-22	Application of Axial [1.2 $L.ha^{-1}$], Biathlon Duo [0.060 $kg.ha^{-1}$], and Actirob B [0.8 $L.ha^{-1}$]	Pinoxaden, cloquintocet-mexyl, florasulam, tritosulfuron, esterified rapeseed oil	Inhibition of acetyl-CoA carboxylase, inhibition of acetolactate synthase	A, B

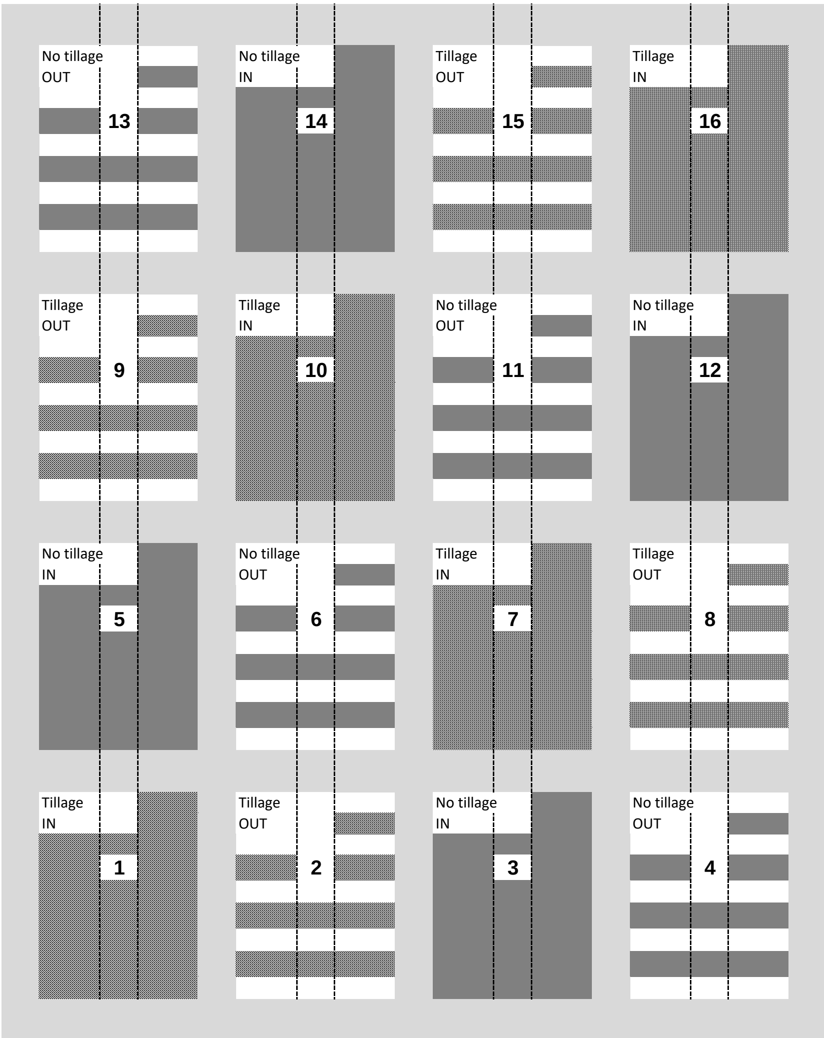

Figure C.1: Experimental plan of long-term soil management trial.

Table C.2: All fitted models made with the glmmTMB R function in the R syntax.

Number	Sampling	Response variable	Explanatory variables	Random efects	family	ziformula
1	Seedbank	Total seedling density	Tillage x Residue exportation * Depth	1| length: width	nbinom2	
2	Seedbank	MATCH seedling density	Tillage x Residue exportation * Depth	1| length: width	nbinom2	
3	Seedbank	ALOMY seedling density	Tillage x Residue exportation * Depth	1| length: width	nbinom2	
4	Seedbank	Shannon diversity index	Tillage x Residue exportation	1| length: width	tweedie	
5	Seedbank	Species Richness	Tillage x Residue exportation	1| length: width	gaussian	length+ width
6	winter wheat crop	weed density	Tillage x Residue exportation*crop stages	crop stages| ID /length: width	nbinom1	
7	winter wheat crop	ALOMY density	Tillage x Residue exportation*crop stages	1| ID /length: width	nbinom2	
8	winter wheat crop	MATCH density	Tillage x Residue exportation*crop stages	1| ID /length: width	nbinom2	
9	winter wheat crop	log(Weed biomass+1)	Tillage x Residue exportation	1| length: width	gaussian	
10	winter wheat crop	log(MATCH+1)	Tillage x Residue exportation	1| length: width	gaussian	
11	winter wheat crop	log(ALOMY+1)	Tillage x Residue exportation	1| length: width	gaussian	
12	winter wheat crop	Biomass ALOMY^{-1}	Tillage x Residue exportation	1| length: width	gaussian	
13	winter wheat crop	Yield	Tillage x Residue exportation	1| length: width	gaussian	

