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Analysing the effects of cover crops on emergence rates and vigor, seedling establishment and verticillium wilt development of sunflower varieties

RUEDA Bertille
MSc Agroecology

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1. Introduction

1.1. Sunflower crop

Sunflower *(Helianthus annus L.)* is consistently labelled as an agroecological crop providing ecosystem services in diverse cropping systems (Ceccon *et al.,* 2005; Debaeke *et al.* 2017). Its potential for sustainable intensification of agriculture relies on its agronomic features allowing it to grow under reduced input systems and/or under diverse crop management practices (Debaeke *et al.* 2017). Foremost, sunflower is characterized by a taproot able to extract water from deep soil horizons (Cabelguenne *et al.,* 1998). It also has the capacity to limit its leaf area and water potential during early water stress, inducing a decrease in transpiration level and thus in water loss (Blanchet *et al.*, 1990; INRAE, 2020). Therefore, sunflower is considered drought tolerant (Champolivier *et al.* 2011; Morizet et Merrien, 1990). Sown during the spring season, the crop does not require irrigation, and 95% of its area in France is grown under rainfed conditions (Debaeke *et al.*, 2017). Able to reach an optimum yield performance with only 75% of its water needs, sunflower uses a moderate volume of irrigation water of 80 mm during the entire crop cycle, compared to corn that requires more than 200 mm (Lecomte and Nolot, 2011). Furthermore, sunflower is an ecological crop that needs little phytosanitary interventions and has low nitrogen fertilization requirements (56kg.ha^{1}) (Agreste, 2014; Occitanie DRAAF, 2019). The treatment frequency index of sunflower is 1.7, which makes it among the least pesticide-dependent field crops, compared to, for example, durum wheat and oilseed rape having a treatment frequency index of 3.8 and 5.7, respectively (Occitanie DRAAF, 2019; Butault *et al.*, 2010). Sunflower yield in organic systems may reach up to 90% of the conventional one (Martin-Monjaret *et al.*, 2019; Lieven and Wagner, 2012). Moreover, sunflower crop has the capacity to improve soil and reduce weeds for the next crop by breaking up winter crop successions. It has a short crop cycle, occupying the soil for a short period of time. It especially represents a favorable preceding crop for cereals such as durum wheat (Ceccon *et al.,* 2005; Lecomte and Nolot, 2011), resulting in an average yield increase of 15% in the following wheat compared to wheat without sunflower as a previous crop (Martin-Monjaret, 2019).

Sunflower is part of the major oilseed crops grown worldwide (Ceccon *et al.,* 2005; Bret-Mestries *et al.*, 2016), reaching 57.1 million tons of seeds produced in 2019–2020 (FAO, 2021). France, in particular, is the third largest oilseed sunflower producer in Europe after Ukraine and Romania (FAO, 2021; Semae, 2021). The French surface grown to sunflower is 550,000 ha (i.e.,16% of the total European surface) for a total production of 1.2 million tons. Nevertheless, its productivity greatly varies from year to year, and mean yields in France have barely exceeded 2 tons per ha^{-1} for the last 10 years (Desbois and Legris, 2007; Franceagrimer, 2021). The underlying reason is that sunflower crop is sensitive to a wide range of biotic and abiotic factors including birds (Sausse *et al.,* 2021), fungal diseases (Jouffret *et al.,* 2011), high temperatures and soil conditions (Alberio *et al*., 2015; Debaeke *et al*., 2017; Harris *et al*., 1978) affecting its growth cycle and final yield. In France, sunflower is mainly grown in the southwestern parts (e.g., Occitanie and Nouvelle Aquitaine regions) characterized by a warm climate. Sunflower crop is often grown in clay hilly areas, sensitive to soil erosion because of steep slopes greater than 10% (Lecomte and Longueval, 2013). The crop is therefore subjected to seedling establishment problems (Lecomte and Nolot, 2011). Besides, sunflower is grown in France under conventional tillage with a frequent return of the crop in the rotation, and after a long fallow period during which soils are left bare. This increases nutrient leaching, and reinforces the presence of weeds and pests, becoming more complex to control (Lecomte and Longueval, 2013).

Growing sunflower in more sustainable cropping systems, with no- or minimum-tillage could help reduce the negative environmental impacts of conventional tillage while improving the soil fertility level and ensuring productivity in water-limited environments (Ordóñez Fernández *et al*., 2007; Celik *et al*., 2013). But classical management of no-till has several environmental and economic drawbacks (such high cost of herbicides, degradation of soil physical properties, etc.). The implementation of cover crops (CCs) before sunflower crop may counteract those issues and help sunflower cropping systems to address the dual challenges of production and environmental preservation (Durru *et al.,* 2015; Therond *et al.,* 2017; Tilman *et al*., 2002; Tittonell, 2014). Cover crops (CCs) are non-cash crops that are grown as a sole crop or as a mixture during the fallow period between the previous harvest and sowing of the subsequent primary crop (Justes and Richard, 2017). The relevance of such a practice is based on an agroecological paradigm to intra-field and intra-farm diversification in space and time (Pelzer *et al.,* 2012), and allowing an increased reliance on ecosystem services[1] (Altieri and Rosset, 1996; Duru *et al*, 2015; Lamichhane and Alletto, 2022; Wezel *et al*., 2014).

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[1] *Ecosystem services are natural capital assets that provide life-support services. Agriculture supplies all three major categories of ecosystem services (provisioning, regulating and cultural services) while it also demands supporting services that enable it to be productive. Ecosystem services from agriculture include regulation of water and climate systems, aesthetic, and cultural services, as well as enhanced supporting services, such as soil fertility (Swinton et al., 2007).*

1.2. Sunflower emergence and establishment quality

Sunflower crop is particularly demanding in terms of seedbed preparation and its seedlings are often prone to damage by slugs, birds, and wild animals (Lecomte and Longueval, 2013; Lecomte and Nolot, 2011). The crop establishment consists of three sub-phases; sowing to seed germination; seed germination to seedling emergence; and seedling emergence to initial competition among young plants (Lamichhane*,* 2022 (Editorial)). The crop's establishment quality (i.e., high rate of uniform healthy-looking young plants compared to the sowing density) under different field conditions is determined with key indicators such as seedling emergence vigor (i.e., the speed of seedling emergence) and final rates (Maguire, 1962).

Seedling emergence is one of the most important factors to the success of optimum plant density. But this early phase is vulnerable and can be significantly altered by interacting abiotic components and biotic stresses; the seedbed[2] structure in particular (i.e., biological, physical, and chemical components), but seeds and seedlings characteristics as well (Creamer and Finney, 2008; Doran*,* 1980; Lamichhane *et al.,* 2018; Glen *et al.,* 1989; Otten and Gilligan, 2006; Melander and Kristensen, 2011, Brown and Morra*.,* 1996; Håkansson *et al*., 2002). A poor seedbed structure generally impedes a seedling's emergence due either to mechanical forces exerted on seedlings, or soil moisture availability and aeration for the crop (Lamichhane *et al.,* 2018). Large soil aggregates for instance, reduce soil-seed contact and restrict water movement (Braunack and Dexter, 1989). This may cause poor crop emergence, particularly in clay and clay loam soils, where the seedbed often becomes coarse and non-uniform and dries out quickly under drought (Håkansson *et al*., 2022). Ferraris (1992) and Onemli (2011) report that sunflower seedling emergence increases with soil organic matter content as it increases soil moisture content.

Seedling vitality is also an essential trait for a successful crop establishment (Ellis, 1992), and is inherent to seed characteristics. Seedling vigor is defined as a complex aspect of seed performance influencing emergence level, rate and growth uniformity and sometimes lower vegetative and reproductive yield (Moses Kamanga *et al.,* 2021; Perry, 1981). Under non-optimal conditions, sown seeds may show contrasting abilities to establish plants due to differences in their vigor (Finch-Savage and Bassel, 2016). In the field, it corresponds to the emergence speed of the seedlings. A seedling that takes longer to establish will be more sensitive to early biotic stresses (Marcos-Filho, 2015).

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[2] *The seedbed constitute the upper layer of soil which has been tilled (or not) to a condition to promote germination, emergence, and growth of seedlings (Braunack and Dexter, 1989).*

1.3. Sunflower verticillium wilt development

Fungal diseases are another constraint for the stability of sunflower yield. Sunflower verticillium wilt (SVW) specifically, caused by the soilborne ascomycete fungus *Verticillium dahliae (V. dahliae),* has become increasingly invasive the past 10 years in southwestern France (Debaeke *et al*, 2017; Bret-Mestries *et al.,* 2022; Mestries, 2019). This pathogen survives in the soil as microsclerotia, a long-lasting surviving structure that constitute the main potential infective inoculum of *V. dahliae* in field soils, where it can survive for up to 14 years in the absence of a susceptible host (Fradin and Thomma, 2006; Wilhelm, 1955). The percentage of farmers' fields with SVW in the southwest of France has increased since 2013 from 20% of the plots to more than 40% of the plots affected (Martin-Monjaret *et al.,* 2019), causing up to 30% yield losses (Mestries and Lecomte, 2012). The first symptoms of SVW appear on the lower leaves at the base of the plants, starting from 40 days after sowing, when the plant approaches the flowering stage (González-Thuillier *et al.,* 2015; Schneiter and Miller, 1981), and going up progressively towards the upper leaves. From small spots, the disease progresses to large, intense yellow chlorosis that develops between the veins. These lesions rapidly evolve into large brown necroses surrounded by a golden yellow margin (Supplemental Figure 2).

In France, no active chemical substance is registered for farmers to control sunflower verticillium wilt. Among different management levers, use of resistant or tolerant genotypes is the most important way to reduce SVW (Inderbitzin *et al.,* 2011; Klosterman and Hayes*,* 2009; Quiroz *et al.,* 2008; Yadeta *et al.,* 2013). Moreover, crop management such as no-tillage may affect the inoculum retention, as infected stubbles remain in the soil surface. Roots confined to or growing near the soil surface may be prone to pathogen attack. Nevertheless, Quiroz et al (2008) showed that after sunflower cultivation under a no-tillage system, verticillium microsclerotia were reduced compared to sunflower plots under conventional tillage. No-till increases soil microbial activity which would provide a highly competitive environment and increase root density and plant root activity (Lynch and Panting, 1980, Carter and Rennie, 1984). It appeared that the combination of no-tillage and highly resistant cultivars is a promising tool to manage *V. dahliae* in sunflower (Quiroz *et al.,* 2008). Additionally, fertilization strategy could impact verticillium development as well (Elmer, 2000; Elmer and Ferrandino, 1994). Green manures appeared to be negatively correlated with verticillium infections (Davis *et al*., 2010).

1.4. Cover crops

The long interculture period between a wheat and a sunflower is particularly suitable for cover cropping. In most cases, CCs provide and enhance ecosystem services (Schipanski *et al.*, 2014). Legume cover crops (*Fabaceae*) are able to biologically fix atmospheric nitrogen. Thus, they generally have a low C/N ratio and mineralize rapidly after incorporation, increasing soil mineral nitrogen available for the next crop (Couëdel *et al.,* 2018), and increasing soil organic matter content (Smith *et al.,* 1987). They are used as a green manure to improve soil nutrition for the subsequent primary crop, avoiding in most cases nitrogen pre-emptive competition[3]. Crucifer (Brassicaceae) have a deep and dense root system allowing them to reach far into the soil to capture nutrients (Couëdel *et al.* 2018). Among other, they absorb excess nitrate from the soil and improve soil conservation services (e.g., preserving soil aggregate stability, soil erosion control) (Finney *et al.,* 2016; Couëdel *et al.,* 2018). They also reduce pests and fungal diseases (Couëdel *et al*., 2018; Justes and Richard, 2017; Lavergne *et al*., 2021) thanks to allelopathic mechanisms[4].

Allelopathy occurs when one plant species releases chemical compounds, either directly or indirectly through microbial decomposition of residues. For weed management purposes, allelopathy is considered a nonherbicide strategy of control (Liebman and Dyck, 1993). Crucifer in particular, release several potentially biocidal hydrolysis products for fungus such as isothiocyanates, from secondary metabolites known as glucosinolates present in their tissues. Termed as biofumigation (Kierkegaard and Sarwar, 1998), this process and method involves crushing and burying of crucifer's biomass at the flowering stage (Michel *et al.,* 2007), inducing allelopathic effects during the decomposition of the crop, which would, among other, help fighting against V. *dahliae*. Glucosinolates were detected in 11 dicotyledonous plant families and can be present in very different numbers and amounts (Reau *et al.,* 2005). For instance, white mustard contains only two types, whereas some others have more than 30 types like horseradish, with contents ranging from less than 0.02 Imol/g to more than 100 Imol/g (Fenwick *et al*., 1983). Within species, there can also be significant diversity. Seeds, leaves, roots or flowers do not contain exactly the same glucosinolates concentration (Kirkegaard and Sarwar, 1997). Additionally, conversion of precursor glucosinolates to isothiocyanates is not complete and yields of only 15% have been observed (Gimsing and Kirkegaard, 2009). The amounts of isothiocyanates metabolized from glucosinolates are thus

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[3] *Pre-emptive competition happens when the nitrogen uptake by the catch crop occurs in a type of competition with the succeeding crop (Thorup-Kristensen, 1993).*

[4] *Allelopathy is defined as a direct or indirect interaction, whereby allelochemicals released by one organism influence the physiological processes of other neighboring organisms (Graeber et al., 2017)*

very small, but these compounds may have efficient antifungal and antibacterial activities. In vitro experiments of biofumigation showed probing results on the reduction of SVW whereas this alternative disinfection in field conditions still appear to be very contrasted (Morris *et al.,* 2020). The effectiveness of Brassicaceae for biofumigation ultimately depends on several factors such as the plant's parts used, the biomass produced and its glucosinolates concentration, the timing and efficiency of incorporation (to achieve peak glucosinolates presence), losses due to volatilization, leaching, and microbial degradation (reviewed by Brown and Morra, 1997; Kierkegaard and Sarwar, 1998) as well as mild temperatures and water availability (Morris *et al.,* 2020). The biochemical and physical characteristics of crop residues also determine their decomposition kinetics, the proportion of compounds likely to diffuse to the soil, the mode of microbial colonization and the nature of the microbial populations involved in the decomposition process. The nitrogen content (or carbon/nitrogen ratio) is a criterion that allows the overall prediction of the net effect of residue incorporation on soil mineral nitrogen dynamics (Nicolardo and Recous, 2001).

One of the only study available to date, indicates that cover cropping enhances sunflower yields, more after Fabaceae mixtures than after fallow in a reduced-tillage system, and more after Brassicaceae in a no-tillage system (Rosner *et al.,* 2018). The potential utility of growing Brassicaceae CCs to regulate soilborne diseases such as V. *dahliae* could thus be supported by other cover crops species, such as Fabaceae. Contrasting traits and functional groups within CCs mixtures generally help maximize ecosystem services and avoid disservices[5] (Lavergne *et al*., 2021). Couëdel *et al.,* (2018) fund that mixtures of CCs tended to have more biomass per plant than for sole crops, both for shoots and roots, providing better green manure effect and maintaining or even increasing total glucosinolates production per plant.

