Yearly report on activities in demonstration sites Year 3

C.2 – Demonstration in trial areas

LIFE RESILIENCE

LIFE17 CCA/ES/000030

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Deliverable Name: Yearly report on activities in demonstration sites Year 3

Action C.2: Demonstration in trial areas

Compiled by: BALAM

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Content table

1. Summary

The following report describes all the activities that have been carried out throughout year 2021 on the demo farms. Due to the differences between the farms, each one has been described separately. The objective of this report is to collect all the information on the activities that have been carried out by the project partners to achieve the proposed objectives.

2. Introduction

In all the farms described bellow 5 treatments were established in order to achieve best agricultural strategies against XF.

All farms have a control treatment in which standard procedures needed were done as irrigation, prune, harvest, etc. Four treatments were also established in which the same standard procedures were done and additionally a specific treatment was implemented. In each farm every treatment will be described.

3. Chapters + Results

This report includes the following chapters:

- Chapter I: El Valenciano Farm (Carmona, Sevilla - Spain)
- Chapter II: Herdade do Charqueirão Farm (Alandroal, Évora - Portugal); and
- Chapter III: La Traversagna Farm (Pisa, Toscana - Italy).

Chapter I. El Valenciano Farm

(Carmona, Sevilla - Spain)

Chapter developed in collaboration with the External Agronomist: Ideagro

1. Treatments

On the table 1 description of the standard procedures in each crop and the additional application depending on the treatment.

Table 1 El Valenciano Farm Standard procedures and treatments dates

Action C2	T0 - control	T1	T2 - Deficit irrigation	T3- Cover crops	T4 (combination T1-T2-T3)
	Traditional management	**T1 - IBNP +ISR**			
El Valenciano - SP	**1.Soil:** tillage (3 times/year) and herbicide (3 times/year). **2.Irrigation**: on demand (March-November; 2-3 days/week) **3.Pests:** Prays oleae, Bactrocera oleae, Palpita unionalis. Total treatments 5/year. **4.Diseases:** Spilocea oleagina, total treatments 3/year. **5.Nutrition:** Soil (19/year) and Foliar (8/year). **6.Pruning:** Formation pruning (1/year) and Top and bottom pruning (2 times/year) **7.Harvest:** Nov/Dic (1 year)	**1.Soil**: tillage (3 times/year), herbicide (3 times/year) and **IBNP (1 time/year; dose: 1 kg/ha) 29/06/2021 + ISR (2/year; dose 0.5 L/ha): 13 and 27/07/2021** **2.Irrigation:** on demand (March-November; 2-3 days/week) **3.Pests:** Prays oleae, Bactrocera oleae, Palpita unionalis. Total treatments 5 times/year. **4.Diseases**: Spilocea oleagina, total treatments 3 times/year. **5.Nutrition:** Soil (19 times/year) and Foliar (8 times/year). **6.Pruning**: Formation pruning (1 time/year) and Top and bottom pruning (2 times/year) **7.Harvest:** Nov/Dic (1 year)	**1.Soil:** Tillage (3 times/year) and herbicide (3 times/year). **2.Irrigation**: **On demand (March-June)-(September-November) and deficit irrigation (July-August).** **3.Pests:** Prays oleae, Bactrocera oleae, Palpita unionalis. Total treatments 5 times /year. **4.Diseases:** Spilocea oleagina, total treatments 3/year. **5.Nutrition:** Soil (19 times/year) and Foliar (8 times/year). **6.Pruning:** Formation pruning (1/year) and Top and bottom pruning (2 times/year) **7.Harvest:** Nov/Dic (1 year)	**1.Soil:** tillage (1/year), herbicide (2/year), **cover crop: sow (11/2021), and weed (1/year).** **2.Irrigation:** on demand (March-November) **3.Pests**: Prays oleae, Bactrocera oleae, Palpita unionalis. Total treatments 5/year. **4.Diseases:** Spilocea oleagina, total treatments 3/year. **5.Nutrition**: Soil (19/year) and Foliar (8/year). **6.Pruning:** Formation pruning (1/year) and Top and bottom pruning (2/year) **7.Harvest**: Nov/Dic (1 year)	**1.Soil:** tillage (1/year), herbicide (2/year), **cover crop: sow (1/year), weed (1/year), IBNP (1/year) 29/06/2021 and ISR (4/year) 13/07/2021; 27/08/2021.** **2.Irrigation**: **On demand (March-June)-(September-November) and deficit irrigation (July-August).** **3.Pests:** Prays oleae, Bactrocera oleae, Palpita unionalis. Total treatments 5/year. **4.Diseases**: Spilocea oleagina, total treatments 3/year. **5.Nutrition:** Soil (19/year) and Foliar (8/year). **6.Pruning:** Formation pruning (1/year) and Top and bottom pruning (2/year) **7.Harvest**: Nov/Dic (1 year)

Five treatments were established, Table 2 describes each treatment, product, dose, timing and mode of application. The schedule and doses described in table 2 will be done in the entire project. Moreover, in Image 1 is located treatment's distribution in the farm El Valenciano. In Table 3 treatments and corresponding products are described, dates of applications in 2021 is also included.

Table 2 Treatments, products, dose, timing and mode of applications

Treatment	Product	Dose	Date	Mode
T0	Conventional management			
T1	MST -NP	1 kg/ha	June	Irrigation
	ProCrop ISR	0.5 l ha/week	15 days after MST-NP	Irrigation
T2	Deficit irrigation			
T3	Cover crops	30 kg/ha	September	
T4	T1 + T2 +T3			

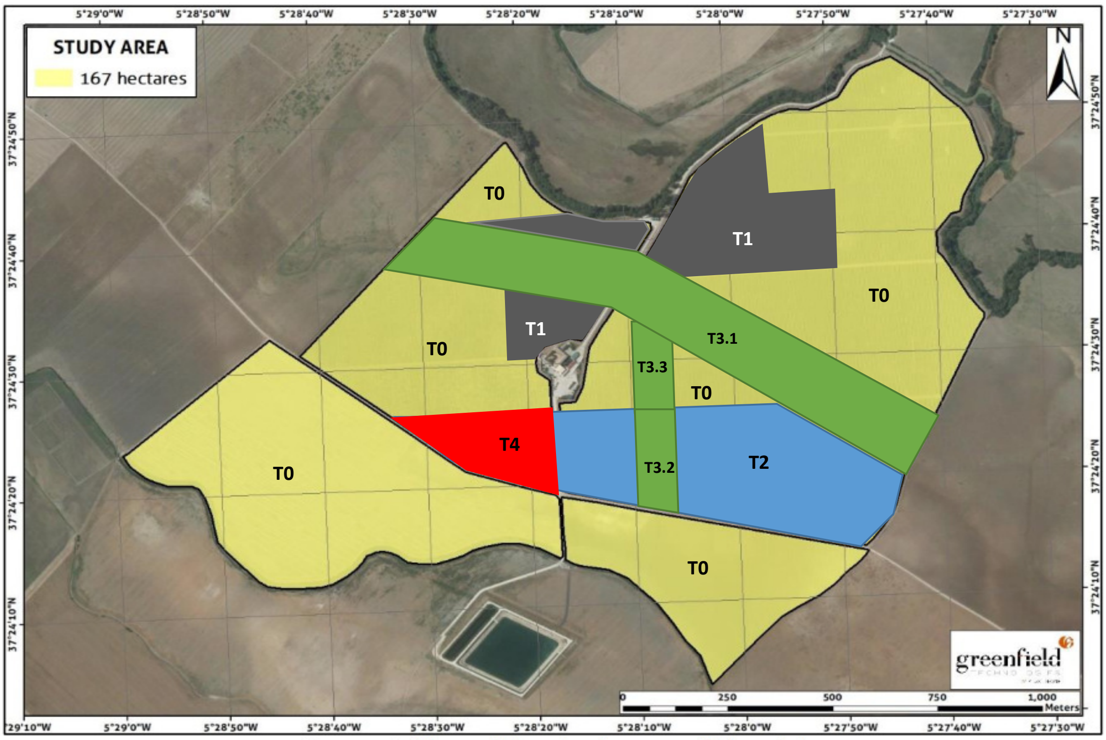


Image 1 Distribution of the treatments in El Valenciano Farm

1.1 Applications

Table 3 Treatments, products, dose and dates in El Valenciano Farm

Treatment	Products	Dose	Date
T0	Control (Conventional methods)		
T1	IBNP	1 kg/ha	29/06/2021
	Procrop ISR	0.5 l/ha/week	13/07/2021 27/07/2021
T2	Deficit irrigation		July - August 2021
T3	Cover crops		2021
T3.1	Plant Cover Mixture	30 kg/ha	2021
T3.2	Farm Cover Crop	-	2021
T3.3	Tilling	-	2021
T4	T1+T2+T3	IBNP (1 kg/ha) Procrop ISR (0,5 l/ha/week)	(MST-NP) 29/06/2021 Procrop ISR 13/07/2021 27/07/2021

2. Results

In A1 action (preparatory actions) the parameters of the project performance indicators were described and the descriptions of the methods were established. After that, in Action D1 each indicator was measured and evaluated every year. Indicators were evaluated visiting El Valenciano farm every week to verify the correct implementation of the treatments and its evolution. The results in the third season (2021) obtained are described below.

I. Tree Health

I.1. Nutrient state through foliar analysis

Foliar analysis is a technique that determines the content of nutrients at a specific time or stage of development in plant tissues. In order to determine foliar nutrient content leaves were sampled in preharvest (early September 2021) at El Valenciano. In order to determine differences between treatments one foliar analysis per treatment was done.

In early September 2021 foliar analysis was done on El Valenciano. The results are described in the following table (Table 4 and Table 5). Measured parameters had normal levels for the crop. However, T1 treatment increased nutrient assimilation by the crop in comparison to T0 treatment. In fact, T1 nutrient assimilation as phosphorus and potassium increment was measured. However, T2 treatment increased calcium, magnesium and potassium levels due to probably to water stress situation. In this sense T4 was also deficit irrigation treatment sodium was increased.

With respect to microelements, increases in all microelements levels were observed in all the treatments respect to control

Table 4 Microelements per treatment in El Valenciano Farm

Macroelements	T0	T1	T1_to T0	T2	T2 to T0	T4	T4 to T0
N	1.92	1.84	-4%	1.66	-14%	1.87	-3%
P	0.15	0.19	27%	0.17	13%	0.12	-20%
K	1.45	1.48	2%	1.22	-16%	0.99	-32%
Ca	1.65	1.66	1%	2.27	38%	2.08	26%
Mg	0.15	0.15	0%	0.16	7%	0.15	0%
Na	0.02	0.01	-50%	0.02	0%	0.03	50%
S	0.14	0.12	-14%	0.11	-21%	0.14	0%
Cl	0.1	0.1	0%	0.08	-20%	0.05	-50%

Table 5 Microelements per treatment in El Valenciano Farm

Microelements	T0	T1	T1 to T0	T2	T2 to T0	T4	T4 to T0
Fe	36.48	47.78	31%	48.90	34%	79.58	118%
Mn	52.19	55.15	6%	73.21	40%	90.87	74%
Zn	24.88	30.07	21%	15.76	-37%	31.64	27%
Cu	159.45	173.45	9%	157.70	-1%	299.62	88%
B	42.3	44.74	6%	36.20	-14%	29.3	-31%
Mo	0.92	1.07	16%	<0,30	-68%	1.53	66%

I.2. Tree temperature

Image 2 shows the temperature of the crop cover (once the soil has been removed) in pre-harvest in the 2021 campaign at Finca El Valenciano. The results of the thermal flight presented a low variability in the temperature recorded by the thermal sensor, with results between 28 and 32 ºC (Table 6). Very similar temperatures in all the treatments make it difficult to establish comparisons regarding the degree of hydric stress, being more useful the spatial variability of the temperature.

Regarding the spatial variability of the temperature of the tree crown, no patterns are observed when the treatments are compared with each other or with the control treatment T0, where the variability of the temperature is around 1ºC to 2ºC of difference. In this sense, the crop appears to be highly homogeneous, which is convenient for crop management control and, above all, for a homogeneous harvest in terms of production and quality.

Table 6 Canopy mean temperature (Cº) El Valenciano Farm

	Tree temperature Pre-Harvest 2021 (ºC)			
Treatment	**Mean**	**Desv. St.**	**Min**	**Max**
T0	29.77	0.40	28.01	31.71
T1	30.03	0.34	29.18	30.96
T2	30.15	0.33	28.99	30.99
T3	30.10	0.36	28.37	30.97
T4	30.06	0.38	29.02	30.99

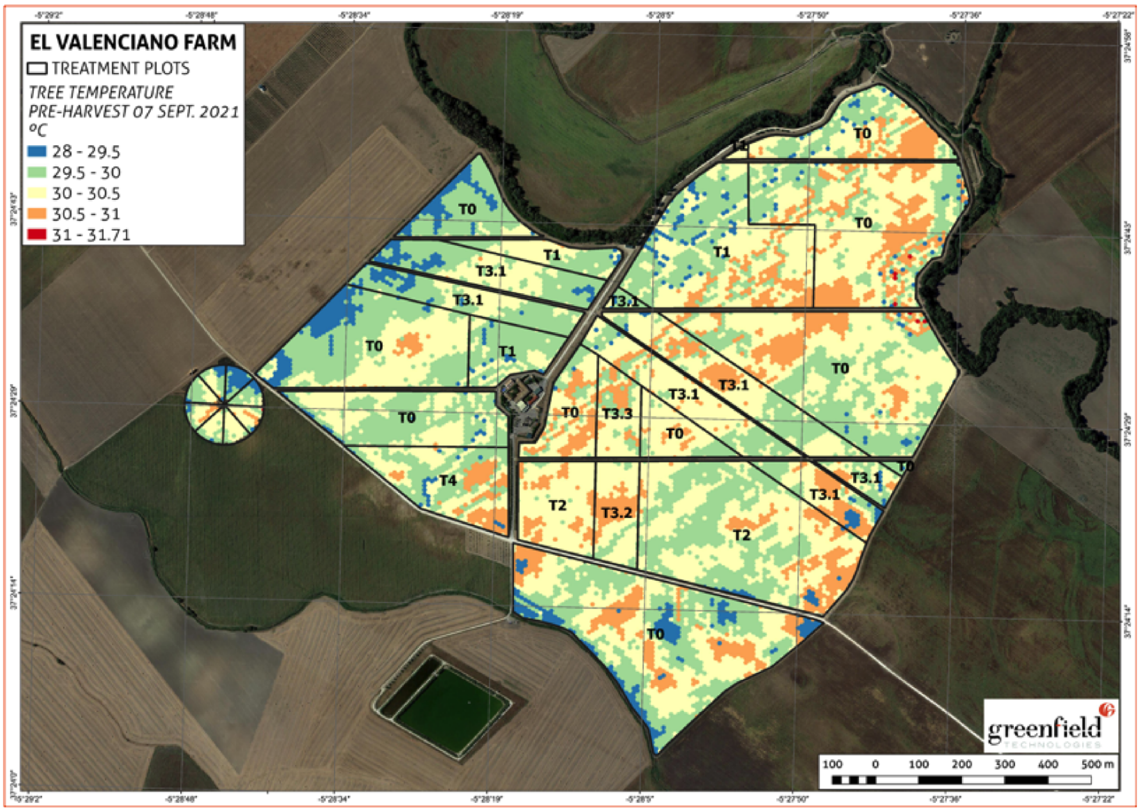


Image 2 Pre harvest canopy temperature spatial variability in El Valenciano Farm

I.3. Vegetative development

Image 3 to Image 12 shows the states of vegetative development and moisture content in different phenological phases of the 2021 campaign. In general terms, the NDVI and NDWI vegetation indices gradually increase until the pit hardening, where they remain stable until post-harvest, where they rise. Regarding the behaviour between treatments of vegetative development and moisture content, no different evolutions are appreciated. There is heterogeneity between the different treatments that mean that specific behaviours are not visually appreciated.

Image 3 and Image 4 shows the intra-plot and inter-plot variability, respectively, of the state of the crop in the phenological stage of flowering. In this period, the spatial variability in vegetative development and moisture content are the lowest of the entire campaign. However, they are very homogeneous, only possibly varying due to soil effects (traces of an old stream can be seen). Homogeneity at the beginning of the campaign allows a better assessment of the changes produced throughout it. Regarding intra-parcel variability, differences in vegetative development and moisture content are recognized within some treatments that are maintained throughout the growing season, making it difficult to contrast the differences between treatments.

Image 3 Vegetative development relative spatial variability at flowering stage in El Valenciano Farm

Image 4 Vegetative development absolute spatial variability at flowering stage in El Valenciano Farm

Image 6 and Image 7 shows the intraplot and interplot variability, respectively, of the state of the crop in the phenological stage of pit hardening. In this period, the values of the NDVI and NDWI indices have increased throughout the farm in a very homogeneous way. It is worth highlighting punctual areas of greater growth in the northern part of the farm, as well as an area of low value for both indices in treatment T0 located to the north, which may be influenced by the soil, since a similar area is observed in the water retention capacity (AWC) plan generated at the beginning of the project.

Image 5 Vegetative development relative spatial variability at pit hardening stage in El Valenciano Farm

Image 6 Vegetative development absolute spatial variability at pit hardening stage in El Valenciano Farm

Image 7 and Image 8 shows the intra-plot and inter-plot variability, respectively, of the state of the crop in the phenological stage of the beginning of fat formation. In this phenological period, patterns of behaviour between treatments remain undistinguished.

In addition, the indices have slightly increased their values, where it seems that they have stabilized and that the most delayed areas in terms of vegetative development and moisture content are equating, showing more homogeneous images.

Image 7 Vegetative development relative spatial variability fat formation stage in El Valenciano Farm

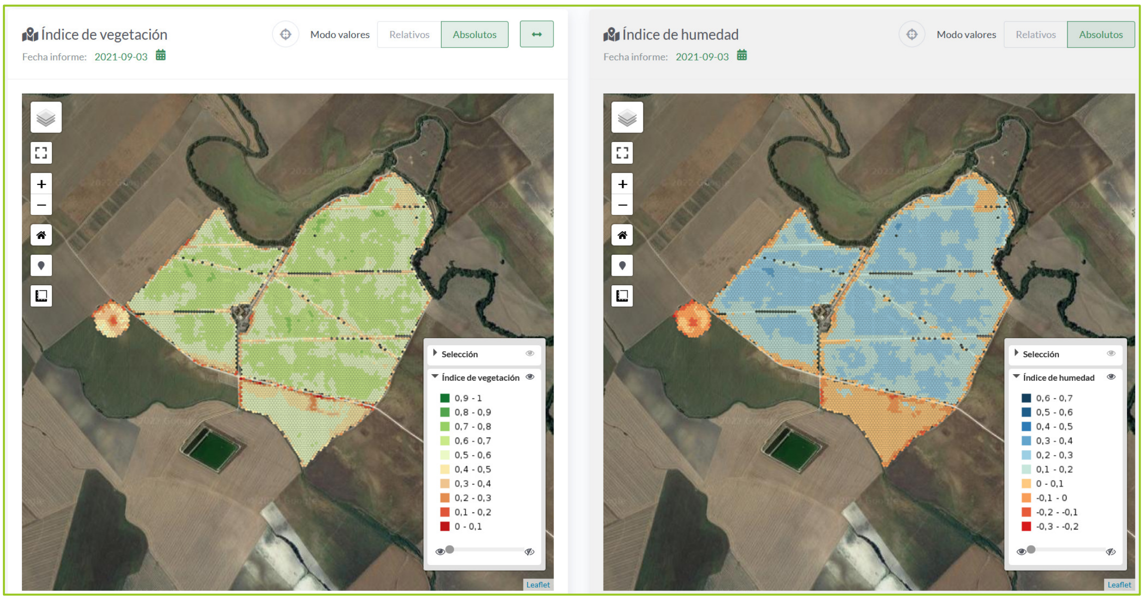


Image 8 Vegetative development absolute spatial variability at fat formation in El Valenciano Farm

Image 9 and Image 10 shows the intra-plot and inter-plot variability, respectively, of the state of the crop in the pre-harvest phenological stage. In this state, the NDVI and NDWI vegetation indices show totally homogeneous interplot variability in almost the entire farm. This result is consistent with the low temperature variability observed in the drone flight with a thermal camera carried out in the same period. Regarding the intraplot variability, it is observed that there have been differences within the different treatments or specific areas of the farm that have remained more or less stable throughout the campaign.

