

Deliverable 2.2: LL methodology for conventional farming (version 2 – M18)

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WP1 WP2 WP3 WP4 WP5 WP6 WP7 WP8
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Version number	Implemented by	Notes
1.0 (M6)	CTIFL	with CICYTEX
2.0 (M18)	CTIFL	with CICYTEX

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1. Introduction

GOOD project will foster Living Labs (LLs) aimed at promoting agroecological weed management strategies and reducing the use of herbicides in different European crops. Co-creation activities and experimental trials will be developed in conventional, organic and mixed systems for the testing of Agroecological Weed Management (AWM) strategies.

Work Package (WP2) is oriented to the development of experimental activities in pilot sites in conventional systems.

The objectives of this WP are:

- O2.1:** To design and test AWM strategies within LLs, for both annual and perennial crops.
- O2.2:** To reduce or eliminate herbicide use.
- O2.3:** To provide standards and typologies for the agroecological transition of conventional farming systems adopted at national level.

The main innovation points of GOOD will be to use **cover crops** combined with various practices in an AWM context, to reduce weed pressure and reliance on herbicides.

The Task 2.1 establishes the creation of guidelines and protocols to be followed in a common and coordinated way by all the experimental sites in order to implement the AWM strategies and trial designs and to assess the most relevant indicators. These protocols will be updated up to three times throughout the project.

The description of Task 2.1, as written in the Grant Agreement (GA) is the following¹:

Task 2.1: Establishment of guidelines and protocols for the conventional sites of each LL.

This Task will develop the framework of the pilot operations along with the guidelines for establishing and managing the conventional experimental sites of LLs providing instructions about the methods and tools (D2.1). It will also include the template received from T1.1 and protocols received from T1.2 (key timelines, cover crop species, AWM strategies, termination techniques of cover crops). The LL boards established (T1.1) will monitor, update, and provide feedback for improving the guidelines and protocols of the pilot farms annually.

The Task 2.1 is led by CTIFL ([María-Martha Fernandez](#)).

The following partners are participating in Task 2.1: UC, LSSV, AUA, COSMOCERT, AIAB, CNR, UNICT, CICYTEX, USC, CTIFL, CUT, MRZIP, HUMOFERT, DELPHY and LLKC.

The Deliverable D2.1 “LL methodology for conventional farming (version 1)” is due in Month 6 of the project (i.e., 31 October 2023).

1.1. Objective of the task.

The overall objectives of Task 2.1 are:

- Encourage discussion among WP2 partners on the best AWM techniques and the most appropriate methodologies.
- Develop guidelines on the methodologies to be applied in each LL for experimental designs and measurement of effectiveness indicators of the different strategies for weed control, crop development, environmental and socioeconomic indicators.
- Include inputs and protocols coming from other WPs in successive versions.
- Successful implementation of pilot sites and co-creation activities.

Target numbers and Key Performance Indicators (KPI) related to the establishment of conventional sites are given in Table 1.

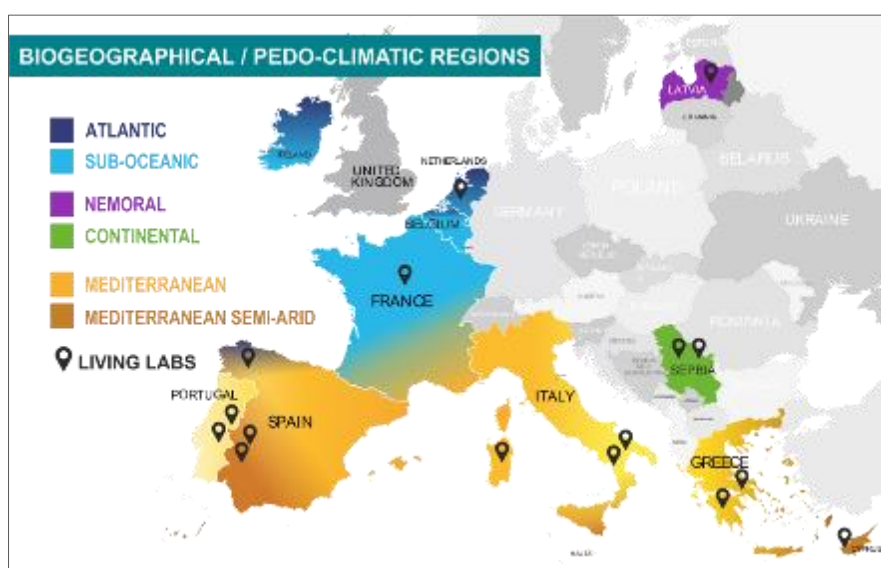
¹ (GA, p.81, or Proposal-Part-B, p.11/49)

Table 1: Expected results and target values in conventional pilot sites.

Result	KPI – Target value
Design, assess and demonstrate combinations of AWM strategies in conventional farming systems to enhance user acceptance	20 assessed cover crop species combined with 15 main crops & 48 assessed AWM solutions combined with cover crops (3 per LL)
	At least 14 assessed cover crops combined with inoculation (1 per LL) & at least 40 weed species identified using AI from the drone images
	32 Best combinations of AWM practices (2 per LL)
	N° of crops on which AWM solutions will be tested:15
	N° of AWM strategies included in the repository:40
Stakeholders engaged	N° of stakeholders engaged in the co-creation of LL boards: 160 (10 per LL)
	N° of stakeholders engaged in the AWMN: 1600 (100 per LL)
Reporting	16 Life Cycle Assessment report (incl. Social, Economic and Environmental LCA results) (1 per LL)
	80 Factsheets (5 per LL)
	80 Practice Abstracts (5 per LL)

1.2. Experimental sites in conventional systems

GOOD will develop, test and demonstrate context-specific AWM strategies in conventional systems through the establishment of 16 large field scale LLs in six different pedo-climatic conditions.



In accordance with the European Union's designation which is without prejudice to positions on status, and is in line with UNSCR 1244/1999 and the ICJ opinion on the Kosovo declaration of independence.

Emphasis will be given on the diversity of farming systems and the wide range of crops (15), both annual (9) and perennial (5) (Table 2).

The main crops selected for testing in each LL belong to the most economically important ones in the LL's country and/or those considered vulnerable to weed infestations and difficult to manage. The combinations of AWM solutions will be designed considering factors like the respective weed suppression, the technical and economic feasibility, the societal acceptability, the operational capacity, and the potential impact on soil health properties and diversity.

Table 2: Experimental sites of conventional systems

Annual crops			Perennial crops		
Crop	Country/Partner	LL code number	CROP	Country/Partner	LL code number
RYE/PEA	Latvia/LLKC	LV_rye-pea/11	APPLE	France/CTIFL	FR_apple /21
ONION	Netherlands/DELPHY	NL_onion/12	OLIVES	Portugal/ LSSV	PT_olives/22
SOYBEAN	Serbia/MRIZP	RS_soybean/13	CITRUS	Italy/AIAB	IT_citrus/23
MAIZE	Serbia/MRIZP	RS_maize/14	GRAPES	Italy/AIAB	IT_grapes/24
TRITICALE	Italy/CNR	IT_triticale/15	GRAPES	Greece/AUA	GR_grapes/25
WHEAT	Greece/AUA	GR_wheat/16	OLIVES	Cyprus/CUT	CY_olives/26
COWPEA	Portugal/LSSV	PT_cowpea/17	CHERRY	Spain/CICYTEX	ES_cherry/27
RICE	Spain/CICYTEX	ES_rice/18	APPLE/GRAPES	Spain/USC	ES_apple-grapes/28

1.3. Connection with other Work Packages

The overall activities developed in WP2 and in task T2.1 especially, will foster several AWM strategies that will be defined by the co-creation processes of the LLs and will be complemented with the information, protocols and knowledge gathered in other WPs. Likewise, the activities developed in the pilot sites will serve to provide inputs and evidence to other WPs in order to assess the soil health indicators, Life Cycle Assessment, weed mapping and to feed the AWM Toolbox.

In detail, WP2 and Task 2.1 will feed and be fed by other WPs (WP1, WP4, WP5, WP6 and WP7).

1.3.1. Establishment of LLs and monitoring protocols (WP1).

WP1 → WP2

- Generate typologies and methodologies for the experimental research to be conducted throughout the project in the conventional farming sites of the LLs, based on farmers' decision-making and perception of AWM and barriers and needs for the agroecological transition of agricultural systems.
- Knowledge on needs, barriers, gaps, and opportunities for AWM that will be used for the establishment of the LLs and the experimental design
- List of weed management innovations and strategies based on the combinations of preventive, cultural, biological, digital and mechanical non-chemical weed control method
- Templates and Protocols (T1.1, T1.2) about key timelines, cover crop species, AWM strategies and termination techniques.
- Templates and Protocols for establishing LLs and LLs boards as well as for experimental results reporting.
- The LL boards established (T1.1) will monitor, update and provide feedback for improving the guidelines and protocols of the pilot farms annually.

WP2 → WP1

- Data and content from the R&I activities to be used in the co-creation activities
- Needs, barriers, gaps and opportunities for AWM implementation and adoption to be discussed in the cooperation meetings with other projects

1.3.2. Arbuscular mycorrhizal fungi (AMF) analyses and soil health indicators (WP4).

Seed inoculation with beneficial microorganisms (AMF) will be a strategy to guarantee cover crop establishment and crop productivity and favor their competitive ability against weed species. Additionally, effects of the strategies tested on WP2 on crop productivity, weed diversity and soil health (including chemical, physical and biological parameters) will be assessed in WP4.

Beyond the proposed soil parameters for all LLs, each partner may conduct additional studies that are of interest to them (*nutrients and water availability, endo and meso-fauna, etc.*).

WP4 → WP2

- Protocols for soil sampling (AMF identification, soil health indicators)
- Protocols for seed inoculation of cover crops
- Native AMF identification, reproduction and delivering the inocula for seed inoculation in the second and third year

WP2 → WP4

- Send pooled soils samples (AMF identification, soil health indicators)
- Send to UNIPi root samples to assess mycorrhizal colonization of inoculated and non-inoculated cover crop plants and to evaluate the symbiotic competence of native AMF in the second and third year.

1.3.3. Weed identification and mapping with drone flights. (WP5)

In all the LLs, UAVs will be used annually to acquire photographs of the weed flora. This imagery will enable the production of weed prescription maps to prioritize the dominant and invasive plant species (using AI – T5.1) and proceed to termination activities. Protocols and assessment about weed mapping will be developed by WP5.

All the LLs will deploy UAVs equipped with high-resolution cameras to perform flights once or twice per season, based on the cropping type (once for annuals, and if possible, twice for perennials).

WP5 → WP2

- Protocols for technical implementation and troubleshooting through sessions and training
- Development a software interface to exchange information with LLs' weed experts.
- AI algorithms for weed identification and mapping
- Provision of actionable information of UAV data and AI models for weed management in the field.

WP2 → WP5

- Information about areas/facilities of UAV flights, the annual calendar of field operations, plantation properties and field characteristics in each site for the successful pilot operation.
- LLs' weed experts will annotate all relevant data through a user-friendly software interface, which EDEN will develop.
- Evidence-based data coming from the experimentation within the LLs to feed the Agroecological Weed Management Toolbox.

1.3.4. Life Cycle Assessment. Societal, economic and environmental impact (WP6)

Several assessments will be done in WP6 regarding the social, economic, and environmental impacts of Agroecological Weed Management, as well as the Life Cycle Assessment (LCA) of these strategies at farm level. The LCA will allow the evaluation of the impacts generated by the different crops, treatments, and approaches used within the LLs and consequently build an articulated picture of the impacts of cover crop-based rotations in different environments.

WP6 → WP2

- LCA Common protocol to all partner countries, with a focus on soil health and the impact of agroecological solutions on wildlife (e.g., earthworms) and soil properties

WP2 → WP6

- Relevant evidence-based data from LLs to feed the LCA assessment.

1.3.5. Dissemination, communication and demonstration

All relevant information from WP2 will be communicated and disseminated to stakeholders in participating countries.

WP7 → WP2

- Guidelines to provide useful information from LLs activities to feed the Communication and Dissemination plan.

WP2 → WP7

- Demo-farmers of the LLs will share their experiences with other practitioners through different field events.
- Reporting of demo activities
- Dissemination of the adapted materials through different channels, depending on the characteristics of their LL.

1.3.6. Agroecological performance evaluations: TAPE and OASIS tools

Two existing frameworks will be used for GOOD R&I activities to assess the agro-ecological performance of solutions. The first is the Tool for Agroecology Performance Evaluation (TAPE) developed by FAO and the second is the Original Agroecological Survey and Indicator System (OASIS) developed by the Agroecology Europe initiative.

All Living Labs will apply these tools in the **third experimentation year** for all proposed weed control strategies. The data collection to feed other previous assessments in GOOD (LCA, costs, stakeholders interviews, workshops...) will be useful to complete the TAPE and OASIS assessments.

OASIS (Original Agroecological Survey and Indicator System)

The OASIS Methodology (Škorjanc et al, 2021) addresses five main dimensions in the framework where each LL will assess the AWM performance.

1. Agroecological farming practices
2. Economic viability
3. Socio-political aspects
4. Environment and biodiversity
5. Resilience

A set of indicators will be collected through a two-and-a-half-hour interview per farm. They look at agricultural activities from the farmer's point of view. This methodology will be applied, at least, in the experimental farms of the GOOD project (organic sites of each LL). The analysis can be extended voluntarily in each LL to other farms in the territory that are applying AWM in order to have a territorial vision of the agroecological transition performance. The OASIS assessment will be done **twice**: the first one to characterize the usual practices in place and the second one to assess the application of AWM practices and their contribution to the agroecological transition in comparison with the previous practices

Examples of some indicators measured with the OASIS tool through online software.



The authors of OASIS have provided a Guidebook to follow this methodology step by step. An online tool is also available (<https://oasis.agroecology-europe.org/>) in order to make the assessment easier. The person carrying out the study in each LL must register in this free application.

Specific instructions will be provided to the LLs to carry out this study well in advance.

TAPE (Tool for Agroecology Performance Evaluation)

The TAPE evaluation approach reaches not only the farm dimension but also the territorial context and the communities living on it, thus providing an assessment of how the practices are contributing to the agroecological transition and to achieve the Sustainable Development Goals (SDGs). It will also generate information for policy makers. The 10 principles of Agroecology are used to characterize production systems by assessing their level of transition to agroecology (*Recycling, Responsible Governance, Synergies, Diversity, Co-creation & sharing of knowledge, Resilience, Human & social values, Culture & food tradition, Efficiency, Circular & Solidarity Economy*). This diagnostic can be used to identify strengths and weaknesses of the systems assessed, but also to monitor and evaluate projects or to establish entry points for activities (FAO, 2019). Moreover, it is a participatory methodology that will have to be validated by all stakeholders in the LL.