While many studies report positive effects of cover crops (sole or in mixture) on the subsequent cash crop, there is not a consensus on the potential of CCs in improving sunflower yields in the literature (Ait Kaci Ahmed *et al*., 2022; Bolandi *et al.,* 2015; Viguier *et al.,* 2021). More specifically, little is known on the effects of CCs with long cycles on sunflower performance as most studies are focused to date on cover crops grown for a shorter period of time (Schipanski *et al*., 2014). As well, the impact of mechanical stresses due to CCs residues and soil characteristics within a no-tillage system (changes in seedbed structure, seed-soil contact, and plant growing environment and pathosystems etc.) is unknown on

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[5] *Disservices are the ecosystem generated functions, processes and attributes that result in perceived or actual negative impacts on human wellbeing (Vaz et al., 2017).*

sunflower successful emergence. Additionally, most sunflower varieties available to date belong to late maturity groups which were bred for conventional cropping, enhancing risk for crop establishment failure (Lamichhane *et al.*, 2022). Also, literature generally focus on a single service, which may limit the definition, management, and use of CCs for their complete panel of ecosystem services. Complementary ability of CCs may as well reflect different efficiencies of seed germination and seedling emergence, and the rapidity of soil cover, that are important for an enhanced level of ecosystem services. Finally, field biofumigation by Brassicas supported by legumes is still very little studied. Understanding species choice to successfully combine nutrient cycling benefits of legumes with pest suppressive potentials of crucifers is important to design appropriate mixtures that can achieve maximum production of bioactive compounds for pest suppression without generating related disservices within the agroecosystem.

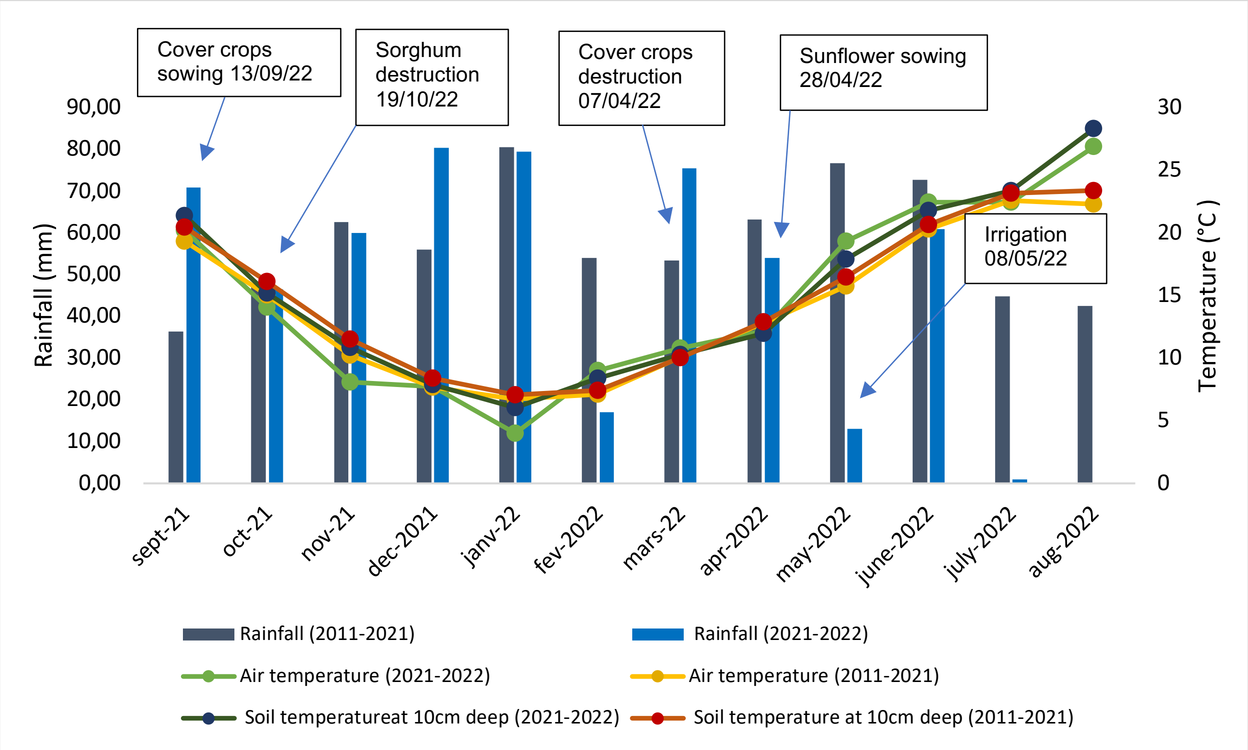
Considering the current lack of knowledge on the effect of CCs on sunflower emergence and establishment, and verticillium wilt development we performed field experimentations to understand the potential effect of CCs mixtures on 4 sunflower genotypes and measured key variables that affect the crop performance viz. emergence vigor and final rates and verticillium wilt incidence and severity were tested.

2. Material and methods

2.1 Experimental design and crop management system

2.1.1. Field site

A field experiment was carried out from September 2021 to August 2022 at INRAE Auzeville-Tolosane, southwestern France (43.528◦ N, 1.501◦ E). The experimental site was characterized by a temperate oceanic climate (*cfb.*), according to the Köppen-Geiger climate classification. The soil type of the experimental plot was clay-loam (composed of 35% of clay, 36% of silt, and 29% of sand), calcareous and hydromorphic from 50 cm and deeper. The site receives less than 750 mm of precipitation annually, and Auzeville-Tolosane meteorological station recorded mean monthly temperatures between 7°C (in January) to 22°C (in June and August), the past ten years (Climatik, 2022). Figure (1) exhibits the cumulated rainfall and monthly average air and soil (at 10cm depths) temperatures of the last 10 years and during the experiment, on the field site.



Cover crops sowing 13/09/22

Sorghum destruction 19/10/22

Cover crops destruction 07/04/22

Sunflower sowing 28/04/22

Irrigation 08/05/22

Figure 1 : Weather data at Auzeville experimental site. From growing seasons of cover crops (September 2021) until sunflower maturity (August 2022), compared to mean tendancies of the last 10 years

2.1.2. Seedbed characterization

Seedbed conditions were characterized using photographical observations of the seedbed, and identifying the seedbed type like showed Lamichhane *et al.,* (2021). A cracked soil surface and relatively large soil aggregates were observed (Figure 2a), testifying of a quite dry and compact soil. The seed furrow did not close properly at the time of sowing (Figure 2b). The seed line was left open, and the soil dried quickly, potentially leaving seeds visible to the naked eye and seedlings planted in a hole (Figure 2c).

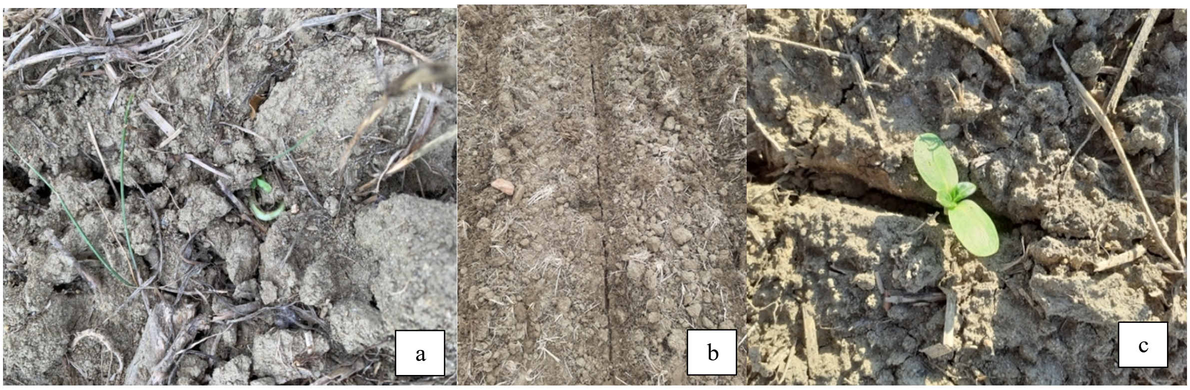


Figure 2 : Seedbed photographies

2.1.3. Cover crops choice and management system

Seven cover crops treatments were sown in a completely randomized plot (0,58 ha), with four replicates. Cover crops mixtures ranged in diversity from one to three species and included a no cover crop control (Table 2 and Appendix 1). Individual cover crops characteristics are summarized in Table (1). Mixtures were designed to include services defined by two characteristics: N fixing function (*Fabaceae*, legume) and biofumigation potential (*Brassicaceae*, crucifer). Chosen species are commonly grown in southwestern France. They were selected for their contrasting characteristics considering complementary root system and occupation of the airspace. Cover crops with a high biomass potential and those having long cycle were prioritized.

Table 1 : Cover crops used in this study

Family	Species	Scientific name	Variety	Root System*
Legume	Winter field bean	*Vicia faba* L.	Irena	Pivot
	Hairy vetch	*Vicia villosa* L.	Savane	Pivot
	Purple vetch	*Vicia americana* L.	Titane	Pivot
	Field pea	*Pisum sativum* L.	Arkta	Pivot
Crucifer	Brown mustard	*Brassica juncea* L.	Vitasso	Pivot
	Fodder radish	*Raphanus sativus L.* var. *oleiformis*	Terranova	Pivot
	White mustard	*Sinapis alba* L.	Abraham	Pivot
	Rye	*Secale cereale* L.	Wastauro	Fasciculate
Hydrophyllaceae	Phacelia	*Phacelia L.*	Maja	Pivot
Poacae	Forage sorghum	*Sorghum bicolor L.* var. *Moench*	Pipper	Fasciculate

**Information were retrieved from Arvalis (no date) and Semences de France (no date).*

Table 2 : Composition of cover crops treatments

Treatment n°	*Composition*
1	Forage sorghum, winter field bean and brown mustard
2	Purple vetch and fodder pea
3	Fodder radish, white mustard, and hairy vetch
4	Rye
5	Purple vetch, rye, and field pea
6	Brown mustard, winter field bean, and phacelia
7	Bare soil

Cover crops were sown using a cereal seed drill (Kuhn, Saverne, France) on 09/07/2021 for sorghum forage and on 13/09/2021 for the others. Species grown in sole crop were sown at densities recommended by the cover crop seed distributor and breeder (notably, 150kg/ha for winter field bean, 1.5kg/ha for brow mustard and 14kg/ha for forage soghum). In a mixture of two or three species, the sowing density for each species was 1/2 or 1/3 of its corresponding sole-crop density. Cover crops were not fertilized. The destruction and biofumigation was performed by crushing and dethatching close to the flowering of the Brassicaceae on 07/04/2022, except for sorghum forage on 19/10/2021. Cover crops were chopped using a flail mower (Kverneland, Klepp, Norway) and quickly incorporated into the soil using a cultivator (Agrisem International, Ancenis-Saint-Géréon, France).

2.1.4. Sunflower choice and management system

Sunflower was sown 3 weeks after CCs destruction on 28/04/2022 and arranged in completely randomized microplots (6m x 3m each), allowing the seeding of six sunflower rows at 50 cm distance. The four sunflower genotypes sown were Carrera CLP, MAS86OL, MAS89M, and MAS98K. Characteristics of sunflower genotypes are summarized in Table (3). For each genotype, data of the Thousand-Seed Weight (TSW) and germination capacities were retrieved from the seed companies' indications.

Late maturity group were favored to better benefit of the effects of the buried CCs residues. Indeed, CCs degrade in the soil releasing mineral elements from 18 days after destruction and burial. Two thirds of the nitrogen requirements of sunflower are generally supplied by nitrogen residues at sowing and mineralization of soil organic matter, as sunflower has a deep root system. The highest nitrogen requirements of the crop are between flower bud and flowering, i.e., 120 kg/ha. Its nitrogen needs are therefore relatively late in the crop cycle (Enrique *et al.*, 2015). Sunflower genotypes were then selected on the basis of two parameters to evaluate water stress tolerance: the leaf expansion and the transpiration thresholds. Carrera CLP and MAS98K were chosen for their low leaf expansion rates and contrasting transpiration rates Table (3). MAS86OL and MAS89M were chosen for their high leaf expansion rates and contrasting transpiration rates. MAS89M is one of the few varieties that have very low transpiration level.

Table 3 : Key characteristics of the 4 oilseed sunflower varieties

Genotype	Oil type	Maturity group	TSW (g)	Germination capacity (%)	Breeder
Carrera CLP	Linoleic	Late	76	86	Masseeds
MAS86OL	Oleic	Mid-late	77	89	Masseeds
MAS89M	Linoleic	Mid-early	57	96	Masseeds
MAS98K	Linoleic	Mid-early	63	98	Masseeds

Genotype	Leaf expansion rate	Transpiration rate	Drought resistance	Verticillium resistance
Carrera *CLP*	- 4,55	- 5,4	Tolerant	Non sensitive
MAS86OL	- 2,4	- 7,64	Tolerant	Non sensitive
MAS89M	- 2,15	- 13,98	Tolerant	Non sensitive
MAS98K	- 3,68	- 8,68	Tolerant	Non sensitive

Stubble tillage before sunflower sowing was performed with a rotary harrow, at less than 15 cm deep. Sunflower seeds were not treated. Sunflower was sown using an experimental seeder at a depth of 3 cm, seeding density being 8.8 seeds per m^2. Protection nets (F1070 DIATEX) protecting against birds were placed on 29/04 on the plot. Pre-emergence herbicide (Mercantor Gold and Racer) were applied 4 days after sowing on 02/05/2022 to control weeds during the growing season. No fertilizer or glyphosate was applied. Sunflower was irrigated 10 DAS with 30mm of water.

2.2. Cover crops sampling and measurement

For an estimation of the incorporated residues into the soil, approximately 15 days prior cover crop termination (week of 21/03/2022), samples of the shoot and root biomass of Brassicaceae and Fabaceae (sole crops and mixtures) were collected. Samples were performed from 0.5 m^2 replicates in 4 microplots for each CCs treatments per block. Samples were collected from the middle of the plots to avoid edge effects between treatments. They were separated by plant species (weeds were also sampled), and aerial and root parts of every species were dissociated. Fresh samples were weighed, dried at 80°C for 48 h and weighed again to estimate the total dry biomass produced per species.

2.3. Seedling emergence dynamics and rates

Some factors related to the seed affect germination and emergence (seed viability, germination power and vigor) (Gonzalez Belo *et al.*, 2014, section crop management). Laboratory experiments were performed to calculate the sunflower varieties germination capacities. Seeds were sterilized with bleach for 10 minutes, rinsed with clear water and dried. The experiment was done on 25 seeds per variety which were deposited into 8 millimeters of water in petri dishes. It was replicated 4 times. They were placed in an incubator at 26°C. Germination was monitored 3 and 7 days after incubation.

Thermal time (cumulative degree-days (°Cd)) was calculated from sowing (time 0) as the sum of the average daily air temperature minus the base temperature of each sunflower variety (base 6) using the following equation:

- $Thermal\ time\ (TTi) = \sum_{d=1}^{d=i}(Tmd - Tbgerm)$

Where TTi is the cumulative thermal time on i^{th} day since the initialization day (d=1) and Tmd is the daily average air temperature, and Tbgerm is the germination base temperature (4.55 for sunflower crop, based on Lamichhane et al., 2022).