Image 9 Vegetative development relative spatial variability at Pre harvest stage in El Valenciano Farm

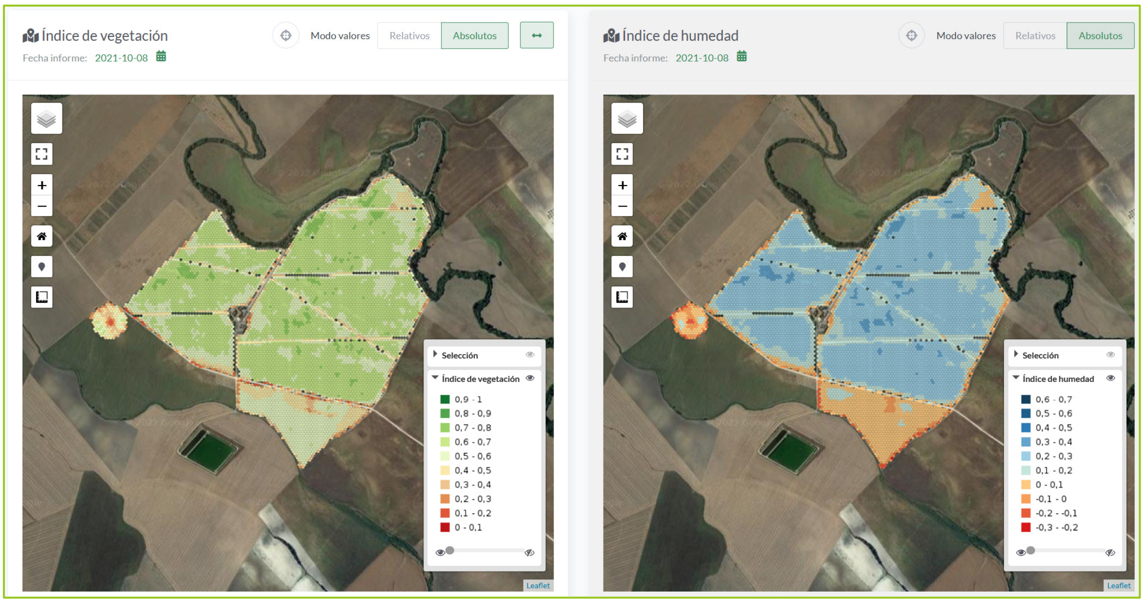


Image 10 Vegetative development absolute spatial variability at Pre harvest stage in El Valenciano Farm

Image 11 Vegetative development relative spatial variability at post harvest stage in El Valenciano Farm

Image 11 and Image 12 shows the intra-plot and inter-plot variability, respectively, of the state of the crop in the postharvest phenological stage. In this period, the homogeneity of the entire crop plot, in absolute terms, has been maintained. However, the values of the indices have increased with respect to the pre-harvest phenological state. It is possible that the spontaneous vegetation cover is influencing this rise in value in the respective indices, since the crop would be entering a certain winter shutdown, lowering its levels of photosynthetic activity and development.

Image 12 Vegetative development absolute spatial variability at post harvest stage in El Valenciano Farm

II. Soil quality

II.1. Available Water Capacity (AWC)

Available Water Capacity vas measured at the beginning of the project.

The results of the soil analyses carried out in 2021 at the El Valenciano farm show a reduction in the organic matter content compared to the results of 2018, not exceeding a reduction of 0.5%. These results have not caused a visible reduction of the AWC in the different treatments, which may be mainly due to the high clay content of the soils of this demonstration farm.

Table 7 Evolution of AWC in El Valenciano Farm (2018-2021)

Treatment	T0	T1	T2	T3	T4
Mean AWC (mm/m) 2018	140	130	130	130	140
Mean Organic Matter (%) 2018	1.43	0.95	1.26	1.45	1.65
Mean AWC (mm/m) 2021	140	130	130	130	140
Mean Organic Matter (%) 2021	1.1	0.8	1.1	1.1	1.2

II.2. Soil microbiological activity

Soil samples were taken from georreferenced points and analyzed in laboratory to determinate fertility biological index and enzymatic activity such as, B-glycosidase, dehydrogenase, catalase, phosphodiesterase (phosphatase) and urease activities (Image 13).

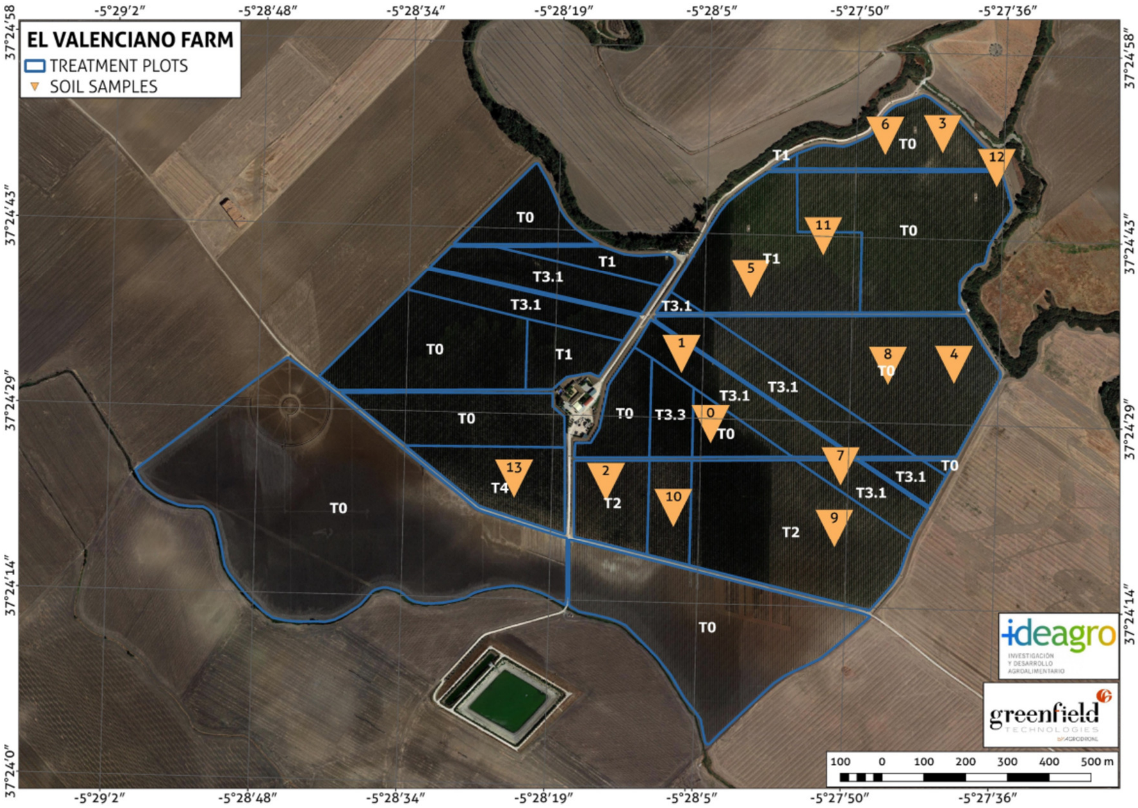


Image 13 Treatments and georeferenced soil samples location in El Valenciano Farm

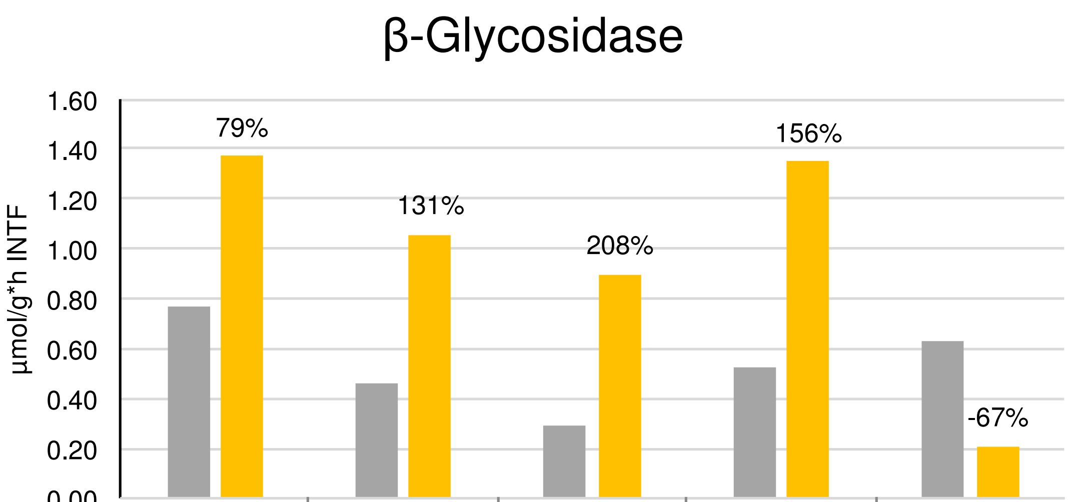
Table 8 Soil chemical analysis El Valenciano Farm 2021.

	T0	T1	T2	T3	T4
E.C. (dS/m) (extract 1:5)	0.8	0.6	1.0	1.2	0.6
Macronutrients					
%N Total	0.1	0.1	0.1	0.1	0.1
NO_3^- (mg/kg)	9.2	5.2	**34.7**	28.9	11.5
P (ppm)	4.6	6.2	5.5	6.9	**18.8**
K (meq/100 g)	1.4	1.3	1.5	1.4	**1.6**
Ca (meq/100 g)	14.7	11.2	**17.6**	15.7	8.8
Mg (meq/100 g)	**4.3**	3.7	2.5	**4.5**	3.8
SO_4^{-2} (meq/100g)	1.4	1.1	1.4	**2.8**	0.8
Micronutrients					
Fe (ppm)	2.6	**5.1**	2.0	3.3	2.1
Mn (ppm)	5.0	**6.7**	5.3	**6.7**	2.4
B (ppm)	1.5	1.6	**1.9**	1.8	1.1
Cu (ppm)	5.6	5.8	4.9	**8.7**	6.6
Zn (ppm)	0.4	0.6	0.4	**0.9**	0.6
Cl^- (meq/100 g)	1.7	1.1	**2.9**	2.5	0.8
Oligoelements					
Na (meq/100 g)	3.3	2.2	3.4	**4.8**	1.7
Organics					
% O.M. Oxidable	1.1	0.8	1.1	1.1	1.2
%C Total	0.8	0.6	0.8	0.8	0.9
C/N	9.5	10.0	9.6	8.7	**11.7**
Carbonates					
% $CaCO_3$ Total	22.1	27.8	9.8	21.1	27.8
% $CaCO_3$ active	9.3	8.3	2.3	9.1	5.6

Regarding the nitrogen content, all the treatments showed very similar values. On the contrary, as far as NO_3 is concerned, a much higher value was observed in T2 with respect to the remaining treatments. The phosphorus content in the soil was much higher in the T4 treatment, the same was observed for the potassium content. Very similar values were observed between treatments regarding the content of calcium, magnesium and sulfur. About micronutrients, a slightly higher content of Fe and Mn was observed in T1 treatment.

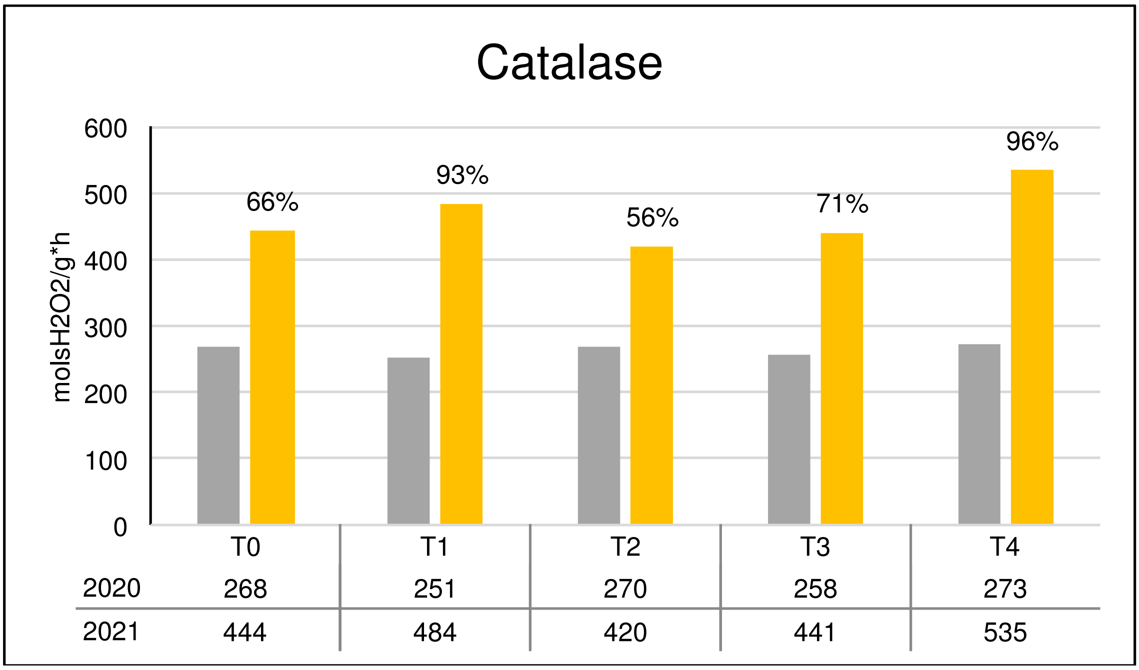
Regarding soil enzymatic activity, ß-glycosidase activity was greater in all the treatments respect to 2020 season. However in T4 treatment a decrement in the enzyme activity was observed in comparison to 2020. The dehydrogenase activity is linked to the metabolic activity of the soil; it indicates that soil fertility that is due to the activity of microorganisms in the soil. A slightly decrease was observed in 2021 season in comparison to season 2020. The treatment that experienced a slighter reduction in dehydrogenase activity was T1, probably because the greater metabolic activity in the soil due to bioestimulant treatment. In comparison with previous seasons catalase activity increased in all the treatments in El Valenciano Farm in season 2021. Treatments with a larger increment were T1 and T4 in which biostimulants were applied.

Regarding to phosphatase activity a higher activity of this enzyme indicates a greater mobilization of phosphorus in inorganic form in the soil and a greater availability of this element to the plant. An increased in phosphatase activity was observed in all the treatments in 2021 respect to 2020, in El Valenciano Farm (Graph 4).

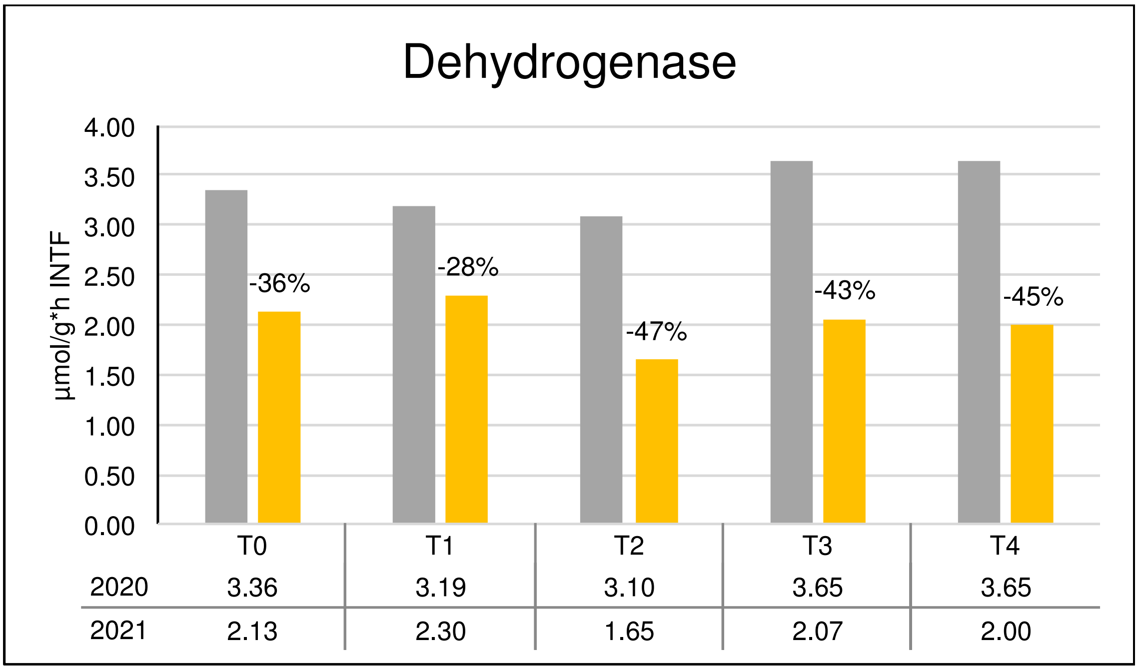


	T0	T1	T2	T3	T4
2020	0.76	0.46	0.29	0.53	0.63
2021	1.37	1.06	0.90	1.35	0.21

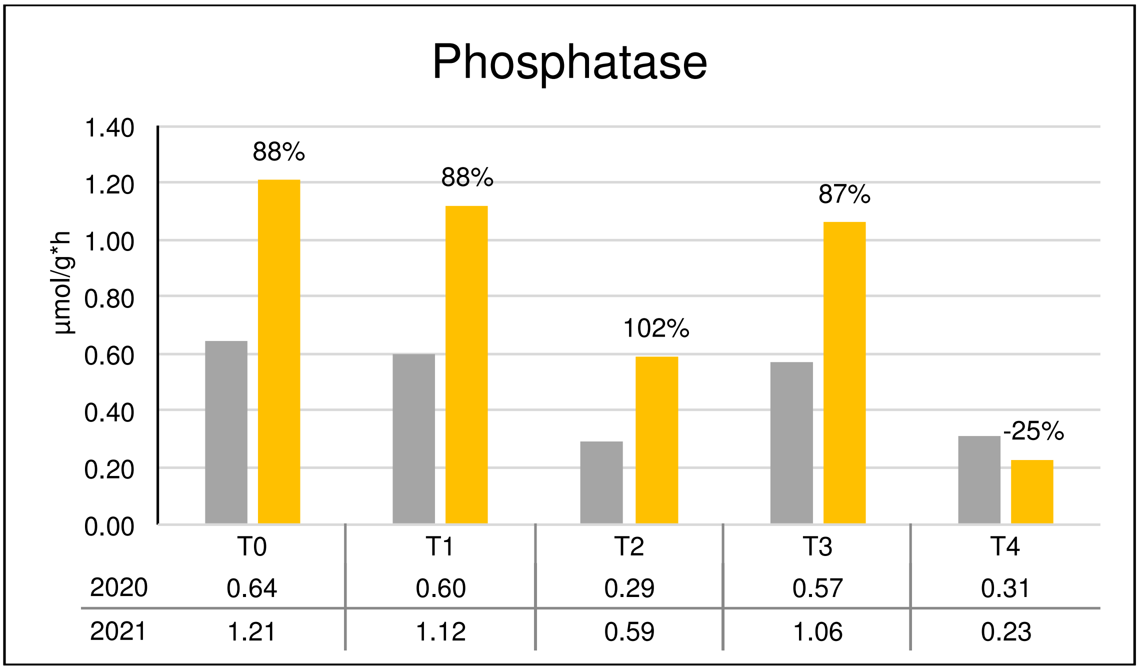
Graph 1 El Valenciano Farm ß-Glycosidase activity