It is composed of 4 steps:

1. Step 0: Information about systems and context. Collection of all relevant context information, from territorial and higher scales, including the descriptions of production systems and agroecosystem and the enabling local and regional environment.

2. Step 1: Characterization of Agroecology Transition by identifying strengths and weaknesses of the systems assessed. An on-farm survey will be conducted to characterize the current managements before the application of AWM practices and the performance of GOOD activities following the TAPE criteria.
3. Step 2: The 10 core criteria of performance quantify the impact of the level of transition to agroecology assessed in STEP 1 by informing various dimensions of sustainability. An on-farm survey will be done to measure progresses in the experimental farms after the application of AWM practices and the performance of GOOD activities.
4. Step 3: A participatory analysis of the results, where the multidimensional performances (STEP 2) are reviewed in the light of the level of transition to agroecology (STEP 1) and the context and enabling environment (STEP 0). **This phase could be part of the LLs workshops.**

Steps 0, 1 and 2 can be completed together in an online survey form. Many of the previous data collection carried out in GOOD (LCA, costs, interviews and questionnaires, etc) could be useful to feed the TAPE surveys and can be used to fill them in.

Guidelines and templates adapted to GOOD farms will be provided to all LLs in the third year. More information about TAPE methodology can be found [here](#).

1.4. Technical and environmental context

Weed control is essential for maintaining productivity, profitability and sustainability of plant productions, and also to ensure the sanitary quality of crop products.

The harmfulness of weeds species is defined in relation to a given crop. It can operate at several levels: competition for water, nutrients and light (direct primary harmfulness), impact on crop pests, on cultural or post-harvest operations, risk of toxicity in harvested products (indirect primary harmfulness), or at the farm or territorial scale through the dispersal of seeds and invasive species (secondary harmfulness) (CORDEAU, 2018).

Today, in conventional agriculture, weed control is essentially based on chemical herbicide use, due to their high efficacy, their simplicity of use and their low cost. Thus, herbicides constitute the second most-widely sold category of pesticides in the EU-27, accounting for 35% of all pesticide sales in 2020 (EUROSTAT, 2023).

But herbicide molecules are also among the active substances most frequently found in surface waters, lakes and rivers, where they present a risk of toxicity for aquatic organisms. Some may also be harmful to soil health, or dangerous to human health. For the last two decades, farmers have been faced with a significant reduction in the number of herbicide active substances approved by European or national regulations, and more recently, with a strong pressure from society against the use of these active ingredients.

Reducing agriculture's dependence on herbicides has become a policy priority in Europe, and sustainable and effective non-chemical alternatives must be deployed to reduce or eliminate their use. The potential of agroecology could be one of the levers to achieve this goal, in combination of other non-chemical means.

2. Agroecological weed management strategies

Agroecology is a holistic approach that relies on and maximizes the use of natural ecosystem functionalities to support agricultural production. Applied to weed control, it will consist in preventive or curative means of breaking the development cycles of weeds, and thus preventing their harmfulness to the main crop. These means will rely on natural regulatory mechanisms between plant species, among each other, or with the soil microbiome, or even by inducing a temporal shift in weeds emergence. Other alternative methods (mechanical, physical, digital, biocontrol) can also be combined with these natural levers, which, alone, are likely to be only partially effective.

2.1. Agroecological methods

2.1.1. Cover-crops

2.1.1.1. Principles

The use of intercropping cover crops is one of the cornerstones of conservation agriculture applied to arable crops. First developed in response to the European Nitrates Directive, their role has gradually evolved to exploit the diversity of ecosystem services they can provide: crucifers for their "nitrate trap" effect, legumes for their "green manure" effect, grasses for their ability to restructure the soil, etc. (JUSTES, 2017). The idea of using them for weed management is more recent, and the way to use them for this purpose has yet to be perfected.

The use of ground covers for weed management is based on the dominance relationships that exist between plant species, based mainly on two types of mechanism: **competition** and, probably to a lesser extent, **allelopathy**.

Competition for resources, and especially for light, is the main mechanism involved in the regulation of weeds by cover crops. Competition will give the advantage to species with a high biomass, sown densely and able to grow very quickly. This will smother weed emergence, thus eliminating weeds in the cover crop, but also with an expected long-lasting effect to limit weeds emergence on the following main crop.

Allelopathy is a mechanism by which a plant (a living plant or its residues in decomposition) affects the growth of neighboring plants through the production of chemical compounds released into the environment. Root exudates in particular can be released, inhibiting the germination of other species.

Under natural conditions, with no human intervention, this is particularly evident in the development of some large monospecific lawns, even consisting of very small species (*Hieracium pilosella*, wild strawberries, etc.). This is also confirmed by many laboratory studies. But the possible use of species with allelopathic properties to control weeds in the field has yet to be demonstrated and developed.

In practice, several approaches can be considered for using cover crops for weed management:

- **On annual crops**, cover crops are used as intercropping crops:
 - 1) The cover crop (monospecific or mixture of species) is sown before the main crop (or unless otherwise possible at the same time as the main crop).
It is recommended to sow the cover crop in early autumn in the case of winter main crops (wheat, triticale, etc.) or in late autumn or winter in the case of spring main crops (soybean, onion, maize, rice, etc.)
 - 2) This cover crop is destroyed at the end of its cycle (either naturally by frost, or by other methods), and then the main crop is sown and cultivated.

In this case, both the cover crop and the main crop are managed as annual crops.
- **On perennial crops (fruit trees or vine)**, the cover crops can be used on the inter-rows of the orchard or vineyard (as annual or perennial crops), or directly on the tree- (or vine) rows.
 - On the inter-rows, in addition to improving weed management, the functions to be targeted are the improvement of the soil's bearing capacity and its physical and biological properties.
 - On the rows, the aim is to help controlling the weeds, while limiting the competition between the cover crop and the trees or vines. Therefore, the objective will rather be to keep the cover crop to a minimum height, either by choosing short species or by applying additional management methods to the cover crop (such as mowing).
 - In all cases (interrows or rows), a "green manure" function can also be considered, by using legume species (which can be with high biomass, or at the contrary, dwarf cultivars). This can be particularly interesting in the case of organic production systems.

2.1.1.2. Soil enrichment with AMF (Arbuscular Mycorrhizal Fungi)

Arbuscular mycorrhizal fungi (AMF) are obligate root endophytes which rely on getting carbohydrates from plants. In return, they can provide multiple ecosystem services to their host plant: enhancement of soil nutrients uptake (especially phosphorus, zinc and copper), help to resist drought, protection against root pathogens, etc. However, AMF are not only beneficial, and interactions between plants and AMF can range from highly mutualistic to antagonistic (SÅLE, 2022; RINAUDO, 2010). This could be exploited to contribute to control the weeds in the agroecosystems: thus, AMF may have a direct suppressive effect on the growth of many weeds belonging to families that are not usual hosts for AMF (non-mycorrhizal species); they also could act indirectly by enhancing the competitive ability of crop species to the detriment of some mycorrhizal or non-mycorrhizal weed species (EL OMARI, 2021).

In these recent years, many research studies have been conducted on this subject, mainly on arable crops. They conclude to varying levels of effectiveness depending on the crop species, the weed species and the taxonomic diversity of the AMF applied. They also encourage further studies in field conditions, especially under agroecological production systems.

In practice, the simplest way to enrich the soil with AMF is to inoculate them to the seeds before sowing. This can therefore be applied to the cover crops, with two types of expected positive effects on weed control:

- Help to suppress the growth of most undesirable weeds in the cover crop, and thus, also later on the main crop.
- Give the advantage to the cover crop to the detriment of the weeds, favoring their rapid soil coverage and growth, to allow them to smother the weeds, through competition for light.

AMF are naturally present in the soils, but their abundance or taxonomic diversity may differ depending on a multitude of factors (pedological, climatic, agricultural practices intensity, etc.).

In GOOD project, AMF will be isolated from soil samples taken from the LL's experimental sites, to be sure of their adaptation to local conditions. These samples should come from both the conventional and organic fields, to maximize the diversity of taxa collected. They are then multiplied in laboratory conditions, and later inoculated to the seeds of the selected cover crops.

2.1.2. False seed bed practice

The false seed bed technique is a cultural practice that can be implemented before the main crop is sown, with the objective of reducing the weed seeds stock already present in the soil.

After the previous crop has been destroyed, it consists in a succession of shallow tillage operations (less than 10 cm) performed to favor the germination of the weed seeds already present in the soil, and then to destroy the weeds plantlets after their emergence. Several series of false seedings are necessary to reduce the seed stock present in the superficial layers of the soil. The success of the technique relies on a good choice of shallow tillage equipment (harrow, tine or vibrashank cultivator, etc.) and on suitable climatic conditions (soils wet enough for the emergence of weeds).

This technique only concerns the case of annual main crops and is nowadays quite commonly used in biological farming systems.

2.1.3. Crop rotation

Crop rotation is probably one of the oldest techniques (beside manual weeding) for breaking the development cycle of the weeds. With farming intensification, the diversity of crop rotations has been impoverished, to the detriment of this essential function.

Extending crop rotation and introducing intermediate crops is an option which should be re-examined to facilitate weed management in annual crops.

2.1.4. Vegetal mulch

Vegetal mulching is an ecological technique that can be applied on perennial crops. It consists in hindering weed emergence by depriving them of access to light, by covering the soil with a thick layer of plant fragments.

This mulch can be obtained from woody plants present on the plot (e.g., chipped pruning wood in orchards), or from hedge pruning along field or road edges, or even from crops grown outside the field for this purpose (e.g., straw, miscanthus, etc.).

The mulch layer must be thick enough and able to last long enough on the soil. As it is naturally biodegradable, it must be renewed to maintain its effectiveness.

2.1.5. Grazing

Agro-pastoralism is an old farming system that has fallen into disuse in developed countries, with the intensification and specialization of crop production. The promotion of innovative cropping systems, with greater emphasis on agroecology, has led to a reconsideration of this approach, for the mutual benefits that can be generated between livestock and cash crops.

Regarding weed management, grazing can be used:

- on annual crops, as a means of managing and exploiting intercropping plant covers; it is then integrated into the overall management of crops rotation, before cash crops' cultivation.
- on perennial crops, the use of livestock herbivores can be exploited as a direct weed management measure, within the orchard or vineyard itself, while providing welfare benefits for the animals (shade, protection against wind, etc.).

These systems are possible if the farmer is himself a stockbreeder, but also through the establishment of partnerships (possibly under contract) between farmers and local stockbreeders.

General principles and warning points:

- **Annual crops**

Sheep grazing is the most studied example.

The floristic composition of the plant cover must be chosen with a grazing objective and must therefore take into account the feed value of the mix of species to be used, while avoiding the introduction of health risks for the sheep (bloat prevention).

The cover crops used for grazing can be: an intercropping summer plant cover, after a crop harvested early in the end of spring (such as peas); a frost-sensitive cover crop before a winter cereal; or a non-frost-sensitive cover crop before a spring cash crop.

Depending on the cover crops chosen, and on the needs of the farmers, the grazing period can be more or less long (from the end of summer to easter time next year).

For the stockbreeder, this partnership allows to benefit from grazing areas outside his own meadows, with high-quality feed for his herds over a period extending from mating to lambing. For the farmer, this practice allows soils nutrients to be retained, avoiding leaching, and later to be returned to the soil, in a form that is more easily mineralized for the following crop (via animal excrements), and also to reduce the pressure of certain weed species in the field. (RWDR, 2022).

- **Perennial crops**

Sheep, cattle (with particular constraints) or poultry can be used as "natural mowers" to manage grass and weeds inside the orchard.

Because of all the cultural practices to be applied to the orchard, grazing should be considered as a temporary practice, that will consist in introducing the livestock when the grass is high, and then, in removing it later (the number of animals should be reasoned accordingly). This also means being particularly vigilant about compatibility between grazing periods and the spraying calendars applied to the crop (toxicity of copper applied in organic orchards, and of chemical treatments in conventional orchards: a delay of more than three weeks between the treatments and the introduction of animals must be respected).

Animals can impact crop yields by feeding on the branches they can reach. Sanitary risks on fruits, due to animal droppings, must be considered (coliforms, listeria, salmonella, yeasts, molds, etc.). (AREFE, 2009-2014).

In all cases (annual or perennial crops), the practice of grazing requires setting up protective measures against predators (fixed or mobile fences, etc.), and complying with the administrative and regulatory procedures currently in force at national level (registration of animals, no mixing between animal

batches, organic animals in organic crops, veterinary monitoring and risks of parasitism for the animal).

2.2. Mechanical and physical techniques

Mechanical means include tillage and mowing.

- **Tillage** is the very basis of the definition of arable crops, and a very wide choice of equipment is therefore available for annual crops. Various working tools can be combined on the same equipment or used alone at separate times.

For perennial crops, a range of offset tools has been developed over the years for working the soil at very shallow depth along the rows of organic orchards and vineyards. They are equipped with feelers that allow the work assembly to be retracted to avoid obstacles and preserve the trunks (intercept tools).

A wide range of tools are available today for on-the-row mechanical weeding, with different modes of action (weeds pulling, or weeds root system cutting): intercept hoeing blades, rotary tillers, harrows, discs, metallic brushes, finger weeders, etc. Various tools can be used together on the same tool carrier, or separately to adapt to soil conditions.

This kind of equipment is still rarely used in conventional systems.

- **Mowing** is commonly used on the inter-rows of grass-covered orchards (either in conventional or organic farming in some countries), and it can also be applied along the planting rows, either to manage cover crops, or the spontaneous flora.

This practice has developed in some organic fruit crops but is rarely used in conventional orchards. It is also based on the use of various mowing tools (shredders, flail mowers, nylon cords brushes) specifically adapted to orchards or vineyards, capable of cutting the grass along the planting rows and between the trees, thanks to offset equipment and avoidance systems.

Other equipment, based on physical means, has also been developed to control the weeds without herbicides:

- **Thermal weeding** using gas, hot water or water steam. Some equipment is available on the market for weeding orchards along the tree rows, and some tools are still at the prototype stage. They must be used on very young weeds (plantlets stage).
- **Electric weeding; laser weeding**: these techniques are still under development, and their interest on perennial crops has still to be confirmed.
- **Physical barriers** can also be used to avoid the emergence of weeds on perennial crops: **synthetic mulch** (plastic or biodegradable materials) or **prefabricated natural mulch** (hemp or coconut fiber canvas, etc.).