Field emergence dynamics of sunflower genotypes started 7 DAS (from 05/04/2022). Measurements were carried out on 4 microplots per variety per microplots, on 4 linear meters of central rows. The number of seedlings completely out of the soil surface and visible to the naked eye was counted every day until the number of seedlings did not increase anymore for three consecutive days. The seedling was considered emerged when the cotyledons break through the surface and do not touch the surface of the seedbed (BBCH stage 09) (Lancashire *et al.*, 1991). If precipitations were expected in the following days, emergence observations continued as wetting of the crusted surface could promote the emergence of additional seedlings. Emergence rates and vigor were calculated as follows:

- $Emergence\ rate = 100 * \frac{\text{number of emerged seedlings}}{\text{sowing density}}$

- from (Maguire, 1962):

$$\text{Emergence vigor (t)} = \frac{\sum \frac{number\ of\ emerged\ seedlings\ (t)}{DAS}}{\text{number of final emerged seedlings}}$$

Where vigor (t) is the vigor index of emerged seedlings and DAS is the number of days after sowing at t time (in days after sowing or thermal time).

In order to improve data quality and deeper understand seedlings growth, adjusted value of the emergence percentage was determined by fitting a Gompertz function with the observed emergence rates. The Gompertz growth model is commonly used to interpret growth, by understanding or predicting patterns of progression (Jeger, 2004; Tjørve and Tjørve, 2017). Adjustments of emergence dynamics were made in relation to degree-days and DAS:

- $G(t) = Gmax\ e^{\left(\left(-\frac{b}{c}\right)e^{(-c.t)}\right)}$

Where G (t) is the percentage of emerged seeds at t time (in days after sowing or thermal time), Gmax is the final observed percentage of emergence, b, and c parameters of the Gompertz model.

Regarding Gompertz adjustments applied to emergence rates, their accuracies were evaluated through the efficiency (EF) of the model, the root mean square error (RMSE) and the mean error (MD):

$$\mathrm{EF} = 1 - \frac{\sum_{j=1}^{n}(P_j - O_j)^2}{\sum_{j=1}^{n}(O_j - \bar{O})^2} \quad (1)$$

$$RMSE = \sqrt{\sum_{j=1}^{n}\left[\frac{(P_j - O_j)^2}{n}\right]} \quad (2)$$

$$MD = \frac{1}{n} - \sum_{j=1}^{n}(P_j - O_j) \quad (3)$$

With Pj and Oj respectively the fitted and observed values, n the number of observations and Ö the mean of the observed values. The accuracy of the model EF varies from -∞ to 1. The closer EF is to 1, the more accurate the model predictions are. The unit of RMSE is the same as that of the variables analyzed. The model deviation MD estimates *the tendency of the model to under- or over-predict the obtained values compared to the measured values.*

Causes of non-emergence were determined at stages 12 and 14 on the BBCH scale (Lancashire *et al.*, 1991) using the visual diagnostic key adapted from the literature review of pre-emergence damping-off symptoms (Lamichhane, 2022 (Editorial)). Observations were done on the 4 replicates of the 4 genotypes. Empty points along the rows where seedlings did not emerge were selected (2 points per rows, 15 points per varieties). Each observation was associated with a cause of non-emergence, according to the symptoms observed.

Post-emergence damage were analyzed at stage 12 and 14 on the BBCH scale (Lancashire *et al.*, 1991). Observations were made on the 4 replicates of the 4 genotypes and 1m^2 (i.e., 2 linear meters) per row was considered. The total number of emerged plants as well as the number of seedlings with post-emergence damage were counted. The causes of post-emergence damages were identified using the visual diagnostic key adapted from the literature review of post-emergence damping-off symptoms (Lamichhane, 2022 (Editorial)).

2.4. Investigation of verticillium incidence and severity

Typical symptoms of SWV are the formation of bleached and grayish lesions on the main stem, branches, or pods (Supplemental Figure 2). Before SVW appeared, 10 sunflowers were tagged within 5 (out of 7) CCs treatments for the 4 blocks (four replicates per treatment). Overall, 800 sunflowers were recorded. At the first signs of SVW, 40 days after sowing (08/06/2022), symptoms were assessed weekly.

During the growing season, the incidence of verticillium wilt was assessed by noting the presence or absence of verticillium wilt symptoms on plants followed-up. At each observation, the verticillium incidence percentage was calculated for each microplots, using the following equation from Palanga et al., (2017):

- $$Incidence = \frac{Number\ of\ plants\ with\ symptoms}{Number\ of\ plants\ observed\ (here\ 10\ plants)}$$

The severity of symptoms was assessed by visually estimating a severity score. To describe the severity of symptoms on leaves, a rating scale from 0 to 4 was used: 0 = healthy plant, 1 = [1–20%], 2 = [21–50%], 3 = [51–80%] and 4 = >80% of the plant displaying wilt symptoms.

Disease severity was assessed up to 102 DAS, until sunflower physiological maturity, when the sunflowers' ray florets fall. At least 8 observations were made. Disease severity index (DSI) was investigated based on the severity scores, for each microplot, according to the equation from Chaube and Singh (1991):

❖ $DSI\ (\%) = \frac{Sum\ (score\ frequency\ x\ score)}{(Total\ number\ of\ observation\ x\ maximal\ score)} x\ 100$

Growth stages at each rating dates were recorded. Area under the Disease Progress Curve (AUDPC) was calculated according to Shaner and Finney (1977):

❖ $A.U.D.P.C. = \sum_{i}^{n-1}((\frac{Yi+Y(i+1)}{2}) * \ (t(i+1) - ti))$

in which Yi is the disease severity on the i^{th} date, and n is the number of dates on which *Verticillium dahliae* was recorded.

2.5. Statistical analysis

Seedlings emergence and verticillium incidence data obtained was converted into percentage. Arcsine and square root transformations were carried out on all data prior to the application of statistical analysis to improve the homogeneity of variance (Ahrens et al., 1990). An ANOVA was performed when the data followed a normal distribution according to the Shapiro–Wilk test ($p < 0.05$) while a non-parametric Kruskal–Wallis's test was computed when the data did not meet the ANOVA assumptions. Statistical analyses consisted in two one-way ANOVA or two Kruskal-Wallis's test to determine the potential effect of CCs mixtures and sunflower genotypes, on the measured variables under field conditions. In case of significant effect on the measured variables, the ANOVA and Kruskal-Wallis' tests were followed by a Tukey's HSD post-hoc and a Dunn test, respectively, to assess the significant differences between treatments. Cover crops were pooled by mixtures only. For all data analyses, differences among treatments were considered significant at $\alpha = 0.05$.

A potential correlation between final emergence and vigor (quantitative variables) was studied using Spearman's correlation coefficient (non-parametric test). The significance of the correlation was determined using the test of nullity of the correlation coefficient. Only coefficients significantly different from 0 were interpreted. All statistical analyses were applied using R Studio (version 4.2.0).

3. Results

3.1. Cover crops biomass

Data obtained from sampling and laboratory weighing of CCs indicated that treatments that were the most productive (total biomass above and below ground) were the sorghum-faba bean-mustard (SFM), mustard-faba bean-phacelia (MFP) and radish-mustard-vetch (RMV) treatments (with 9.83 t/ha, 5.4 t/ha, 4 t/ha, respectively). Regardless of the mixtures, crops with the highest total biomass were forage sorghum destroyed in October 2021 (3.6 t/ha), brown mustard in the MFP treatment (3.7 t/ha), white mustard in the RMV treatment (1.7 t/ha), faba bean (2 t/h), and rye (0.9 t/ha). Radish also stood out with a high biomass of 1.7 t/ha, since it is composed of a large pivot. On the opposite, brown mustard from the sorghum-faba-bean mustard (SFM) treatment produced a very low biomass of 0.018 t/ha.

3.2. Sunflower seedlings emergence rates and dynamics

Seedlings mean emergence rates under field conditions of the 4 sunflower genotypes are reported in Table (4). Seedlings emergence began 8 DAS (96.7°C-day) and attained its maximum value 18 DAS (224.6°C-day). A significant effect of genotype ($p < 0.05$) was observed on this variable. Throughout the phase, emergence rates were the highest for MAS89M and MAS98K genotypes, whereas the lowest emergence rates were observed for MAS86OL followed by Carrera CLP. The thermal time to reach the 50% emergence ($TT50\%_{emer}$) of sunflower genotypes ranged from 76 °C-day to 77 °C-day. Final emergence rates of sunflower genotypes ranged from 63% to 75% (Figure 5).

Table 4 : Sunflower genotypes emergence rates at each rating dates

	Emergence rate (%)± SD				
Genotype	**8 DAS (96.7°Cd)**	**9 DAS (109.25°d)**	**11 DAS (137,55°Cd)**	**15 DAS (199.7°Cd)**	**18 DAS (250.65°Cd)**
Carrera CLP	43 ±15 ^{a}	51 ± 16 ^{ab}	56 ± 17 ^{ab}	66 ± 15 ^{ab}	67 ± 15 ^{ab}
MAS86OL	34 ± 9 ^{b}	46 ± 9 ^{b}	52 ± 10 ^{b}	61 ± 12 ^{b}	63 ± 12 ^{b}
MAS89M	45 ± 11 ^{a}	56 ± 13 ^{a}	63 ± 13 ^{a}	74 ± 13 ^{a}	75 ±13 ^{a}
MAS98K	46 ± 13 ^{a}	54 ± 14 ^{a}	59 ± 14 ^{ab}	72 ±13 ^{a}	73 ± 12 ^{a}
Kruskal p-value	0.003578	0.02262	0.0009369	0.002178	0.004784

SD= Standard deviations. Mean emergence rates in the same column followed by the same letters are not significantly different (P < 0.05, Kruskal-Wallis test).

Adjustments of seedlings emergence dynamics of sunflower genotypes were performed using the Gompertz function. The quality of adjustments is reported in Figure (3). The determination coefficient R^2 ranged from 0.97 to 0.99.

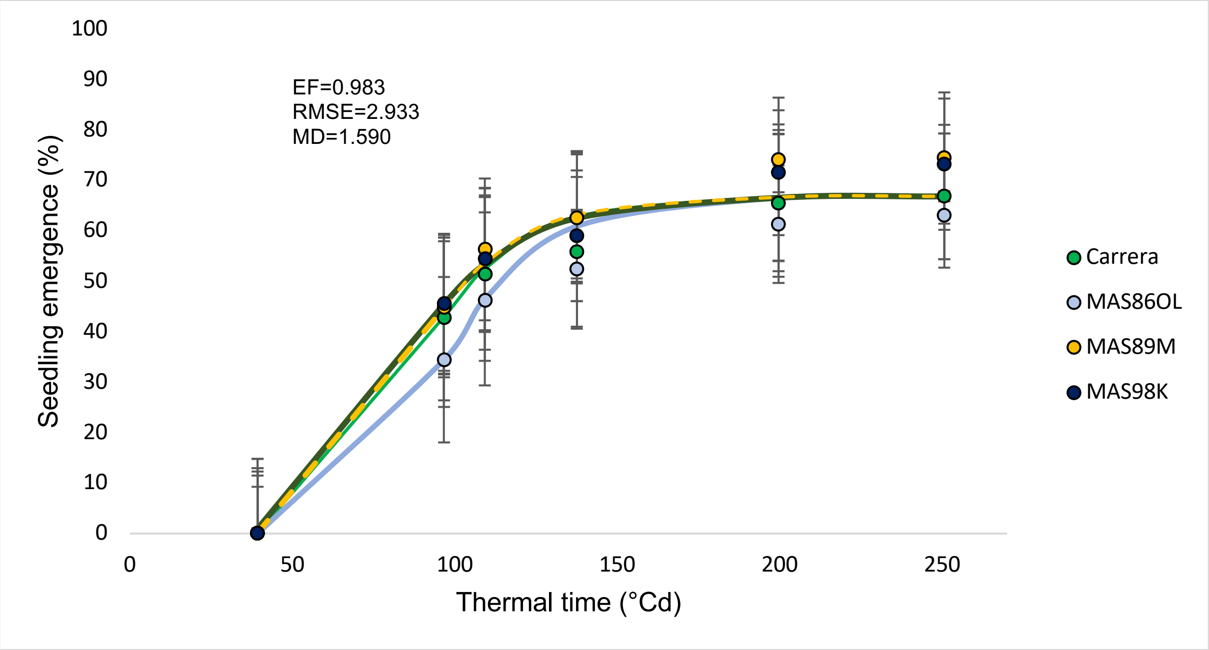


Figure 3 : Graphical representation of Gompertz's adjustments of the emergence dynamics of 4 sunflower genotypes in relation to thermal time

Vertical bars reported in the figure represent standard deviations.

Seedlings emergence rates under field conditions as affected by CCs treatments are reported in Table (5). A significant effect of CCs treatments ($p < 0.05$) was observed on this variable, that was consistent over time. Emergence rates of sunflower were the highest following a bare soil treatment and a sorghum-faba bean-mustard treatment, while lower emergence rates were observed following a radish-mustard-vetch treatment, a rye treatment, and a vetch-pea treatment. The thermal time to reach the 50% emergence ($TT50\%_{emer}$) after CCs treatments ranged from 78 °C-day to 82 °C-day. Final emergence rates ranged from 63% to 77 % (Figure 5).

Table 5 : Seedlings emergence rates after CCs treatments at each rating dates

Treatment	**Emergence rate (%)±SD**				
	8 DAS (96,7°Cd)	**9 DAS (109,25°d)**	**11 DAS (137,55°Cd)**	**15 DAS (199,7°Cd)**	**18 DAS (250,65°Cd)**
Mustard-Faba bean-Phacelia	45 ± 12 ^{ab}	55 ±13 ^{ab}	61 ± 11 ^{abc}	71 ± 11 ^{abc}	72 ± 11 ^{abc}
Radish-Mustard-Vetch	36 ± 11 ^{ab}	44 ± 13 ^{b}	48 ± 13 ^{c}	60 ± 12 ^{c}	61 ± 12 ^{c}
Rye	34 ± 13 ^{b}	43± 13 ^{b}	50 ± 15 ^{c}	62 ± 18 ^{abc}	64 ± 17 ^{c}
Bare soil	48 ± 12 ^{a}	61 ±10 ^{a}	69 ± 9 ^{a}	78 ± 8 ^{a}	79 ±8 ^{a}
Sorghum-Faba bean-Mustard	44 ± 10 ^{ab}	57 ± 8 ^{ab}	63 ± 9 ^{ab}	77 ± 56 ^{ab}	78 ± 6 ^{ab}
Vetch-Pea	42 ± 16 ^{ab}	51 ± 15 ^{ab}	54 ± 15 ^{c}	64 ± 15 ^{abc}	65 ± 15 ^{bc}
Vetch-Rye-Pea	45 ± 14 ^{ab}	53 ± 14 ^{ab}	58 ± 15 ^{abc}	65 ±13 ^{abc}	67 ± 3 ^{bc}
Kruskal p-value	0.02064	0.001305	0.0006077	0.0004202	0.001594

SD= Standard deviations. Treatments correspond to: SFM: Sorghum-Faba bean-Mustard; Rye: Rye; MFP: Mustard- Faba bean-Phacelia; RMV: Radish-Mustard-Vetch; BS: Bare Soil; VP: Vetch-Pea; VSP: Vetch-Rye-Pea. Vertical bars reported in the figure represent standard deviations. Mean emergence rates in the same column followed by the same letters are not significantly different (P < 0.05, Kruskal-Wallis test).