Graph 2 El Valenciano farm Catalase activity



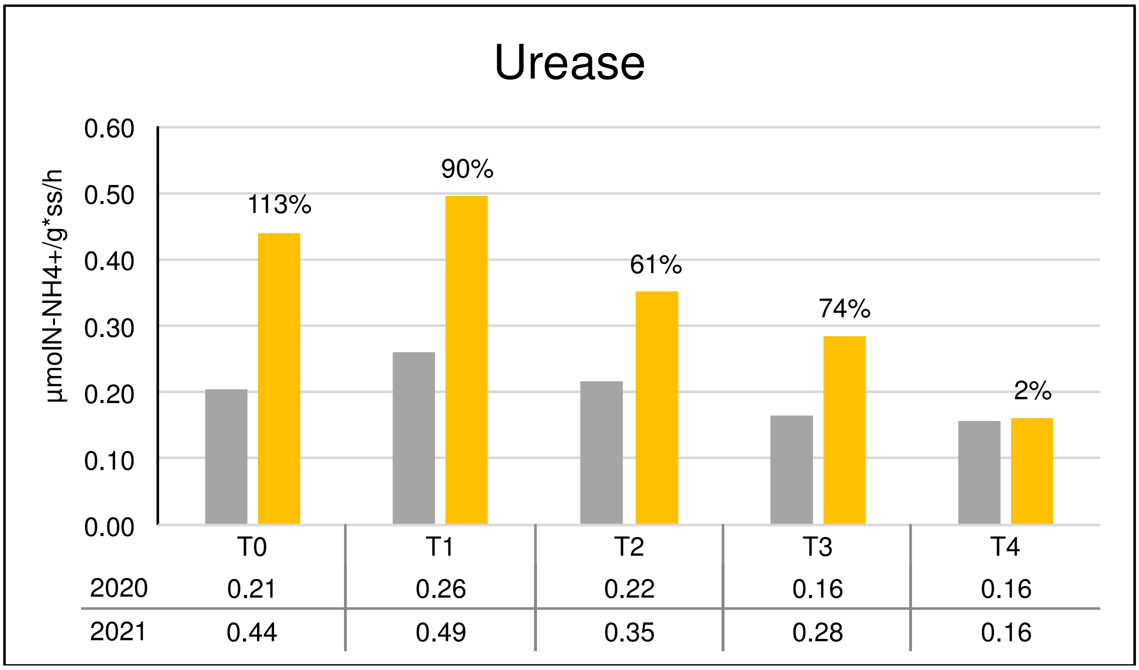
Graph 3 El Valenciano farm Dehydrogenase activity



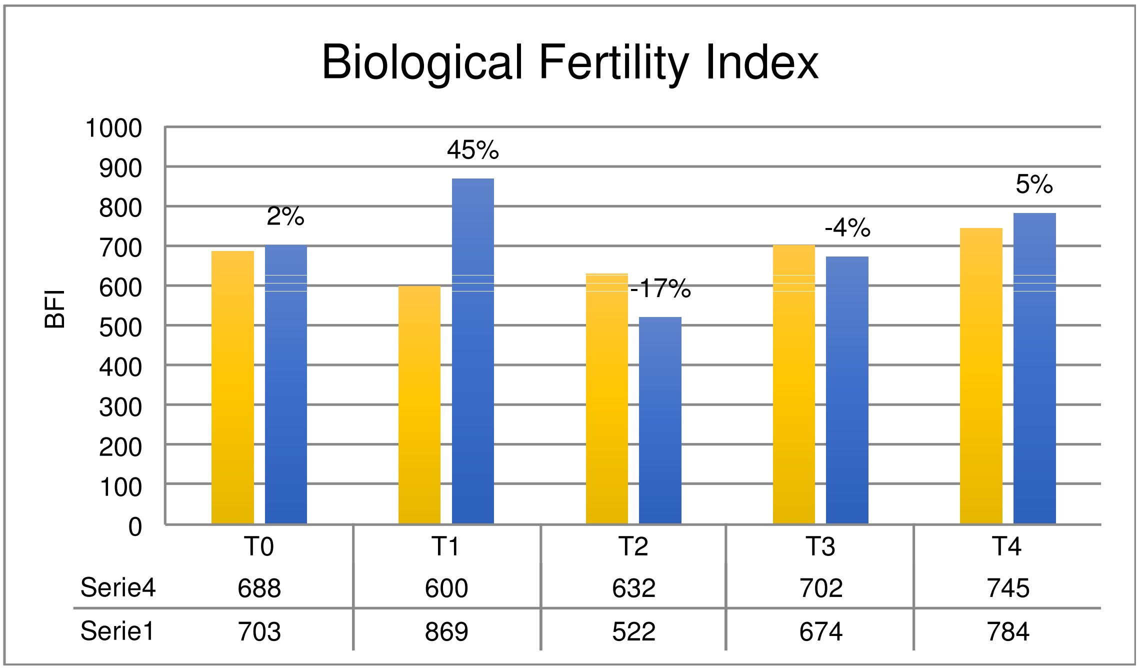
Graph 4 El Valenciano farm Phosphatase activity

Urease activity indicates the conversion of urea to ammonia by soil microorganism. As can be seen in the graph urease activity was strongly increased in season 2021 in all the treatments respect to 2020 campaign (Graph 5).

A great increment in the biological fertility index was observed in T1 treatment in 2021in comparison to previous campaign in El Valenciano Farm (Graph 6). On the contrary, T0, T3 and T4 treatments values were very similar in comparison to 2020 values. Hence, a slight decrement was observed in T2 treatment respect to previous year.



Graph 5 El Valenciano farm Urease activity



Graph 6 El Valenciano farm Biological fertility index

III. Disease prevalence

III.1. *Xylella fastidiosa* disease control

Since no symptoms of disease had been observed in year 2021 in El Valenciano farm and insect vector had not been detected on the farm, these disease control analyses were not carried out yet. Moreover, The Junta de Andalucía is conducting a disease-tracing program throughout the autonomous community. Whenever an alert will be detected in a nearby area, sampling will be carried out in El Valenciano farm to determine the disease as soon as possible.

In case of disease detection plant tissues will be sampled at a specific time or stage of development following the protocol of the laboratory (refer to Guidelines for the survey of *Xf* in the Union territory, European Commission, 2015).

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III.2. Insect vectors trap

Periodically, Nutriprado will evaluate the insects present in the farm. According to the diagnostic protocol adopted by the Standards Committee on behalf of the Commission on Phytosanitary Measures in August 2018 (see Annexes at the end of this document): "*Vectors should preferably be collected with sweeping nets (adults) or aspirators. Sampling for insects should preferably be carried out from late spring until early autumn to maximize the likelihood of detecting the bacterium*". In annex 1 results is shown in detail

IV. Weather

IV.1. Weather conditions

Annual information on the parameters measured in darsky climate stations, including: Temperature Maximum (ºC), Average Wind Speed (m/s), Temperature Time Maximum, Middle Wind Direction (º), Temperature Minimum (ºC), Radiation (MJ/m^2), Temperature Time Minimum, Precipitation (mm), Temperature Average (ºC), ET_0 (mm/day), Maximum Relative Humidity (%), Minimum Relative Humidity (%), Accumulated Precipitation (mm), Relative Medium Humidity (%), and Cumulative ET_0 (mm).

Regarding the climatic data recorded, no extreme meteorological phenomena have been observed that may have affected the treatments applied on El Valenciano farm.

V. Quality

V.1. Organoleptic characteristics (olive oil)

The olive tree fruits and olive oil obtained will be evaluated annually to determine its quality by means of the following parameters: content of fatty acids and volatile compounds present in green olives and their oil; total phenolic compounds; and sensory analysis.

The majority fraction of the oil (95%) is constituted mainly by triglycerides and to a lesser extent by diglycerides, monoglycerides and free fatty acids. The fatty acid composition is characterized by being high in monounsaturated fatty acids, oleic being the main fatty acid, whose concentration varies between 60-80%. Table 9 presents the profile of fatty acids identified and quantified in the 4 oil samples, in which it can be seen that, in all the samples, the majority acid was oleic acid, with values higher than 60%, being slightly higher in the T1 oil, maintaining said difference when adding the two isomers of oleic acid, C18:1c9 and C18:1c11, reaching values close to 66%. The second most important acid is palmitic acid, which appeared in the highest concentration in sample T2 (16.51%). Regarding the groups of fatty acids, the majority were monounsaturated fatty acids, oleic acid being the most important, with a value greater than 65%, followed by saturated fatty acids, with values between 17% and 19 %, and finally polyunsaturated fatty acids, with values between 13% and 15%.

Phenolic compounds are the main responsible for the antioxidant capacity of olive oil, which is why they provide stability against oxidation and autoxidation, in addition to generating benefits for the consumer. After analyzing the oils (
Table 10), significant differences were observed in the antioxidant activity analysis, with sample T1 being the one with the highest total polyphenol content (130.5 mg GAE L^{-1}).

Table 9 Olive oil fatty acid content (%)

Fatty acids	Anova	T0	T1	T2	T4
C14:0	NS	0,01	Trace	0,01	Trace
C15:0	NS	Trace	Trace	Trace	Trace
C16:0 (Palmitic)	NS	16,46	16,42	16,51	16,44
C16:1 (Palmitoleic)	NS	0,14	0,12	0,14	0,13
C16:1 (Palmitoleic)	NS	1,47	1,53	1,53	1,65
C17:0 (Margaric)	NS	0,11	0,10	0,1	0,11
C17:1 *cis*	NS	0,22	0,2	0,21	0,21
C18:0 (Stearic)	NS	1,78	1,86	1,73	1,79
C18:1 *cis*-9 (Oleic)	NS	63,69	63,82	62,65	63,18
C18:1c11	NS	2,77	2,81	3,53	3,48
C18:2 *n6c* (Linoleic)	NS	12,03	11,90	12,19	11,62
C18:3n6 gamma	NS	0,38	0,37	0,37	0,39
C18:3 n6 alfa	NS	0,51	0,44	0,50	0,53
C20:1 n9 cis-11	NS	Trace	Trace	Trace	Trace
C21.0	NS	0,01	0,01	0,01	0,01
C20:3n3	NS	0,11	0,10	0,11	0,12
C20:3n6	NS	Trace	Trace	Trace	Trace
C23:0	NS	0,30	0,30	0,31	0,27
C24:0	NS	0,01	0,01	0,01	0,01
C22:6	NS	Trace	Trace	0,07	0,04
Σ AGS	NS	18,67	18,69	18,67	18,62
Σ AGM	NS	68,29	68,49	68,07	68,68
Σ AGP	NS	13,03	12,81	13,24	12,69

*NS = not significant at p> 0.05, *, ** and *** when p <0.05, 0.01 and 0.001, respectively. The values (mean of 3 repetitions) followed by the same letter within the same row, do not present statistically significant differences (p> 0.05) according to the Tukey Multiple Ranges Test.*

Table 10 Total Phenolic content (mg GAE/L)

	T0	T1	T2	T4
g GAE/ L	71,872 bc	130,493 a	74,215 b	80,809 b

The volatile compounds are responsible for the aroma of the extra virgin olive oil, which helps to differentiate it from the rest of the edible oils, since in its preparation a refining process is not required in which all the volatile compounds are lost. Table 11 shows the volatile profile of the 4 analyzed samples, showing the 11 main volatile compounds, being able to appreciate significant differences in some of these compounds according to the applied treatment. In all samples, the compound with the highest concentration was 1-hexanol, a compound that is related to fruity aromas, reminiscent of tomato, whose highest concentration was found in treatments T1 and T4 (687.62 and 650.51 mg 100 mL $^{-1}$, respectively). The next most important compounds were trans-2-hexenal, which did not show significant differences between treatments, and trans-2-hexen-1-ol, being higher in treatment T4 (202.73 mg 100 mL^{-1}). These compounds, together with hexanal, are bibliographically related to green aromas.

Table 11 Volatile compounds (mg/100 ml oil)

Chemicals	Anova	T0	T1	T2	T4
Hexanal	**	41,59 d	56,93 cd	84,12 b	99,61 a
trans-2-Hexenal	NS	343,42	350,97	345,46	354,54
trans- 2- Hexen 1-ol	**	164,25 b	155,23 b	187,96 b	202,73 a
1-Hexanol	**	549,16 b	687,62 a	556,36 b	650,51 a
2.4 Hexadienal	NS	1,81	1,93	1,64	1,89
Hexanoic acid	NS	1,03	0,43	0,63	0,38
3-Hexen-1-ol-acetate	NS	94,70	84,74	91,77	95,53
Hexyl acetate	NS	35,4	35,06	33,3	47,7
Limonene	*	7,96 bc	8,88 b	8,46 b	9,06 ab
Nonanoic acid	NS	7,99	8,64	8,89	8,29
Ethyl decanoic	NS	0,29	0,15	0,18	0,13

*NS = not significant at p> 0.05, *, ** and *** when p <0.05, 0.01 and 0.001, respectively. The values (mean of 3 repetitions) followed by the same letter within the same row, do not present statistically significant differences (p> 0.05) according to the Tukey Multiple Ranges Test.*

The sensory analysis established by the COI only includes as key attributes to define the commercial category of the oil the total fruity and the presence or absence of defects, in addition to taking into account the basic bitter taste and the pungency of the oil. The last two attributes are attributes that depend on factors such as harvest time and variety. In this case, the oils samples have been analyzed in a more descriptive way, expanding the attributes to be studied, such as the maturity character of the oil.

Table 12 Sensory analysis

Sensory attribute	**Anova**	**T0**	**T1**	**T2**	**T4**
Fruity (olive)	***	2,6 c	2,9 bc	4,0 ab	4,9 a
Fruity (green)	**	3,7 b	4,9 ab	4,0 b	5,9 a
Fruity (ripe)	**	6,3 a	5,1 ab	6,0 a	4,1 b
Floral	NS	0	0	0	0
Green (artichoke)	***	0,0 b	0,1 b	0,0b	2,2 a
Green (grass)	*	0,3 c	0,6 b	1,4 a	0,7 b
Green (pepper)	NS	0,3	0,3	0	0,1
Tomato	***	0,9 b	0,2 b	0,5 b	2,4 a
Bitter	**	1,2 b	1,7 b	2,7 a	1,6 b
Rusty	NS	0	0	0	0
Rancid	NS	0	0	0	0
Fusty	**	0,0 b	0,5 a	0,0 b	0,0 b
Mold	NS	0	0	0	0
Remains	**	2,0 a	1,0 b	0	0
Astringent	NS	0,1 a	0,4 a	0,4 a	0,1 a
Pungent	NS	2,0 b	3,1 a	2,4 b	2,3 b

*NS = not significant at p> 0.05, ** and *** when p <0.01 and 0.001, respectively. ‡ The values (mean of 8 evaluations) followed by the same letter within the same row, do not present statistically significant differences (p> 0.05) according to the Tukey Multiple Range Test*

In Table 12 it can be seen how modifications have occurred in the sensory profile. In the table can be seen that for the attribute "fruity (olive)" the samples with the highest score are those corresponding to treatments T2 and T4, 4.0 and 4.9, respectively, while the lowest value was obtained in the sample corresponding to treatment T0. Regarding the "fruit-ripe" attribute, it can be seen that the oil from the T0 treatment has a more mature character, while the T4 treatment has a greener character, with the "green artichoke" being the one that predominates in this sample. On the other hand, significant differences also appear in the "bitter" attribute, being higher in the T2 sample (2.7), while in the pungency of the oil, the T1 sample stands out, with a score of 3.1. Regarding the presence of defects, in sample T1 the "fusty" defect appears, which is related to the state of the olive at the time of grinding and oil extraction, with a value of 0.5, while in the T0 sample, the defects of "remains" (2.0) appear.

VI. Water use

VI.1. Water use efficiency (WUE)

The WUE is the relation between water consumed (m^3) by the crop and irrigation water applied (m^3). Both values were calculated each year. In Table 13 WUE values are listed. In T2 and T4 treatments deficit irrigation was applied and WUE was increased as expected. On the other hand, in T0 and T1 had the same irrigation and WUE.

Table 13 El Valenciano WUE per treatment

	T0	T1	T2	T3	T4
WUE	1.94	1.94	2.48	_	2.48

VI.2. Irrigation water productivity (IWP)

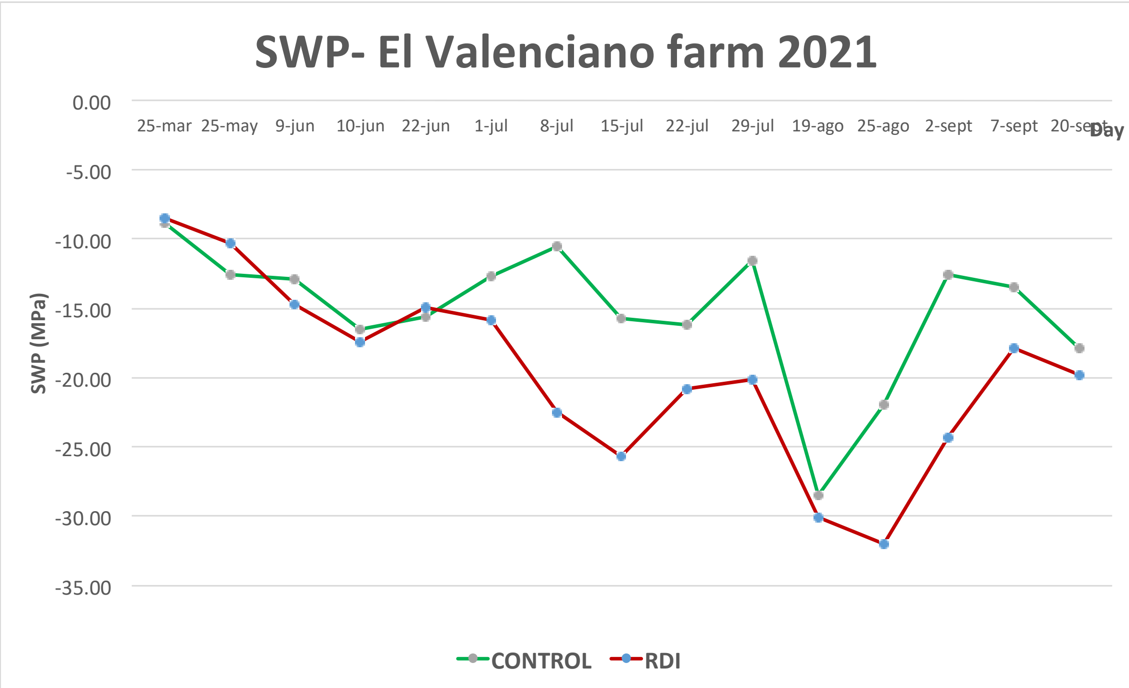
The IWP is the relation between annual yield (kg) and water applied (m^3). Both values were measured each year and IWP was calculated. In **¡Error! No se encuentra el origen de la referencia.** IWP 2021 are listed. T4 treatment was more productive because IWP was the highest, similar situation was observed in T2 treatment. On the other hand, very similar values were measured in T0 and T1.

Table 14 El Valenciano IWP per treatment.

	T0	T1	T2	T3	T4
IWP	4.35	4.61	5.92	-	8.66

VI.3. Stem water potential (SWP)

The SWP was measured by the Scholander pressure chamber on mature leaves to determine tree water status in olive during the growing season. The measures were taken every week to schedule the irrigation following the Hydrosustainable Protocol (BALAM).



Graph 7 SWP in Valenciano Farm 2021 season

VII. Carbon footprint

VII.1. CO_2 emitted during the agricultural processes

Annual CO_2 emitted in each farm will be described at the end of the project. In order to do that, annual CO_2 emitted in each farm will be estimated by Carbon Footprint Assessment ISO/TS 14067:2013.

VIII. Biodiversity

VIII.1. Auxiliary fauna

Nests hotel and insect boxes were installed on 2020 autumn (September; Image 14). Occupancy rate of hotels nests and insect boxes was determined by visual counting in 2021 autumn. Of a total of 40 nest boxes and shelters for fauna installed in 2016, plus 23 nest boxes and shelters installed in 2020 in the El Valenciano farm, activity of the following species has been detected (Graph 8):

- The percentage of unoccupied boxes has been reduced from 18% in the last revision to 13%.

- Of the three locations for kestrels, 2 of them have been occupied, twice as many as in the previous review.

- No domestic honeybee swarm has been found, which means less competitive pressure for native wild bees.