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Table C.2 continued from previous page

Number	Sampling	Response variable	Explanatory variables	Random efects	family	ziformula
14	winter wheat crop	Yield	Weed biomass + Tillage x Residue exportation	1| length: width	gaussian	
15	winter wheat crop	Shannon diversity index at wheat tillering	Tillage x Residue exportation	1| length: width	gaussian	
16	winter wheat crop	Shannon diversity index at wheat flowering	Tillage x Residue exportation	1| length: width	gaussian	
17	winter wheat crop	Species Richness at wheat tillering	Tillage x Residue exportation	1| length: width	compois	
18	winter wheat crop	Species Richness at wheat flowering	Tillage x Residue exportation	1| length: width	gaussian	

Table C.3: Deviance table analysis (Wald chi-square tests) of Type III. If no significant effect was observed in Type III and so no interaction effect was observed an Deviance table analysis of type II was made because of more robust test when no interaction are observed. Significant p-value (<0.05) are highlighted in bold.

Response variables	Explanatory variables	X^2	df	p.value	Type ANOVA
Total seedling density	Tillage	14.8429	1	**0.0001168**	
	Residue exportation	0.252	1	0.615688	
	Depth	5.1946	1	**0.0226577**	
	Tillage X Residue exportation	0.9821	1	0.3216828	
	Tillage X Depth	15.1185	1	**0.000101**	
	Residue exportation X Depth	1.1321	1	0.2873343	
	Tillage X Residue exportation X Depth	3.8653	1	**0.0492949**	
MATCH seedling density	Tillage	6.1542	1	**0.01311**	
	Residue exportation	0.0118	1	0.913547	
	Depth	2.1891	1	0.138989	
	Tillage X Residue exportation	0.4383	1	0.507937	

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Table C.3 continued from previous page

Response variables	**Explanatory variables**	**X²**	**df**	**p.value**	**Type ANOVA**
	Tillage X Depth	10.0855	1	**0.001494**	
	Residue exportation X Depth	0.4194	1	0.517213	
	Tillage X Residue exportation X Depth	2.6192	1	0.10558	
ALOMY seedling density	Tillage	6.4418	1	**0.01115**	
	Residue exportation	3.0136	1	0.08257	
	Depth	4.1367	1	**0.04196**	
	Tillage X Residue exportation	2.9929	1	0.08363	
	Tillage X Depth	5.8087	1	**0.01595**	
	Residue exportation X Depth	0.3508	1	0.55367	
	Tillage X Residue exportation X Depth	0.5923	1	0.44151	
Shannon diversity index (based on seedbank)	Tillage	6.8644	1	**0.008793**	
	Residue exportation	1.4849	1	0.223006	
	Tillage X Residue exportation	1.9941	1	0.157913	
Species Richness (based on seedbank)	Tillage	5.4403	1	**0.01968**	
	Residue exportation	0.1865	1	0.66581	
	Tillage X Residue exportation	0.1377	1	0.71057	
Weed density	Tillage	27.6501	1	**1.45E-07**	
	Residue exportation	0.6646	1	0.415	
	Crop stages	1.207	1	0.2719	
	Tillage X Residue exportation	0.0147	1	0.9036	
	Tillage X Crop stages	0.0828	1	0.7736	
	Residue exportation X Crop stages	0.0451	1	0.8319	
	Tillage X Residue exportation X Crop stages	0.0076	1	0.9306	
ALOMY density	Tillage	7.608	1	**0.005811**	
	Residue exportation	1.1339	1	0.286953	
	Crop stages	0	1	0.998457	
	Tillage X Residue exportation	0.486	1	0.485704	

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Table C.3 continued from previous page