Adjustments of the seedling emergence dynamics after CCs treatments were performed using a Gompertz function (Tjørve and Tjørve, 2017; Wang and Zuidhof, 2004). The quality of adjustments is reported in Figure (4). The determination coefficient R^2 ranged from 0.97 to 0.99.

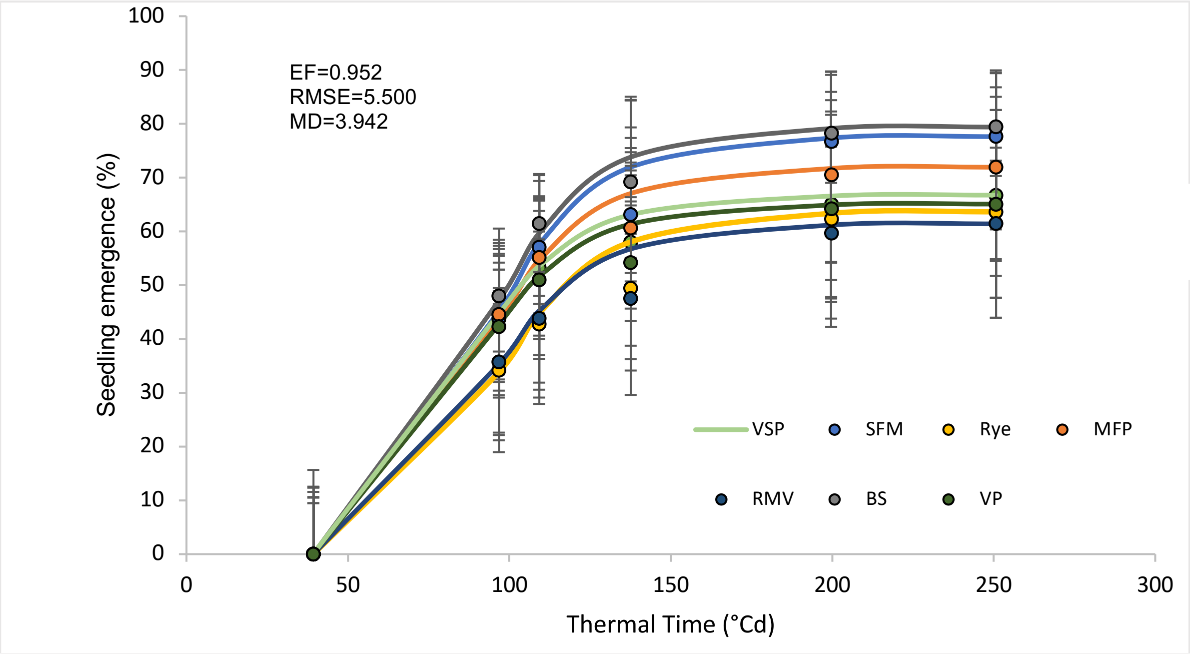


Figure 4 : Graphical representation of Gompertz's adjustments of sunflower seedlings emergence dynamics after cover crops treatments, in relation to thermal time. *Treatments correspond to: SFM:*

Sorghum-Faba bean-Mustard; Rye: Rye; MFP: Mustard- Faba bean-Phacelia; RMV: Radish-Mustard-Vetch; BS: Bare Soil; VP: Vetch-Pea; VSP: Vetch-Rye-Pea. Vertical bars reported in the figure represent standard deviations.

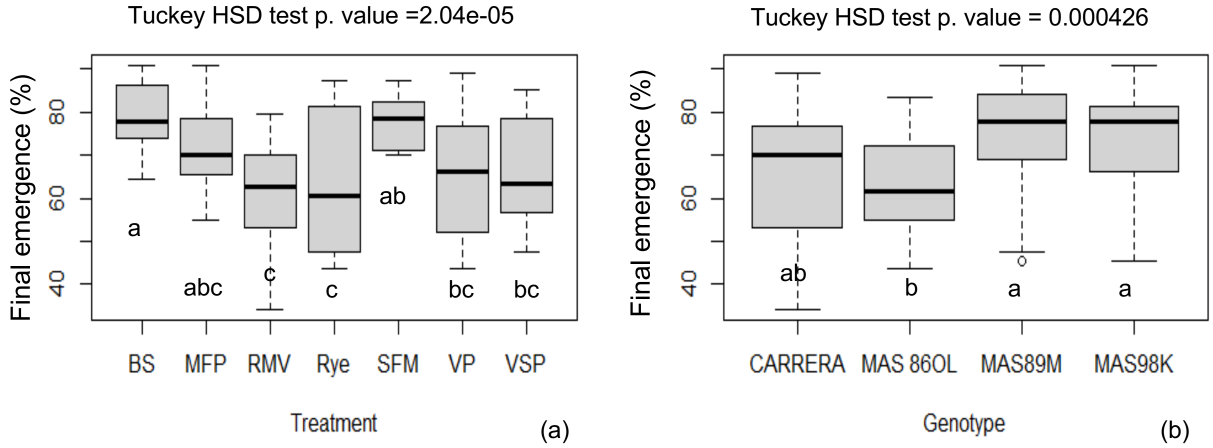


Figure 5 : Final seedling emergence rates (18 DAS) of 4 sunflower genotypes (b) after CCs treatments (a). *Vertical bars reported in the figure represent standard deviations. Treatments correspond to: SFM: Sorghum-Faba bean-Mustard; Rye: Rye; MFP: Mustard- Faba bean-Phacelia; RMV: Radish-Mustard-Vetch; BS: Bare Soil; VP: Vetch-Pea; VSP: Vetch-Rye-Pea.*

3.3. Sunflower seedlings emergence vigor

Seedlings mean emergence vigor index of the 4 sunflower genotypes are reported in Table (6). A non-significant effect of genotype ($p > 0.05$) was observed on this variable, except at the first observation date (for which $p < 0.05$). Mean emergence vigor index ranged from 0.7 (8 DAS) to 0.36 (18 DAS).

Table 6 : Emergence vigor index of the 4 sunflower genotypes

	Vigor (index) ±SD				
Genotype	**8 DAS (96,7°Cd)**	**9 DAS (109,25°d)**	**11 DAS (137,55°Cd)**	**15 DAS (199,7°Cd)**	**18 DAS (250,65°Cd)**
Carrera CLP	0.08 ± 0.02^{a}	0.16 ±0.03^{a}	0.24 ± 0.04^{a}	0.30 ± 0.04^{a}	0.36 ±0.04^{a}
MAS86OL	0.07 ± 0.02^{a}	0.15 ± 0.03^{a}	0.23 ± 0.03^{a}	0.29 ± 0.03^{a}	0.35 ± 0.03^{a}
MAS89M	0.08 ± 0.01^{a}	0.16 ± 0.03^{a}	0.24 ± 0.03^{a}	0.30 ± 0.03^{a}	0.36 ± 0.03^{a}
MAS98K	0.08 ± 0.02^{a}	0.16 ±0.03^{a}	0.23 ± 0.04^{a}	0.30 ± 0.04^{a}	0.35 ± 0.04^{a}
Tukey p-value	0.04746	0.3445	0.6836	0.6230	0.6196

SD= Standard deviations. Mean index in the same column followed by the same letters are not significantly different (P<0.05, ANOVA).

Seedlings mean emergence vigor index after CCs treatments is reported in Table (7). A significant and consistent over time effect of CCs treatments ($p < 0.05$, except at the first observation date) was observed on this variable. Highest values were obtained after a vetch-rye-pea treatment, followed by vetch-pea, bare soil and mustard-faba bean-phacelia treatments whereas the lower values were attained after a radish-mustard-vetch treatment and a rye treatment. Mean emergence vigor index ranged from 0.6 (8 DAS) to 0.30 (18 DAS).

Table 7 : Emergence vigor index of sunflower after CCs treatments.

	Vigor (index) ±SD				
Treatment	**8 DAS (96,7°Cd)**	**9 DAS (109,25°d)**	**11 DAS (137,55°Cd)**	**15 DAS (199,7°Cd)**	**18 DAS (250,65°Cd)**
Mustard-Faba bean-Phacelia	0.06 ± 0.04 ^{a}	0.13 ± 0.07 ^{ab}	0.19 ± 0.11 ^{ab}	0.24 ± 0.13 ^{ab}	0.29 ± 0.15 ^{ab}
Radish-Mustard-Vetch	0.06 ±-0.03 ^{a}	0.12 ± 0.07 ^{ab}	0.18 ± 0.10 ^{ab}	0.23 ± 0.12 ^{ab}	0.27 ± 0.14 ^{b}
Rye	0.05 ± 0.03 ^{a}	0.11 ± 0.06 ^{b}	0.17 ± 0.09 ^{b}	0.22 ± 0.12 ^{b}	0.27 ± 0.14 ^{b}
Bare soil	0.06 ± 0.03 ^{a}	0.13 ± 0.07 ^{ab}	0.19 ± 0.10 ^{ab}	0.24 ± 0.13 ^{ab}	0.29 ± 0.15 ^{ab}
Sorghum-Faba bean-Mustard	0.06 ± 0.03 ^{a}	0.12 ± 0.07 ^{ab}	0.18 ± 0.23 ^{ab}	0.23 ± 0.12 ^{ab}	0.28 ± 0.15 ^{ab}
Vetch-Pea	0.06± 0.04 ^{a}	0.13 ± 0.07 ^{ab}	0.19 ± 0.10 ^{ab}	0.25 ± 0.13 ^{a}	0.29 ± 0.15 ^{ab}
Vetch-Rye-Pea	0.07 ± 0.04 ^{a}	0.14 ± 0.08 ^{a}	0.20 ± 0.11 ^{a}	0.25 ± 0.13 ^{a}	0.30 ± 0.16 ^{a}
Tukey p-value	0.08366	0.0174	0.01364	0.01461	0.01397

SD= Standard deviations. Mean index in the same column followed by the same letters are not significantly different (P<0.05, ANOVA).

3.4. Correlation between sunflower final emergence rates and vigor

A Spearman test and a linear regression were performed to identify a potential correlation between final emergence rates and vigor of sunflower genotypes after CCs treatments (Figure 6). The two variables showed a significant degree of linear association.

Table 8 : Comparison of final vigor and emergence rates of sunflower genotypes

Genotype	**Final vigor index ±SD**	**Final emergence (%)±SD**
CARRERA CLP	0.36 ±0.04 ^{a}	67 ± 15 ^{ab}
MAS 86OL	0.35 ± 0.03 ^{a}	63 ± 12 ^{a}
MAS89M	0.36 ± 0.03 ^{a}	75 ±13 ^{b}
MAS98K	0.35 ± 0.04 ^{a}	73 ± 12 ^{b}
Tukey p.value	0.6196	0.004784

SD= Standard deviations. Mean index in the same column followed by the same letters are not significantly different (P<0.05, ANOVA).

Table 9 : Comparison of final vigor and emergence rates of sunflower seedlings after CCs treatments

Treatment	**Final vigor index ±SD**	**Final emergence (%)±SD**
Mustard-Faba bean-Phacelia	0.29 ± 0.15^{ab}	72 ± 11^{abc}
Radish-Mustard-Vetch	0.27 ± 0.14^{b}	61 ± 12^{a}
Rye	0.27 ± 0.14^{b}	64 ± 17^{a}
Bare soil	0.29 ± 0.15^{ab}	79 ± 8^{c}
Sorghum-Faba bean-Mustard	0.28 ± 0.15^{ab}	78 ± 6^{bc}
Vetch-Pea	0.29 ± 0.15^{ab}	65 ± 15^{ab}
Vetch-Rye-Pea	0.30 ± 0.16^{a}	67 ± 3^{ab}
Tukey p.value	0.01397 *	0.001594

SD= Standard deviations. Mean index in the same column followed by the same letters are not significantly different (P<0.05, ANOVA).

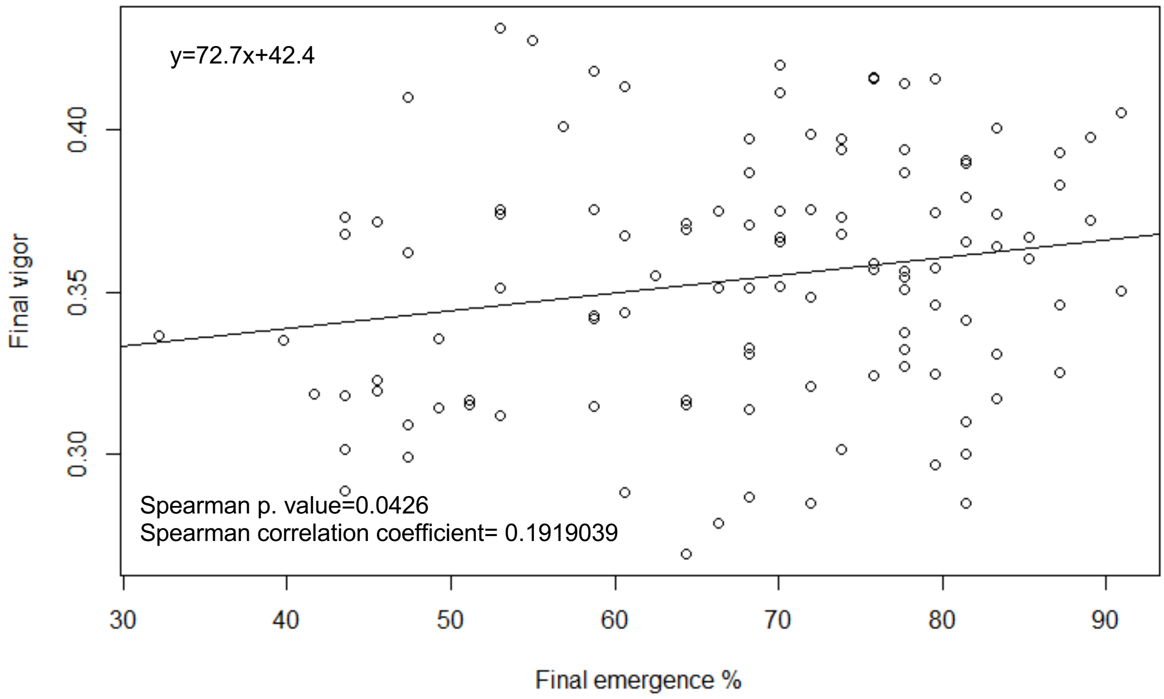


Figure 6 : Linear regression of the final emergence rates and vigor of sunflower seedlings (P<0.05, Spearman test)

3.5. Non-emergence causes

Non-emergence rates of sunflower seedlings due to each related causes are reported in Figure (7). The absence of seed or the presence of damaged seeds due to bird's predation was the key cause of seedlings non-emergence while minor impacts of mechanical stresses (i.e., seedling mortality due to a soil surface crust and soil clods, recognizable with an intact seed content but no germination) and of soil-dwelling pests (presence of holes or larvae in or around seeds) were also observed (Supplemental Figure 3). Non-emergence rates at the plot scale ranged from 20% to 40%.