2.3. Biocontrol of weeds

Biocontrol of weeds includes the use of biological control methods (using living organisms) and the use of natural-based substances. In Europe, while biocontrol has expanded significantly over the last few decades concerning pest and disease control, it remains still very underdeveloped in weed control, and few bioherbicides have reached the stage of commercially available products (SHAW, 2018; HARDING, 2015).

- **Biological weed control agents**

A first option concerns the use of **macro-organisms**, either through conservation biological control, that will consist in promoting existing populations (example of carabid beetles, that are natural weed seeds consumers and whose populations could be favored using cover crops (MOREAU, 2022)), or by introducing phytophagous species (acclimatation biological control), that will target specifically certain invasive weed species (example of *Ophraella communa* used against *Ambrosia artemisiifolia*). This line of research is a promising way of controlling non-native invasive weed species, especially, but the efficiency in the field of these methods needs further demonstration, as well as their harmlessness to non-target crops (IPMWRAISE, 2022).

The second option, which has been widely explored around the world for many years, but very little deployed in Europe, involves the use of **pathogenic microorganisms** (fungi, bacteria, viruses) or **phytotoxins** produced by these kinds of agents. Natural candidates for herbicidal action have been identified in at least 34 fungal genera, five bacterial genera and one virus species (GUILLEMIN, 2018). But to move to the commercial product stage, their safety on useful microflora, on crops, on environment, and on animal and human health must be demonstrated (HASAN, 2021). In 2016, worldwide, only 12 bioherbicides derived from microorganisms were registered on the market (nine derived from fungal microorganisms and three from bacteria) (CORDEAU, 2016).

- **Natural substances with herbicidal action**

Plant-based bio-herbicides include plant extracts, mostly from species with **allelopathic** properties, and **essential oils**, obtained by hydro- or steam-distillation from aromatic plants. Some twenty plant compounds have been identified for their action against certain weed species, with mechanisms of action still to be clarified. At least seven commercial products have been registered on the world market since 2012: one allelopathic substance (pelargonic acid) and six essential oils (VERDEGUER, 2020).

Basic substances, such as vinegar, or other substances derived from by-products from natural sources (mustard seed meal, for example) have also demonstrated an ability to suppress weed growth. Some are also available as registered commercial products (HASAN, 2021). Many of these natural substances are largely nonselective in their herbicidal effect; this constraint could be overcome through localized applications, to preserve the crop plants (LODDO, 2021).

Despite the quite broad range of biocontrol solutions described above, many are still at the research stage, and very few are actually available on the European market. In GOOD project Living Labs, these bioherbicides can only be applied in the field in compliance with European regulation (active substances approved and registered by EFSA), as well as with national regulations (plant protection product authorized for herbicide use and for the crop concerned).

If these conditions are not met, it should be remembered that an experimentation permit is required, and that the testing of these solutions can only be performed within the restricted framework authorized by this permit (Regulation (EC) No 1107/2009, Article 54).

2.4. Precision weeding

The development of **tools for detecting and even identifying weeds** opens up an innovative avenue for reducing weeding operations (chemical or even mechanical) and better targeting the weed control interventions. Based on optical means, imagery and artificial intelligence, these tools are still at a very early stage of study, but some applications already exist in the form of field-usable tools (mostly at the prototype stage). Coupled with precision sprayers, they can target only the weeds present, and thus considerably reduce the quantities of herbicides sprayed per hectare.

Their use is being particularly studied on annual crops, to target weed outbreaks when they appear sparsely in the field, and in particular certain perennial weeds that are particularly harmful or difficult to eliminate.

On perennial crops, they could be used to control perennial weeds (e.g., to reduce the amount of glyphosate applied), but compatibility with national regulations concerning restrictions in the authorized number of spraying interventions has to be verified.

Beyond precision spraying, these detection tools can be useful to farmers (both on annual or perennial crops) to have an overview of the weeds present in their field at a given time, and thus better reason their weeding interventions (better choice of active ingredients, positioning of mechanical operations, etc.).

Several of the levers listed above can also be combined together (at the same time or at different periods) to form **Integrated Weed Management strategies**. This can offer farmers a wider choice of weed management options for a transition towards herbicide reduction use: from **strategies combining the use of alternative methods and herbicide interventions** (with a goal of reducing herbicide use by at least 50%, by using **chemical herbicides at reduced rate**, or even **bio-herbicides**) to **fully agroecological weed management strategies**.

However, for a better harmonization of experiments in GOOD project, a common base of levers to be tested has been proposed (see below), in compliance with the Grant Agreement. If other options can be tested (depending on local conditions, on crops, or even on knowledge advances from previous works), they will yet be considered and discussed in the Project Steering Group (PSG).

2.5. Table of cover crops and AWM strategies experimented in GOOD Living Labs

• Cover crops

More than 34 cover crop species are experimented in GOOD Livings Labs.

On annual crops, they are used as intercropping crops (before the main crop), and destroyed either by ploughing, mowing, or by the winter freeze (Latvia), or even as living mulches between the rows of Triticale in Sardinia (managed by mowing).

On perennial crops, they are used on the interrows as intercropping crops or green manure in most Living Labs (they are then mowed at the end of their cycle); in France, they are established as perennial living mulches along the tree rows, in which case they are not destroyed. All Living Labs test at least 3 cover crops (4 in CICYTEX's living lab in Spain (cherry), and 6 in the French living lab (apple)).

The following table gives the list of species (or mixtures) experimented as cover crops:

Living Labs	Main crops	COVER CROPS
ANNUAL CROPS		
LATVIA LLKC LL11	RYE PEA	CC1. <i>Raphanus sativus</i> + <i>Sinapis alba</i> + <i>Avena sativa</i> CC2. <i>Secale cereale</i> + <i>Raphanus sativus</i> + <i>Phacelia</i> CC3. <i>Secale cereale</i> + <i>Vicia sativa</i> + <i>Fagopyrum esculentum</i> + <i>Phacelia</i>
NETHERLANDS DELPHY LL12	ONION	CC1. Yellow Mustard CC2. Grass CC3. Phacelia
SERBIA MRIZP LL13	SOYBEAN	CC1. Rye CC2. Common vetch CC3. Oat
SERBIA MRIZP LL14	MAIZE	CC1. Rye CC2. Common vetch CC3. Oat
ITALY CNR LL15	TRITICALE	CC1. <i>Medicago truncatula</i> CC2. <i>Trifolium brachycalycinum</i> CC3. <i>Medicago truncatula</i> + <i>Trifolium brachycalycinum</i>
GREECE AUA LL16	WHEAT	CC1. <i>Trifolium alexandrinum</i> CC2. <i>Lolium spp.</i> CC3. <i>Trifolium alexandrinum</i> + <i>Lolium spp.</i>
PORTUGAL LSSV LL17	COWPEA	CC1. Rye CC2. Oat + Lupine + Turnip CC3. Oat + Lupine + Turnip + Rye + Flax + Mustard
SPAIN CICYTEX LL18	RICE	CC1. <i>Avena sativa</i> CC2. <i>Medicago scutellata</i> CC3. <i>Hordeum vulgare</i>

Living Labs	Main crops	COVER CROPS
PERENNIAL CROPS		
FRANCE CTIFL LL21	APPLE	CC1. <i>Lolium perenne</i> + <i>Lolium arundinaceum</i> CC2. <i>Thymus serpyllum</i> (planted as plug plants) + <i>Medicago truncatula</i> CC3. <i>Thymus vulgaris</i> (sown) CC4. <i>Mentha spicata</i> (planted as plug plants) + <i>Medicago truncatula</i> CC5. <i>Lolium perenne</i> + <i>Festuca rubra</i> CC6. <i>Lolium perenne</i> + <i>Festuca rubra</i> + <i>Festuca ovina</i> + <i>Koeleria macrantha</i>
PORTUGAL LSSV LL22	OLIVES	CC1. Rye CC2. Oat + Lupine + Turnip CC3. Oat + Lupine + Turnip + Rye + Flax + Mustard
ITALY AIAB LL23	CITRUS	CC1. <i>Vicia faba</i> L. minor CC2. <i>Trifolium alexandrinum</i> L. CC3. <i>Vicia villosa</i> Roth + <i>Avena sativa</i> L.
ITALY AIAB LL24	GRAPES	CC1. <i>Vicia faba</i> L. minor CC2. <i>Trifolium alexandrinum</i> L. CC3. <i>Vicia villosa</i> Roth + <i>Avena sativa</i> L.
GREECE AUA LL25	GRAPES	CC1. <i>Avena sativa</i> CC2. <i>Vicia sativa</i> CC3. <i>Avena sativa</i> + <i>Vicia sativa</i>
CYPRUS CUT LL26	OLIVES	CC1. <i>Vicia</i> spp. CC2. <i>Pisum sativum</i> CC3. <i>Vicia</i> spp + <i>Pisum sativum</i> + <i>Triticum durum</i>
SPAIN CICYTEX LL27	CHERRY	CC1. <i>Ornithopus sativus</i> CC2. <i>Trifolium subterraneum</i> CC3. <i>Ornithopus sativus</i> + <i>Trifolium subterraneum</i> + <i>Trifolium michelianum</i> + <i>Trifolium resupinatum</i> + <i>Trifolium vesiculosum</i> + <i>Trifolium incarnatum</i> + <i>Lolium multiflorum</i> CC4. <i>Festuca arundinacea</i> + <i>Lolium multiflorum</i> + <i>Lolium perenne</i> + <i>Dactylis glomerata</i>
SPAIN USC LL28	APPLE GRAPES	CC1. <i>Raphanus sativus</i> , <i>Brassica</i> , <i>Brassica juncea</i> , <i>Eruca sativa</i> CC2. <i>Lolium perenne</i> , <i>Lolium multiflorum</i> CC3. <i>Trifolium pratense</i> , <i>Trifolium repens</i>

• AWM strategies

The main AWM strategies experimented by GOOD Living Labs in the conventional sites are: herbicides registered for the main crop (full and half rate), bio-based herbicides (tested on soybean, maize and triticale), intercropping (before the main crop or between the rows), false seed bed (on soybean, maize, and wheat, and before tree-planting on apple), mechanical weeding by tillage or hoeing, mowing, plant mulching (on soybean, maize and olive).

Furthermore, cover crops are experimented as living mulches on triticale and apple (in France), automated control is tested on onion (in Netherlands), precision spraying by drone is experimented on soybean (in Serbia), and hand-weeding is tested on grape and apple in Galicia in Spain, due to non-mechanizable conditions (slopes and terraces).

These weeding methods are mainly tested as single levers (combined with intercropping with cover crops). Strategies based on combinations of methods are experimented on onion (in Netherlands), and on apple (in France).

Depending on the crop, the reference strategies are based on mechanical weeding or herbicide at full rate. No-weeded controls are also considered in the experimental plots of the Living Labs.

The following table summarizes the AWM methods tested in each GOOD Living Lab:

Living Labs	Main crops	HERBICIDE FULL-RATE	HERBICIDE HALF-RATE	BIO-BASED HERBICIDES	INTERCROPPING	FALSE SEEDBED	TILLAGE / HEOING	HAND WEEDING	MOWING	MULCHING	LIVING MULCHES	AUTOMATED CONTROL	DRONE SPRAYING	NO WEEDED
ANNUAL CROPS														
LATVIA LLKC LL11	RYE PEA	X	X		X		X							X
NETHERLANDS DELPHY LL12	ONION	X	X		X		X					X		X
SERBIA MRIZP LL13	SOYBEAN	X	X	X	X	X	X			X			X	X
SERBIA MRIZP LL14	MAIZE	X	X	X	X	X	X			X			X	X
ITALY CNR LL15	TRITICALE	X	X	X	X				X		X			X
GREECE AUA LL16	WHEAT	X	X		X	X	X							X
PORTUGAL LSSV LL17	COWPEA	X	X		X		X		X					X
SPAIN CICYTEX LL18	RICE	X	X		X		X							X
PERENNIAL CROPS														
FRANCE CTIFL LL21	APPLE	X	X			X	X		X		X			X
PORTUGAL LSSV LL22	OLIVES	X	X		X				X					X
ITALY AIAB LL23	CITRUS	X	X		X		X		X					X
ITALY AIAB LL24	GRAPES	X	X		X		X		X					X
GREECE AUA LL25	GRAPES	X	X		X				X					X
CYPRUS CUT LL26	OLIVES	X	X		X		X		X	X				X

Living Labs	Main crops	HERBICIDE FULL-RATE	HERBICIDE HALF-RATE	BIO-BASED HERBICIDES	INTERCROPPING	FALSE SEEDBED	TILLAGE / HEOING	HAND WEEDING	MOWING	MULCHING	LIVING MULCHES	AUTOMATED CONTROL	DRONE SPRAYING	NO WEEDED
SPAIN CICYTEX LL27	CHERRY	X	X		X				X					X
SPAIN USC LL28	APPLE GRAPES	X			X			X						X

3. Experimental designs

The following common base is proposed for experimental designs implementation in all LLs:

A minimum of measurements (cf. section 4.) must be done in a common way in all experimental sites (both annual and perennials).

For each LL's main crop, **three cover crops** and one control plot (without cover crop) will be established **in the first year**. This 1st year will be a pre-selection stage which will allow to select the most suitable cover crop, to be later tested, with or without AMF inoculation (in the following 2nd and 3rd years).

In the second and third years, the best performing cover crop will be inoculated with AMF provided by WP4. The selected cover crop will then be sown twice: inoculated and non-inoculated. A reference with no cover crop ("standard practice") will also be tested.

The chosen cover crops for the first stage of experimentation are known to be adapted to each of the six pedo-climatic conditions of GOOD sites, and will be tested in a broad spectrum of conditions, alone or as mixtures (*Avena sativa*, *Secale cereale*, *Lolium spp.*, *Festuca spp.*, *Sorghum spp.*, *Hedysarum coronarium*, *Lupinus spp.*, *Medicago sativa*, *Medicago truncatula*, *Trifolium alexandrinum*, *T. subterraneum*, *T. repens*, *Vicia spp.*, *Brassica oleracea*, *B. rapa*, *Raphanus sativus*, *Sinapis alba*, *Linum*, *usitatissimum*, *Phacelia tanacetifolia*, *Hordeum vulgare*, *Thymus vulgaris* and *Mentha spicata*).

The use of cover crops will be **combined with other weed control strategies** applied to the main crop. For each pilot site, these treatments will be based on the levers described in section 2., and will include, at least, 1) one cultural, 2) one mechanical, 3) one chemical "standard chemical practice"), 4) one chemical at reduced rate, and 5) one weedy treatment ("no-weeding control").