Response variables	**Explanatory variables**	**X²**	**df**	**p.value**	**Type ANOVA**
	Tillage X Crop stages	0.0056	1	0.94041	
	Residue exportation X Crop stages	0.0606	1	0.805524	
	Tillage X Residue exportation X Crop stages	0	1	0.995959	
MATCH density	Tillage	30.4963	1	**3.35E-08**	
	Residue exportation	1.9161	1	0.16628	
	Crop stages	6.2924	1	0.01213	
	Tillage X Residue exportation	2.0479	1	0.15242	
	Tillage X Crop stages	0.5559	1	0.45592	
	Residue exportation X Crop stages	0.0217	1	0.88292	
	Tillage X Residue exportation X Crop stages	0.0142	1	0.90515	
log(Weed biomass+1)	Tillage	6.8241	1	**0.008994**	Type II
	Residue exportation	0.4253	1	0.51432	
	Tillage X Residue exportation	0.0142	1	0.905149	
log(ALOMY+1)	Tillage	1.8074	1	0.1788	Type II
	Residue exportation	0.2648	1	0.6069	
	Tillage X Residue exportation	0.1602	1	0.689	
log(MATCH+1)	Tillage	10.1092	1	**0.001475**	Type II
	Residue exportation	0.0032	1	0.95477	
	Tillage X Residue exportation	0.2089	1	0.647641	
Biomass $ALOMY^{-1}$	Tillage	1.1294	1	0.2879	Type II
	Residue exportation	1.3937	1	0.2378	
	Tillage X Residue exportation	2.5038	1	0.1136	
Yield	Tillage	4.0851	1	**0.04326**	Type II
	Residue exportation	0.086	1	0.7693	
	Tillage X Residue exportation	0.0237	1	0.87773	
Yield	Weed biomass	7.7916	1	**0.005249**	

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Table C.3 continued from previous page

Response variables	**Explanatory variables**	**X²**	**df**	**p.value**	**Type ANOVA**
	Tillage	1.05	1	0.305518	
	Residue exportation	0.3287	1	0.566435	
	Tillage X Residue exportation	0.0046	1	0.946005	
Shannon diversity index at wheat tillering	Tillage	0.9762	1	0.3231	Type II
	Residue exportation	0.0005	1	0.9815	
	Tillage X Residue exportation	0.0039	1	0.9503	
Shannon diversity index at wheat flowering	Tillage	3.4112	1	0.06476	Type II
	Residue exportation	0.2364	1	0.62685	
	Tillage X Residue exportation	0.3346	1	0.56296	
Species Richness at wheat tillering	Tillage	5.8203	1	**0.01584**	
	Residue exportation	0.8549	1	0.35518	
	Tillage X Residue exportation	0.054	1	0.81616	
Species Richness	Tillage	19.407	1	**1.06E-05**	
	Residue exportation	0.7763	1	0.3783	
	Tillage X Residue exportation	0.097	1	0.7554	

Table C.4: Weed density per species (expressed per m²) at wheat tillering and at wheat flowering as well as the percentage of total weed density.

Tillering stage				**Flowering stage**			
Species EPPO code	Species Latin names	weed density	% of total weed density	Species EPPO code	Species Latin names	weed density	% of total weed density
ALOMY	*Alopecurus myosuroides* Huds.	106.75	50.5	ALOMY	*Alopecurus myosuroides* Huds.	115	66.9
MATCH	*Matricaria chamomilla* L.	94.75	44.9	MATCH	*Matricaria chamomilla* L.	41.8	24.3
POLAV	*Polygonum aviculare* L.	3.4	1.6	POLAV	*Polygonum aviculare* L.	11.9	6.9
GALAP	*Galium aparine* L.	3.3	1.6	VERHE	*Veronica Hederifolia* L.	1.35	0.8
VERHE	*Veronica Hederifolia* L.	1.8	0.9	GALAP	*Galium aparine* L.	0.6	0.3
CIRAR	*Cirsium arvense* (L.) Scop.	0.45	0.2	CIRAR	*Cirsium arvense* (L.) Scop.	0.3	0.2
POAAN	*Poa annua* L.	0.25	0.1	POAAN	*Poa annua* L.	0.25	0.1
CAPBP	*Capsella bursa-pastoris* (L.) Medick	0.15	0.1	EQUAR	*Equisetum arvense* L.	0.25	0.1
PAPRH	*Papaver rhoeas* L.	0.15	0.1	SONAR	*Sonchus arvensis* L.	0.2	0.1
BRSNN	*Brassica napus* L.	0.1	0.0	PAPRH	*Papaver rhoeas* L.	0.15	0.1
LAMPU	*Lamium purpureum* L.	0.05	0.0	VIOAR	*Viola arvensis* Murray	0.1	0.1
POATR	*Poa trivalis* L.	0.05	0.0	CAPBP	*Capsella bursa-pastoris* (L.) Medick	0.05	0.0
VERAR	*Veronica arvensis* L.	0.05	0.0				