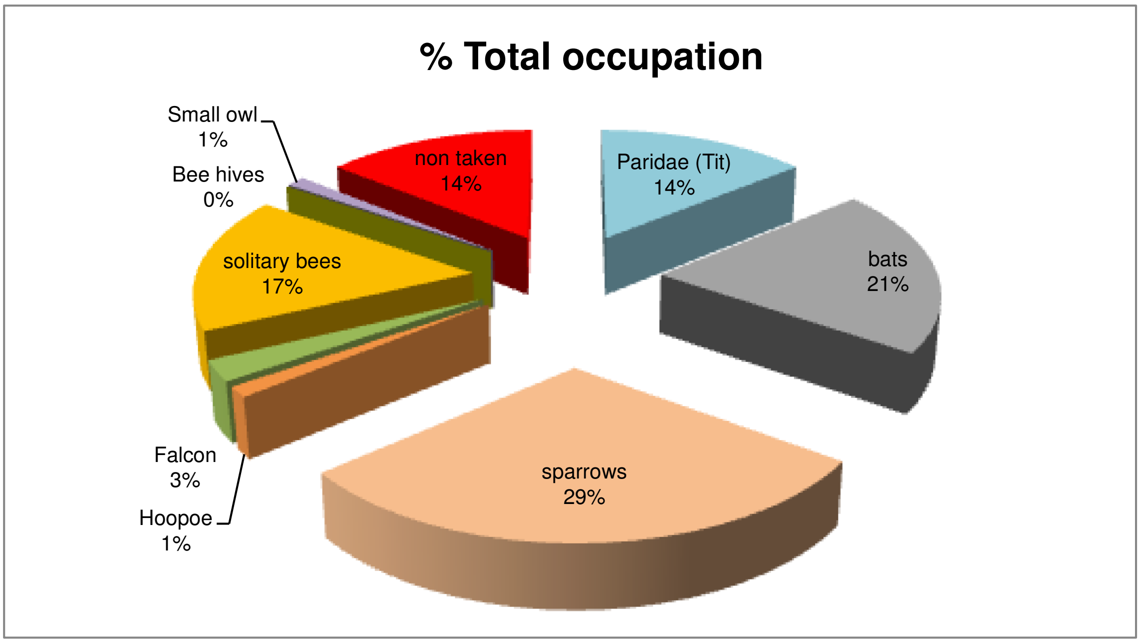
- The occupation by sparrows, probably *Passer montanus* in all cases, has increased from 22% to 30%.

- Occupancy by tits, probably *Parus major* in all cases, has remained stable at 14%.

- The occupation by bats (*Pipistrellus pygmaeus)* is detected in 100% of the installed shelters, including those of the last installation in 2020.

- All the insect hotels installed in 2016 and 2020 have been occupied to a greater or lesser extent by solitary bees and some species of solitary wasps, to a lesser extent.

- The presence of Dormouse (*Eliomys quercinus*) hibernating in one of the nest boxes has been detected for the first time.



Graph 8 Total occupation (%)

Image 14 Installation of bug hotels and nest boxes

Nest boxes insectivorous birds:

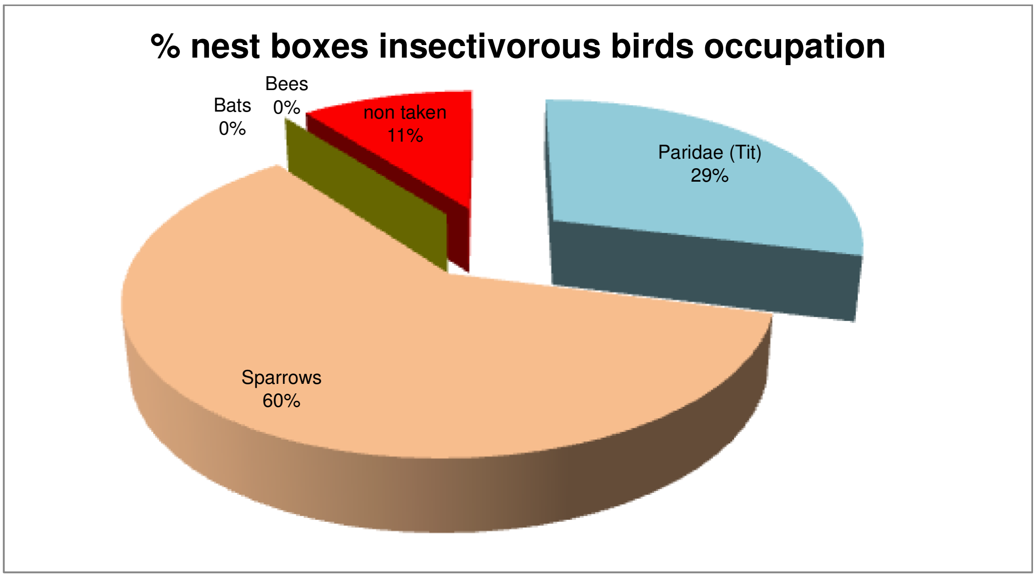
The population of titmouse (29%) in the area has remained stable compared to the last review, it can be seen that competition with sparrows (60%) is strong (Graph 9). Taking into account these data, if new nest boxes are installed they should have the smallest entrance to limit the entrance of Passer and favour the titmouse.

On this occasion there have been no boxes occupied by honey bees, perhaps the drought has influenced, the use of some type of pesticide or the distance from the location of nearby hives by beekeepers, the latter perhaps being the most likely variable (Graph 9).

Wryneck clutches have not been detected, but that does not mean that there were not, they are simply difficult to detect if it is not with the remains of eggs since they do not build nests, and the remains of eggs or feces are cleaned by other species that can nest after them, such as sparrows or great tit, eliminating the clues or traces that make it easier for us to know which species nested before, the same thing happens with other species that do not build nests, it is difficult to detect them if it is not at the time of occupation.

Most of the boxes are used by Geckos (*Tarentola maurtanica*) to spend the winter. This year we have not detected wintering bats in the bird boxes, but we have detected them in the bat shelters.

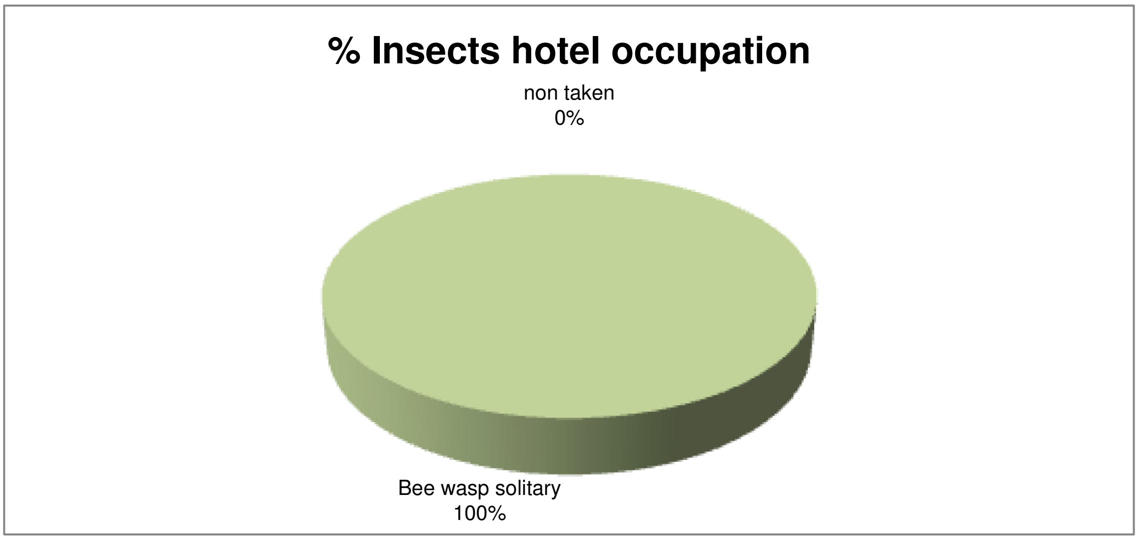
One of the nesting boxes for birds installed in 2016 in the vicinity of the farmhouse was occupied by a pair of hibernating dormouse, this is the first time that the species has been detected in El Valenciano.



Graph 9 Nest boxes insectivorous birds occupation

Insect's hotel occupation

Of the 4 hotels installed in 2017, 3 of them show activity of solitary bees and/or wasps, (*Rhynchium* sp. *Ancistrocerus* sp. *Megachile* sp.), undetermined microbes. The fourth hotel no longer exists, the three hotels have fulfilled their function for 6 years and they are quite old, it would be advisable to remove them to reduce the impact of fungi, mites and kleptoparasites. The technical hotels (holes 15 cm deep) installed in 2020, all of them have activity, mainly from megachylids, but also from other solitary hymenoptera.



Graph 10 Insects hotel occupation

Big nest boxes occupation

Large hotels have higher parasitoid activity, so smaller hotels are preferable from the point of view of solitary bee survival. Even so, they can serve as a refuge for geckos and lizards, although for this it would be better to use rock cages or dry stone walls (or simple piles of rocks on unused margins).

References

Chapter II. Herdade do Charqueirão Farm (Alandroal, Évora - Portugal)

Chapter developed in collaboration with the External Agronomist: Ideagro

1. Treatments

Table 15 describes standard procedures for almond crop that was done in 2020 in Herdade do Charqueirão Farm and treatments dates.

Table 15 Herdade do Charqueirão Farm Standard procedures and treatments dates

Action C2	T0 - control	T1	T2 - Deficit irrigation	T3- Cover crops	T4 (combination T1-T2-T3)
	Traditional management	T1 - IBNP +ISR			
H. Charqueirao - PT	**1.Soil:** tillage (3/year) and herbicide (3/year). **2.Irrigation:** on demand (March-November) **3.Pests**: Myzus persicae, Jacobiasca lybica, Monosteira unicostata, Panonychus ulmi, Capnodis tenebrionis and Anarsia lineatella . Total treatments 12/year. **4. Diseases:** Tranzschelia pruni-spinosae, Coryneum beijerinckii and Polystigma amygdalinum. Total treatments 6/year. **5.Nutrition:** Soil (17/year) and Foliar (18/year). **6.Prunning:** Formation pruning (1/year) and Top and bottom pruning (2/year) **7.Harvent:** August/September (1 /year)	**1.Soil:** tillage (3 times/year) and herbicide (3 times/year) and **IBNP (1/year; dose: 1 kg/ha) 30/07/2021 + ISR (2 times/year; dose: 0.5 L/ha) 13/08/2021.** **2.Irrigation:** on demand (March-November). **3.Pests:** Myzus persicae, Jacobiasca lybica, Monosteira unicostata, Panonychus ulmi, Capnodis tenebrionis and Anarsia lineatella . Total treatments 12/year. **4.Diseases**: Tranzschelia pruni-spinosae, Coryneum beijerinckii and Polystigma amygdalinum. Total treatments 6/year. **5.Nutrition:** Soil (17/year) and Foliar (18/year). **6.Pruning:** Formation pruning (1/year) and Top and bottom pruning (2/year) **7.Harvest:** August/September (1/year)	**1.Soil:** tillage (3 times/year) and herbicide (3 times/year) **2.Irrigation**: **On demand (March-June)-(September-November) and deficit irrigation (July-August).** **3.Pests:** Myzus persicae, Jacobiasca lybica, Monosteira unicostata, Panonychus ulmi, Capnodis tenebrionis and Anarsia lineatella . Total treatments 12/year. **4.Diseases**: Tranzschelia pruni-spinosae, Coryneum beijerinckii and Polystigma amygdalinum. Total treatments 6/year. **5.Nutrition:** Soil (17/year) and Foliar (18/year). **6.Pruning:** Formation pruning (1/year) and Top and bottom pruning (2/year) **7.Harvest:** August/September (1/year)	**1.Soil:** tillage (1/year), herbidide (2/year), **cover crop: sow (10/2019), and weed (1/year).** **2.Irrigation**: on demand (March-November) **3.Pests**: Myzus persicae, Jacobiasca lybica, Monosteira unicostata, Panonychus ulmi, Capnodis tenebrionis and Anarsia lineatella . Total treatments 12/year. **4.Diseases**: Tranzschelia pruni-spinosae, Coryneum beijerinckii and Polystigma amygdalinum. Total treatments 6/year. **5.Nutrition**: Soil (17/year) and Foliar (18/year). **6.Pruning:** Formation pruning (1/year) and Top and bottom pruning (2/year) **7.Harvest:** August/September (1/year)	**1.Soil:** tillage (1/year), herbidide (2/year), **cover crop: sow (1/year), weed (1/year), IBNP (1/year) 30/07/2021 and ISR (13/08/2021)** **2.Irrigation**: **On demand (March-June)-(September-November) and deficit irrigation (July-August).** **3.Pests:** Myzus persicae, Jacobiasca lybica, Monosteira unicostata, Panonychus ulmi, Capnodis tenebrionis and Anarsia lineatella . Total treatments 12/year. **4.Diseases:** Tranzschelia pruni-spinosae, Coryneum beijerinckii and Polystigma amygdalinum. Total treatments 6/year. **5.Nutritionn**: Soil (17/year) and Foliar (18/year). **6.Pruning:** Formation pruning (1/year) and Top and bottom pruning (2/year) **7.Harvest:** August/September (1/year)

Due to Herdade do Charqueirão Farm almond cultivar location a special treatment design was done in order to have comparable results. Image 16 shows plot distribution on Herdade do Charqueirão Farm.

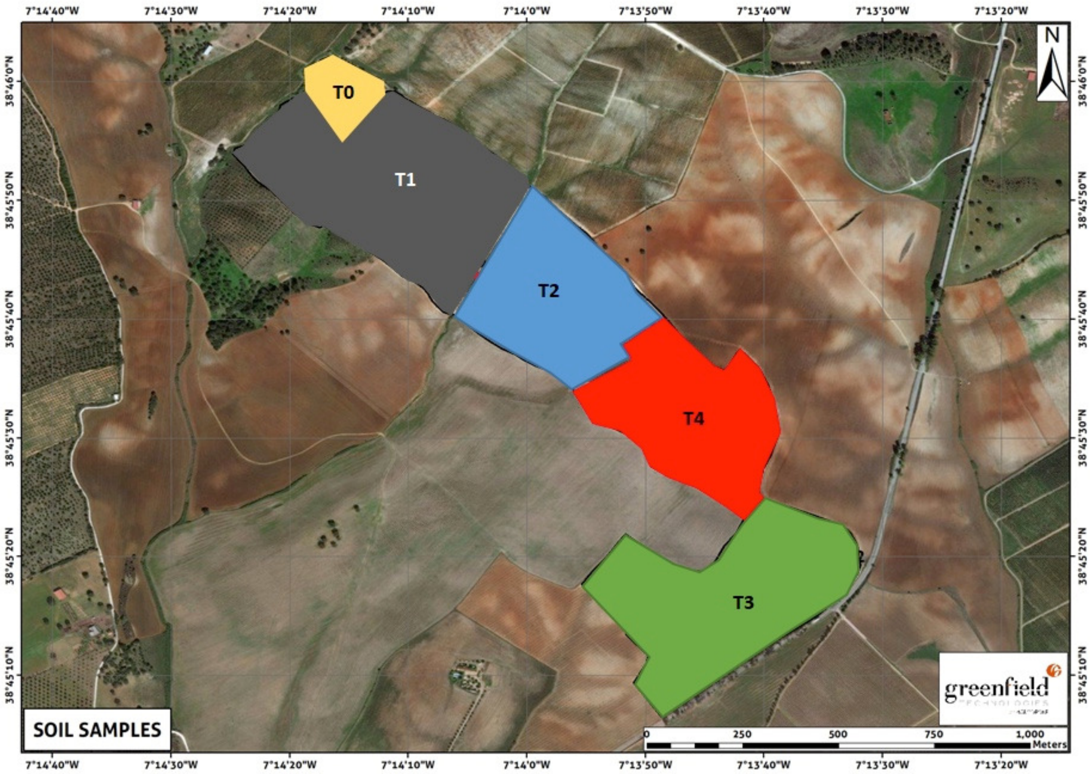


Image 15 Herdade do Charqueirão Farm treatments location

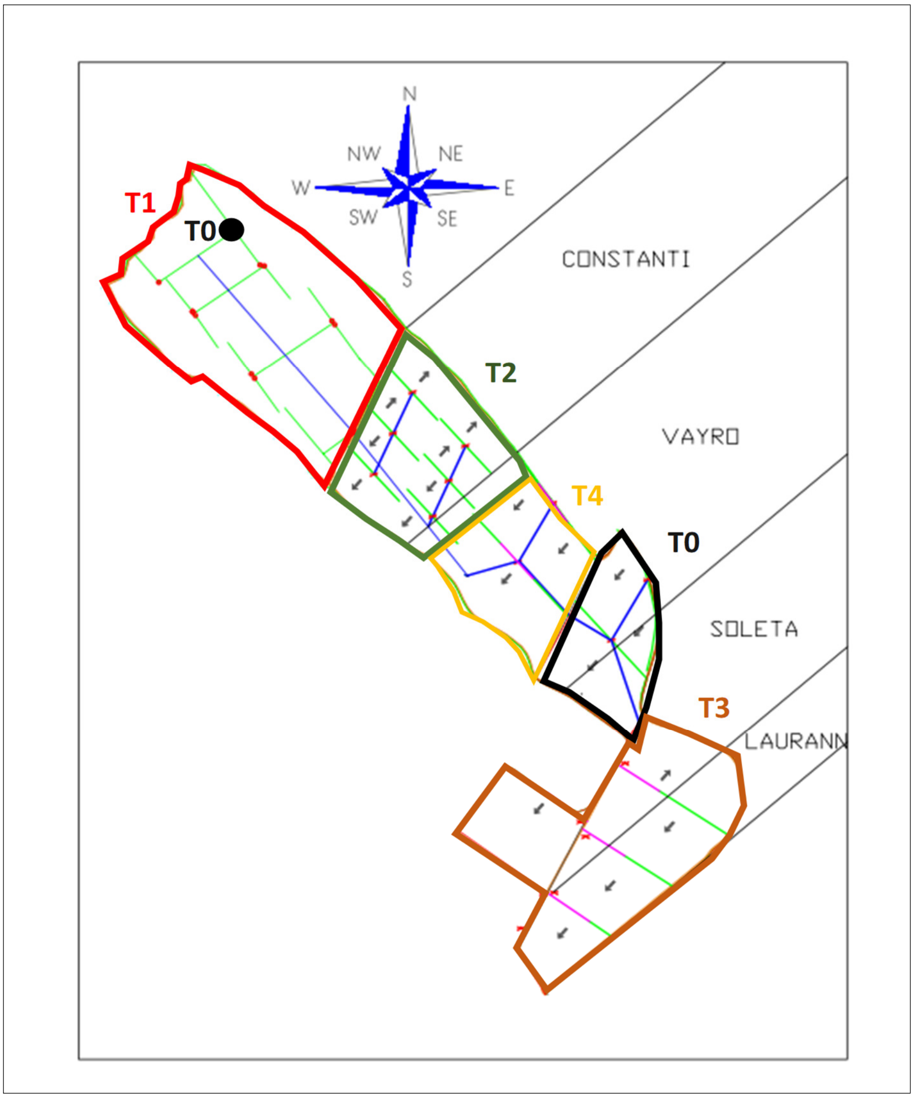


Image 16 Herdade do Charqueirão Farm Almond varieties location

1.1. Applications

Dose, timing and mode of application of each treatment are described in Table 16. The schedule and doses described in table 15 will be done in the entire project.

The first biostimulant IB-NP was applied on July 30th in a dose of 1 kg/ha. According to the protocol ProCrop ISR should be applied on early July, however due to a problem with fertirrigation system in the farm was applied on September 18 as shown in Table 16.

Table 16 Treatments; products, dose and date on Herdade do Charqueirão Farm

Treatment	Products	Dose	Date
T0	Control (Conventional methods)		
T1	IBNP	1 kg/ha	30/07/2021
	Procrop ISR	0.5 l/ha/week	13/08/2021
T2	Deficit irrigation		
T3	Cover crops		2020
T3.1	Plant Cover Mixture	30 kg/ha	2020
T3.2	Farm Cover Crop	-	2020
T3.3	Tilling	-	2020
T4	T1+T2+T3	IBNP (1 kg/ha) Procrop ISR (2 l/ha/week)	(MST-NP) 30/07/2021 Procrop ISR 13/08/2021

2. Results

I. Tree Health

I.1. Nutrient state through foliar analysis

As described on A1 action, crop nutrient state was determined through foliar analysis. In 2021 season a problem with fertirrigation system delayed T1 and T4 bioestimulant application. Although the treatments were applied later after almond harvest, the delay would prevent seeing the results of the treatments in the foliar nutrient content of the leaves. For this reason, foliar analyzes were not carried out in the 2021 campaign. However foliar nutrient content will be determined in 2022 when almond new leaves budding. They could be carried out later in April or May 2022 in order to determine the effect of the treatments on the nutrient content of almond leaves.