In all LLs, except the French one, experiments will be conducted on both conventional and organic plots (cf. D3.1); it is recommended to choose the conventional plot close to the organic plot (similar soils).

The experimental designs proposed in this protocol comply with the minimum requirements established in the Grant Agreement. Other designs or additional treatments, more complex or more appropriate for each situation, can be developed by partners as long as the minimum requirements of the GA are met (if needed, cf. section 9.).

3.1. Annual crops

3.1.1. First year

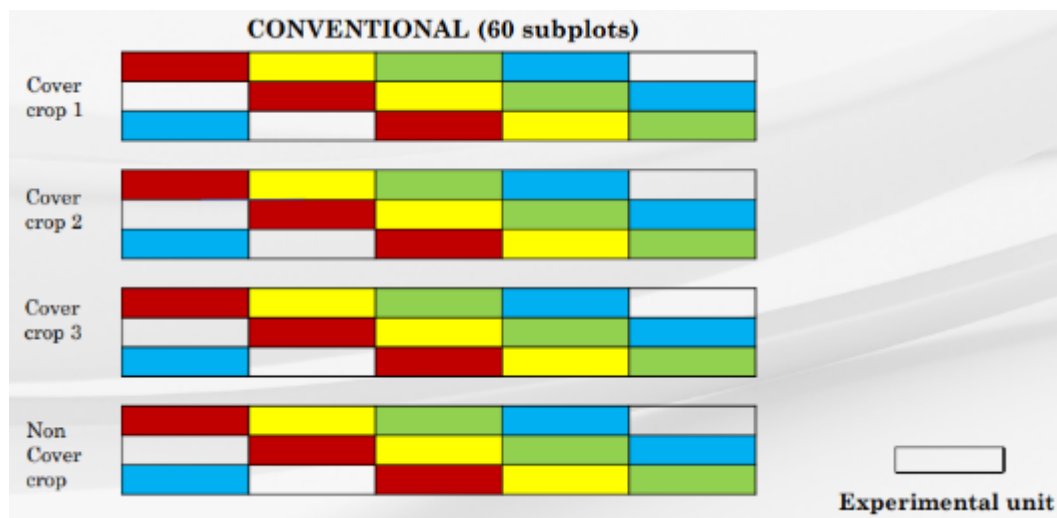
Three cover crops or mixtures have to be sown in the first year; a **reference** is kept without cover crop ("standard practice").

Sowing of the main crop after the termination of the cover crops and applying weed management treatments on the main crop.

The minimum weed management treatments (5) will be: **herbicide (full rate)**, **herbicide (half rate)**, **mechanical weeding**, **cultural practice** (false seedbed, grazing, bio-based herbicides, intercropping, roller crimper), plus an **untreated control** (weedy).

Each experimental unit has to be repeated at least **three times** in a randomized block design; the size of each subplot must be of at least 10-20 m².

DESIGN EXAMPLE (the five colors represent five weed management treatments):



3.1.2. Second and third year

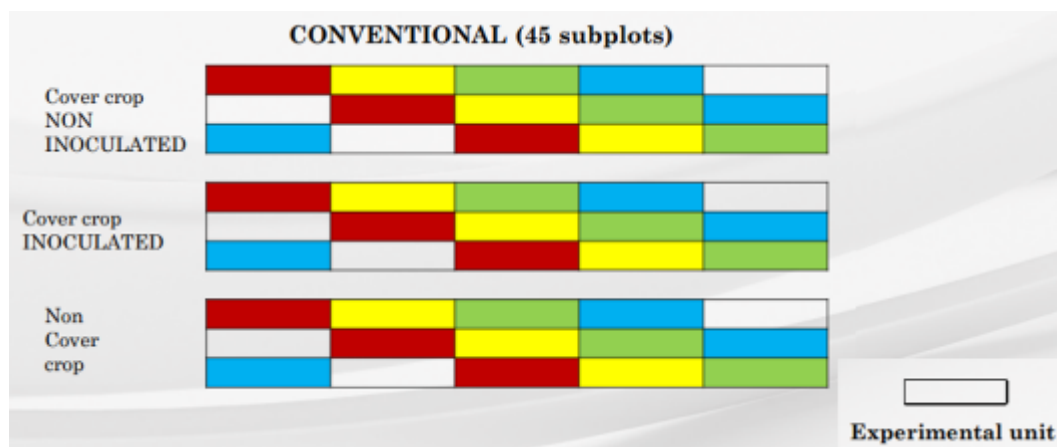
The best cover crop has to be sown twice (**with** or **without AMF inoculation**); a **reference** is kept without cover crop (“standard practice”).

Sowing of the main crop after the termination of the cover crops and applying weed management treatments on the main crop.

The minimum weed management treatments (5) will be: **herbicide–full rate**, **herbicide–half rate**, **mechanical weeding**, **cultural practice** (false seedbed, grazing, bio-based herbicides, intercropping, roller crimper), plus an **untreated control** (weedy).

Each experimental unit has to be repeated at least **three times** in a randomized block design; the size of each subplot must be of at least 10-20 m².

DESIGN EXAMPLE (the five colors represent five weed management treatments):



3.2. Perennial crops

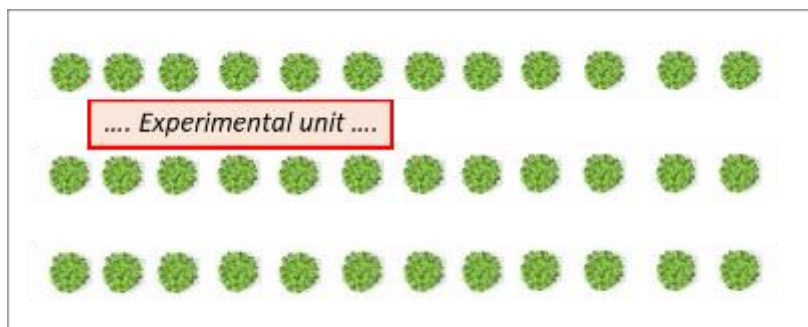
3.2.1. First year

Three cover crops (single species or mixtures: legume, grass, cereals, etc.) has to be sown on the interrows (corridors) of the orchard or vineyard; a **reference** is kept without cover crop (“interrows standard practice”).

Four additional practices have to be experimented on the interrows: **herbicide–full rate**; **herbicide–half rate**; one **nonchemical practice** (e.g., mulching, mowing, etc.), plus an **untreated control** (weedy).

There will be 7 treatments in total for the conventional pilot sites with a minimum of 3 replications per treatment (**21 subplots**).

In this 1st year, no mandatory measurements have to be done on the main perennial crop. *But partners can add other treatments of their interest (e.g., testing additional cover crops, starting with AWM practices on the tree- or vine-rows, etc.).*



3.2.2. Second and third year

A single cover crop is chosen (as a minimum) according to the results obtained in the first year. From year two, this will be used as a companion crop on the interrows of the perennial crop, in a new experimental design, which will combine this cover crop with various AWM practices applied on the tree- or vine-rows.

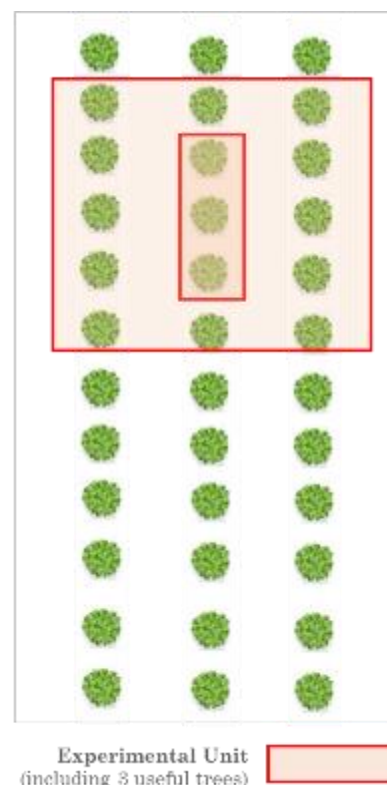
At the beginning of the “2nd year” stage, and at the most suitable time, this cover crop is sown twice (**with or without AMF inoculation**); a **reference** is kept without cover crop on the interrows.

On the rows, **a minimum of five different AWM practices** must be applied, among the following: no-weeding (as a control), herbicide–full rate (as a chemical reference), herbicide–half rate, chemical precision weeding, bio-based herbicides, intercropping, shallow tillage, mowing, organic mulch, synthetic mulch, manual weeding. This experimental scheme will be maintained throughout the 2nd and 3rd years.

- **Unit plot minimal size:** the best compromise between:
 - At least 3 “useful” trees (or vines): i.e., trees (or vines) that will be harvested (for yield quantification); they can be surrounded by 2 border trees (or vines)
 - At least a portion of “weeded strip along the row” of approximately 9 m² (+ the related portion on the interrow).
- **Number of replicates:**
 - Each experimental unit plot must be replicated at least 3 times.
 - This can be multiplied by n blocks (2 for instance), if possible (and even more so if there's a good reason for it: for example, two different soil profiles well identified in the orchard).
 - If needed (to minimize the size of the experimental field, or to simplify the protocol implementation), a split-plot design can also be used.

Therefore, the minimal experimental design will comprise **a total of 45 subplots** (5 AWM practices on the rows x 3 treatments on the interrows x 3 replicates).

A design example is given in [Appendix I](#).



3.3. Some general recommendations

3.3.1. Chemical treatments

- Herbicide applications must comply with the national regulations currently valid in country of each pilot site.
- The "herbicide-half rate" strategies are intended to achieve a 50% reduction in the use of herbicides. This can be obtained by reducing the size of the sprayed area (e.g. by reducing the width of the weeded strip in perennial crops), or by reducing the number of herbicide applications as part of an overall strategy. Note that, for several chemical herbicide active ingredients, half-dose spraying may result in resistances developing.

3.3.2. Size of the experimental design

- Floristic diversity is often quite aggregated (i.e., weed species may differ from zone to zone). So, the larger the observation zones, the lower the risk of making false conclusions.
- Several types of observations will need to be made, and not all of them in exactly the same place (to avoid interfering with the development of the weed flora). Thus, it is important to reserve enough space when determining the size of the unit plots.

On the other hand:

- The more repetitions there are, the more observations need to be made, and this can quickly become very time-consuming: so stay reasonable.
- The protocol must also be quite easy to apply in practice (especially if farmers/tractorists are involved): this needs to be taken into consideration in the experimental design. It's better if it's not the strongest in statistical terms, but if it is feasible (within the limits of the minimum instructions given above). The risk is that the farmer or tractorist will get discouraged, and the protocol won't be properly applied.

3.4. Designs of conventional pilot sites in each LL.

The experimental designs applied to the CONVENTIONAL sites are presented in APPENDIX III.

4. Observations to be made in the Living Labs

Experiments undertaken in the Living Labs should enable to assess and demonstrate the feasibility of various AWM strategies in context-specific conditions, their effectiveness, and their sustainability (economic and environmental).

A set of observations and monitoring will be realized to measure the ability of each strategy to:

- Control the weeds
- Maintain the productivity and growth of the main crop
- Limit competition for soil water and nutrients
- Maintain or improve the biological and physical soil properties
- Maintain or improve the economic and environmental sustainability

The following indicators will be the minimum to be done in common in order to compare LLs results, but others could be proposed and measured by each partner according to its interests and conditions (Indicators measured in each LLs will be collected in the SharePoint).

4.1. Floristic indicators

4.1.1. Plant coverage percentage (*for weeds on perennial crops / for cover crops on annual crops and on the interrows of perennial crops*)

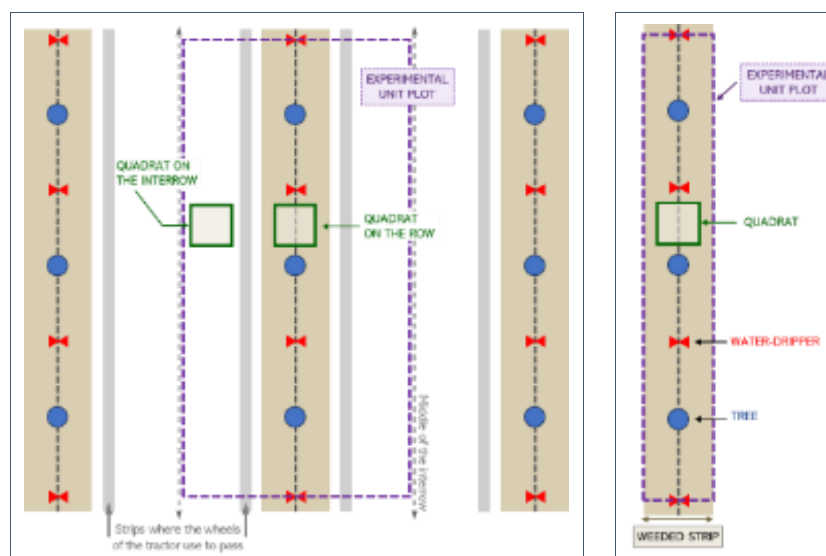
In perennial crops (orchard or vineyard), the plant coverage percentage is the best indicator to either measure weeds extension and cover crops' ability to establish and maintain over time, and to allow the comparison between AWM strategies by simple statistical analysis methods.

To enable follow-up over time, the plant coverage rate must be quantified at the same locations throughout the project.

Measures are done within a frame-quadrat, with a minimal size of 50 cm x 50 cm, to 1 m x 1 m. This quadrat is positioned on the row, between two trees (or vines), but, if possible, avoiding the zone directly under a water dripper. The position of the quadrat is chosen (and repaired) at the first observation date, and it will not be changed further.

For measurements on the interrows, the quadrat is positioned between the middle of the interrow (which is the limit of the experimental unit plot) and the border of the "weeded strip" but avoiding the zone where tractor wheels use to pass (Figure 1). When rows and interrows are concerned, two quadrats must be observed per unit plot ("Experimental design-1", which is the most common case in GOOD project, in accordance with the basic experimental design described in section 3.). Whereas a single quadrat is observed per unit plot in case of "Experimental design-2" (standard practice on the interrows / no additional cover crop tested on the interrows).

Figure 1: Quadrat position in case of *Experimental design-1* (left: rows and interrows are concerned) or in case of *Experimental design-2* (right: only the rows are concerned).



The plant coverage percentage is a visual estimation of the percentage of ground covered by vegetation, including weeds, cover crops (where relevant) and mosses. BRAUN-BLANQUET diagrams can be used to help approximate these measurements (Figure 2).