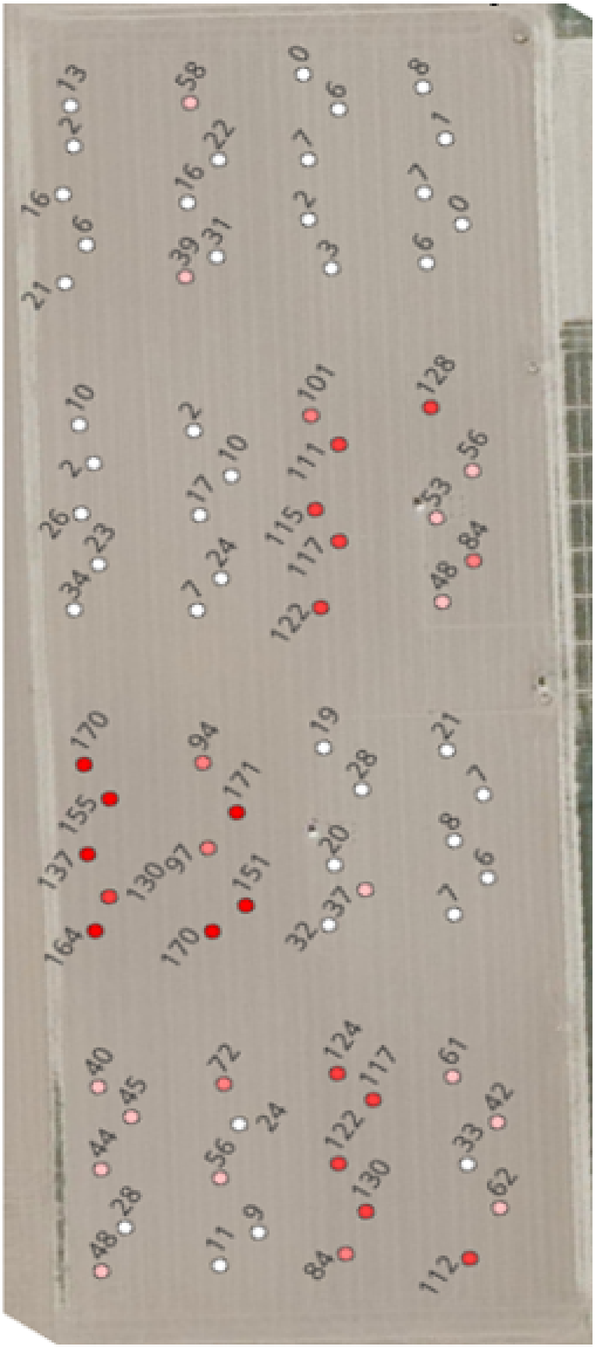

Figure C.2: Spatial representation of weed density per quadrat. The more intense the red, the greater the weed density.