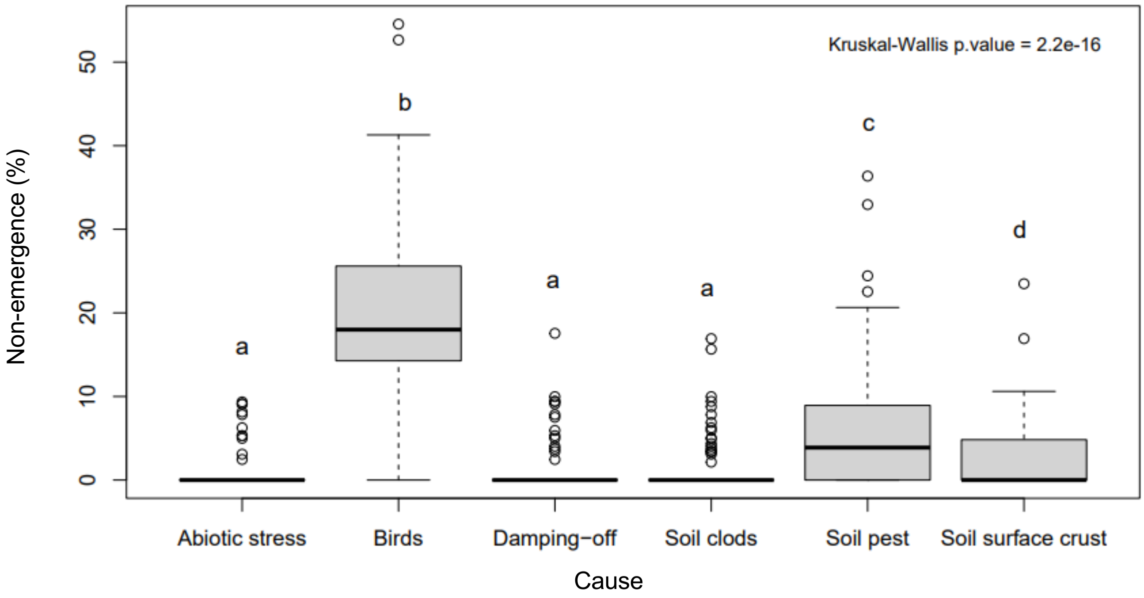


Figure 7: Percentage of sunflower seedlings non-emergence due to related causes *from the visual diagnostic key adapted from the literature review of pre-emergence damping-off symptoms (Lamichhane, 2021 (Editorial)). Vertical bars represent standard deviations.*

A non-significant effect of genotype ($p > 0.05$) was observed on this variable (Figure 8) whereas a significant effect ($p < 0.05$) of CCs treatment was fund (Figure 9). Sunflower grown after rye appeared to be the most impacted by bird's predation, whereas after a bare soil, birds caused the lowest losses. Soil pest such as seed maggots, wireworms, symphylans, millipedes were more harmful after radish-mustard-vetch and a vetch-pea treatments. Lowest values for this same cause were obtained after the bare soil and the vetch-rye-pea treatments. Non-emergence of sunflower seedlings was also observed because of damping-off diseases (rotten seed content and no germination), especially after a radish-mustard-vetch treatment.

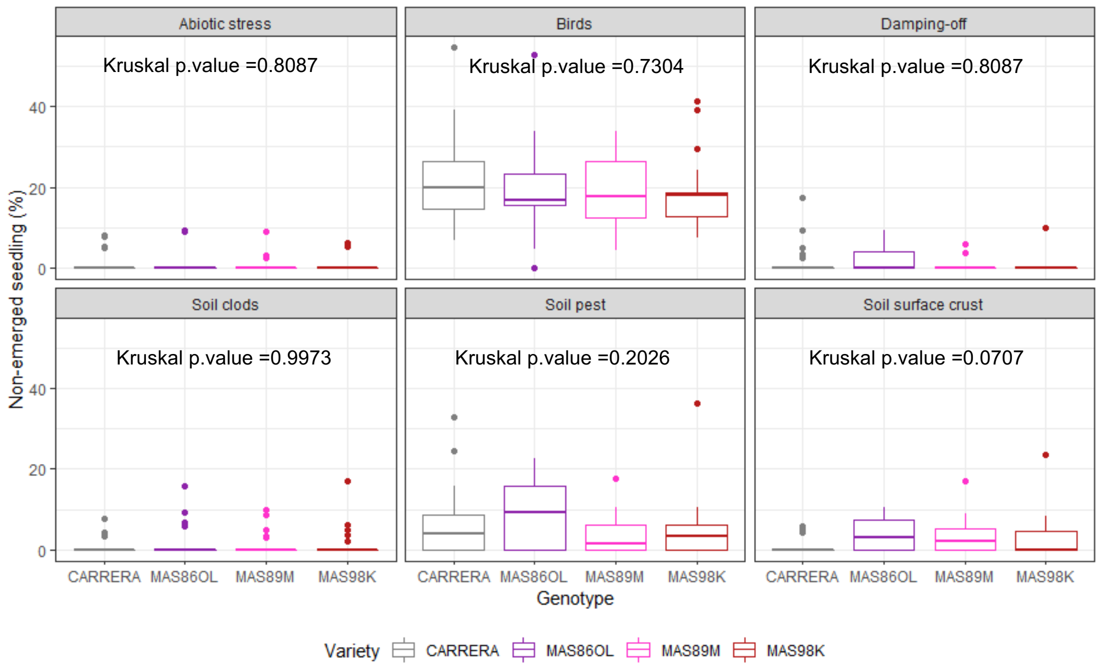


Figure 8: Non-emergence percentage of sunflower genotypes seedlings related to causes from the visual diagnostic key adapted from the literature review of pre-emergence damping-off symptoms (Lamichhane, 2022 (Editorial)). Vertical bars represent standard deviations.

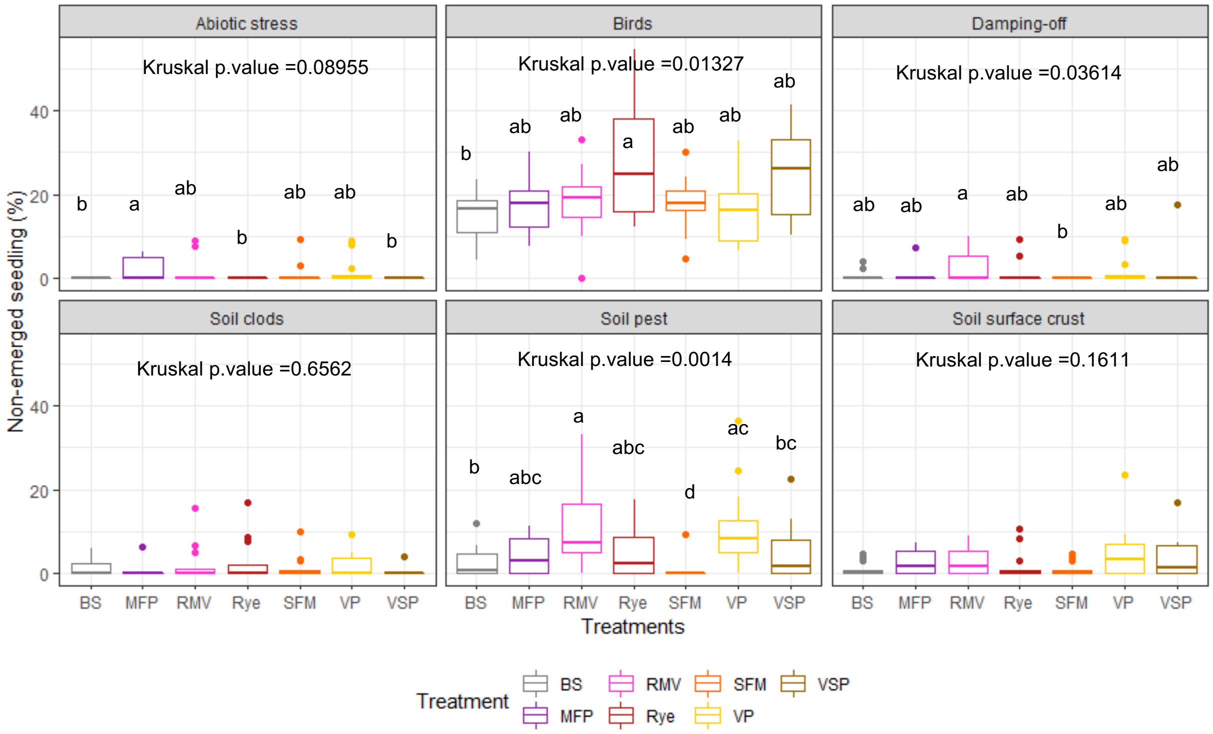


Figure 9: Non-emergence percentage of sunflower seedlings after CCs treatments related to causes from the visual diagnostic key adapted from the literature review of pre-emergence damping-off symptoms (Lamichhane, 2022 (Editorial)).

Vertical bars represent standard deviations. SFM: Sorghum-Faba bean-Mustard; Rye: Rye; MFP: Mustard- Faba bean-Phacelia; RMV: Radish-Mustard-Vetch; BS: Bare Soil; VP: Vetch-Pea; VSP: Vetch-Rye-Pea

3.6. Post-emergence damages

No post-emergence damages were observed on the sunflower seedlings at the time of observations.

3.7. Verticillium incidence

First verticillium leaf symptoms appeared 43 DAS (655.3°d). Verticillium mean incidence percentage on sunflower genotypes under field conditions are reported in Table (10). A significant effect of sunflower genotypes ($p < 0.05$) was observed on this variable throughout the rating phase. The number of infested plots varied from 10% (43 DAS) to 100% (from 78 DAS). Verticillium incidence percentage was the higher for MAS98K from 43 DAS to 64 DAS, and the lower for MAS89M at the last rating date.

Table 10 : Verticillium incidence percentage on 4 sunflower genotypes from first symptoms until the final evaluation

Incidence (%)±SD

Genotype	**43 DAS (655.3°d)**	**50 DAS (809.4°d)**	**58 DAS (968.0°d)**	**64 DAS (1050.8°d)**	**71 DAS (1180.5°d)**	**78 DAS (1334.5°d)**	**85 DAS (1491,0°d)**	**92 DAS (1625,2°d)**
Carrera CLP	10 ±13 ^{a}	52 ±39 ^{a}	55 ±40 ^{ab}	55 ±40 ^{ab}	94 ±18 ^{a}	94 ±18 ^{a}	97 ±12 ^{b}	99 ±4 ^{a}
MAS86OL	11 ±14 ^{a}	45 ±36 ^{ab}	60 ±39 ^{ac}	60 ±39 ^{ac}	95 ±22 ^{a}	95 ±22 ^{a}	100 ±0 ^{a}	100 ±0 ^{a}
MAS89M	10 ±14 ^{a}	4 ±25 ^{b}	53 ±35 ^{b}	53 ±35 ^{b}	93 ±17 ^{a}	93 ±17 ^{a}	93 ±22 ^{b}	96 ±11 ^{b}
MAS98K	17±20 ^{b}	50 ±37 ^{ab}	64 ±40 ^{ac}	64 ±40 ^{ac}	93 ±20 ^{a}	93 ±20 ^{a}	93 ±23 ^{b}	100 ±0 ^{a}
Kruskal p.value	0.004607	0.03837	0.00295	0.002557	0.6083	0.4082	1.623e-06	8.75e-09

SD= Standard deviations. Means in the same column followed by the same letters are not significantly different (P<0.05, Kruskal-Wallis test).

In the same way, a consistent and significant effect of CCs treatments ($p < 0.05$) was fund on verticillium incidence percentage (Table 11). Values ranged from 4% (43 DAS) to 100%

(from 78 DAS). Until 64 DAS, verticillium incidence was the highest after a sorghum-faba bean-mustard and vetch-pea mixture, followed *by a* mustard-faba bean-phacelia mixture. The bare soil treatment appeared to be the less infected by verticillium at symptoms apparition. From 78 DAS, sorghum-faba bean-mustard and vetch-pea mixtures attained the highest values with the bare soil treatment. The lowest final incidence was observed after a radish-mustard-vetch treatment.

Table 11 : Verticillium incidence percentage on sunflower plants after cover crops treatments from first symptoms until the final evaluation

Incidence (%)±SD

Treatment	**43 DAS (655.3°d)**	**50 DAS (809.4°d)**	**58 DAS (968.0°d)**	**64 DAS (1050.8°d)**	**71 DAS (1180.5°d)**	**78 DAS (1334.5°d)**	**85 DAS (1491,0°d)**	**92 DAS (1625,2°d)**
Mustard-Faba bean-Phacelia	12 ±15^{b}	52 ±36^{b}	53 ±36^{c}	53 ±36^{c}	92 ±17^{b}	92 ±17^{b}	98 ±8^{a}	98 ±7^{ab}
Radish-Mustard-Vetch	5 ±10^{c}	35 ±34^{c}	41 ±37^{dc}	40 ±36^{dc}	78 ±35^{c}	78 ±35^{c}	81 ±33^{b}	96 ±11^{b}
Bare soil	4 ±11^{c}	31 ±28^{c}	39 ±36^{d}	39 ±36^{d}	100 ±0^{a}	100 ±0^{a}	100 ±0^{a}	100 ±0^{a}
Sorghum-Faba bean-Mustard	19 ±15^{a}	64 ±34^{c}	86 ±32^{a}	86 ±32^{a}	100 ±0^{a}	100 ±0^{a}	100 ±0^{a}	100 ±0^{a}
Vetch-Pea	19 ±19^{a}	53 ±31^{b}	70 ±30^{b}	70 ±30^{b}	99±5^{a}	99 ±5^{a}	99 ±5^{a}	100 ±0^{a}
Kruskal p.value	2.2e-16	2.2e-16	2.2e-16	2.2e-16	2.2e-16	2.2e-16	2.2e-16	2.2e-16

SD= Standard deviations. Means in the same column followed by the same letters are not significantly different (P<0.05, Kruskal-Wallis test).

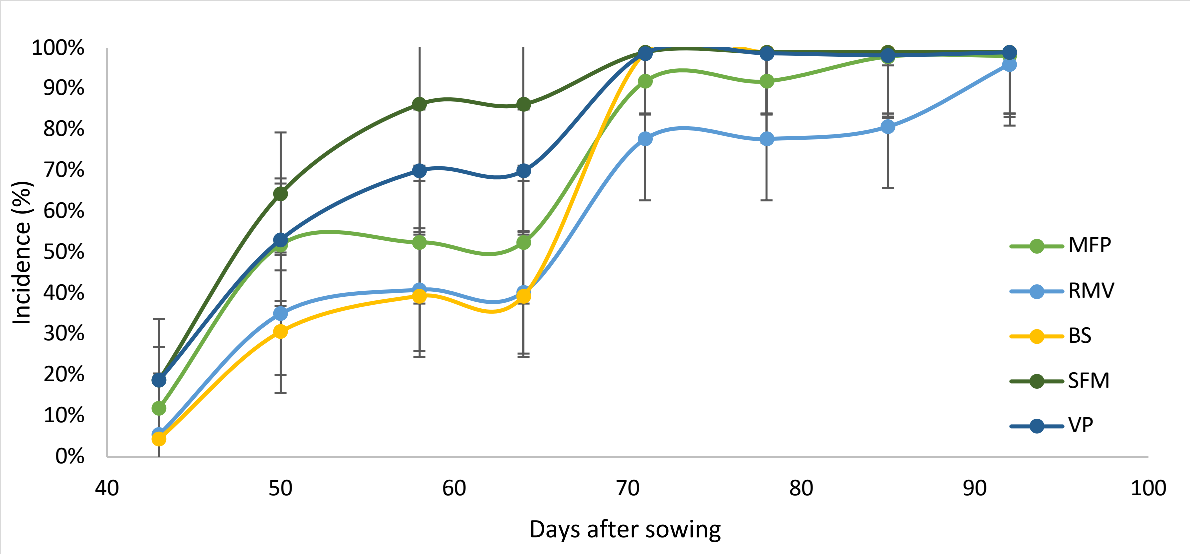


Figure 10: Verticillium incidence dynamics on sunflower plants after cover crops treatments in relation to days after sowing

Treatments correspond to: SFM: Sorghum-Faba bean-Mustard; Rye: Rye; MFP: Mustard- Faba bean-Phacelia; RMV: Radish-Mustard-Vetch; BS: Bare Soil; VP: Vetch-Pea; VSP: Vetch-Rye-Pea. Vertical bars reported in the figure represent standard deviations.