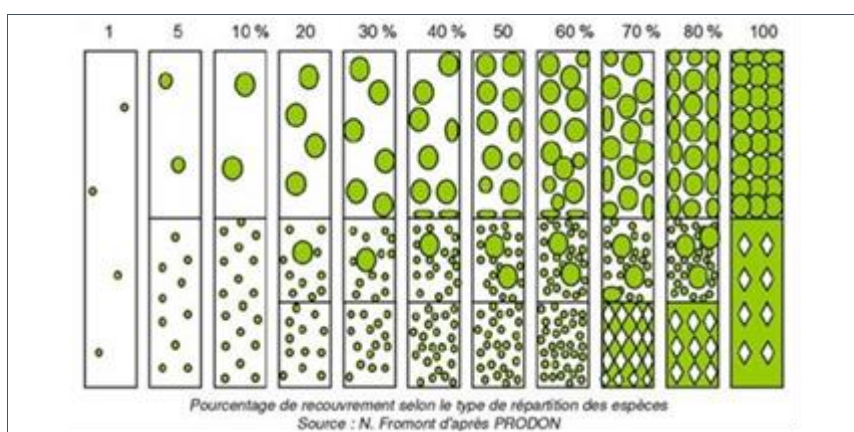


Figure 2: Diagrams to help visual estimation of plant coverage percentage (inspired by the Braun-Blanquet method).

The coverage percentage must be measured for:

- **Weeds:** at the species level as much as possible. When species recognition is too difficult for the observer, identification to gender or even family level is possible (i.e., “Rumex sp.”, “Poaceae”).
- **Cover crops:** at the species level if relevant.
- **Bryophytes** (mosses), as a single group (no distinction at species level is required).

The percentage of **bare soil** (which can include stones or pebbles in the case of gravelly soils) is deducted by subtracting the sum of all these categories from the maximum rate of 100%.

Identifying the angiosperm flora at species level will enable to deduce another important indicator: **the floristic richness**, which is defined as the number of species present per observation unit.

The estimation of plant coverage percentages by vegetation groups can be done in the field, or later, based on pictures of the quadrats (in this case, a prior inventory of the species present in the field is recommended, to avoid misidentification on the photos). **In all cases, it is highly recommended to take pictures of each quadrat, properly referenced, to allow visualization of the evolution of the flora over time.**

These observations are recommended at a **minimal frequency** of at least once during winter rest and three times between April and October. When weed control measures are applied to certain plots,

measurements of plant coverage percentage must be done systematically shortly before weeding, and this on all the plots (even non-weeded ones), to allow comparisons.

4.1.2. Plant density (for weeds on annual crops)

Plant density is defined as the **number of plants per surface unit** (commonly per m²).

This indicator is not well suited to weed management studies on perennial crops, where the plant coverage percentage must be preferred.

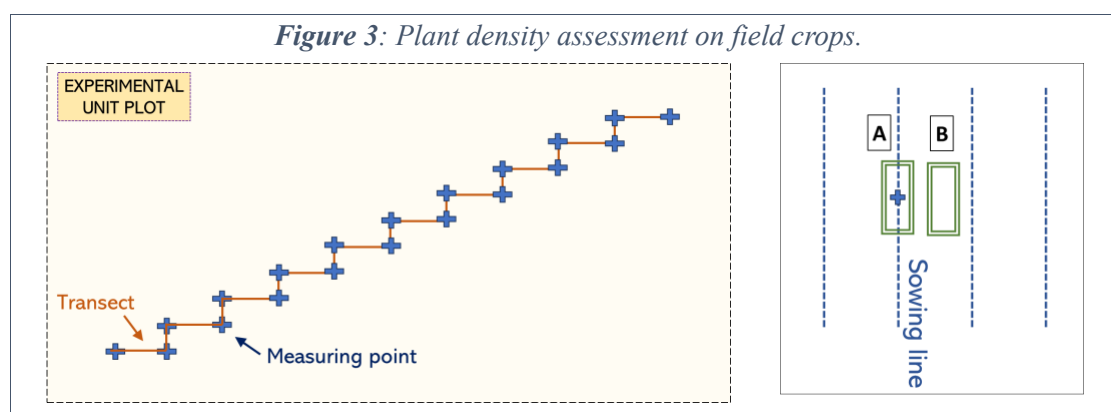
However, on annual crops, which cover large areas and where the weeds often emerge sparsely, plant density is the indicator most commonly used.

At each observation date, measurements are taken along a walked path, priorly defined inside each experimental unit plot (Figure 3). Along this transect, 20 measuring points, evenly distributed, are repaired with stakes (when the crop is in place, these markers are placed on the sowing line to not be crushed by the tractors).

At each point, measurements are taken within a 20 cm x 50 cm frame-quadrat (0,1 m²). Weed plants are either counted (when numbers are low), or their abundance is estimated, using the BARRALIS density scale (Table 3a). These 20 points measurements (corresponding to 2 m²) are added together, after conversion according to Table 3b), to obtain the plant density per m², for the given elementary plot.

These counts must be done **at species level**, each time when possible; identification to genus or species is allowed, when taxa are too complex for the observer to distinguish. By this method, the **floristic richness** can also be deduced.

Note that the plant density indicator can also be used in case of perennial crops, if the pre-planting stage is studied. In this specific case, the orchard has not yet been planted, and the plot is still an open field.



Left: An example of walked path with 20 measuring points per experimental unit plot. *Right:* In case of row-seeded crops, it can be relevant to double the observations for each point (on the sowing line (A), and on the interline (B)).

Table 3a: Plant density classes (modified Barralis scale)

CLASS	PLANT DENSITY
1	$0,1 < D \leq 1 \text{ pl/m}^2$
2	$1 < D \leq 3 \text{ pl/m}^2$
3	$3 < D \leq 10 \text{ pl/m}^2$
4	$10 < D \leq 20 \text{ pl/m}^2$
5	$20 < D \leq 50 \text{ pl/m}^2$
6	$50 < D \leq 100 \text{ pl/m}^2$
7	$100 < D \leq 250 \text{ pl/m}^2$
8	$D > 250 \text{ pl/m}^2$

Table 3b: Plant coverage estimation from Barralis density classes (*)

Class density	Estimated plant coverage
1	$pc < 0,5\%$
2	$0,5 < pc \leq 2\%$
3	$2 < pc \leq 5\%$
4	$5 < pc \leq 20\%$
5	$20 < pc \leq 50\%$
6	$50 < pc \leq 70\%$
7	$70 < pc \leq 90\%$
8	$pc > 90\%$

(*) Depending on the shape of the species, this estimation may be quite imperfect.

4.1.3. Plant biomass *(for cover crops and weeds on annual and perennial crops, and for main crop in annuals)*

Plant biomass is measured by cutting the vegetation at ground level from selected quadrats. Weeds and cover crops (or main annual crop) are then separated into two samples, and the plants are placed into a drying oven at 60°C for 24 hours. The biomass value is expressed in kg of dry matter per m² (or per ha). For each date, three samples must be taken per experimental unit plot (using square frames 50 x 50cm).

Measurement frequency:

- On perennial crops: at least one sampling at the beginning of the spring, and another at the beginning of the summer (and additional samples before mowing or mechanical weeding interventions)
- On annual crops: at least one sample in winter and another just before the cover crop termination, and the main crop harvesting.

4.1.4. Others *(optional)*

Weed height measurement could be an option on perennial crops, when observations are done on the tree-rows. This indicator is not mandatory.

4.2. Impact on the main crops

4.2.1. Crop productivity

Total production has to be harvested on each experimental plot, and per replicate, to allow reliable statistic comparisons.

- Total grain yield per ha for annual crops
- Total fruits or grapes production per ha for perennial crops.

Other relevant parameters can also be measured according to the crops.

4.2.2. Others *(optional)*

- *Main crop growth (trunk cross section area for perennial crops / biomass for annual crops: see above)*
- *Grain quality*
- *Fruit quality and size*
- *Root development*
- *Injuries and mortality (in case of perennial crops)*
- *Phytosanitary aspects*

4.3. Impact on the soil *(optional except for those related to WP4)*

- *Nutrients and water availability*
- *Soil physical properties (water infiltration rate)*
- *Beneficial endo- and meso-fauna*
- **Soil microbiome** *(cf. WP4, if the LL is involved in WP4)*

4.4. Economic and environmental sustainability

- **Cost and profits of the weed control strategies** *(cf. WP6, T6.4)*
- **Carbon impact** *(cf. WP6, T6.2)*
- *Impact on water pollution (optional)*

4.5. Table of indicators measured per LL and methodologies

The main core indicators listed in the guideline are measured on the experimental sites of the Living Labs, sometimes with some adaptations.

Thus, weeds or cover crops biomass are not measured in the French LL, where these indicators are replaced by measurements of the height of the cover crops and of the most dominant weeds (so as not to interfere with the establishment of the living mulches, which is slow and difficult, by a destructive method).

To assess weeds' abundance and diversity, and the cover crops establishment, the measurement of coverage percentage on quadrats is the method most frequently employed by the LLs (but weed diversity is not always qualified at species level, depending on weeds stages or weeds botanical families, when too difficult for the experimenter). In the French LL, visual notations of weed abundance, done on surface units larger than the quadrats (using an internal scale), also complement the measurements of the coverage on the quadrats, to give a broader appraisal of species frequency.

Some other indicators are also assessed in addition in some LLs' sites:

- measurement of vegetation index (Normalized Difference Vegetation Index) in the Serbian and Greek LLs;
- assessment of soil water status, using a camera with multispectral sensors in the Cypriot LL, or tensiometers in the Spanish (cherry) and French LLs (at least);
- measurement of water infiltration capacity of the soil, determination of Nitrogen (Nmin) soil balance, counting of earthworms, assessment of the above-ground beneficial arthropod fauna abundance, in the French LL;
- (and maybe others?)

The following table gives a list (maybe not exhaustive) of the indicators collected in GOOD Living Labs:

Living Labs	Main crops	Weeds density	Weeds coverage percentage	Weeds biomass	Weeds height	Floristic richness	Cover crops biomass	Cover crops height	Main crop biomass	Tress / vines growth	Main crop productivity	Complementary indicators
LATVIA LLKC LL11	RYE PEA		X	X		X	X		X		X	
NETHERLANDS DELPHY LL12	ONION		X	X		X	X		X		X	
SERBIA MRIZP LL13	SOYBEAN		X	X		X	X		X		X	X
SERBIA MRIZP LL14	MAIZE		X	X		X	X		X		X	X
ITALY CNR LL15	TRITICALE		X	X		X	X		X		X	
GREECE AUA LL16	WHEAT		X	X		X	X		X		X	
PORTUGAL LSSV LL17	COWPEA						X		X		X	
SPAIN CICYTEX LL18	RICE	X		X		X	X		X		X	
FRANCE CTIFL LL21	APPLE		X		X	X		X		X	X	X
PORTUGAL LSSV LL22	OLIVES		X	X		X	X			X	X	

<i>Living Labs</i>	<i>Main crops</i>	Weeds density	Weeds coverage percentage	Weeds biomass	Weeds height	Floristic richness	Cover crops biomass	Cover crops height	Main crop biomass	Tress / vines growth	Main crop productivity	<i>Complementary indicators</i>
ITALY AIAB LL23	CITRUS		X	X			X			X	X	
ITALY AIAB LL24	GRAPES		X	X			X			X	X	
GREECE AUA LL25	GRAPES		X	X			X			X	X	X
CYPRUS CUT LL26	OLIVES		X	X			X			X	X	X
SPAIN CICYTEX LL27	CHERRY		X	X		X	X			X	X	X
SPAIN USC LL28	APPLE GRAPES		X			X	X			X	X	

5. Field days and demo events.

Each LL will organize at least 2 field demonstration events throughout the project lifetime to show the results of AWM in practice. Around 60 participants are expected in total in the demo events of each LL.

These events will be adapted to the agriculture sector of each LL and they will allow to show the results of the field research after the first and second year of experimentation. It could be open field days, practical demonstrations or any other format.

They will be addressed to LLs and non-LLs stakeholders in order to expand the use of AWM practices. These events will contribute to knowledge input in the co-creation activities of the LLs.

The following table gives the calendar of demo events (DE) or field days (FD) already held, or planned, by the GOOD Living Labs in 2024 and 2025:

<i>Living Labs</i>	Jun. 2024	Jul. 2024	Aug. 2024	Sep. 2024	Oct. 2024	Nov. 2024	Dec. 2024	Jan. 2025	Feb. 2025	March 2025	Apr. 2025	May 2025	Jun. 2025	Jul. 2025	Aug. 2025
LATVIA (LLKC) LL11 / RYE & PEA		DE													
NETHERLANDS (DELPHY) LL12 / ONION	DE		DE												
SERBIA (MRIZP) LL13 & LL14 / SOYBEAN & MAIZE															
ITALY (CNR) LL15 / TRITICALE							FD				FD				
GREECE (AUA) LL16 / WHEAT												DE			
PORTUGAL (LSSV) LL17 / COWPEA				DE											

Living Labs	Jun. 2024	Jul. 2024	Aug. 2024	Sep. 2024	Oct. 2024	Nov. 2024	Dec. 2024	Jan. 2025	Feb. 2025	March 2025	Apr. 2025	May 2025	Jun. 2025	Jul. 2025	Aug. 2025
SPAIN (CICYTEX) LL18 / RICE															FD
FRANCE (CTIFL) LL21 / APPLE						DE								FD	
PORTUGAL (LSSV) LL22 / OLIVE						OL									
ITALY (AIAB) LL23 & LL24 CITRUS & GRAPE					DE					DE					
GREECE (AUA) LL25 / GRAPE											DE				
CYPRUS (CUT) LL26 / OLIVE						DE					FD				
SPAIN (CICYTEX) LL27 CHERRY											FD				
SPAIN (USC) LL28 / GRAPE & APPLE															

6. Reporting of R&I results

The LL manager will be responsible for collecting all required info and data, and for monitoring its LL. The obtained research results will be included in Deliverable 2.3 “Implementation and assessments in conventional farming systems” and updated three times throughout the project (M18, M30, M42).

This information will also be used to feed the activities developed in WP1, WP4, WP5, WP6 and WP7.

A wide dissemination will be made to stakeholders through the different tools, platforms and protocols established in WP7.

Concerning all the activities carried out in the LLs related to conventional sites (meetings, workshops, demo events, dissemination activities...) a reporting protocol and calendar is described in D1.2 (“Establishment of GOOD LLs and LL boards”). The LL manager should use the template in Annex 6 of this document (D1.2).