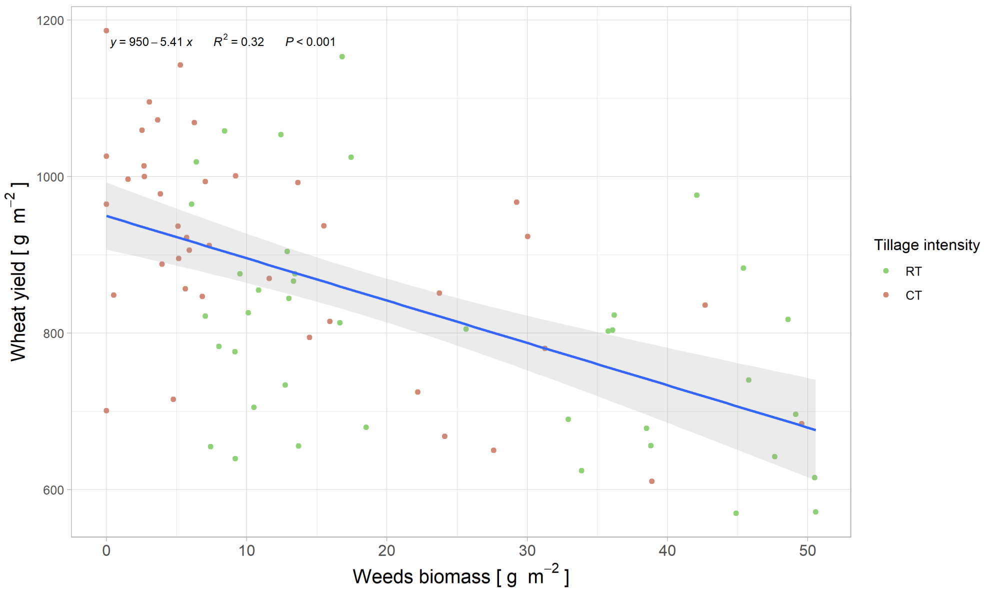

Figure C.3: Wheat yield biomass in function of weeds biomass at wheat flowering.

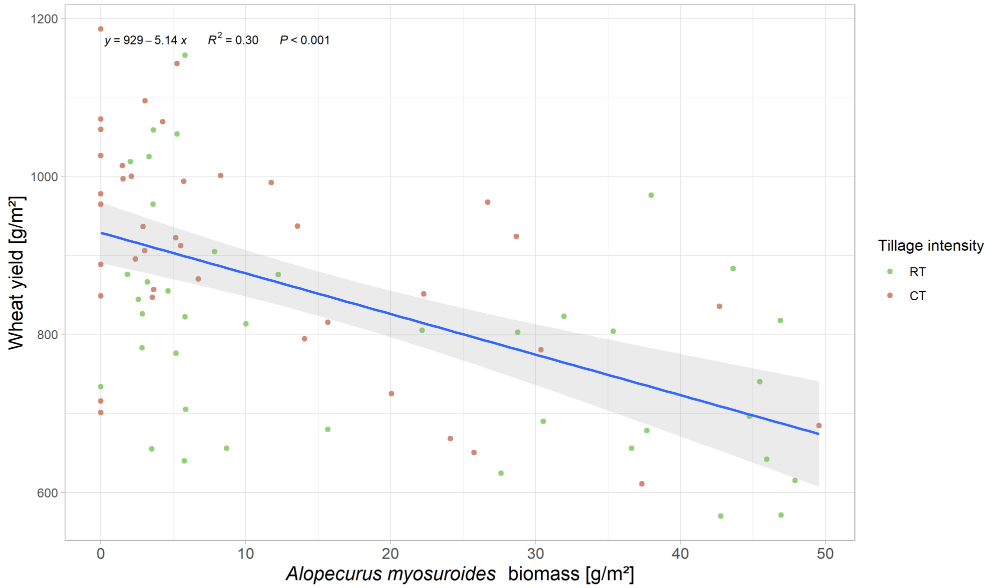

Figure C.4: Wheat yield biomass in function of *Alopecurus myosuroides* at wheat flowering.