3.8. Verticillium severity

The disease severity index allowed to evaluate the severity of verticillium symptoms expressed on the sunflower plants. A significant effect of sunflower genotypes (p<0.05) was observed on the DSI (Table 12). The DSI varied from 2.5 (43 DAS) to 53.3 (92 DAS) (Figure 11). At the first and last rating dates, the DSI attained the highest values for the MAS98K genotype compared to the 3 other genotypes. This quite high severity may be due to a high initial density of inoculum.

Table 12 : Disease Severity Index of verticillium on 4 sunflower genotypes from first symptoms until the final evaluation

Disease Severity Index ±SD

Genotype	**43 DAS (655.3°d)**	**50 DAS (809.4°d)**	**58 DAS (968.0°d)**	**64 DAS (1050.8°d)**	**71 DAS (1180.5°d)**
Carrera CLP	2.5 ± 3.4^{b}	13.1 ± 9.8^{a}	14.0 ± 10.2^{ac}	13.9 ± 10.1^{ac}	25.5 ± 6.4^{b}
MAS86OL	2.8 ± 3.5^{b}	11.2 ± 8.9^{ab}	$15,9 \pm 11.1^{ab}$	15.9 ± 11.1^{ab}	26.8 ± 8.8^{a}
MAS89M	2.5 ± 3.5^{b}	10.3 ± 6.3^{b}	13.2 ± 8.7^{c}	13.2 ± 8.7^{c}	26.3 ± 8.3^{ab}
MAS98K	4.1 ± 5.0^{a}	12.4 ± 9.4^{ab}	16.3 ± 10.3^{a}	16.4 ± 10.5^{a}	26.8 ± 9.7^{ab}
Kruskal p.value	0.004607	0.03837	0.004133	0.003589	0.2156

Genotype	**78 DAS (1334.5°d)**	**85 DAS (1491,0°d)**	**92 DAS (1625,2°d)**

Carrera CLP	31.3 ± 11.5 ^{a}	36.0±12.7 ^{a}	43.4±18.8 ^{a}
MAS86OL	28.0 ± 9.8 ^{ab}	36.3±11.8 ^{a}	45.9±19.3 ^{a}
MAS89M	27.8 ± 9.9 ^{b}	32.5±14.8 ^{b}	46.0±25.5 ^{a}
MAS98K	27.5 ± 10.9 ^{b}	35.3±16.8 ^{ab}	53.3±21.1 ^{b}
Kruskal p.value	*0.004878*	0.03081	0.0001173

SD= Standard deviations. DSI in the same column followed by the same letters are not significantly different (P>0.05, Kruskal-Wallis test).

A highly significant effect of CCs treatments (p<0.05) was observed on the DSI (Table 13). It varied from 1.1 to 4.7 (43 DAS) and from 28.9 to 67.5 (92 DAS) (Figure 11). Throughout the rating dates, no tendencies were identified. At the last rating date (92 DAS), each treatment was significantly different from the others. The bare soil treatment attained the highest DSI followed by the sorghum-faba bean-mustard mixture and the vetch pea mixture. The mustard-faba bean-phacelia mixture obtained the lowest DSI followed by the radish-mustard-vetch mixture.

Table 13 : Disease Severity Index of verticillium on sunflower plants after CCs treatments from first symptoms until the final evaluation

	Disease Severity Index ±SD				
Treatment	**43 DAS (655.3°d)**	**50 DAS (809.4°d)**	**58 DAS (968.0°d)**	**64 DAS (1050.8°d)**	**71 DAS (1180.5°d)**
Mustard-Faba bean-Phacelia	3.0 ±3.6 ^{c}	13.0 ± 9.0 ^{b}	13.1 ± 9.1 ^{c}	13.1 ± 9.1 ^{c}	23.3 ± 4.7 ^{b}
Radish-Mustard-Vetch	1.3 ± 2.6 ^{c}	8.8 ± 8.6 ^{c}	10.2 ± 9.3 ^{d}	10.1 ± 9.1 ^{d}	19.6 ± 9.0 ^{c}
Bare soil	1.1 ± 2.7 ^{c}	7.7 ± 7.0 ^{c}	10.2±9.6 ^{d}	10.3 ± 9.9 ^{d}	28.4 ± 4,1 ^{a}
Sorghum-Faba bean-Mustard	4.7 ± 3.9 ^{a}	16.1 ± 8.6 ^{a}	23.0 ± 9.1 ^{a}	23.0 ± 9.1 ^{a}	31.6 ± 9.7 ^{a}
Vetch-Pea	4.7 ± 4.8 ^{a}	13.3 ± 7.9 ^{b}	17.5 ± 7.4 ^{b}	17.5 ± 7.4 ^{b}	28.4 ± 7.2 ^{a}
Kruskal p.value	2.2e-16	2.2e-16	2.2e-16	2.2e-16	2.2e-16

Treatment	**78 DAS (1334.5°d)**	**85 DAS (1491,0°d)**	**92 DAS (1625,2°d)**

Mustard-Faba bean-Phacelia	23.4 ± 4.8 ^{c}	26.4±5.9 ^{b}	28.9±8.9 ^{e}
Radish-Mustard-Vetch	20.4 ± 9.8 ^{d}	28.4±16.0 ^{b}	38.6±18.6 ^{d}
Bare soil	34.4 ± 8,1 ^{b}	42.2±11.6 ^{a}	67.5±16.8 ^{a}
Sorghum-Faba bean-Mustard	32.2 ± 9.6 ^{ab}	39.1±15.3 ^{b}	57.0±20.8 ^{b}
Vetch-Pea	32.5 ± 10.9 ^{b}	38.8±12.8 ^{b}	45.8±16.0 ^{c}
Kruskal p.value	2.2e-16	2.2e-16	2.2e-16

SD= Standard deviations. DSI in the same column followed by the same letters are not significantly different (P>0.05, Kruskal-Wallis test).

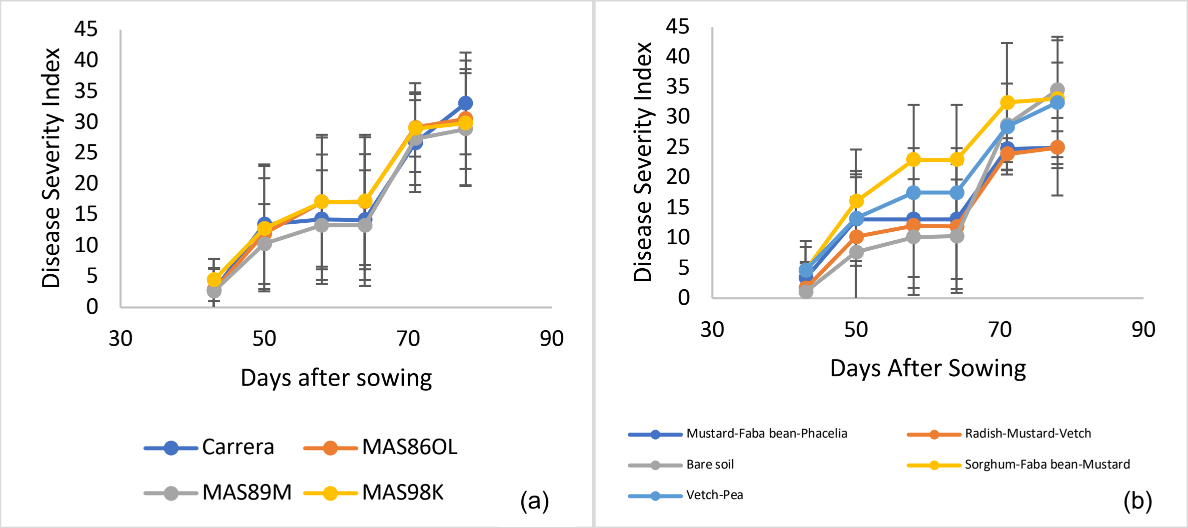


Figure 11: Verticillium disease severity index of sunflower genotypes (a) and after CCs treatments (b) related to days after sowing.

Vertical bars reported in the figure represent standard deviations

Area under the Disease Curve (AUDPC) was performed for each sunflower plant. On the one hand, a non-significant effect (P>0.05) of sunflower genotypes was observed on this variable. On the other hand, a significant effect (P<0.05) of CCs treatment was observed on this variable (Table 14). AUDPC varied from 3.1 (43 DAS) to 388.3 (92 DAS). Throughout the rating dates, a higher AUDPC was observed after a sorghum-faba bean-mustard treatment and a vetch-pea treatment than other treatments. At the first rating date (42 DAS), the bare soil treatment followed by the radish-mustard-vetch treatment presented the lowest AUDPC values. At the last rating date (92 DAS) and for the total AUDPC, sorghum-faba bean-mustard treatment and vetch-pea treatment along with the bare soil treatment attained the highest AUDPCs (Figure 12). The AUDPC was the lowest for the mustard-faba bean-phacelia and radish-mustard-vetch treatments.

Table 14 : Area under the Disease Curve (AUDPC) of verticillium disease on sunflower plants from first symptoms until the final evaluation

AUDPC (per plant) ±SE

Treatment	**43 DAS (655.3°d)**	**50 DAS (809.4°d)**	**58 DAS (968.0°d)**	**64 DAS (1050.8°d)**	**71 DAS (1180.5°d)**
Mustard-Faba bean-Phacelia	8.3 ±4.4 ^{ab}	44.6 ±10.0 ^{b}	73.1 ±13.3 ^{b}	73.5 ±13.3 ^{b}	101.9 ±9.3 ^{c}
Radish-Mustard-Vetch	4.1 ±3.0 ^{bc}	28.8 ±9.4 ^{c}	53.7 ±13.4 ^{c}	57.6 ±13.2 ^{c}	83.8 ±10.3 ^{c}
Bare soil	3.1±1.9 ^{c}	24.5 ±9.5 ^{c}	49.9 ±14.8 ^{c}	57.3 ±14.1 ^{c}	108.5 ±11,4^{d}
Sorghum-Faba bean-Mustard	13.1 ±7.6 ^{a}	58.2 ±12.4 ^{a}	109.4 ±9.1 ^{a}	128.6 ±6.4 ^{a}	152.7 ±5.0 ^{a}
Vetch-Pea	13.1 ±6.3 ^{a}	50.3 ±12.6 ^{ab}	86.2 ±13.6 ^{b}	98.0 ±12.6 ^{a}	128.6 ±9.1 ^{b}
Kruskal p.value	1.299e-05	3.93e-11	2.2e-16	2.2e-16	2.2e-16

Treatment	**78 DAS (1334.5°d)**	**85 DAS (1491,0°d)**	**92 DAS (1625,2°d)**	**Total AUDPC**
Mustard-Faba bean-Phacelia	130.8 ±6.3 ^{b}	143.5 ± 8.8^{c}	164.3 ± 7.2^{e}	741.6 ± 63.5^{c}
Radish-Mustard-Vetch	112.8±8.4 ^{c}	151.1 ± 11.8^{c}	221.5 ± 9.3^{d}	715.7 ± 68.0^{c}
Bare soil	175.4±11.5 ^{a}	250.9 ±10.9^{a}	388.3 ±17.2^{a}	1082.6 ± 70.9^{b}
Sorghum-Faba bean-Mustard	178.5 ±5.8 ^{a}	229.3 ± 4.9^{b}	331.4 ± 8.7^{b}	1237.5 ± 49.9^{a}
Vetch-Pea	170.6 ±6.1 ^{a}	230.1 ± 4.2^{b}	285.5 ± 8.5^{c}	1083.9 ± 61.3^{b}
Kruskal p.value	2.2e-16	2.2e-16	2.2e-16	2.2e-16

SE= Standard Error. Means in the same column followed by the same letters are not significantly different (P>0.05, Kruskal-Wallis test).

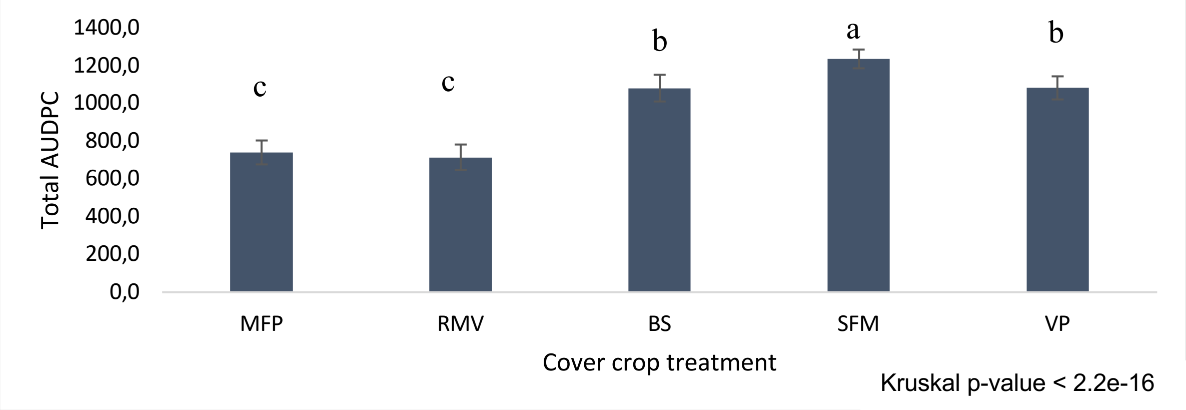


Figure12: Total Area Under the Disease Progress Curve (AUDPC) of verticillium impact evaluations after CCs treatments

Treatments correspond to: SFM: Sorghum-Faba bean-Mustard; Rye: Rye; MFP: Mustard- Faba bean-Phacelia; RMV: Radish-Mustard-Vetch; BS: Bare Soil; VP: Vetch-Pea; VSP: Vetch-Rye-Pea. Treatments with the same letter above are not statistically different according to Tuckey HSD test at the a=0.05 level. Vertical bars reported in the figure represent standard deviations

4. Discussion

This study aimed to assess the seedbed conditions at the time of sunflower seedlings emergence as well as the level of verticillium damage during sunflower growing season, after Brassicaceae and Fabaceae cover crops grown in sole crop or in a mixture of two or three species. At the time of CCs crushing, the total biomass of the CCs treatments as well as the individual biomass of the CCs within the treatments, showed great variability. The reasons for such differences are not relevant to the study. Nevertheless, it was expected to observe more pronounced effects on the variables considered in the study after a high amount of biomass left on the ground, either positive or negative, than a low or no biomass. CCs residues left on the soil were able to create seedbed conditions either conducive or detrimental for the emergence rates and vigor of sunflower seedlings and by the same token for the development of V. *dahliae*.