I.2. Tree temperature

Image 17 shows the temperature of the crop cover (once the soil has been removed) in pre-harvest in season 2021. In general terms the results of the thermal flight present a low variability in the tree canopy temperature recorded by the thermal sensor, between 29.02 ºC and 35.64 ºC. The spatial distribution of temperature shows the lowest values in the center of the farm, with the temperature increasing as we move towards the extreme north and south. Table 17 shows the mean temperature results for each of the treatments.

The treatment that registered the highest average temperature was the control treatment T0 cv. Vayro, with 32.82 ºC. On the other hand, the dispersion of the results obtained in this treatment was the lowest recorded, with 1.73 ºC, indicating that the temperature variability within the same treatment was the lowest. These results indicate that crop management by the farmer presents the greatest water stress on the farm.

The T2 treatment showed the lowest average temperature, with 31.87 ºC. However, it showed one of the greatest variability in temperature, only surpassed by the T0 cv Constantí (located above treatment T1). Regarding the spatial variability of temperature within a treatment (Image 17), it can be seen how T2 is the most homogeneous.

The treatment T1, T3 and T4 presented very similar temperature values to the control treatment T0 cv. Vayro, as far as average, minimums and maximums are concerned. Regarding the spatial variability of temperature, T3 and T4 seem to present spatial patterns of behavior. Treatment T3 shows a slight pattern of change in the middle that runs through the surface from East to West, with a lower temperature being observed on the south side of the farm. It also shows a low temperature zone with very straight cuts, at the lower end of the treatment. The T4 treatment presents a temperature behavior similar to the variability of the water retention capacity (AWC) resulting from the preliminary study of the farm's soil carried out in 2018. On the other hand, T1 showed a more diffuse temperature variability, in which a higher temperature is observed on its western side, which corresponds to the Vayro variety

Table 17 Herdade do Charqueirão Farm Tree temperature (Cº) pre harvest

	Tree temperature Pre-Harvest 2021 (ºC)			
Treatment	**Mean**	**Desv. St.**	**Min**	**Max**
T0_Constanti	32.41	2.15	30.97	34.30
T0_Vayro	32.82	1.73	30.83	35.59
T1	32.69	1.81	29.07	35.45
T2	31.87	2.11	29.48	35.03
T3	32.46	1.88	30.79	34.79
T4	32.27	2.02	30.78	35.63

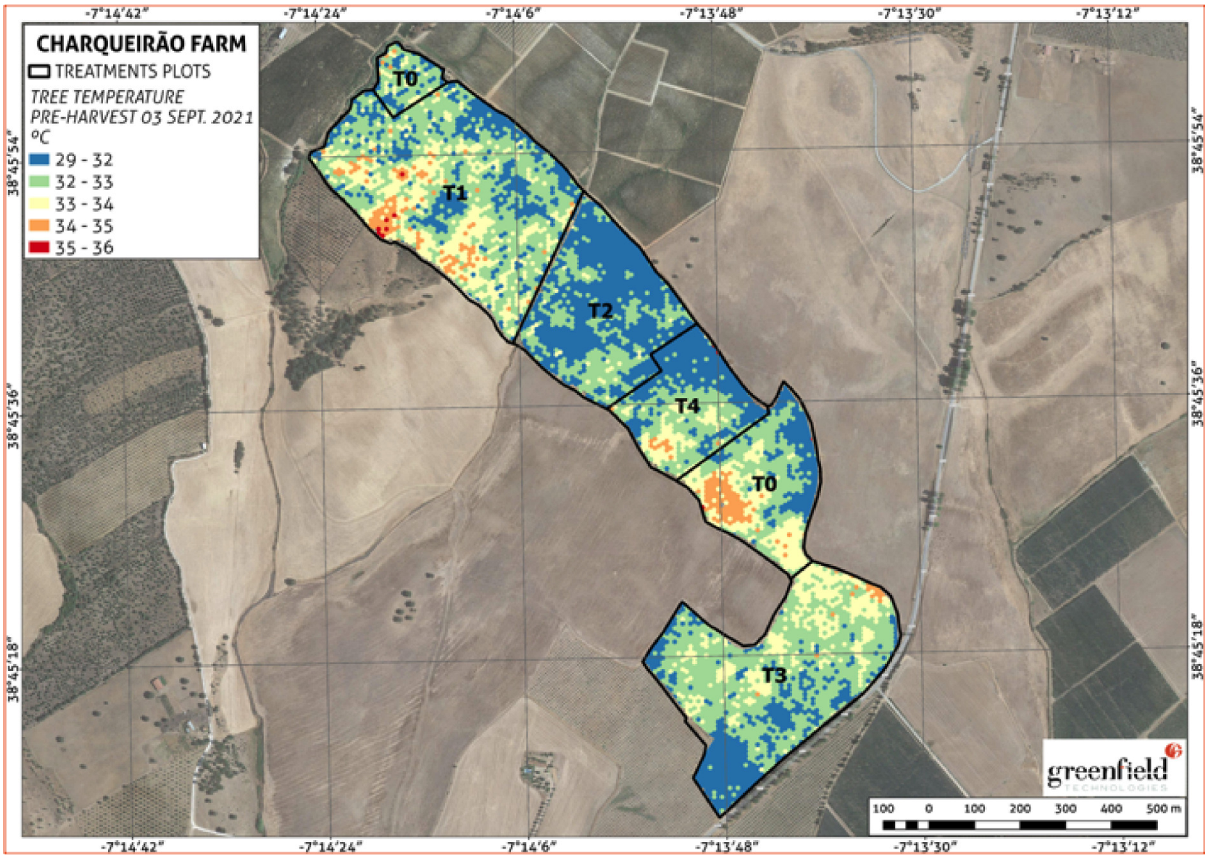


Image 17 Tree temperature spatial variability on pre-harvest Herdade do Charqueirão Farm

I.3. Vegetative development

Image 19 to Image 28 shows the stages of vegetative development and moisture content in different phenological phases of the 2021 season. In general terms, a reduction in vegetative development and moisture content is observed as the campaign went on, being more pronounced in the upper half of the farm, where the Constantí and Vayro varieties are found, as well as the T0 cv Constanti, T1 and T2 treatments. At the end of the season, in the post-harvest stage, the vegetative development by the NDVI index, manages to rise slightly, while the moisture content by the NDWI index, continues to decrease. This result seems to indicate that the vegetation between the tree rows may be beginning to develop and influence the pixel value of the Sentinel 2 satellite.

Image 19 shows the intra-plot spatial variability of the state of vegetative development and moisture content at the time of post-flowering through the NDVI and NDWI vegetation indices, respectively. It is worth noting the high relative value of the indices in the upper half of treatment T3, where the Soleta variety occurs, and a rectangular area with a high NDWI value in treatment T0. Straight cuts in indices are often supported by crop management actions.   Image 20 shows the same parameters at the interplot level, where a behavior pattern of both indexes is observed from the north of the farm to the south, where there is a gradual increase. In this pattern of variability, the treatments T0 cv. Constanti, T1 and part of the T2 treatment showed a vegetative development and a lower moisture content than the rest of the farm, while the T3 treatment showed the highest moisture content.

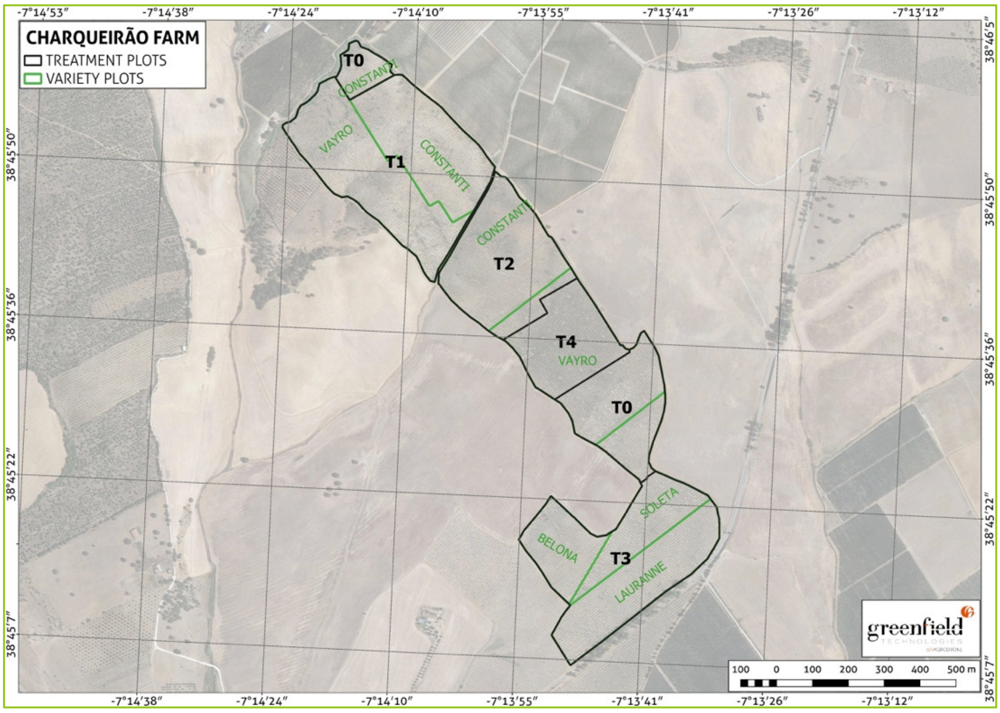


Image 18 Treatments and almond varieties in Herdade do Charqueirão Farm

Image 19 Post flowering Vegetative development relative spatial variability Herdade do Charqueirão Farm

Image 20 Post flowering Vegetative development absolute spatial variability Herdade do Charqueirão Farm

Image 21 and Image 22 shows the intraplot and interplot variability of pit hardening phenological stage. Through these satellite images, the general trend of greater vegetative vigor and higher moisture content in the T0 cv Vayro, T2, T3 and T4 treatment continues to be observed, against the treatments T0 cv. Constanti and T1. Regarding the intraplot variability, it is worth highlighting the change of the Belona variety in treatment T3, becoming the one with the most humidity of the three varieties present in the treatment. The Soleta and Laureanne varieties of T3 treatment appear more homogeneous in terms of vegetation indices. Regarding the interplot variability, lower NDVI and NDWI values are still observed on the surface in the T0 cv. Constanti and T1, while T0 cv. Vayro, T2 and T3 maintain similar mean values. For its part, it is in this phenological stage where the T4 treatment shows the trend of higher values of vegetative development and moisture content of the entire farm.

Image 21 Pit hardening relative Spatial variability on vegetative development in Herdade do Charqueirão Farm

Image 22 Pit hardening absolute Spatial variability on vegetative development in Herdade do Charqueirão Farm

Image 23 and Image 24 show the intraplot and interplot variability, in the phenological stage of ripening. The influence of the climatological parameters typical of the summer period in the southwest of the peninsula causes the surface to increase with values that tend to fall in terms of vegetative development and moisture content. Two well-differentiated areas of the farm are found in this period, where T0 cv Costanti, T1 T2, showing lower values than T0 cv. Vairo, T3 and T4. This behavior supposes the division of the farm in terms of the behavior shown by the indices. This fact may be due to the fact that the upper half of the farm is largely made up of the Costanti variety, whose maturation period is longer as it has a later harvest than the rest of the varieties present on the farm. However, the Vayro variety, which has an earlier harvest, has a different behavior between that present in treatment T1 and that present in adjacent treatments T0 and T4.

Image 23 Ripening relative spatial variability Herdade do Charqueirão Farm

Image 24 Ripening absolute spatial variability Herdade do Charqueirão Farm

Image 25 and Image 26 show the intra-plot and inter-plot variability, respectively, of the state of the crop in the pre-harvest phenological period. At this phenological moment, satellite data shows a slight reduction in crop activity (NDVI) and moisture content (NDWI).

Image 25 Pre harvest relative spatial variability Herdade do Charqueirão Farm

Image 27 and Image 28 show the intraplot and interplot variability, respectively, in the postharvest phenological stage. In this stage, all treatments raise the value of the NDVI index, being more pronounced in treatments T2 and T3. There is a possibility that values are influenced by spontaneous vegetation between the tree rows, as it is an image from the beginning of October. However, the moisture content continues to decrease throughout the farm, with low values extending towards the T3 treatment, up to the division line of the change from Soleta to Laureanne variety. In treatment T1 the lowest humidity values are observed, while the rest of the treatments show average values and in a homogeneous way.

Image 26 Pre harvest absolute spatial variability Herdade do Charqueirão Farm

Image 27 Post harvest relative spatial variability Herdade do Charqueirão Farm

Image 28 Post harvest absolute spatial variability Herdade do Charqueirão Farm

II. Soil quality

II.1. Available Water Capacity (AWC)

Available water capacity was measured at the beginning of the project, before treatments application, and measured again at the end of the project. The results of the soil analyzes carried out at the Herdade do Charqueirao farm show a general increase in the average content of organic matter in the different treatments, being the majority in treatments T1 and T3. These increases have caused increases in the water retention capacity of around 10 mm of water per meter of soil depth. That is, an increase of 0.75% of organic matter in treatment T1 and an increase of 0.73% in T3 have increased the retention capacity by 10 liters per 1000 liters of soil volume.

The rest of the treatments have also increased the organic matter content, but they have not brought about significant changes in the AWC.

Table 18 Evolution of AWC in Charqueirao Farm (2018 – 2021)

Treatment	T0	**T1**	T2	**T3**	T4
Mean AWC (mm/m) 2018	140	140	150	130	150
Mean Organic Matter (%) 2018	1.6	1.55	1.24	1.47	1.76
Mean AWC (mm/m) 2021	140	**150**	150	**140**	150
Mean Organic Matter (%) 2021	1.9	**2.3**	2.1	**2.2**	1.8

II.2. Soil microbiological activity

Soil samples were taken from georeferenced points (Image 29) and analyzed in laboratory to determinate fertility biological index and enzymatic activity such as, B-glycosidase, dehydrogenase, catalase, phosphodiesterase (phosphatase) and urease activities.

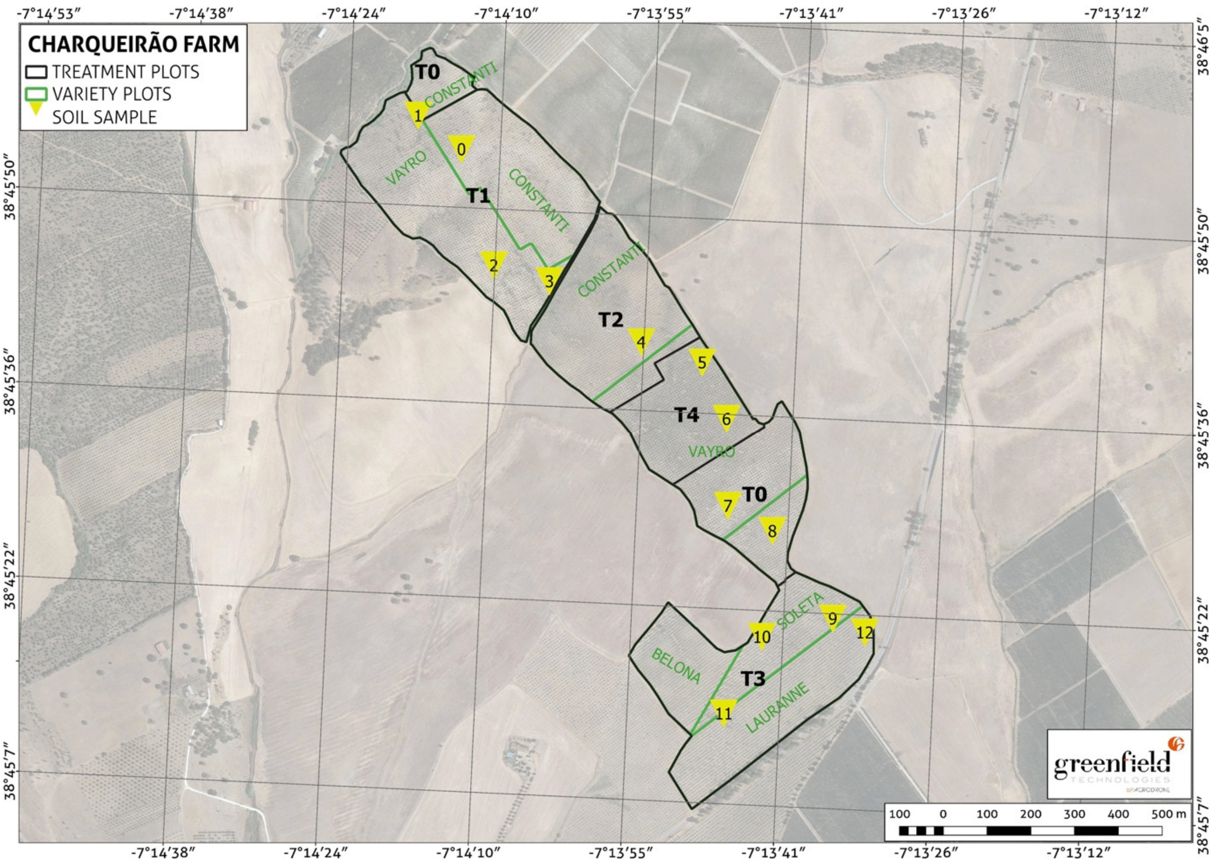


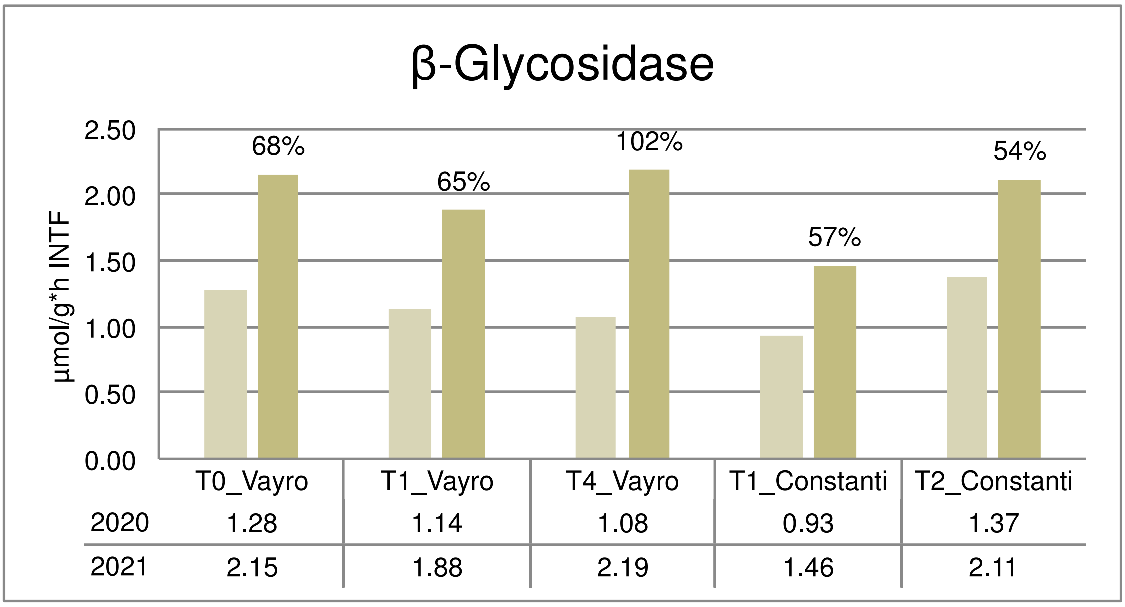
Image 29 Herdade do Charqueirão Farm soil samples point