D

Appendix of chapter 6

1. Supplementary materials

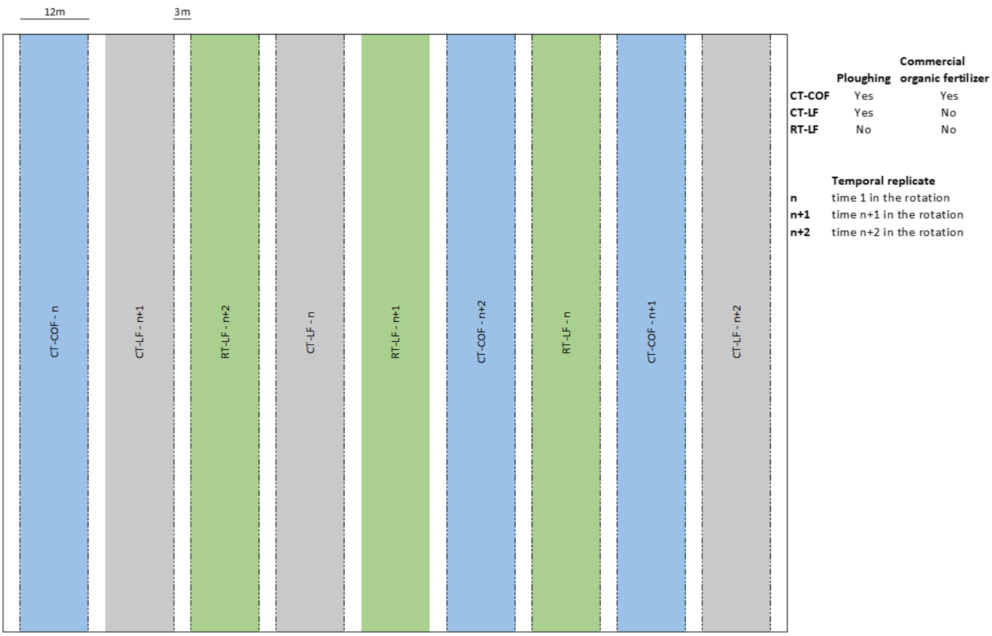

Figure D.1: Plan of experimental design. CT-COF refers to the cropping system under conventional tillage with commercial organic fertilizer input. CT-LF indicates the system under conventional tillage with low fertilizer input, while RT-LF refers the cropping system with reduced tillage and low fertilizer input.

Table D.1: Cultural operation in maize crop in 2021, 2022 and 2023, CT-COF refers to the cropping system under conventional tillage with commercial organic fertilizer input. CT-LF indicates the system under conventional tillage with low fertilizer input, while RT-LF refers the cropping system with reduced tillage and low fertilizer input.

Year	Date	Operation	Detail	CT-COF	CT-LF	RT-LF
2021 (n+2)	04-08-20	Spreading composted cattle manure	20T/ha	X		
	11-01-21	Plowing	With Pottinger plow	X		
	12-02-21	Destruction of cover crop	FACA roller and compact disc harrows		X	X
	01-03-21	Spreading comercial organic fertilizer	6+6+12+2Mgo+14So3 (1T ha^{-1}, 60UN) Rauch Aero	X		

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Table D.1: (continued)

Year	Date	Operation	Detail	CT-COF	CT-LF	RT-LF
	18-03-21	Plowing	With Pottinger plow		X	
	18-03-21	False seedbed preparation	Perugini harrow		X	
	01-04-21	Destruction of first false seedbed	Cambridge rollers,ASKEL, Kuhn harrow	X	X	
	01-04-21	Destruction of regrowth cover crop	Kuhn harrow			X
	20-04-21	Destruction of regrowth cover crop	Kuhn harrow			X
	20-04-21	False seedbed preparation	Dual cultivator (Jadin) in front and kuhn harrow			X
	29-04-21	Seedbed preparation	Dual cultivator (Jadin), cambridge rollers,ASKEL, harrow kuhn	X	X	X
	29-04-21	Sowing (7cm depth, interrow:75cm)	Kuhn optima	X	X	X
	08-06-21	Weeding	Hoe Carre Econet	X	X	X
	17-06-21	Weeding	Hoe Carre Econet	X	X	X
	08-11-21	Harvest				X
	10-11-21	Harvest		X	X	
2022 (n+1)	31-08-21	Spreading composted cattle manure	10.38T/ha	X		
	31-08-21	Incorporation of manure	Dual cultivator (Jadin)	X		
	22-12-21	Plowing	With Pottinger plow	X	X	
	07-03-22	Spreading comercial organic fertilizer	6+6+12+2Mgo+14So3 (1T ha^{-1}, 60UN) Rauch Aero	X		
	22-03-22	Destruction of cover crop	Compact disc harrows Kuhn			X
	25-03-22	Destruction of cover crop	Rotokillers			X

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Table D.1: (continued)

Year	Date	Operation	Detail	CT-COF	CT-LF	RT-LF
	19-04-22	False seedbed preparation				X
	25-04-22	Destruction of first false seedbed	Rotokillers			X
	04-05-22	Seedbed preparation	First pass seedbed cultivator (Hermoye) and dual cultivator (Jadin) in the first pass, in second pass dual cultivator (Jadin), cambridge rollers, ASKEL, harrow Kuhn	X	X	X
	04-05-22	Sowing (7cm depth, interrow:75cm)	Kvenerland optima	X	X	X
	25-05-22	Weeding	Hoe Carre Econet	X	X	X
	13-06-22	Weeding	Hoe Carre Econet	X	X	X
	05-10-22	Harvest		X	X	X
2023 (n)	13 and 14-12-2022	Destruction of cover crop	Shredder Kuhn VKR 305	X		X
	13-12-22	Plowing	With Pottinger plow	X		
	05-01-23	Plowing	With Pottinger plow		X	
	06-03-23	Spreading comercial organic fertilizer	Orgafertibio (1t/ha)	X		
	17-04-23	Destruction of regrowth cover crop	Rotokillers		X	X
	19-05-23	Seedbed preparation	First pass Harrow perrugini, second pass seedbed cultivator (Hermoye) and dual cultivator (Jadin)	X	X	X
	19-05-23	Sowing (7cm depth, interrow:75cm)	Kverneland Optima	X	X	X

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Table D.1: (continued)

Year	Date	Operation	Detail	CT-COF	CT-LF	RT-LF
	08-06-23	Weeding	Hoe Carre Econet	X	X	X
	21-06-23	Weeding	Hoe Carre Econet	X	X	X
	22-11-13	Harvest		X	X	X

Table D.3: Deviance table analysis (Wald chi-square tests). Significant p-value (<0.05) are highlighted in bold.