4.1. Sunflower seedlings emergence dynamics and vigor

Laboratory experiments of sunflower varieties germination capacity provided high germination rates results for all varieties, except for MAS86OL which only reached 40% emergence. The hypothesis of an issue with the MAS86OL seed lot was raised as germination rates were inconsistent with indications of the seed company (86%).

Seedling emergence dynamics are an important indicator of plant performance, including the final yield. A high final emergence rate is not sufficient for sunflower seedlings to successfully establish. For instance, a low emergence speed of sunflower seedlings is detrimental because it will result in a low emergence vigor. Indeed, a slow and uneven emergence is more susceptible to bird, slug, and soil pest damage and a non-optimal initial vigor will lead to poor growth and stunted young plants (Lamichhane, 2022 (Editorial)). Field emergence rates of the sunflower varieties were not all considered acceptable according to Terres Inovia, especially for MAS86OL and Carrera CLP, whose mean final emergence rates were below 70%. Treatments with the lowest emergence rates were correspondingly the ones that stood out in the causes of non-emergence, showing that they were more sensitive to biotic and abiotic stresses. Emergence vigor index also appeared to be lower than expected. Masseed (the breeder) indicates that selected genotypes had a level 7 emergence vigor, which corresponds to genotypes that are "tolerant" to early stresses, and which should therefore have a relatively high vigor. A seedling vigor under optimal conditions would be 1 (Miller and McDonald, 1975; TeKrony and Egli, 1991). However, in the field conditions, the vigor did not exceed 0.35 for all sunflower genotypes. There was a positive relationship between emergence vigor and final emergence rates; 20% of the emergence rates explained the final vigor. The reason for increase in emergence rates and emergence vigor is due to favorable environmental factors. Relatively low final emergence rates and vigor thus testified of stressful conditions that prevented seedlings from emerging and developing rapidly.

On the one hand, the sowing date close to the crushing of CCs residues could have been a physical factor limiting seedlings emergence. Indeed, CCs residues left on the soil raised the question of mechanical obstacles that could hinder sunflower seedlings emergence, especially in a no-till system. Increasing seed-soil contact is one of the major objective of tillage operations (Blunk *et al.,* 2021). The presence of too large amount of crop residues in the same way as large soil clods, reduces soil-seed contact (Lamichhane *et al*., 2021). The soil-seed contact is necessary for rapid seed imbibition, germination, and seedling emergence, allowing the seed to get access to the soil moisture it needs. The degree of contact increases as soil macroporosity decreases (Brown *et al*., 1996). Consequently, the time interval between the seed germination and seedling emergence phase in a seedbed comprising crop residues and soil aggregates is longer, due to the increased tortuosity of the seedling path before reaching the soil surface (Boiffin *et al.,* 1992). This could explain relatively low vigor and non-optimal emergence rates. Also, during the early stages of crops cycle, emergence and seedling establishment do not depend on the quantity of available nitrogen in the soil. It is therefore not a limiting or impacting factor for seedling emergence. The emergence and establishment phase is influenced by the nature and amount of reserves contained in the seed (Gardarin *et al.,* 2016). Seed mass, for instance, is positively correlated with final emergence rates and germination speeds (Eriksson, 1999; Gómez, 2004; Tamet *et al*., 1996). The ability of the seedling to bypass or cross obstacles depends on the emergence force it develops (Sinha and Ghildyal, 1979). This force is itself dependent on stem diameter, which in turn is correlated with seed mass (Gardarin *et al.,* 2016). To this extend, low final emergence rates could be explained also by a low vigor whereby seedling may have exhausted the seed reserves during hypocotyl elongation without being able to emerge under the effect of stronger mechanical constraints such as the presence of clods or a soil crust. This would also be particularly true after CCs with an important biomass that were more difficult to crush, such as radish. Bare soil had the advantage of not having any mechanical obstacles due to crop residues, thus offering a better soil-seed contact, as confirmed with the study results.

On the other hand, CCs biomass left on the soil most probably modified the seedbed temperature and humidity, creating more humid and therefore cooler sowing conditions.

Temperature and humidity play an important role during crop early phases as they condition good water supply for the seed and the seedling. This could explain the relatively better emergence and establishment quality of sunflower seedlings after a sorghum-faba bean-mustard treatment which presented a high biomass. Spring and summer 2022 were particularly dry and hot, with mean air and soil temperatures higher than the average of the last ten years. The seedbed dried very quickly after sowing, leaving a small soil crust, and cracked soil surface. Around 10 DAS especially, the observed emergence rates were much lower than the predictions of the Gompertz model. According to Lamichhane *et al.,* (2022), sunflower has a basic water potential of 1 for germination and, in a soil with texture as the one we had in our experimental site, the crop undergoes a water stress below a threshold corresponding to ~12% of soil gravimetric moisture. This threshold was most likely exceeded around 10 DAS (130°C-days), creating non-optimal conditions for emergence. Irrigation at 10 DAS may have facilitated emergence of seedlings, but due to its high volume (between 25 and 40 mm) it could also have accentuated the soil surface crust formation under high air temperatures. Thus, higher emergence rates and vigor could have been expected under less dry and warm conditions.

Additionally, soil incorporation of fresh CCs roots and shoots could have been a source of release of compounds implicated in the allelopathic growth inhibition or promotion of the sunflower seedlings. It has been documented a lot that, exudates generated from particular CCs such as alfalfa, vetch, or rye, can inhibit the seed germination of cash crop species (Aguilar-Franco *et al*., 2019). Ercoli et al., 2006 mentioned allelopathy mechanisms impacting seedling's growth reductions. However, there is no report if allelopathy could actually also impact the emergence phase. Sunflower generally showed lower emergence rates and vigor index following a rye, a vetch, and a mustard treatment, possibly due to allelopathic inhibition potentials of Brassicaceae. Higher emergence rates occurred with sorghum-faba bean-mustard treatments. This is consistent with the fact that the mustard biomass was very small for this specific treatment. Thus, these results confirm studies conducted by Barnes and Putman (2017), Aguilar-Franco *et al*., (2019), Geddes *et al.* (2015), or Ercoli *et al.,* (2006) who showed that CCs such as rye, brown mustard or hairy vetch are toxic to target species such as weeds, suggesting the release of phytotoxins from plant biomass. But it also assumed that the sorghum specie used did not have a high allelopathic potential, as it was the case for faba bean specie. The efficiency of allelopathy would depend among other on sowing densities (Aguilar-Franco *et al*., 2019). The combination of mustard and vetch probably increased the allelopathic effect on the sunflower. In the case of the mustard-faba bean-phacelia treatment which resulted in a moderate emergence and vigor of sunflower seedlings, phacelia and faba bean probably helped dilute the potential negative impact of mustard on seedlings emergence. Nevertheless, the impact of phacelia could not be identified as positive, negative, or neutral.

With a low C/N, legumes such as faba bean and vetch could be crushed more easily and degraded quickly in the soil. It appeared they have counteracted the negative effects of Brassicaceae on seedling emergence, since sorghum- faba bean-mustard and mustard-faba bean-phacelia treatments had moderate emergence and vigor. On their own, however, with vetch-pea treatment, they did not have a positive effect on emergence.

Sunflower crop is not able to compensate pre- and post-emergence damage and/or losses due to several factors (McMaster *et al.* 2012). As seedling emergence rate is a major component of final crop yield, it was important to identify the primary causes leading to non-emergence to better understand plot phenomenon, and later control biotic and abiotic factors responsible for such damages. In the study, biotic stresses were the main factors for emergence failure. Sunflower seeds appeared to be particularly susceptible to birds and seeding problems, even though these two causes seem at first glance of contrasting nature. Indeed, when sowing in good conditions the seed is normally well buried in the soil and therefore not accessible to birds. The absence of seeds is therefore sometimes more of a seedling problem, rather than birds coming in because the seed is accessible. On a side of the experimental plot, during seeding, some seed furrows did not close properly. It was hypothesized that there was an increased moisture due to weather conditions in the days before seeding, as well as due to crop residues. The non-emergence caused by birds must thus be put into perspective.

Wireworms were also a significant cause of non-emergence. It today represents one of the most important soil-dwelling pests from an economic point of view, both in a conventional or no-till system. These pests cause severe damage on crops, especially at the crop establishment phase (Lamichhane, 2022 (Editorial)). Their broad host range including vegetables such as peas and radishes could explain a relative lower emergence rates after radish-mustard-vetch and vetch-pea treatments. On the contrary, wireworms had a lower impact after a bare soil and a vetch-rye-pea mixture, explained respectively by the absence of crops before sowing (wireworms are usually found in the topsoil layers, eating crop roots) and on the other hand because generally low infestation of wireworms are observed in crop rotations thanks to biofumigation potential of Brassicaceae, such as rye (Dierauer, 2017). In general, a diverse crop rotation promotes various beneficial organisms and lowers the infestation of wireworms (Dierauer, 2017). No attack of slugs was observed due to the very low rainfall.

It would have been interesting to additionally determine germination rates and causes of non-germination. The causes of non- germination could have helped refine the emergence and vigor analysis since they are related and confirm or not the hypotheses of mechanical or thermal stress, allelopathy potential of Brassicaceae, as well as bird's predation. However, this step was too destructive on the microplots of the experiment since this requires digging up the seeds, which is risky on a quite small experiment.

No post-emergence damages were observed while bird predation is generally mostly observed in post-emergence. Sausse *et al.*, (2021) found that 10% of the plots they studied were severely affected, with more than 20% of the seedlings totally destroyed, and leading to reseeding and significant yield losses. This shows the effectiveness of the bird net installed on the plot during the emergence phase (6 DAS), as well as the different methods used such as the scarecrow, bird cannon and stakes.

4.2. Verticillium wilt incidence and severity

The outbreak of verticillium disease started early in the sunflower growth cycle (40 DAS) and occurred in all the experimental micro-plots although seed companies classified studied genotypes as “non-sensitive” to sunflower verticillium wilt (SVW).

Soil and climatic factors may have influenced the incidence and severity of SVW. Indeed, temperature is an important driver of pathogen incidence, and humidity has even a prevailing role in predicting fungal plant disease outbreaks (Romero *et al.*, 2022). High temperatures as well as an increased humidity could provoke or facilitate fungal diseases (Romero *et al.,* 2022). Seasonal temperatures of 2022 were relatively high compared to the last ten years, and the soil warmed up with an average maximum temperature of 23°C at 10 cm deep in July 2022. The optimal soil temperature for the production of microsclerotia and verticillium infection is estimated in a range of 15 to 25°C (Calderon Madrid *et al.,* 2014; Soesanto ,2001), which is also the average soil temperature at 10 cm depth between May and August 2022. An earlier incidence is indeed linked with microsclerotia density and could cause more severe damage to the crop (Erreguerena *et al.,* 2019). On the opposite, very little rainfall occurred from seeding to the end of the observations compared to the previous ten years, and irrigation was only performed once. Consistently, Gimsing and Kirkegaard (2006), found no effect on either glucosinolates or isothiocyanates concentrations with a single irrigation. This study showed a low verticillium incidence after a bare soil treatment at the beginning of the verticillium outbreak. Cover crops residues left on the soil increased soil moisture compared to the bare soil and thus would confirm the hypothesis of higher moisture favoring the early attack of verticillium on sunflower plants. However, since the experimental plot is also located next to a river and on hydromorphic deep soil horizons, it can be assumed that the deeper soil horizons were wet, providing in all cases a favorable environment for microsclerotia to multiply.

In order to be effective against soil borne pathogens, biofumigation needs a minimum of 0.53 t ha−1 of sole crop total dry matter biomass production (Morris et al., 2020). A high biomass production do not lead to a dilution of glucosinolates concentration (Gimsing and Kirkegaard, 2006). This biomass threshold for effective biofumigation has been exceeded with CCs residues biomass and should not have been a limiting factor for biofumigation succeed. Biofumigation also depends on many factors such as the timing of incorporation, the efficiency of incorporation, activity of the specific enzymes, and losses of isothiocyanates due to volatilization, leaching, and microbial degradation (reviewed by Brown and Morra, 1997). Residues were left to the soil shortly before sowing of the sunflower, which probably made it possible to reduce isothiocyanates losses by volatilization. The other parameters could not be evaluated.

Overall, after a radish-mustard-vetch and a mustard-faba bean-phacelia treatment, the incidence and severity of verticillium was lower than after sorghum-faba bean-mustard and vetch-pea treatments. The most feasible explanation of Verticillium wilt diminution was by the Brassicaceae CCs, especially mustard and radish (since the sorghum-faba bean-mustard treatment presented a very low mustard biomass).

White and brown mustard are particularly used in biofumigation for their content in isothiocyanate precursors and also for their agronomic features (Reau *et al.,* 2005). Isothiocyanates present in these species are characterized by their low volatility and thus persistence in the soil as they are degraded. In a general way, isothiocyanates in white mustard are less volatile than those in rapeseed, for example, and should be slower acting. In brown mustard, glucosinolates are present in significant amounts in the aerial parts and are hydrolyzed to volatile isothiocyanates that are likely to have a rapid action, giving it the most potent action. In contrast, white mustard is capable of releasing slower acting isothiocyanates (Reau *et al.,* 2005). Also, the reduction in SVW achieved by forage radish in our study supports previous results showing a significant reduction in germination or microsclerotia development following exposure to chopped forage radish under laboratory conditions (Neubauer *et al*., 2014). However, it was complex to deduce exactly which mechanisms acted on the decrease of verticillium wilt or not, as no data was obtained on soil concentrations of glucosinolates and isothiocyanates. Glucosinolates production differ between shoots and roots of the crops, and among the major hydrolysis products of glucosinolates, isothiocyanates are generally considered the most toxic, however they also vary in their toxicity to different organisms (reviewed by Brown and Morra, 1997). However, the results showed that mustards would present an enhanced biofumigation potential than other Brassicaceae such as radish and rye. This is confirmed among others by Larkin *et al.,* (2011) who, for instance, stipulated that mustard blends resulted in slightly better disease control than rye.

It also appeared that SVW incidence and severity would be positively correlated with seedlings emergence rates and vigor. Indeed, while MAS86OL genotype had the lowest emergence rates and vigor index, it was less affected by verticillium. On the contrary, MAS98K genotype which had the highest emergence rates and vigor, was the most rapidly infested. No explanation was found for this, as more vigorous seedlings are normally less susceptible to fungal attacks. However, since V. *dahliae* enters through the roots, and as the vigorous seedlings will develop their root system more quickly, it was hypothesized that verticillium could have more impact, since it infected these roots first.

In the same way, most affected sunflower plants were after a bare soil, sorghum-faba bean-mustard and vetch-pea treatment. Data of soil nitrogen residues between 0 and 90 cm depth obtained from CCs sampling showed higher amounts of nitrogen after the vetch-pea, bare soil and sorghum-faba bean-mustard treatments. Conversely, the radish-mustard-vetch, rye and mustard-faba bean-phacelia treatments had the lowest soil mineral nitrogen residues. This did not seem to be correlated with the CCs biomass left on the ground. Soil mineral nitrogen could be explained by the previous crop as well as by the lower C/N ratio of legumes, such as vetch and its ability to take up nitrate from the soil and fix atmospheric nitrogen (Couëdel *et al.,* 2018).