Table 19 Soil Chemical analysis

	T0_Vayro	**T1_Vayro**	**T4_Vayro**	**T1_Constanti**	**T2_Constanti**
E.C. (dS/m) (extract 1:5)	0.3	0.3	0.3	0.2	0.2
Macronutrients					
%N Total	0.1	0.2	0.1	0.2	0.1
NO_3^- (mg/kg)	59.9	**65.6**	36.6	54.2	34.4
P (ppm)	137.0	126.4	123.9	**212.0**	28.8
K (meq/100 g)	0.6	0.3	**0.9**	0.6	0.3
Ca (meq/100 g)	12.0	7.0	**17.5**	7.0	9.4
Mg (meq/100 g)	**7.5**	3.2	5.2	2.8	5.8
SO_4^{-2} (meq/100g)	0.1	0.3	0.1	0.1	0.1
Micronutrients					
Fe (ppm)	11.1	13.6	7.0	**26.8**	12.1
Mn (ppm)	11.5	12.9	8.4	6.8	**27.4**
B (ppm)	0.9	0.7	**1.1**	0.8	0.6
Cu (ppm)	6.2	6.5	4.8	**7.2**	3.6
Zn (ppm)	1.2	4.3	1.9	**6.4**	0.5
Cl^- (meq/100 g)	0.4	0.7	0.4	0.3	0.4
Oligoelements					
Na (meq/100 g)	0.6	0.9	0.6	0.6	0.4
Organics					
% O.M. Oxidable	1.9	**2.3**	2.1	**2.2**	1.8
%C Total	1.5	**1.8**	1.6	1.6	1.3
C/N	10.0	10.7	10.6	8.9	**11.3**
Carbonates					
% $CaCO_3$ Total	5.8	5.9	**7.7**	6.4	6.4
% $CaCO_3$ active	<1.00	<1.00	<1.00	<1.00	<1.00

About macronutrients a slight increment in NO_3 content was observed in T1 Vayro. Regarding phosphorus

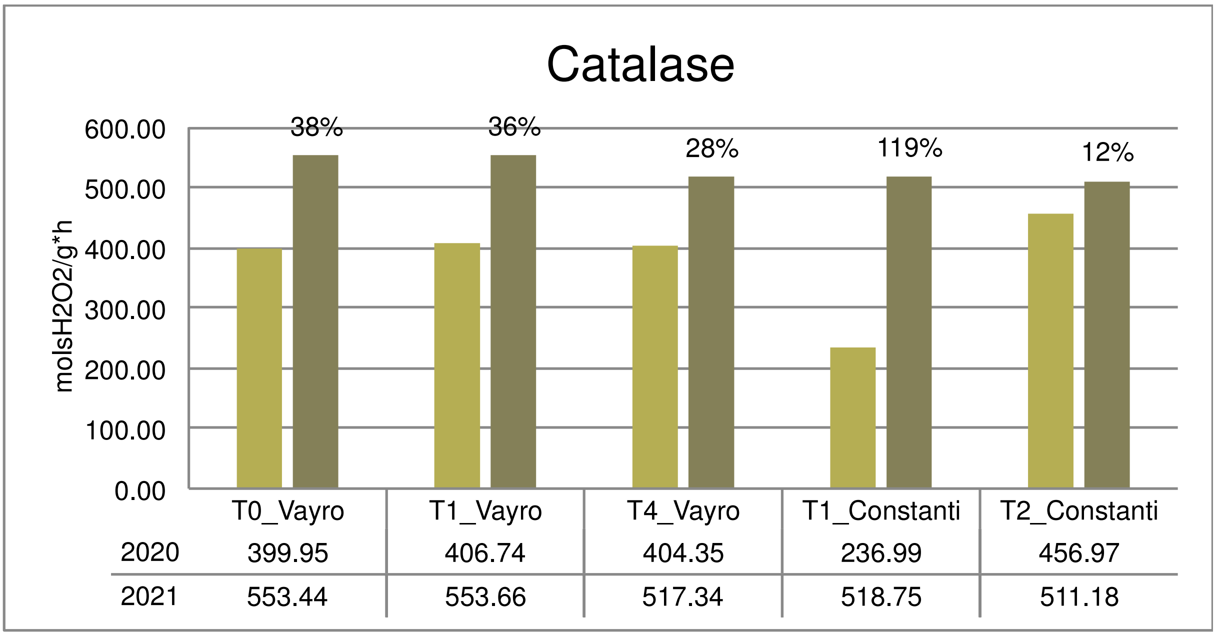
ß-glycosidase is related to soil organic carbon content and organic matter degradation. What it is mean a contribution of micronutrients to the crop. An important increment is observed in ß-glycosidase activity in 2021 respect to 2020 in T0, T1 and T4 Vayro treatments and T1 and T2 Constanti (Graph 11).



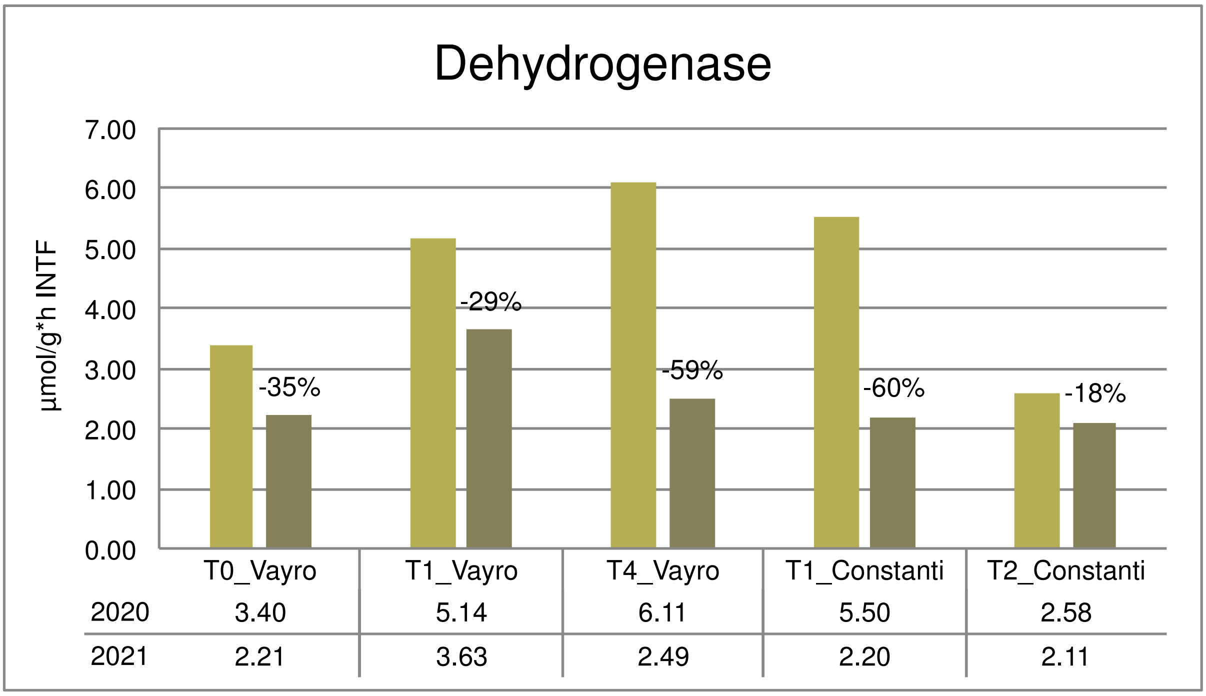
Graph 11 Herdade do Charqueirão Farm ß-Glycosidase activity

Catalase enzyme activity increased generally in all treatments. The increment was more pronounced in Constanti variety, where a increment of 119% was observed in treatment T1 in comparison to previous year. With respect to the Vayro variety, a very homogenous response was observed in al the treatments were an increment of approximately 30% was observed including control treatment (Graph 12), this seems to indicate that the increment in the activity of the enzyme was not due to the treatments.

Dehydrogenase activity was markedly reduced in the all the treatments respect to previous year in both varieties (Graph 13).

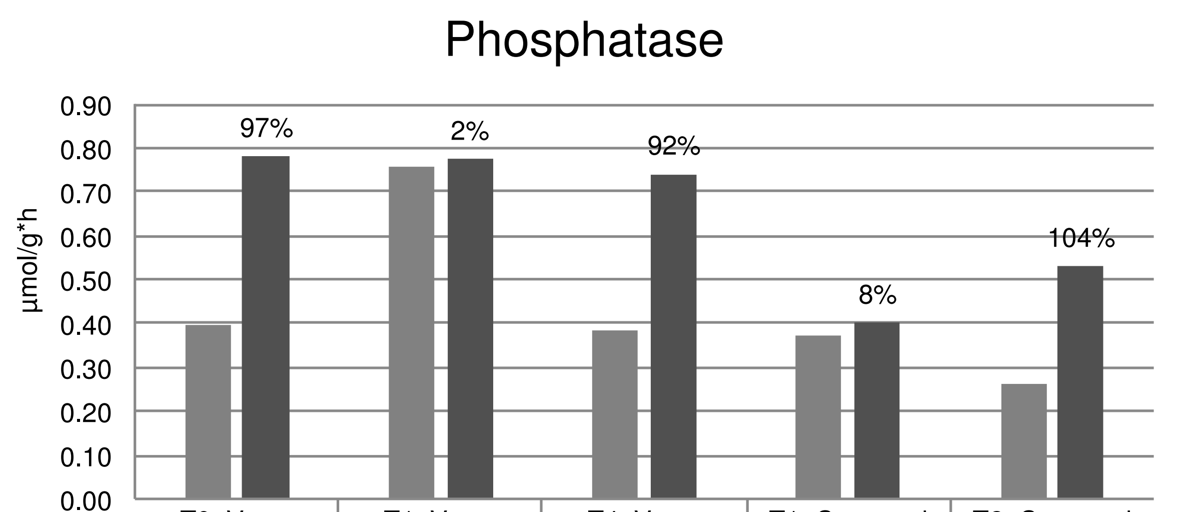


Graph 12 Herdade do Charqueirão Farm catalase activity



Graph 13 Herdade do Charqueirão Farm dehydrogenase activity

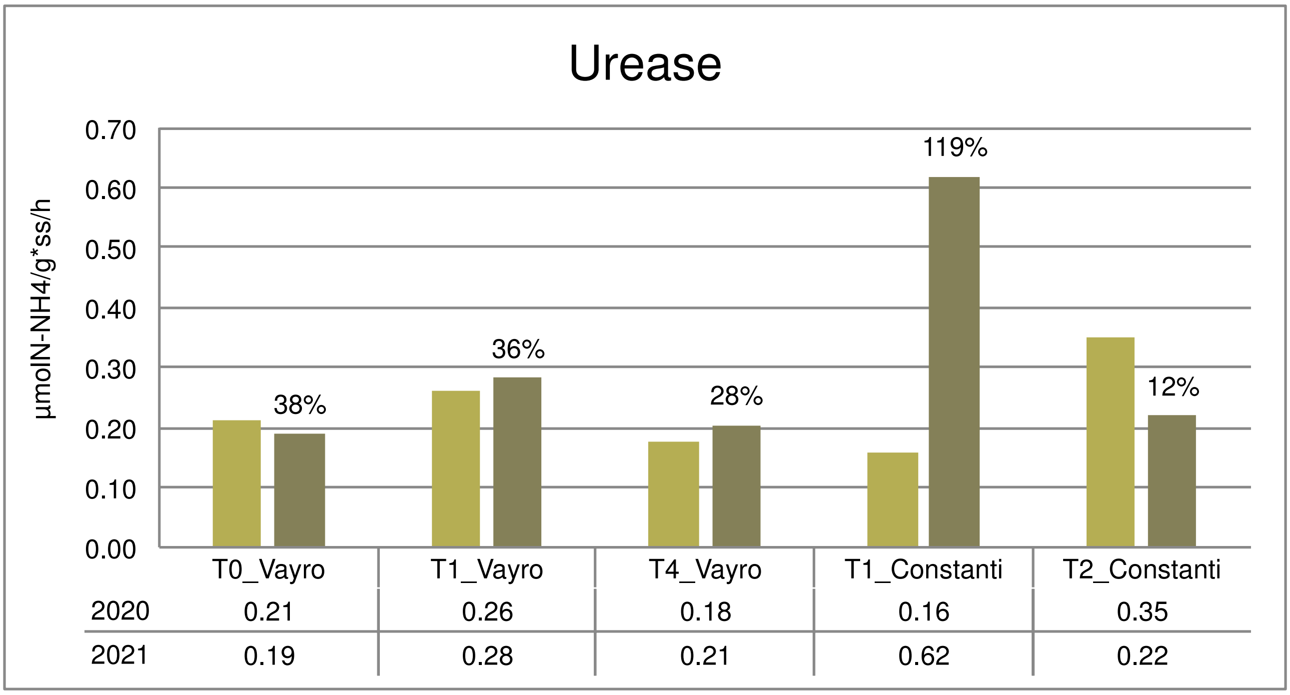
The phosphatase activity was considerably increased in the T4 treatment in the Vayro variety and in T2 Constanti (Graph 14).



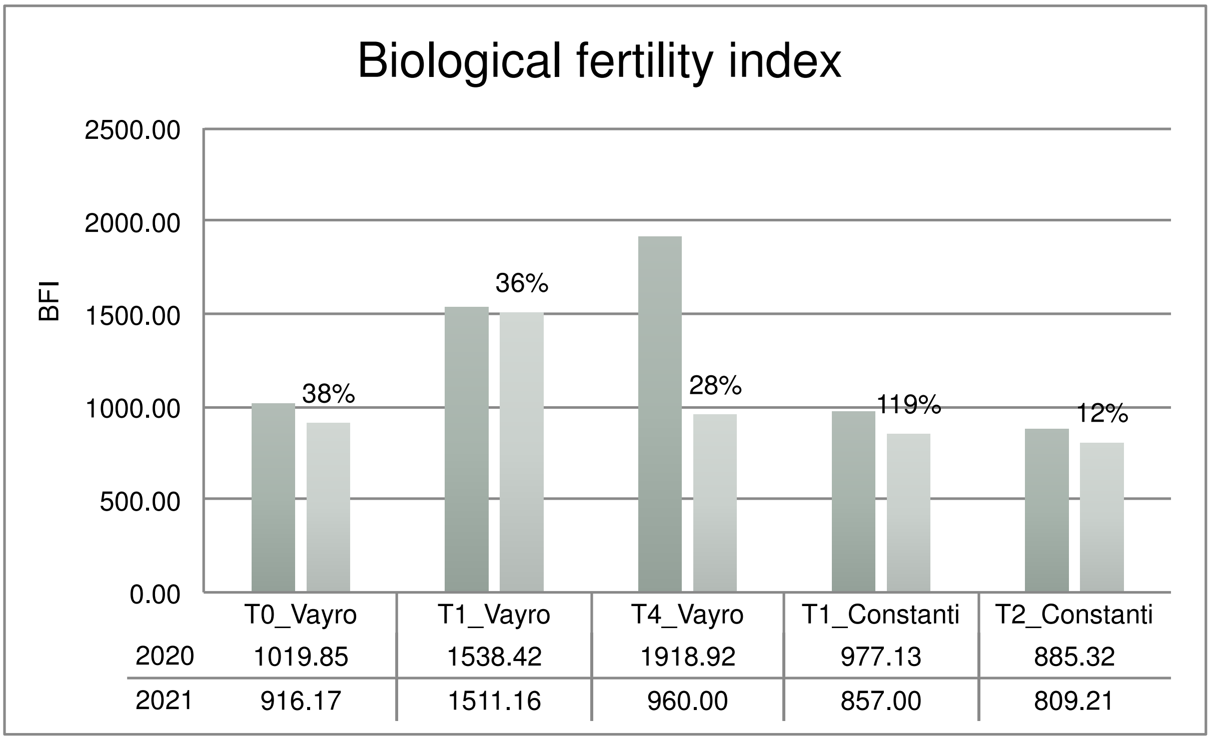
	T0_Vayro	T1_Vayro	T4_Vayro	T1_Constanti	T2_Constanti
2020	0.40	0.76	0.39	0.37	0.26
2021	0.78	0.77	0.74	0.40	0.53

Graph 14 Herdade do Charqueirão Farm Phosphatase activity

The urease activity experienced a considerable increase in all treatments regardless of the variety (Graph 15).



Graph 15 Herdade do Charqueirão Farm Urease activity



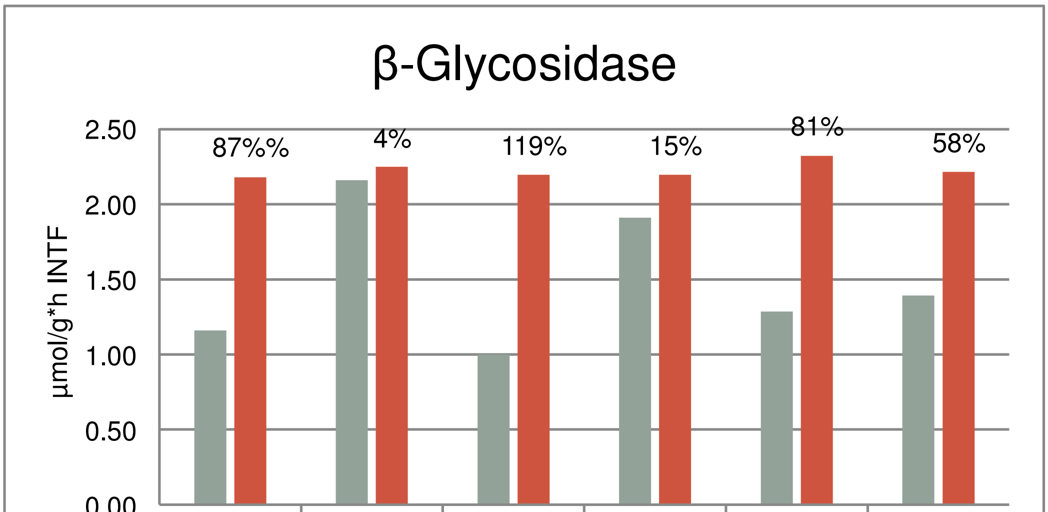
Graph 16 Herdade do Charqueirão Farm biological fertility index

T4 treatment include an application of bioestimulants in a similar way than in T1, also a deficit irrigation period and cover crops that were sowed on 2021 (Table 16). In order to be able to contrast the effect of the plant cover crops on soil biological and microbiological activity, soil samples were taken from each of the cover crops and analysed separately at Herdade do Charqueirão Farm (Image 30).

Image 30 Cover crops locations in Herdade do Charqueirão Farm

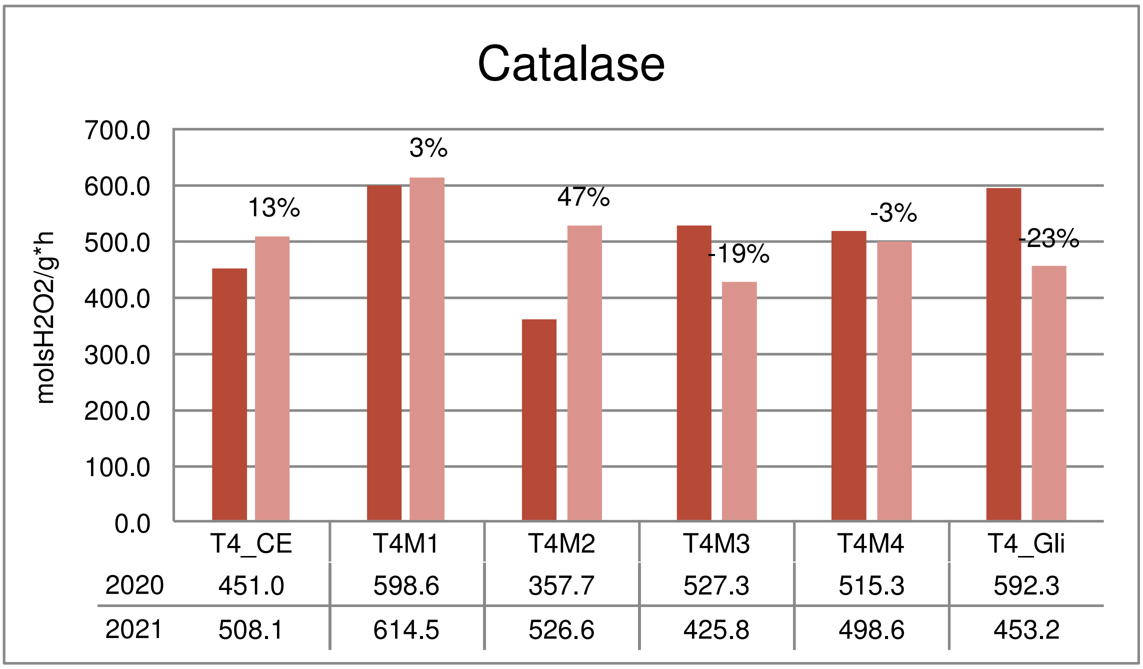
ß-glycosidase is related to soil organic carbon content and organic matter degradation. What it is mean a contribution of micronutrients to the crop. Respect to the activity of the ß-glycosidase enzyme, a higher enzymatic activity was observed in treatment cover crop called mix 1(T4-M1) indicating a greater metabolic activity in that treatment (Graph 17).