All along GOOD project, the LL manager will collect, monitor, retain, analyze, and report the data, results, outcomes, impacts of the Research and Innovation activities conducted in the conventional sites of the LL.

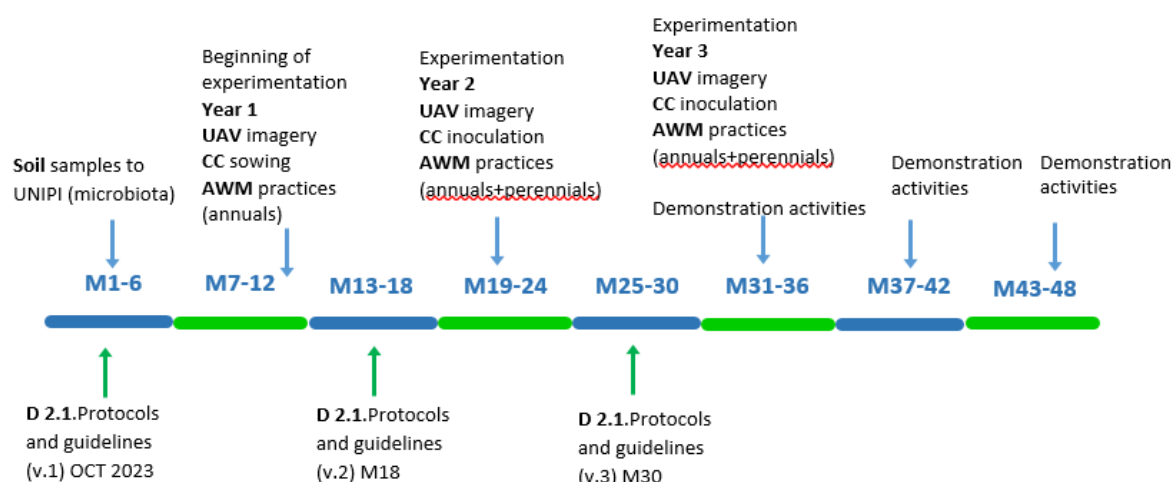
A summary of these R&I results will be reported to the leader of Task T2.2 (AUA, [Ilias Travlos](#)) at the end of every growing season following the template that will be provided to partners.

Reporting dates: October 2024; October 2025; October 2026.

7. Calendar of LLs activities throughout the project

7.1. Key timelines

The general timeline for WP2 and Task 2.1 is the following:



Task 2.1 has one deliverable (D2.1) with three (3) updates. Specifically:

- Living Lab methodology for conventional farming (version 1) [due to M6 – October 2023]
- Living Lab methodology for conventional farming (version 2) [due to M18 – October 2024]
- Living Lab methodology for conventional farming (version 3) [due to M30 – October 2025]

7.2. Calendar of LLs activities

Calendars of LLs' activities, for the years 2023 and 2024 are presented in APPENDIX IV.

8. Possible Risks and mitigation measures

There are some critical risks which have been identified a priori, with regard to the LLs establishment and functioning. A risk mitigation plan has already been designed for them and is presented below:

Table 4: Critical risks and risk mitigation measures associated with WP2 and Task 2.1

Risk No. (from GA)	Description	Proposed Mitigation Measures
1	Delays in establishing the LL boards, the LLs and the AWMN	A strict time schedule will be organized at the beginning of the project to ensure early adaptations in case any delays are foreseen. Each LL will appoint a responsible person.
2	Climatological risks due to climate change to crops and cover crops establishment	The Knowledge Base interviews & questionnaires, and the literature review will help the LL boards to choose the most optimal time windows for all operations. In case of failure of establishment, alternatives will be also proposed by LL boards.
8	Low interest and feedback from stakeholders to participate in LLs	Scheduling of demonstration and dissemination activities based of farmers' calendar. Early contact with more stakeholders than needed will be conducted to ensure engagement.
9	Proposed solutions do not meet farmer needs and ambitions	The information-in stage (WP1, WP6, WP7), the development of knowledge base and the demonstration activities and workshops will allow early communication with stakeholders, identify their needs and ambitions, and design tailor-made solutions.
14	Failures to follow the experimental design in the LLs	In case of failure in following the experimental design, then the PSG and the relevant LL boards will decide on the experimental design that is scientifically sound and allows the implementation of demonstration activities.

If a LL identifies a foreseeable risk, then it should contact the WP leader, the PSG and the Project Coordinator at least two months before the start date of the risk, to co-design mitigation measures. If needed, a communication with the GOOD Project Officer could be carried out in cases of deviations from the GA.

A template letter, to address to GOOD Project Officer, is given in Appendix II.

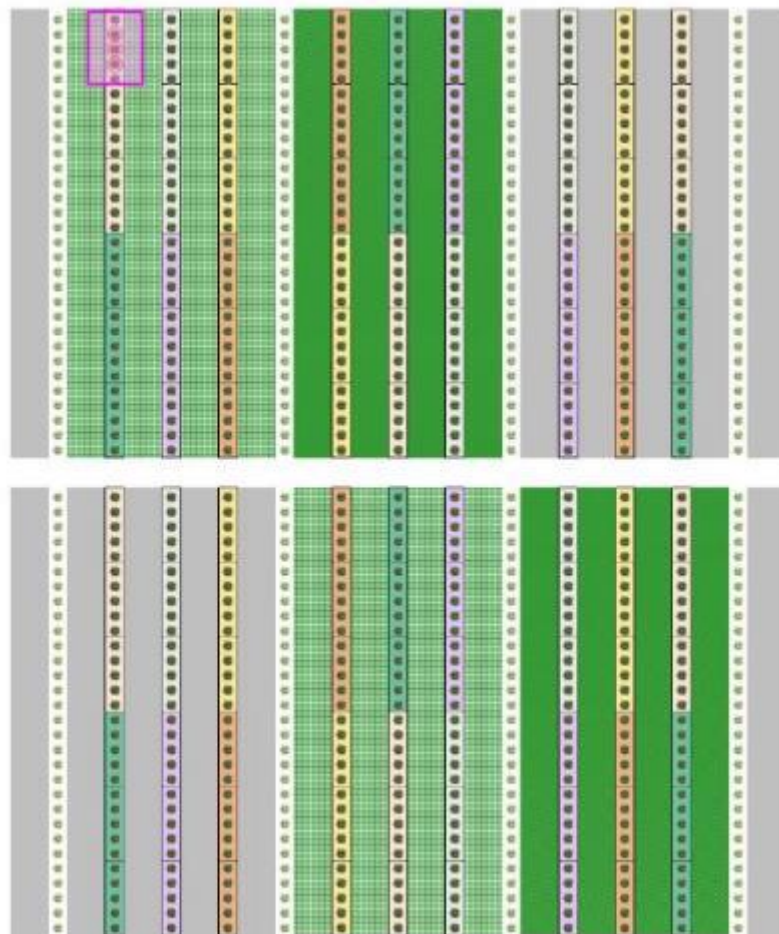
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
— APPENDIX I —

Design example for perennial crops experiments in 2nd and 3rd year of GOOD Project (with 6 AWM treatments tested on the tree-rows)







A single cover crop (selected in year 1) is tested on the interrows (with or without AMF) in comparison to an interrow-reference, and in combination with 6 weed management treatments on the rows. Beforehand, the AMFs were propagated from the soil samples collected in situ, and then inoculated to the seeds. Experimentation is repeated in two blocks



Cover-crops on the interrows :

-  Cover-crop alone (sown)
-  Cover crop from seeds inoculated with AMF
-  REFERENCE (standard practice on the interrows)



Weed management treatments on the rows :

-  Treatment 1
-  Treatment 2
-  Treatment 3
-  Treatment 4
-  Treatment 5
-  STANDARD PRACTICE ON THE ROWS

(One of these treatments can be a no-weeding reference)

One cover-crop, with or without AMF
6 weed management treatments on the rows, including a reference
--> **18 weed management strategies in comparison**
3 replicates per WM strategy ; 5 trees (vines) per repetition
2 blocks

--> **108 experimental unit plots**
(could be reduced to 54 unit plots if 1 block alone)

 **Experimental unit plot for combined weed management strategies (rows & interrows)**
Each of these unit plots must include at least 3 "harvestable" trees or vines: 



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— APPENDIX II —

Template letter to be used to inform and argue about deviations to Grant Agreement

To be sent to GOOD Project Officer by the Project coordinator after its co-preparation with the respective Living Lab

For individual participants in the Living Lab boards in the Agroecology for weeds – GOOD project (GA: 101083589), funded by the European Union's Horizon Europe research and innovation programme.

Living Lab

Annual crops			Permanent crops		
Country	Code number		Country	Code number	
Latvia	LV_rye-pea/11	<input type="checkbox"/>	France	FR_apple-plum/21	<input type="checkbox"/>
Netherlands	NL_onion/12	<input type="checkbox"/>	Portugal	PT_olives/22	<input type="checkbox"/>
Serbia	RS_soybean/13	<input type="checkbox"/>	Italy	IT_citrus/23	<input type="checkbox"/>
Serbia	RS_maize/14	<input type="checkbox"/>	Italy	IT_grapes/24	<input type="checkbox"/>
Italy	IT_triticale/15	<input type="checkbox"/>	Greece	GR_grapes/25	<input type="checkbox"/>
Greece	GR_wheat/16	<input type="checkbox"/>	Cyprus	CY_olives/26	<input type="checkbox"/>
Portugal	PT_cowpea/17	<input type="checkbox"/>	Spain	ES_cherry/27	<input type="checkbox"/>
Spain	ES_rice/18	<input type="checkbox"/>	Spain	ES_apple-grapes/28	<input type="checkbox"/>

Dear [PROJECT OFFICER]

[NAME OF THE PARTNER] is participating in the Agroecology for weeds- GOOD project through the establishment of a Living Lab (LL) where various research and innovation activities will be carried out with different stakeholders.

The LL activities include the implementation of pilot sites where some Agroecological Weed Management strategies will be evaluated, such as the use of cover crops in combination with other cultural, digital and mechanical practices for weed control.

Common protocols have been proposed for the establishment of the LLs, the experimental designs and the indicators to be measured in each LL according to the Grant Agreement (GA). However, some obstacles have been detected that could lead to a deviation of the GA from the LL performance.

Therefore, a modification with respect to what is established in the GA is requested for the detected issue and only for this LL based on the following arguments,

[DETECTED DEVIATION]_____

[ARGUMENTS JUSTIFYING THE DEVIATION AND ALTERNATIVE PROPOSALS]