The results of this study seemed to be correlated with soil nitrogen supply. It does not allow to confirm the negative correlation that would exist between soil nitrogen supply and incidence and severity of verticillium found by Davis *et al.,* (2010), since we observed a positive correlation. Davis *et al*., (2010) mentions that disease reduction with green manures would be strongly correlated with both non-specific microbial activity and major changes in microbial populations. He also states that green manure treatments often do not reduce pathogen populations but reduce pathogen infections based on increased general microbial activity. Compared to all other treatments, the bare soil was affected later but more consistently. This confirms that the microbial activity following the CCs residues could reduce verticillium attacks later in the growth cycle. Also, in the case of our study, where it is the first time that cover crops were grown, one can think that the green manure effect could not be yet observed in the soil. We could thus expect with time, higher bacterial populations and microbial activity and lower fungal populations. In contrast, these observations support a previous study (Seassau *et al.,* 2010) showing a strong positive correlation between high nitrogen supply and premature maturation caused by Phoma, another sunflower fungal disease. Higher nitrogen could have led to an increase in the development of P. macdonaldii, which eventually would have caused an overexpression of V. dahliae symptoms. Like on the opposite, a significant reduction in SVW after purple vetch was measured by Ait Kaci Ahmed *et al.,* 2021, and Phoma symptoms were rare. These results are still poorly understood at this time (Wheeler *et al*., 2012).

Concerning associations with legumes and given the results it was not possible to conclude on an improved performance of mixtures. Notably because the radish-mustard-vetch and mustard-faba bean-phacelia treatments contained legumes that did not present a significant biomass and therefore a high nitrogen composition. The pea vetch treatment was strongly affected.

Finally, the severity of verticillium wilt must be put into perspective. The evaluation of the severity of the symptoms was potentially overestimated since the scoring includes a scale with only few stages. A plant that is in the score 2, presented symptoms that affected 20% to 50% of the plant, which is very broad. Symptom severity was thus probably lower in the field than calculated (the highest percentage was taken to calculate the DSI). Also, since the studied plots were microplots, one can also ask to what extent the "border effect" was excluded. Indeed, with six rows of sunflowers, only two rows in the center of the plot were considered, although they were very close in distance to the edge of the microplot. Many sunflowers did not emerge, and microplots had gaps, which probably concentrated the verticillium attack on the neighbouring plants. Also, the plot borders were not totally respected because of the lack of plants, which sometimes forced to observe the plot border rather than the center of the microplot.

5. Conclusion

This study gives some evidence that cover crops could provide ecosystem services to sunflower by improving the crop establishment and by controlling verticillium wilt compared to a bare soil during the fallow period. In only one year of experimentation, CCs had a consistent and strong impact on the parameters studied. In particular, CCs biomass played a key role in the effectiveness of CCs to provide ecosystem service. Nevertheless, results sometimes showed contrasted and antagonistic reactions of sunflower to CCs, concerning emergence quality and verticillium control.

On the one hand, and despite that weather conditions (drought and heat creating a soil surface crust) were not optimal, the sunflower showed a quite high emergence after CCs, which seemed to be favored by a higher seedbed moisture. However, a possible allelopathic effect at emergence after Brassicaceae has been noted, potentially preventing seedlings from emerging optimally and rapidly. Seedlings vigor was also impacted by CCs residues acting as mechanical obstacles and preventing an optimal soil-seed contact. Seedlings most probably exhausted the seed reserves without being able to emerge under the effect of such stronger mechanical constraints. The presence of Fabaceae may have diluted the negative effects of Brassicas at emergence, but further studies are needed to understand their effects in the mixtures. The results are encouraging, especially in a no-till system. For the establishment of CCs in cropping systems, attention should be paid to the soil practices and the crushing method of the residues in order to obtain a seedbed with a fine texture.

On the other hand, although they might have reduced the final seedling emergence rates, mustard and radish cover crop species showed a high biofumigation potential and seemed to be the most suitable species to provide this targeted ecosystem service. Biofumigation by crucifer did not appear to be supported by legume CCs on the one year of experimentation, since the soil mineral nitrogen content was possibly positively correlated with the incidence and severity of verticillium. Soil mineral nitrogen might have promoted other microorganisms, weakening sunflower plants, and leading to overexposure of verticillium. Since this is the first year of the study, subsequent years may see a positive effect of microbial activity as previously demonstrated. In all cases, the early verticillium attack associated with high temperatures and a seedbed moisture at the time of sowing, caused significant damages. Further studies are needed to fill the current knowledge gap concerning the mechanisms that drive biofumigation in field conditions and the benefits provided by cover crops mixtures mutualization. Even though Brassicaceae were likely to contribute to pathogen suppression, they do not represent a solution for a total elimination of SVW.

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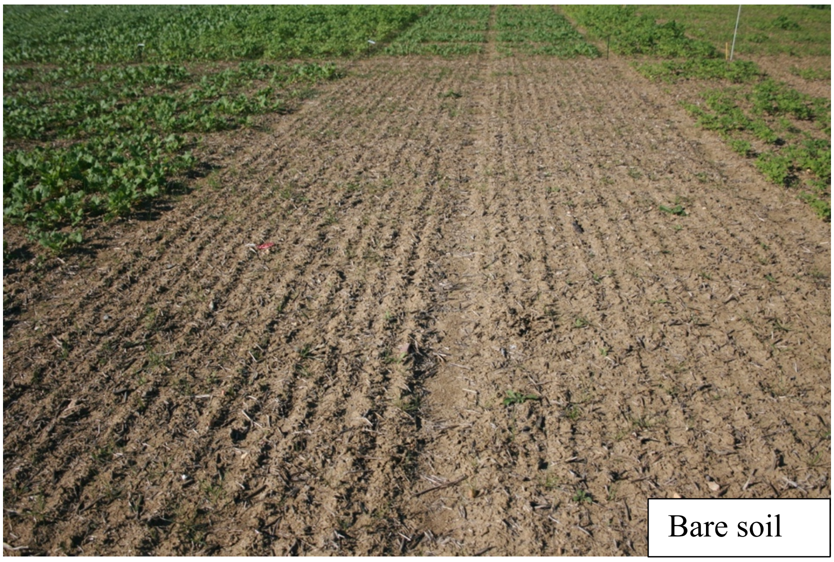
Appendix

Supplemental Figure 1: Cover crops treatments before their destruction (photographies taken by UMR-AGIR).



Radish-mustard-vetch

Rye

Vetch-rye-pea

Mustard- faba bean-phacelia

Vetch-pea

Sorghum- faba bean-mustard

Supplemental Figure 2: Sunflower verticillium wilt leaf symptoms (photographies taken by Bertille Rueda).

Supplemental Figure 3a: Visual diagnostic key describing major causes affecting crop establishment and descriptions of their symptoms/characteristics.

Sub-phase	Symptoms or characteristics			Causes of no crop establishment	References
Sowing - seed germination	Seeds fail to germinate (pre-germination)	Absence of the intact seed or presence of seed parts	No presence of seed or seed parts	Technical problem of sowing or predation of seeds by pests[a]	Carabajal-Capitán et al. (2021); Tschumi et al. (2018)
			Outer seed coat altered, presence of empty seed coat	Damage caused by granivores (e.g. slugs, earthworms or rodents)	Carabajal-Capitán et al. (2021); Tschumi et al. (2018)
		Presence of non-germinated and germinated seeds	Intact seed content but no germination	Abiotic stress (heat water or mechanical) or dormancy problem or seed death	Lamichhane and Aubertot (2021)
Seed germination - seedling emergence	Seedlings fail to emerge (pre-emergence)		Rotten seed content and no germination	Pre-emergence damping-off (seed- and soil-borne pathogens)	You and Barbetti (2017)
			Presence of holes or larvae in or around seeds	Soil-borne pests (e.g. seed maggots, wireworms, symphylans, millipedes)	Ebregt et al. (2005)
			Occurrence of germination but no emergence, twisted seedlings, abnormal radicle growth, absence of necrosis and/or rot, presence of crust and/or soil compaction in the seedbed	Mechanical stress such as soil compaction, soil crust formation	Gallardo-Carrera et al. (2007); Lamichhane et al. (2020); Lamichhane and Aubertot (2021)
			Occurrence of germination but no emergence, twisted seedlings, absence of necrosis and/or rot or crusting, presence of clods, crop residues or stones above the seedling in the seedbed	Mechanical stress such as soil clods, crop residues and stones (depending on the type of (no)tillage	Dürr and Aubertot (2000); Lamichhane et al. (2020)
			Occurrence of germination but no emergence, normal seedlings, absence of necrosis and/or rot or crusting, no clods in the seedbed	Too high sowing depth (technical problem of sowing that leads to a poor soil-seed contact in no-till systems)	Blunk et al. (2021); Kirby (1993); Mahdi et al. (1998)
			Occurrence of germination but no emergence, dried or desiccated seedlings, absence of necrosis and/or rot, no soil crust, neither compaction or large clods in the seedbed.	Post-germination drought stress	Boureima et al. (2011)
Seedling emergence – initial competition among young plants	Seedlings have well-emerged (post-emergence)	Seedling falls down due to the damaged stem at the ground level and remains on the seedbed	Seedling wilting, reddish root necrosis, hypocotyl and seedling collar rot, absence or few secondary roots	Post-emergence damping-off (seed- and soil-borne pathogens)	Rojas et al. (2016); Serrano and Robertson (2018); You et al. (2017)
			Holes and presence of larvae in cotyledons, stem and/or roots; swelling of seedling collar; shrinking and gradual disappearance or entangled seedlings; accumulation of filamentous brown debris	Soil-borne animal pests (e.g. seed maggots, wireworms, symphylans)	Douglas et al. (2017); Furlan et al. (2021b); Vea and Eckenrode (1976)
		Partial or total damage of leaves, damaged stem, uprooted seedlings	Damaged cotyledons and/or part of the hypocotyl/epicotyl at or just after seedling emergence; uprooting of seedlings and their spread in or complete disappearance from the seedbed; pitting and shot-holing; leaf perforation; stunting and poor plant vigour; chewed leaves or entire seedlings	Herbivory related to animal pests (e.g. flea beetles, slugs, birds, rodents, mammals)	Douglas et al. (2017); Douglas and Tooker (2012); Furlan et al. (2021a, 2021b); Lamichhane (2021); Sausse et al. (2021b)
		Seedling falls down and die	Leaf scorching, sudden wilting of the entire plant, pale brown patches in the plot	Frost damage	Brandsæter et al. (2000); Chen et al. (2005); Pescador et al. (2018)
		Seedlings do not fall down on the seedbed	Modification of seedling colors, deformations, abnormal chlorotic or necrotic symptoms but different than those caused by soil-borne pathogens	Problem related to chemical stress (phytotoxicity) or water logging and subsequent root asphyxia	Lamhamdi et al. (2013); Loose et al. (2017); Yasumoto et al. (2011); Zaman et al. (2018)

[a] An absence of the intact seed could be also related to a rapid seed rotting, especially for small-seeded plant species, under high moisture conditions in the seedbed or when the field diagnosis is performed too late.

Supplemental Figure 3b: Examples of non-emerged seedlings due to different causes

Soil surface crust

Soil clods

Damping-off disease

Wireworms

Author: RUEDA Bertille **Year: 2022**

Topic category:
(Do not write in this box)

TITLE: Analysing the effects of cover crops on emergence rates and vigor, seedling establishment and verticillium wilt development of sunflower varieties

Keywords: *Helianthus Annuus - Cover crops - Brassicaceae – Fabaceae -* Verticillium *dahliae – Agroecological protection*

Mots clés : *Helianthus Annuus – Cultures Intermédiaires - Brassicacée – Fabacée -* Verticillium *dahliae – Protection agroécologique*

Résumé:

Le tournesol (Helianthus Annuus) est confronté à d'importants problèmes de production dans le sud-ouest de la France. Il est principalement cultivé dans les zones vallonnées, au sein de rotations courtes et suivant une longue période de jachère pendant laquelle les sols sont laissés nus. Les problèmes d'implantation de la culture et les attaques fongiques sont récurrents et provoquent des pertes de rendement importantes. Les effets de l'implantation de cultures intermédiaires ont été évalués sur les taux d'émergence et la vigueur des plantules de tournesol, et sur le développement du flétrissement verticillien. Quatre génotypes de tournesol ont été semés dans un champ expérimental, après sept traitements de cultures intermédiaires contenant divers mélanges de Fabacées et Brassicacées. Les services écosystémiques fournis par ces familles de cultures semées seules ou combinées ont été évalués sur les variables d'intérêt, dans un système sans labour et sans glyphosate. Les cultures intermédiaires ont eu un impact significatif dans cette expérience d'une année. Les conditions météorologiques ont fortement influencé la diminution de l'émergence et de la vigueur des plantules de tournesol, ainsi que l'apparition précoce de la maladie du verticillium. En général, les résidus de cultures intermédiaires ont inhibé l'émergence des plantules en réduisant le contact sol-graine et en épuisant les réserves de graines. Les Brassicacées telles que le radis et la moutarde ont également montré un impact négatif sur l'émergence des plantules de tournesol par des mécanismes d'allélopathie, alors qu'elles ont réduit le développement du flétrissement verticillien par rapport aux autres traitements. L'association avec les Fabacées n'a pas permis de conclure à une mutualisation des services rendus, si ce n'est qu'elle a peut-être dilué les effets négatifs des Brassicacées lors de l'émergence. Une corrélation positive entre l'azote minéral du sol et la sévérité de la verticilliose a été observée. L'hypothèse d'une augmentation de l'activité microbienne affaiblissant les plantes de tournesol a été émise. Des études complémentaires sont nécessaires pour comprendre comment les Brassicacées et les Fabacées interagissent lors des différentes étapes du cycle de culture du tournesol, afin de cibler et de maximiser les services écosystémiques qu'elles fournissent.

Abstract:

Sunflower (*Helianthus Annuus)* faces major production challenges in southern-western France. It is mostly grown in hilly areas, in short rotations and after a long fallow period during which soils are left bare. Crop establishment issues and fungal attacks are recurrent, causing significant yield losses. The effects of agroecological cover crops (CCs) implementation were assessed on the sunflower emergence rates and vigor, its seedling establishment and on the verticillium wilt development. Four sunflower genotypes were sown in an experimental field, following seven CCs treatments containing diverse mixtures of Fabaceae and Brassicaceae. Ecosystem services provided by these crops families sole or combined were evaluated on the measured variables, in a no-till system and without glyphosate. CCs had a significant impact in this one-year experiment. Weather conditions strongly influenced the decrease in emergence and vigor of sunflower seedlings, as well as the early outbreak of verticillium disease. In general, cover crop residues inhibited seedling emergence by reducing soil-seed contact and depleting the seed reserves. Brassicaceae such as radish and mustard also showed a negative impact on sunflower seedling emergence through allelopathy mechanisms, whereas they reduced verticillium wilt development compared to other treatments. The association with Fabaceae did not allow to conclude on a mutualization of the services provided, except that it possibly diluted the negative effects of Brassicaceae at emergence. A positive correlation between soil mineral nitrogen and verticillium was observed. The hypothesis of an increase in microbial activity weakening the sunflower plants was made. Further studies are needed to understand how Brassicaceae et Fabaceae interact at the different stage of the crop cycle, in order to target and maximize ecosystem services provided by them.

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