In the case of catalase activity, a higher activity of this enzyme was observed in the M1 cover again, the cover crop with the lowest activity of this enzyme was T4 M2 (Graph 18).



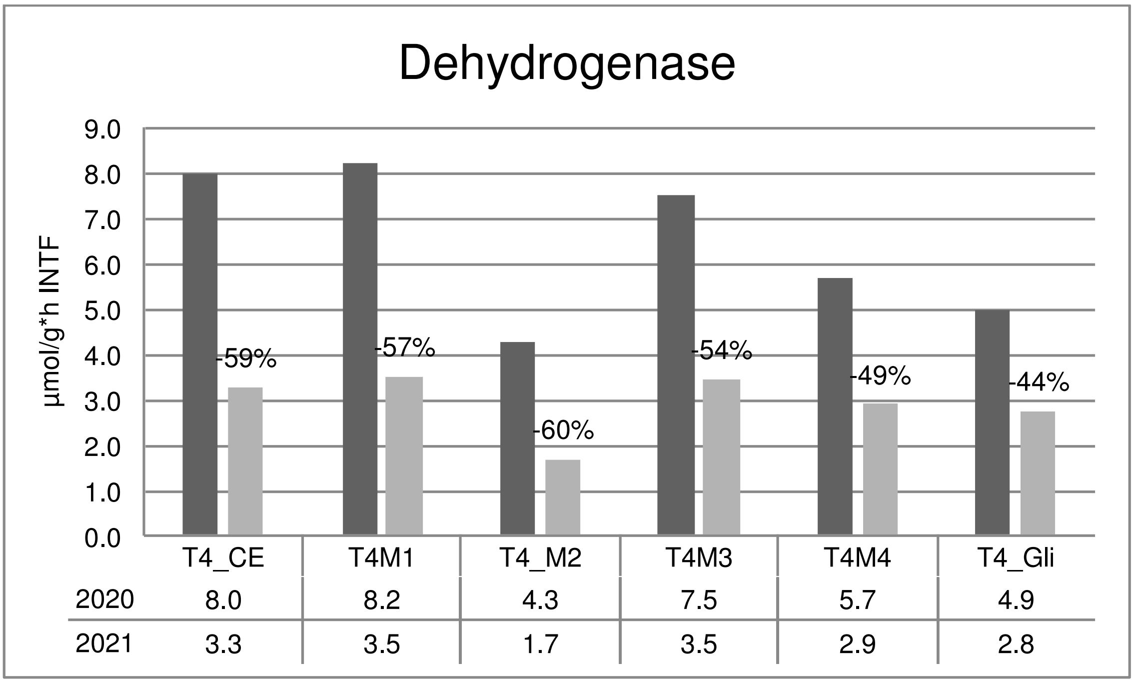
	T4_CE	T4M1	T4M2	T4M3	T4M4	T4_Gli
2020	1.16	2.16	1.00	1.91	1.28	1.40
2021	2.18	2.24	2.19	2.19	2.32	2.21

Graph 17 ß-glycosidase activity in each cover crop T4 treatment Herdade do Charqueirão Farm

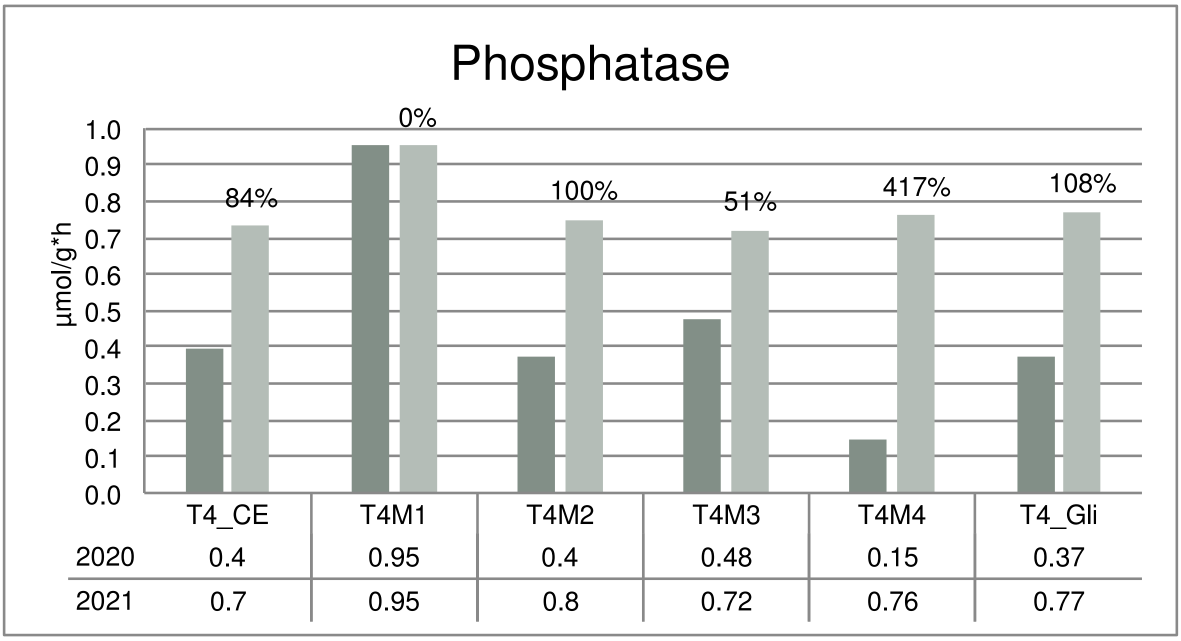


Graph 18 Catalase activity in each cover crop T4 treatment Herdade do Charqueirão Farm

On the one hand, dehydrogenase activity was quite similar in the spontaneous cover crop (T4-CE) and the T4-M1 cover crop. On the other hand, cover crop that presented the lowest dehydrogenase activity was again mix 2 (T4-M2; Graph 19). Regarding to phosphatase activity a higher activity of this enzyme shows a mobilization of phosphorus in inorganic form in the soil and a greater availability of this element to the plant. Phosphatase activity increased again in the M1 cover, being very low in the M4 cover (Graph 20).



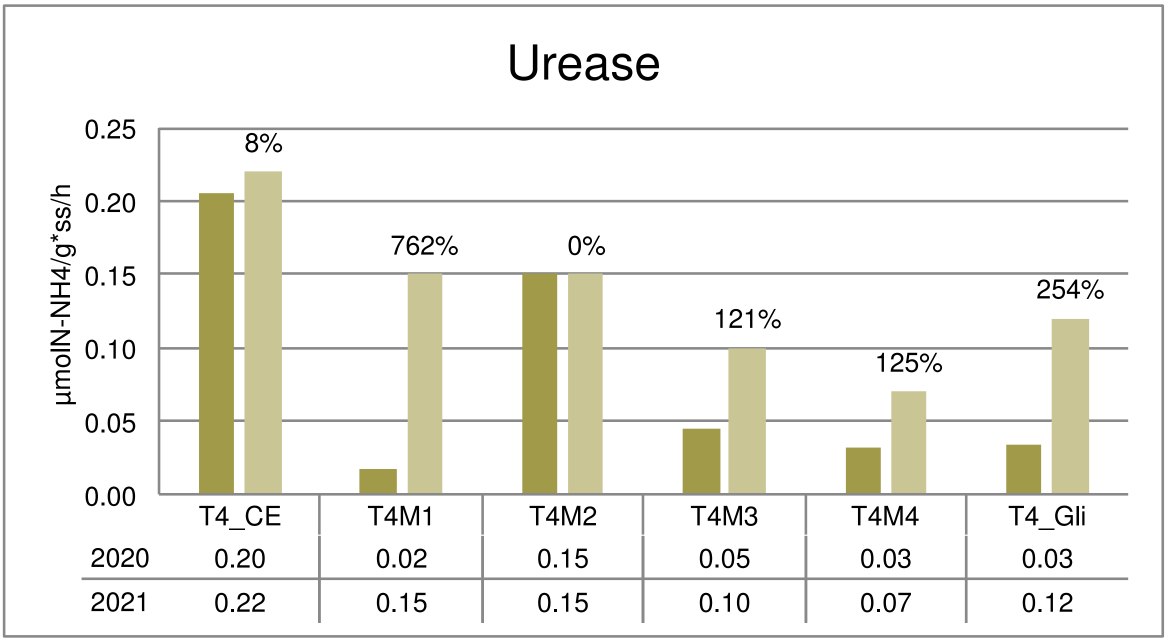
Graph 19 Dehydrogenase activity in each cover crop T4 treatment Herdade do Charqueirão Farm



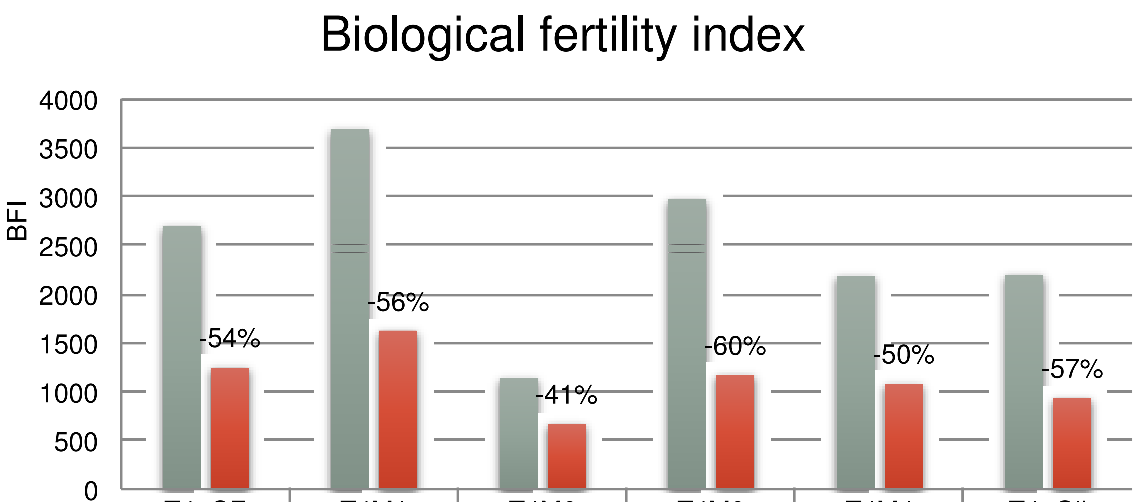
Graph 20 Phosphatase activity in each cover crop T4 treatment Herdade do Charqueirão Farm

Urease activity indicates urea transformation to ammonia by soil microorganism. Urease activity was higher in the spontaneous cover (T4-CE) and in the M2 (T4-M2). The lowest urease activity was observed in mix M1 (T4-M1; Graph 21).

Regarding biological fertility index, this was increased in the case of M1 cover crop (T4-M1; Graph 22) in comparison to remaining treatments.



Graph 21 Urease activity in each cover crop T4 treatment Herdade do Charqueirão Farm



	T4_CE	T4M1	T4M2	T4M3	T4M4	T4_Gli
2020	2698	3692	1140	2972	2190	2198
2021	1250	1623	670	1175	1086	937

Graph 22 Biological fertility index in each cover crop T4 treatment Herdade do Charqueirão Farm

III. Disease prevalence

III.1. *Xylella fastidiosa* disease control

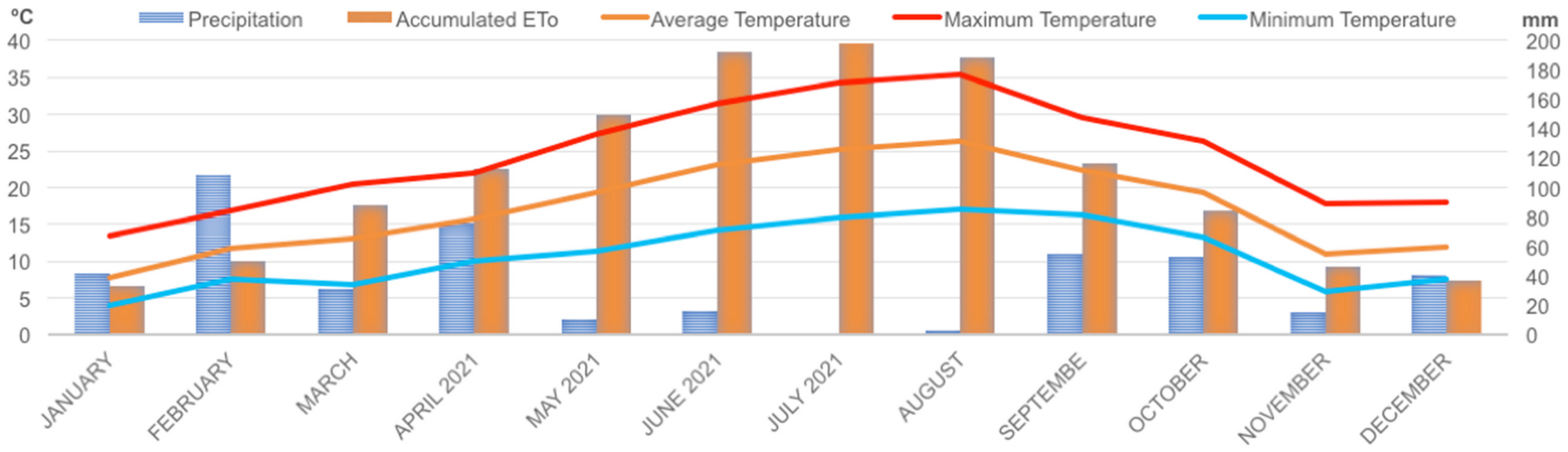
Plant tissues will be sampled at a specific time or stage of development following the protocol of the laboratory (refer to Guidelines for the survey of *Xf* in the Union territory, European Commission, 2015) in case of alarm about XF will be detected.

IV. Weather

IV.1. Weather conditions

Annual information was collected from darksky including: Temperature Maximum (ºC), Average Wind Speed (m/s), Temperature Time Maximum, Middle Wind Direction (º), Temperature Minimum (ºC), Radiation (MJ/m^2), Temperature Time Minimum, Precipitation (mm), Temperature Average (ºC), ET$_0$ (mm/day), Maximum Relative

WEATHER PARAMETERS OF HERDADE DO CHARQUEIRAO FARM SITE												
	Maximum Temperature	Average Temperature	Minimum Temperature	Average Wind Speed	Average Wind Bearing	Precipitation	Accumulated Precipitation	Maximum Relative Humidity	Minimum Relative Humidity	Average Relative Humidity	ETo	Accumulated ETo
	ºC	ºC	ºC	m/s		mm	mm			%	mm/day	mm
JANUARY 2021	13.2	7.8	3.8	3.3	South	41.9	41.9	88.2	65.3	78.5	1.1	33.0
FEBRUARY 2021	16.8	11.7	7.6	3.0	South	109.0	150.9	92.0	67.3	80.6	1.7	50.5
MARCH 2021	20.3	13.0	6.8	2.8	SouthEast	31.1	182.0	80.7	48.3	65.9	2.9	88.4
APRIL 2021	21.9	15.6	10.0	2.5	South	76.0	258.0	83.3	49.6	67.3	3.6	112.5
MAY 2021	27.0	19.3	11.3	3.2	South	10.0	268.1	78.7	34.6	55.4	5.2	149.6
JUNE 2021	31.3	23.1	14.2	2.9	SouthEast	16.4	284.4	84.2	16.5	47.5	6.2	192.1
JULY 2021	34.2	25.2	15.9	3.5	SoutWest	0.0	284.4	82.2	13.3	44.0	6.6	198.2
AUGUST 2021	35.3	26.3	17.0	2.9	SouthWest	3.3	287.7	80.3	12.7	42.7	6.1	189.1
SEPTEMBER 2021	29.3	22.4	16.2	2.8	West	54.7	342.4	86.1	26.8	57.1	3.9	116.6
OCTOBER 2021	26.2	19.2	13.2	2.8	West	53.5	395.9	84.1	31.4	59.3	2.7	84.2
NOVEMBER 2021	17.7	10.9	5.9	2.9	West	15.2	411.1	87.8	39.2	67.9	1.5	46.5
DECEMBER 2021	17.9	11.9	7.6	3.2	SouthWest	41.1	452.2	93.2	54.5	77.9	1.2	37.3



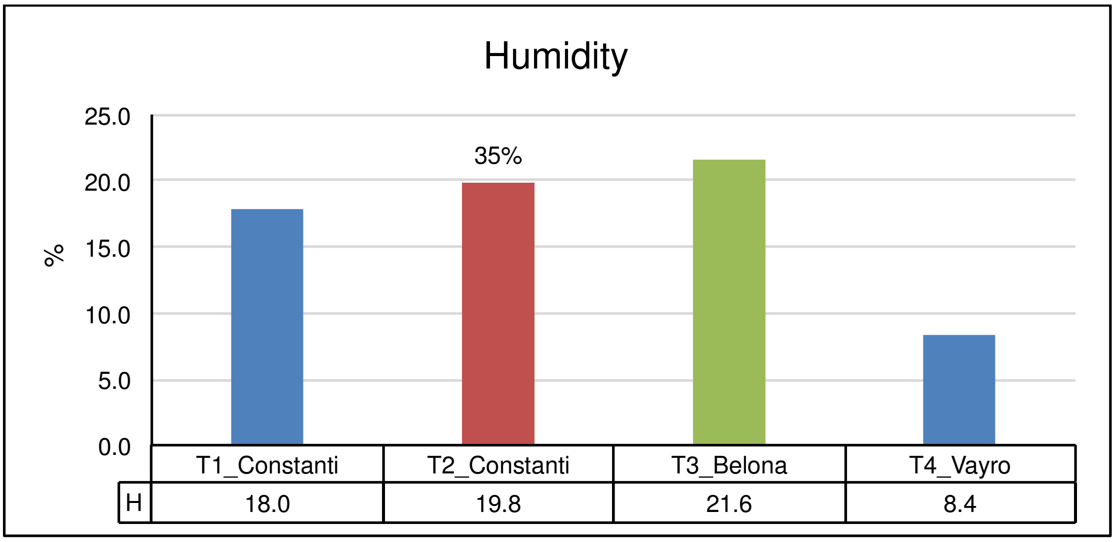
Humidity (%), Minimum Relative Humidity (%), Accumulated Precipitation (mm), Relative Medium Humidity (%), and Cumulative ET_0 (mm).

Regarding the climatic data recorded, no extreme meteorological phenomena have been observed that may have affected the treatments applied on Herdade do Charqueirão farm.