Project coordinator

Signature

Date

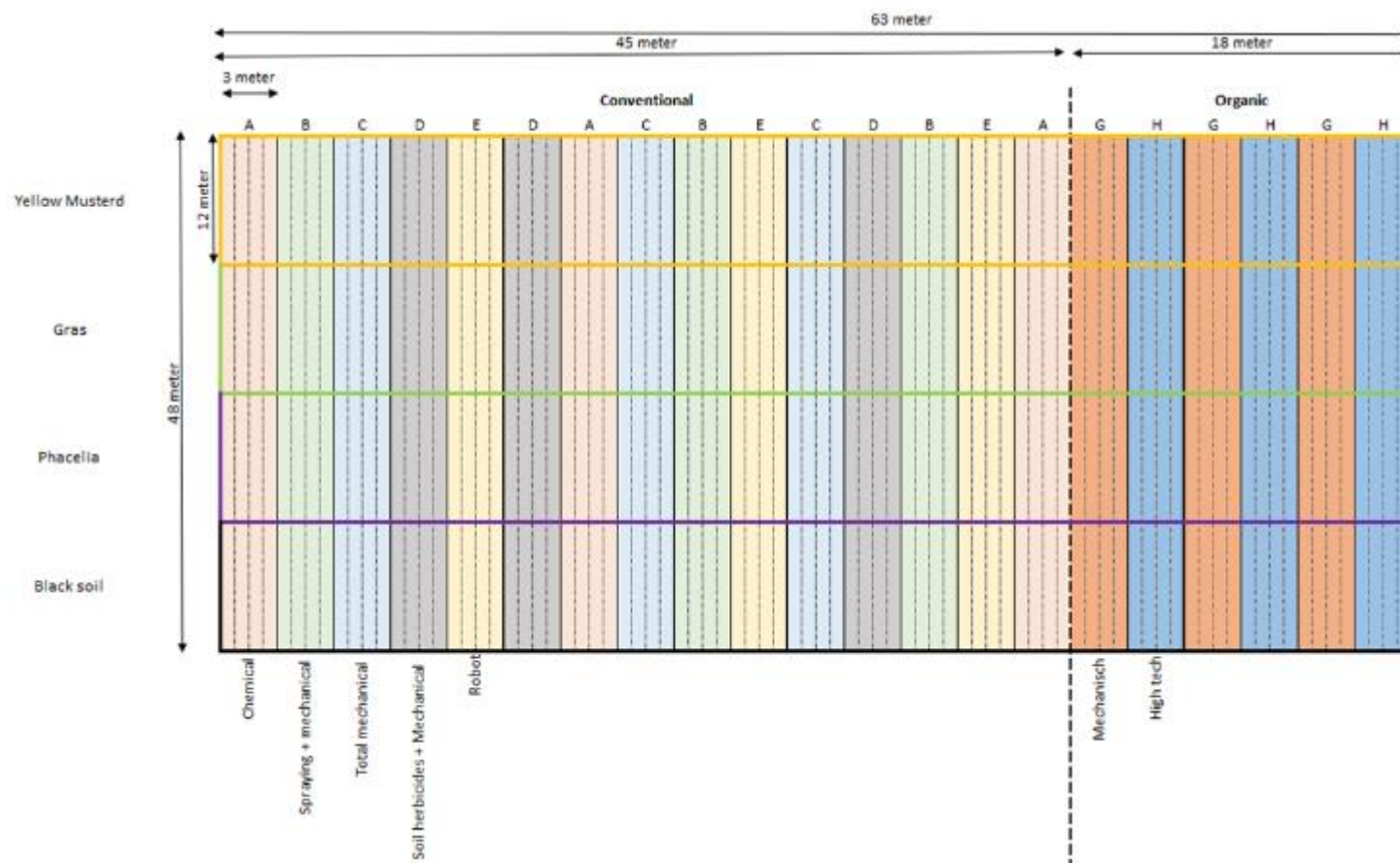
Name of LL manager

Signature

Date

Experimental designs applied on the CONVENTIONAL sites of GOOD Living Labs

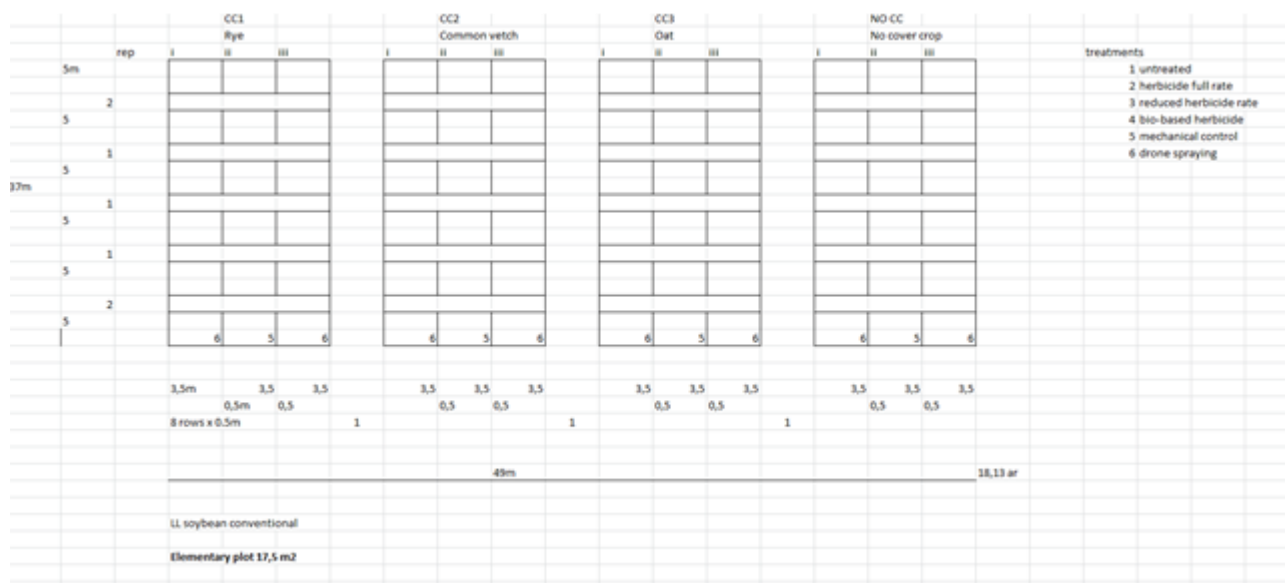
[illegible]



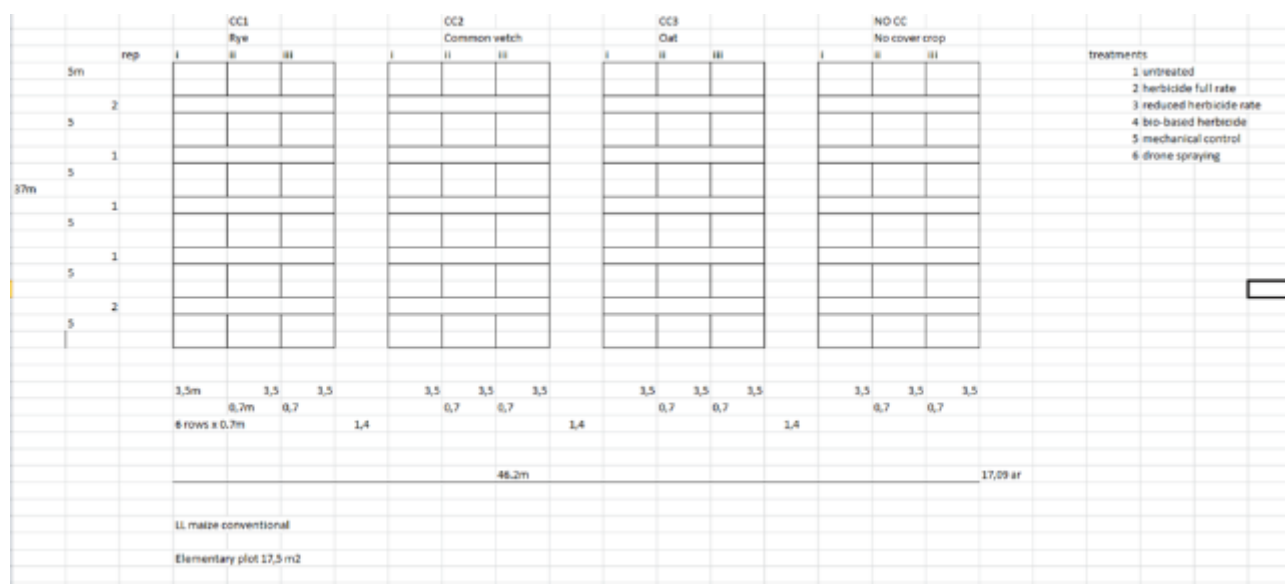
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RS_soybean / 13



RS_maize / 14



IT_triticale / 15

7m	Cover crop1	Cover crop2	Cover crop3	Untreated Control	Chemical reference	Mech. Method	Cultural Method	Digital Method
1m								
7m	Chemical reference	Mech. Method	Cultural Method	Cover crop2	Untreated Control	Cover crop3	Digital Method	Cover crop1
1								
7m	Cultural Method	Cover crop1	Chemical reference	Cover crop3	Digital Method	Untreated Control	Mech. Method	Cover crop2
3m								

GR_wheat / 16

Treatments: T1: control, T2: herbicide recommended rate, T3: herbicide reduced rate, T4: False seedbed, T5: mechanical control

T1	T2	T3	T4	T5	T1	T2	T3	T4	T5	T1	T2	T3	T4	T5	cc 1
T2	T3	T4	T5	T1	T2	T3	T4	T5	T1	T2	T3	T4	T5	T1	cc 2
T3	T4	T5	T1	T2	T3	T4	T5	T1	T2	T3	T4	T5	T1	T2	cc 3
T4	T5	T1	T2	T3	T4	T5	T1	T2	T3	T4	T5	T1	T2	T3	cc 4

Cover crop 1 (cc 1): *Trifolium alexandrinum*

Cover crop 2 (cc 2): *Lolium* spp.

Cover crop 3 (cc 3): *Trifolium alexandrinum* + *Lolium* spp.

Cover crop 4 (cc 4): control (without cover crop)

ES_rice / 18

CONVENTIONAL* (64 PLOTS, 8*2 m)

13	14	15	16
9	10	11	12
5	6	7	8
1	2	3	4

Herbicide (full rate)

29	30	31	32
25	26	27	28
21	22	23	24
17	18	19	20

Herbicide (reduced rate)