Model numbers	Response variables	Explanatory variables	X²	df	p.value
1	Weed density	CS	226.6551	2	**<2.2e-16**
		Year	42.6449	2	**5.49E-10**
		Crop stage	0.0187	1	0.8912
		CS:Year	46.5489	4	**1.89E-09**
		CS: Crop stage	1.6965	2	0.4282
		Year: Crop stage	56.3723	2	**5.74E-13**
		CS: Year: Crop stage	1.8603	4	0.7614
2	Weed biomass	CS	38.5051	2	**4.35E-09**
		Year	39.5204	2	**2.62E-09**
		CS:Year	6.4597	4	0.1673
3	Species Richness	CS	108.4921	2	**< 2e-16**
		Year	8.5997	2	**0.01357**
		CS:Year	11.0714	4	**0.02577**
4	Shannon index	CS	11.2335	2	**0.003636**
		Year	10.0535	2	**0.00656**
		CS:Year	6.6906	4	0.15317
5	Evenness	CS	26.9925	2	**1.38E-06**
		Year	2.0427	2	0.3601
		CS:Year	12.2494	4	**0.01559**
6	Weed biomass	Year	31.6968	2	**1.31E-07**
		Shannon index	1.093	1	0.2958
		Year:Shannon index	5.4622	2	0.06515

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Table D.3: (continued)

Model numbers	Response variables	Explanatory variables	X²	df	p.value
7	Weed biomass	Year	100.093	2	**< 2e-16**
		Species Richness	99.634	1	**<2e-16**
8	Weed biomass	Year	32.2419	2	**9.97E-08**
		evenness	38.0493	1	**6.90E-10**
		Year:evenness	7.0517	2	**0.02943**
9	Crop biomass	Year	20.2474	2	**4.01E-05**
		Weed biomass	33.1426	1	**8.56E-09**
		crop density	2.9734	1	0.08464
		Year:Weed biomass	6.0387	2	**0.04883**
10	Crop biomass	Year	15.9928	2	**0.0003367**
		crop density	1.1592	1	0.2816219
		Species Richness²	30.2657	1	**3.77E-08**
11	Crop biomass	Year	28.023	2	**8.22E-07**
		evenness	27.342	1	**1.71E-07**
		crop density	1.9162	1	0.1663
12	Crop biomass	Year	13.781	2	**0.001017**
		(Shannon index)²	2.6796	1	0.101642
		crop density	7.6831	1	**0.005574**
13	Crop biomass	Year	65.0307	2	**7.56E-15**
		CS	192.8527	2	**< 2.2e-16**
		crop density	5.7308	1	**0.01667**
		Year:CS	12.6802	4	**0.01295**
14	Crop biomass	Year	92.3096	1	**< 2.2e-16**
		CS	252.8698	2	**< 2.2e-16**
		Hand weeding	19.5926	1	**9.58E-06**
		Year:CS	8.5639	2	**0.01382**
		Year:Hand weeding	1.4799	1	0.22378
		CS:Hand weeding	31.8989	2	**1.18E-07**
		Year:CS:Hand weeding	5.0524	2	0.07996
15	Yield	Crop biomass	71.013	1	**< 2.2e-16**
		Year	47.84	2	**4.09E-11**
16	Yield	Year	276.303	2	**< 2.2e-16**

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Table D.3: (continued)

Model numbers	Response variables	Explanatory variables	X^2	df	p.value
		CS	334.297	2	**< 2.2e-16**
		Year:CS	45.192	4	**3.63E-09**
17	Yield	Weed biomass	13.4166	1	**0.0002494**
		Year	1.3651	2	0.5053325