45	46	47	48
41	42	43	44
37	38	39	40
33	34	35	36

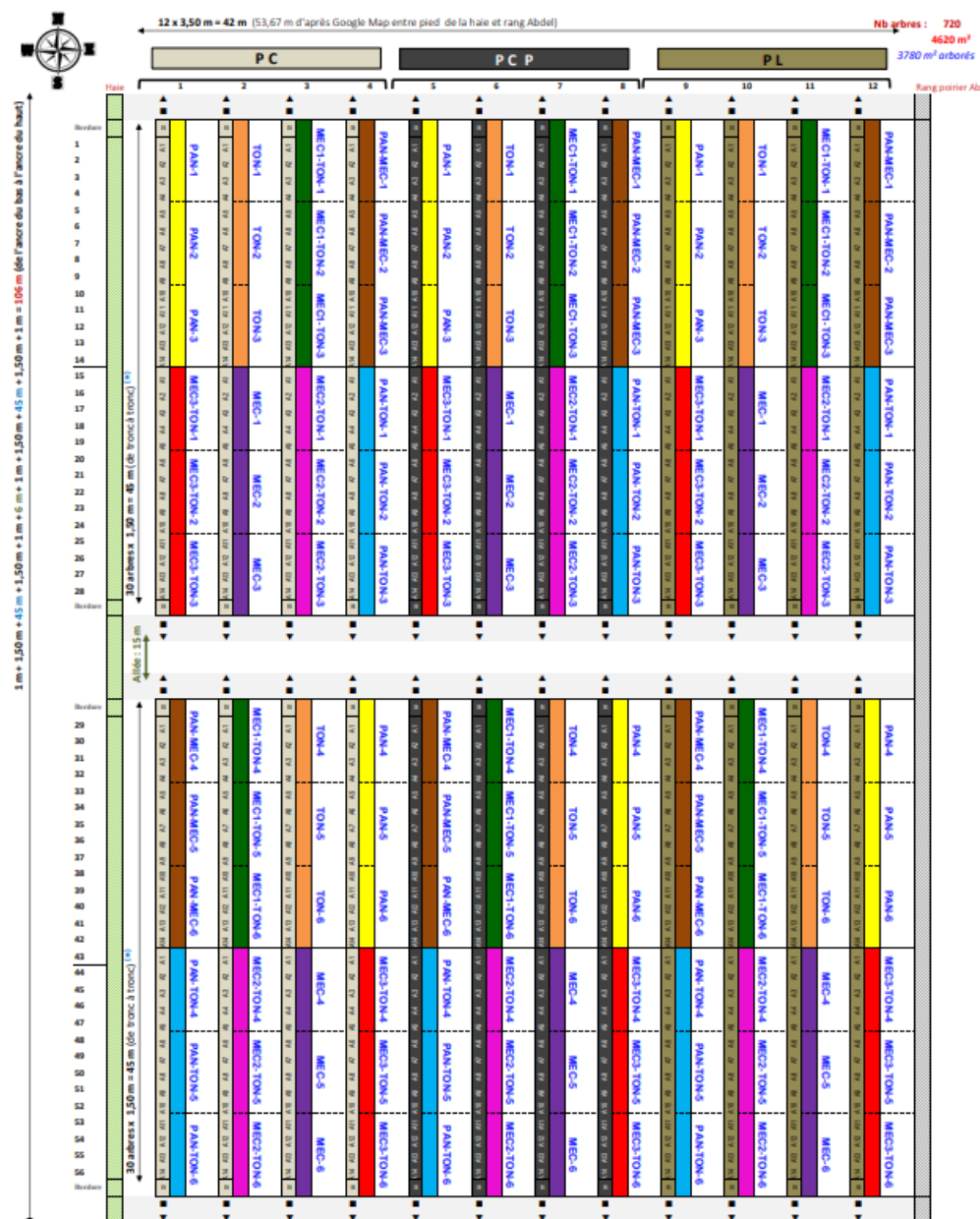
Mechanical control

61	62	63	64
57	58	59	60
53	54	55	56
49	50	51	52

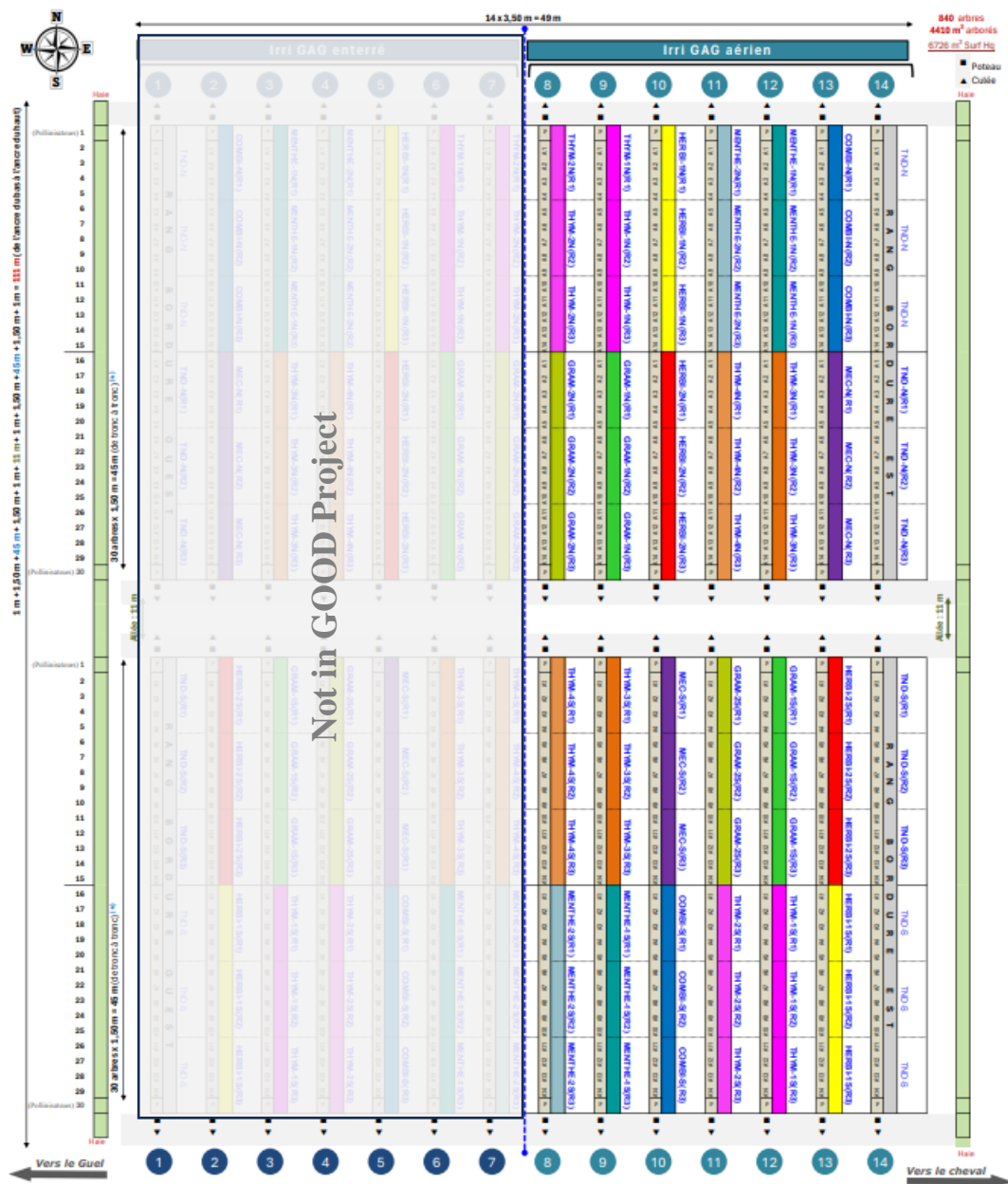
Control

FR_apple-plum/ 21

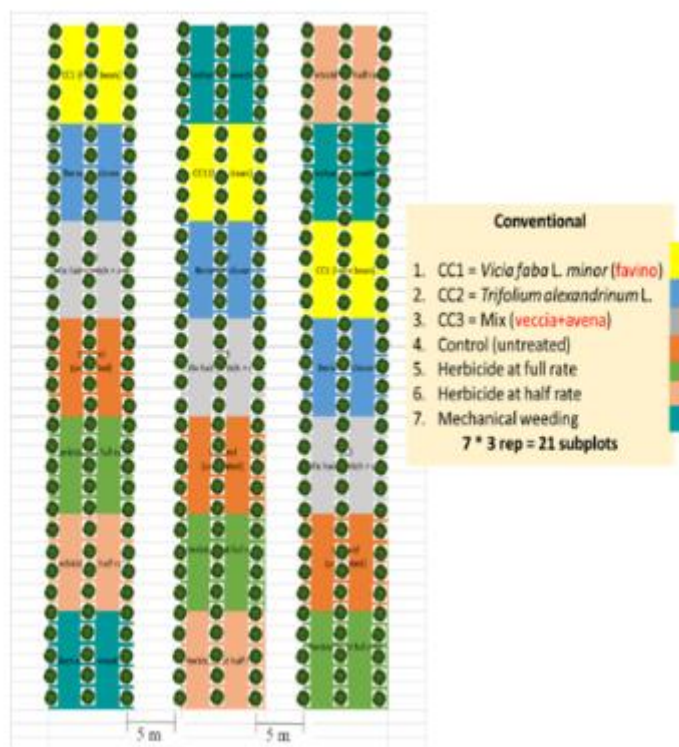
Trial N°1



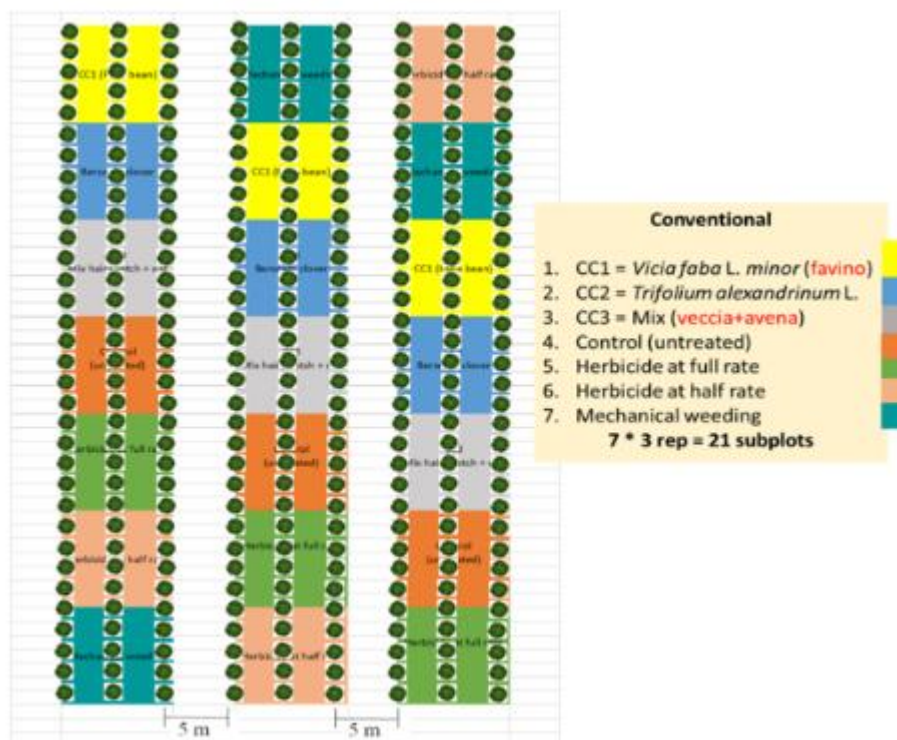
Trial N°2



IT_citrus / 23



IT_grapes / 24



GR_grapes / 25

Treatments: T1: control, T2: herbicide recommended rate, T3: herbicide reduced rate, T4: False seedbed, T5: mechanical control

T1	T2	T3	T4	T5	T1	T2	T3	T4	T5	T1	T2	T3	T4	T5	cc 1
T2	T3	T4	T5	T1	T2	T3	T4	T5	T1	T2	T3	T4	T5	T1	cc 2
T3	T4	T5	T1	T2	T3	T4	T5	T1	T2	T3	T4	T5	T1	T2	cc 3
T4	T5	T1	T2	T3	T4	T5	T1	T2	T3	T4	T5	T1	T2	T3	cc 4

Cover crop 1 (cc 1): *Avena sativa*

Cover crop 2 (cc 2): *Vicia sativa*

Cover crop 3 (cc 3): *Avena sativa* + *Vicia sativa*

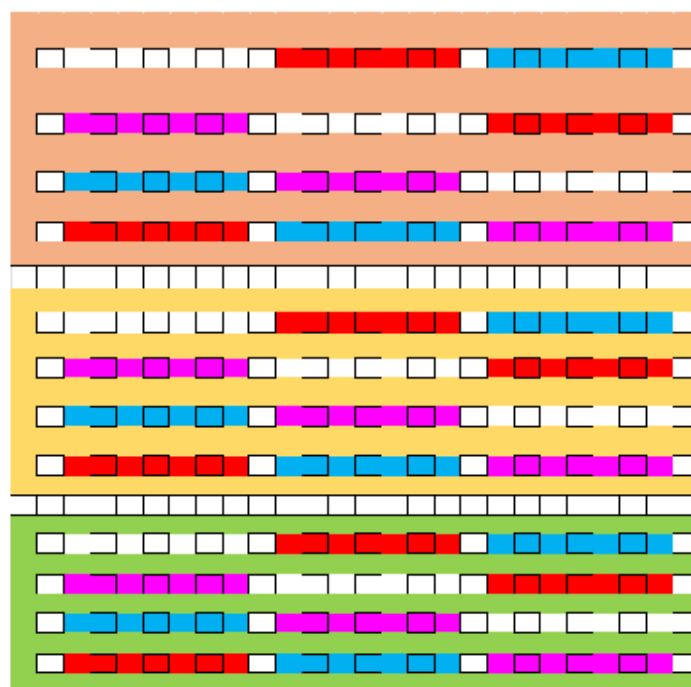
Cover crop 4 (cc 4): control (without cover crop)

CY_olives / 26



ES_cherry / 27

Split-plot design combining cover crops (interrows) with weed control treatments (tree-rows)



NON INOCULATED CC (*Trifolium subterraneum*)

INOCULATED CC (*Trifolium subterraneum*)

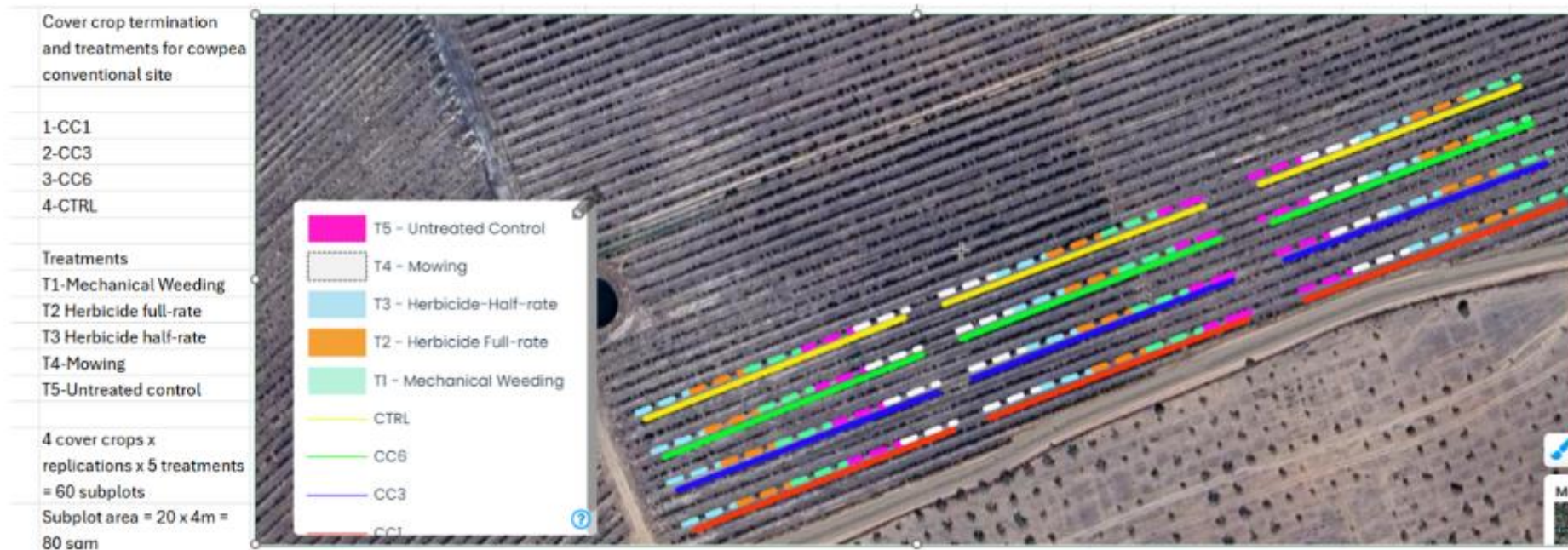
NON COVER CROP (Reference: mowing)

Herbicide Full rate

Herbicide 50% rate

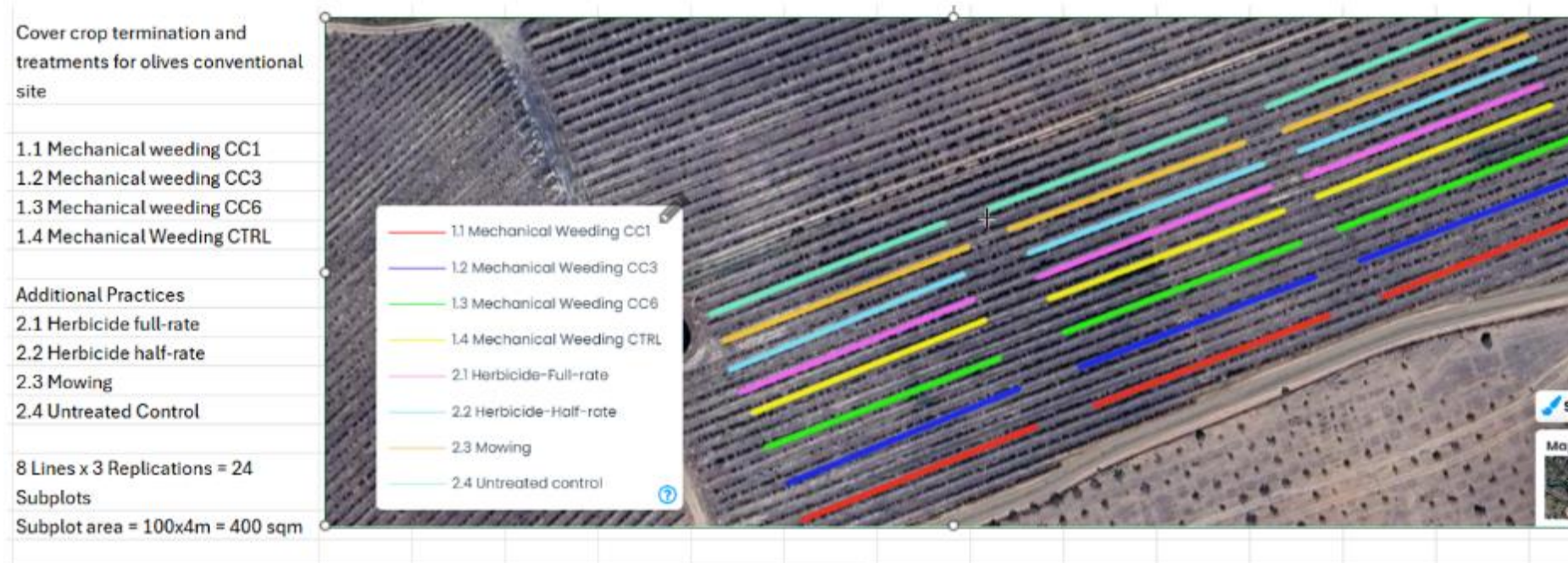
Mowing

Weedy



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— APPENDIX IV —

Calendars of Living Labs' activities, for the years 2023 and 2024

		2023				2024											
		M5	M6	M7	M8	M9	M10	M11	M12	M13	M14	M15	M16	M17	M18	M19	M20
		September	October	November	December	January	February	March	April	May	June	July	August	September	October	November	December
For all Living Labs	Co-creation annual meetings																
	Co-creation workshops																
	National workshop																
Latvia (LV_pea/11) conventional site	Cover crop establishment																
	Cove crop termination																
	Main crop sowing																
	Main crop harvest																
	Drone flights																
	Soil sampling																
	Life Cycle Assessments																
	Demo events (field days)																
Latvia (LV_rye/11) organic site	Cover crop establishment																
	Cove crop termination																
	Main crop sowing																
	Main crop harvest																
	Drone flights																
	Soil sampling																
	Life Cycle Assessments																
	Demo events (field days)																
Netherlands (NL_onion/12)	Cover crop establishment																
	Cove crop termination																
	Main crop sowing																
	Main crop harvest																
	Drone flights																
	Soil sampling																
	Life Cycle Assessments																
	Demo events (field days)																
Serbia (RS_soybean/13)	Cover crop establishment																
	Cove crop termination																
	Main crop sowing																
	Main crop harvest																
	Drone flights																
	Soil sampling																
	Life Cycle Assessments																
	Demo events (field days)																



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[illegible]

	Cover crop establishment	Cove crop termination	Main crop harvest	Drone flights	Soil sampling	Life Cycle Assessments	Demo events (field days)
France (FR_apple-plum/21)	Cover crop establishment						
	Cover crop termination						
	Main crop harvest						
	Drone flights						
	Soil sampling						
	Life Cycle Assessments						
	Demo events (field days)						
Portugal (PT_olives/22)	Cover crop establishment						
	Cover crop termination						
	Main crop harvest						
	Drone flights						
	Soil sampling						
	Life Cycle Assessments						
Italy (IT_citrus/23)	Cover crop establishment						
	Cove crop termination						
	Main crop harvest						
	Drone flights						
	Soil sampling						
	Life Cycle Assessments						
	Demo events (field days)						
Italy (IT_grapes/24)	Cover crop establishment						
	Cove crop termination						
	Main crop harvest						
	Drone flights						
	Soil sampling						
	Life Cycle Assessments						
Greece (GR_grapes/25)	Cover crop establishment						
	Cove crop termination						
	Main crop harvest						
	Drone flights						
	Soil sampling						
	Life Cycle Assessments						

[illegible]