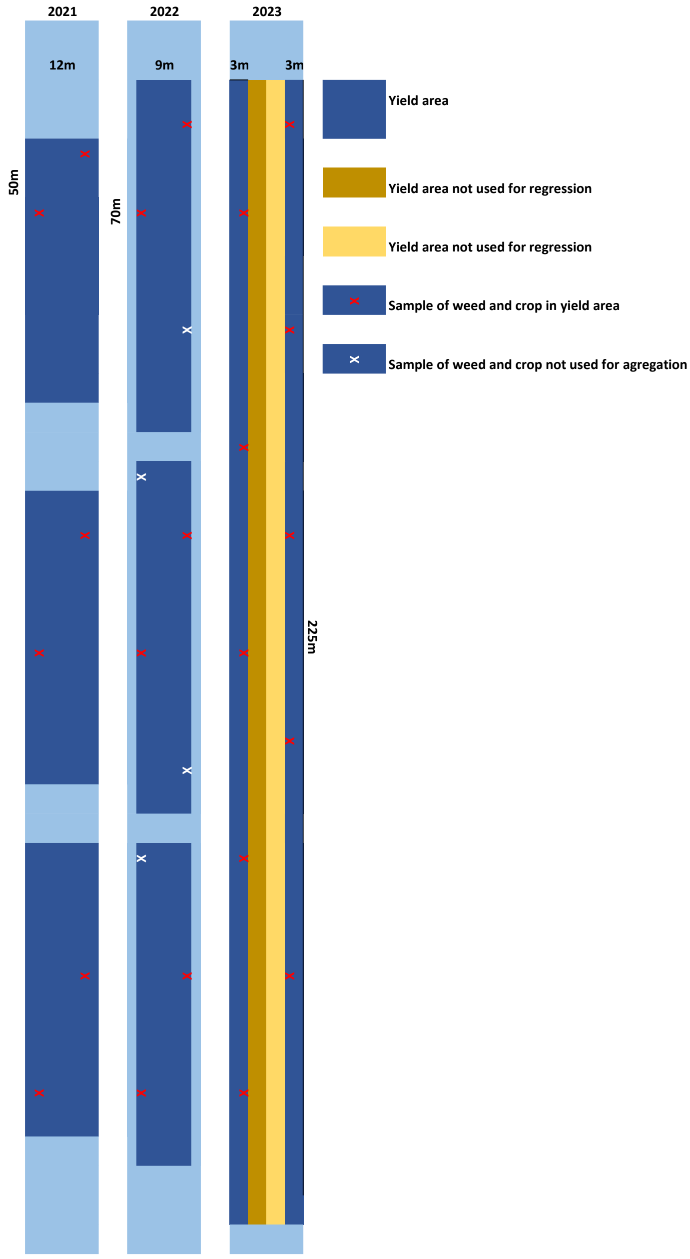

Figure D.2: Plan of sampling in 2021, 2022 and 2023.

Table D.2: All fitted models made with the *glmmTMB* R function in the R syntax.

Model numbers	Response variable	Explanatory variables	Random efects	family	link
1	Weed density	CS*Year*crop stage	1|sample/plot	nbinom2	log
2	Weed biomass	CS*Year		tweedie	log
3	Species Richness	CS*Year		gaussian	
4	Shannon Index	CS*Year		gaussian	
5	Eveness	CS*Year		gaussian	logit
6	Weed biomass	Year*Shannon index		gaussian	
7	Weed biomass	Year+Species Richness		lognormal	log
8	Weed biomass	Year*Evenness		gaussian	log
9	Crop biomass	Year*Weed biomass+crop density		gaussian	log
10	Crop biomass	Year+crop density+(Species Richness)²		gaussian	log
11	Crop biomass	Year+(Shannon index)²+crop density		gaussian	
12	Crop biomass	Year+crop density+Evenness		gaussian	log
13	Crop biomass	CS*Year+ crop density		gaussian	
14	Crop biomass	CS*Hand weeding*Year		gaussian	
15	Yield	Year+Crop biomass		tweedie	log
16	Yield	CS*Year		gaussian	
17	Yield	Weed Biomass+Year		gamma	inverse

Table D.4: Time required for manual weeding of *Cirsium arvense* in 2023 (h ha^{-1}). CT-COF refers to the cropping system under conventional tillage with commercial organic fertilizer input. CT-LF indicates the system under conventional tillage with low fertilizer input, while RT-LF refers the cropping system with reduced tillage and low fertilizer input.

Cropping system	Time required for manual weeding of Cirsium arvense in 2023 [h ha^{-1}]
CT-COF	11.30
CT-LF	30.86
RT-LF	91.30

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