V. Quality

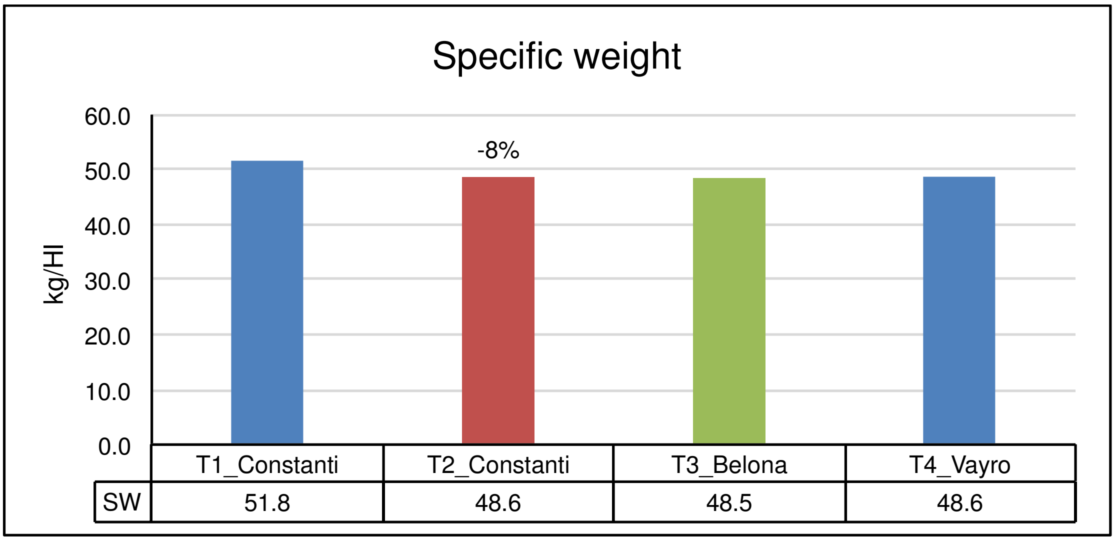
V.1. Humidity, crude fat specific weight

Regarding the quality of the almonds obtained in each of the treatments, no twins kernels was detected. Moreover, humidity parameter was subjected to analysis. The treatment with less humidity content was T4 Vayro cv having just 8% of humidity. However, it is observed that the T2 treatment increased the humidity of the fruits obtained by 35% respect to T1 treatment same cv, while the T3 treatment increased the humidity similarly than T2 treatment (Graph 23).



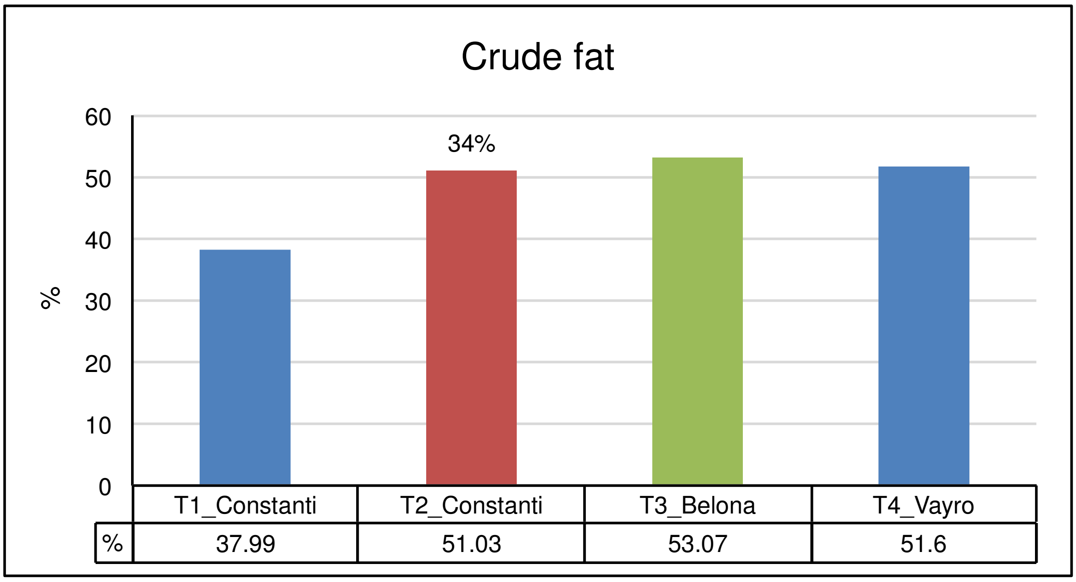
Graph 23 Almond humidity percentage cv. Constanti, Belona and Vayro

Regarding the specific weight of the seeds obtained, a slight increment was observed in T1 treatment respect to T2 treatment. While the T2 treatment, subjected to a hydric deficit, a slight decreased almond specific weight by 8%. A similar decrement was observed in T3 Belona variety and in T4 Vayro cv.



Graph 24 Specific weight Cv Constanti, Belona and Vayro

Regarding crude fat, an increment in T2 treatment by 34% was observed respect to T1 treatment on cv Constanti. In Belona variety similar T2 crude fat content was observed. T4 treatment in which deficit irrigation and biostimulants were applied has a similar response to T2 treatment having very similar crude fat content.



Graph 25 Crude fat Cv Constanti, Belona and Vayro

Respect to mycotoxins in most of the treatments they were below the detection limit (<LD). In the cases in which mycotoxins were detected, all were below the limit allowed in Spain for nuts (Table 20).

Table 20 Mycotoxins content per treatment

Treatment	AFLA B1	DON	T2	FUMONISINA	ZEA
T1_Constanti	<LD	<LD	<LD	<LD	<LD
T2_Constanti	<LD	<LD	<LD	<LD	<LD
T4_Vayro	<LD	<LD	5.2	<LD	<LD
T3_Belona	0.98	513	10.4	<LD	<LD

VI. Water use

VI.1. Water use efficiency (WUE)

The WUE is the relation between water consumed (m^3) by the crop and water applied (m^3). Both values were calculated each year. In Table 21WUE values are listed. In T2 and T4 treatments deficit irrigation was applied and WUE was increased as expected. On the other hand, in T0 and T1 had the same irrigation and WUE.

Table 21 Charqueirao Farm WUE per treatment

	T0	T1	T2	T3	T4
WUE	1.86	1.86	2.5	1.86	2.5

VI.2. Irrigation water productivity (IWP)

The IWP is the relation between annual yield (kg) and water applied (m^3). Both values were calculated each year. In Table 22 IWP 2021 are listed. T1 treatment was more productive because IWP was the highest. However, T2 has a less irrigation water application but harvest was very low this year. Almond crop experienced alternate bearing, which means that one year the harvest is high and next one harvest is low. This situation is also observed in avocado and olive trees. In conclusion, the result obtained in T2 treatment is not related with treatments applied.

Table 22 Charqueirao Farm IWP per treatment.

	T1	T2	T3	T4
IWP	1.5	0.18	-	-

VII. Carbon footprint

VII.1. CO_2 emitted during the agricultural processes

CO_2 emitted in each farm will be estimated by Carbon Footprint Assessment ISO/TS 14067:2013 at the end of the project.

VIII. Biodiversity

VIII.1. Auxiliary fauna

Nest hotels were installed in September 2020 evaluation and evaluation was done in 2021 autumn (September; Image 31). In this farm a problem has occurred, since the local administration cut the trees next to the road a lot of nest boxes were lost.

Image 31 Installation of bug hotels and nest boxes in Herdade do Charqueirão Farm

References

Chapter III. La Traversagna Farm

(Pisa, Toscana - Italy)

Chapter developed in collaboration with the External Agronomist: Ideagro

1. Treatments

On the table description of the standard procedures in each crop and the additional application depending on the treatment.

Action C2	T0 - control	T1	T3- Cover crops	T4 (combination T1-T2-T3)
	Traditional management	T1 - IBNP +ISR		
La Traversagna - IT	**1.Soil:** tillage (3 times/year) and herbicide (3 times/year). **2.Irrigation:** not irrigation **3.Pests:** Prays oleae, Bactrocera oleae, Palpita unionalis. Total treatments 5/year. **4.Diseases:** Spilocea oleagina, total treatments 3/year. **5.Nutrition:** Soil (19/year) and Foliar (8/year). **6.Pruning:** Formation pruning (1/year) and Top and bottom pruning (2/year) **7.Harvest:** Nov/Dic (1 year)	**1.Soil:** tillage (3 times/year), herbicide (3 times/year) and **IBNP 20/04/2021 + ISR (dose: 0.5 L/ha) 23/04/2021.** **2.Irrigation:** not irrigation **3.Pests:** Prays oleae, Bactrocera oleae, Palpita unionalis. Total treatments 5/year. **4.Diseases:** Spilocea oleagina, total treatments 3/year. **5.Nutrition:** Soil (19/year) and Foliar (8/year). **6.Pruning:** Formation pruning (1/year) and Top and bottom pruning (2/year) **7.Harvest:** Nov/Dic (1 year)	**1.Soil:** tillage (1/year), herbicide (2/year), **cover crop: sow (2021), and weed (1/year).** **2.Irrigation:** not irrigation **3.Pests:** Prays oleae, Bactrocera oleae, Palpita unionalis. Total treatments 5/year. **4.Diseases:** Spilocea oleagina, total treatments 3/year. **5.Nutrition:** Soil (19/year) and Foliar (8/year). **6.Pruning:** Formation pruning (1/year) and Top and bottom pruning (2/year) **7.Harvest:** Nov/Dic (1 year)	**1.Soil:** tillage (1/year), herbicide (2/year), **cover crop: sow (1/year), weed (1/year), IBNP 20/04/2021 and ISR 23/04/2021.** **2.Irrigation:** not irrigation **3.Pests:** Prays oleae, Bactrocera oleae, Palpita unionalis. Total treatments 5/year. **4.Diseases:** Spilocea oleagina, total treatments 3/year. **5.Nutrition:** Soil (19/year) and Foliar (8/year). **6.Pruning:** Formation pruning (1/year) and Top and bottom pruning (2/year) **7.Harvest**: Nov/Dic (1 year)

Image 32 La Traversagna farm treatments distribution

1.1 Applications

Treatments applications was done as described in the following table:

Treatments	Products	Dose	Date
T0	Control (Conventional methods)		
T1	MST-NP	1 kg/ha	20/04/2021
	Procrop ISR	2 l/ha	23/04/2021
T3	Cover crops		2021

T3.1	Plant Cover Mixture	30 kg/ha	2021
T3.2	Farm Cover Crop	-	2021
T3.3	Tilling	-	2021
T4	T1+T3	MST-NP (1 kg/ha) Procrop ISR (2 l/ha)	20/04/2021

2. Results

In A1 action (preparatory actions) the parameters of the project performance indicators were described and the descriptions of the methods were established. After that, in Action D1 each indicator was measured and evaluated. Indicators were evaluated visiting La Traversagna farm every week to verify the correct implementation of the treatments and its evolution. The results in the third season (2021) obtained are described below.

IX. Tree Health

I.1. Nutrient state through foliar analysis

Foliar analysis shows an increase in nitrogen content in treatment T1 with respect to the control treatment. Also, in the T3 treatment, the content of nitrogen increased with respect to the control treatment. However, a reduction in nitrogen content was observed in treatment T4 where the foliar content of elements such as, magnesium and cooper was increased, but to a greater extent compared to the increment observed in treatment T3 and T1 (Table 23).

Table 23 Foliar macroelements content La Traversagna Farm

Macroelements	T0	T1	T1 to T0	T3	T3 T0	T4	T4 T0
Nitrogen (N)	1.50	1.60	7%	1.60	7%	1.40	-7%
Phosphorus (P)	1577.50	1321.25	-16%	1051.46	-33%	1210.00	-23%
Potassium (K)	11.55	9.80	-15%	11.35	-2%	11.01	-5%
Magnesium (Mg)	1.18	1.00	-15%	0.99	-16%	1.22	4%

Table 24 Foliar microelements content La Traversagna Farm

Microelements	T0	T1	T1 to T0	T3	T3 T0	T4	T4 T0
Iron (Fe)	128.10	75.52	-41%	210.87	65%	114.70	-10%
Manganese (Mn)	21.40	19.42	-9%	26.30	23%	15.52	-27%
Zinc (Zn)	22.20	13.35	-40%	20.42	-8%	15.25	-31%
Cooper (Cu)	17.54	30.77	75%	18.15	3%	41.57	137%
Boron (B)	14.00	10.49	-25%	13.15	-6%	12.07	-14%

I.2. Tree temperature

Due to the pandemic situation generated by the Covid-19 virus, the drone flight could not be carried out in Italy. An attempt was made to subcontract the service but due to the aforementioned situation it was not possible either. As soon as the situation returns to normal, the pending flight will take place.

I.3. Vegetative development

In general terms, a reduction in vegetative development and moisture content is observed as the campaign progresses, whose values rise in the post-harvest period. In addition, the differences between the different areas of the farm are quite pronounced, whose NDVI and NDWI index values are very expressive, with fairly straight and rectangular boundaries. In this sense, the results of the processing of the satellite images seem to indicate the high incidence of vegetation covers between the streets on the value of the indices, both in absolute terms (interplot or inter-treatments) and in relative terms (intraplot vision or inter-treatments).

With few exceptions, the variability shown by the indexes corresponds to a greater extent to the treatment sets T0-T1 and T3-T4, where even the repetitions of these sets are shown independently of each other. For this reason, the treatments have been named (Image 34), assigning them to 8 easily distinguishable blocks in the plot, with the aim of referring to the different sets of repetitions in their analysis. In this way, it seems that other factors unrelated to the treatments have a great influence, masking the results of the project's treatments, even when the surface of each treatment is compared individually.

Image 33 Distribution of the treatments in La Taversagna Farm

Image 34 and Image 35 show the intra-plot and inter-plot variability in the phenological stage of flowering. Vegetation indexes show a high variability, both between treatments and within the plots themselves. The beginning of the campaign shows a highly variable treatment T0 and T1, where the repetitions of blocks B1 and B5 are very similar, which does not happen in blocks B4 and B8, where large changes are observed with straight delimitations. However, no clear differences are observed between treatments T0 and T1 within the same block. A similar behavior can be observed in the differences between treatments T3 and T4 of blocks B3 and B7 compared to Blocks B2 and B6. The higher value of the NDVI and NDWI indices of block B7 stands out.

Image 34 Vegetative development relative spatial variability flowering stage La Traversagna Farm

Image 35 Vegetative development absolute spatial variability flowering stage La Traversagna Farm

Image 36 and Image 37 show the intraplot and interplot variability of the state of the crop in the phenological stage of pit hardening. In general, the treatments increase the NDVI and NDWI values, homogenizing between them, although the high value of the B7 block treatments stands out. In treatments T0 and T1 of blocks B4 and B8 there is a marked decrease in the vegetation indices, indicating that vegetation has been eliminated from the plant covers or weeds between the streets. On the other hand, treatments T3 and T4 of block B2 also show two well-differentiated zones when intra-parcel variability is observed.

Image 36 Vegetative development relative spatial variability pit hardening stage La Traversagna Farm

Image 37 Vegetative development absolute spatial variability pit hardening stage La Traversagna Farm

Image 38 and Image 39 show the intra-plot and inter-plot variability the start of oil production. In this phenological state, the vegetation indices show a decrease in their NDVI values in the treatments, with the exception of block B8. Everything seems to be homogenized; showing some differences whose extension does not seem to follow a pattern of behavior due to specific actions on the crop. However, in a relative aspect, intraplot variability continues to be observed, which gives rise to behaviors with very regular areas within the blocks, indicating specific agricultural actions that are not related to the project's treatments, such as in blocks B3 and B7.

Image 38 Vegetative development relative spatial variability in oil initiation La Traversagna Farm

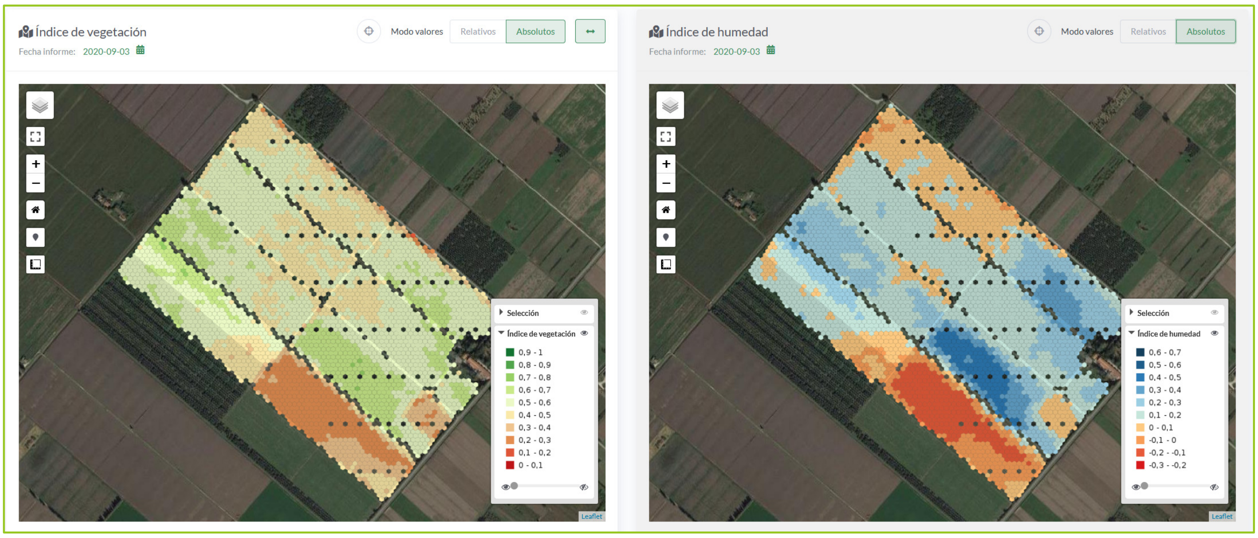


Image 39 Vegetative development absolute spatial variability in oil initiation La Traversagna Farm

Image 40 and Image 41 show the intra-plot and inter-plot variability in the pre-harvest phenological stage. In relative terms, the vegetative development based on the NDVI data is more heterogeneous compared to the previous phenological phase, not observing specific patterns between treatments or between blocks. The increase in treatments T0 and T1 of block B5 and the low value in block B8 stand out, as has been happening on previous dates.

However, in absolute terms, differences between treatments and blocks are observed. The T4 treatment of block B7 has drastically reduced the vegetative development and the moisture content on its entire surface, while the T3 treatment of the same block has maintained it. It is, without a doubt, a result of actions at the field level. Treatments T3 and T4 of block B3 also show variability in the northern third of the block, with greater vigor and moisture content compared to the rest of the farm, only comparable with treatment T3 of block B7.

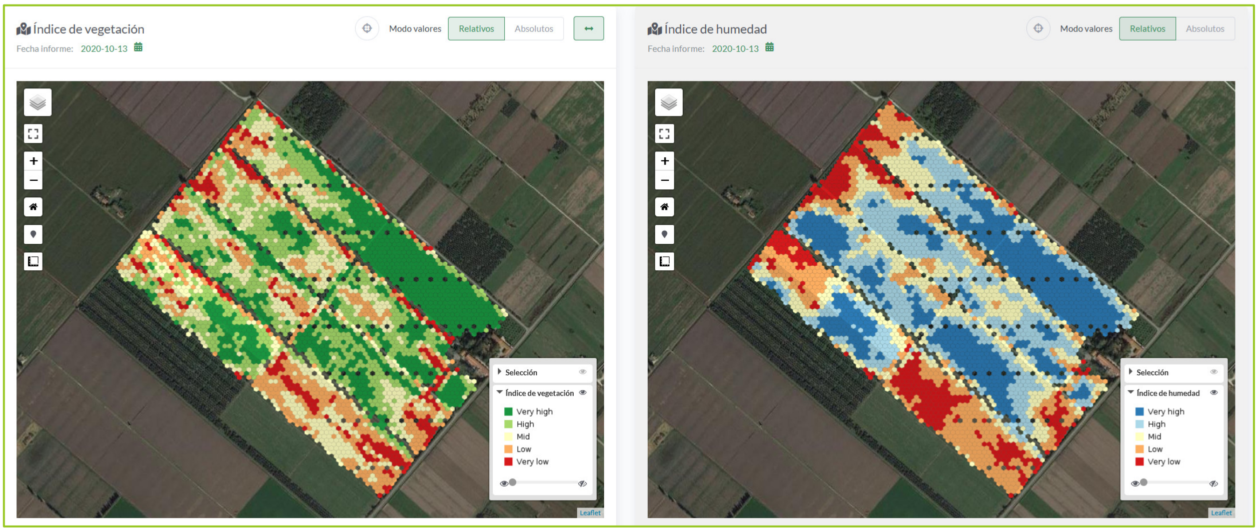


Image 40 Vegetative development relative spatial variability at preharvest stage in La Traversagna

Image 41 Vegetative development absolute spatial variability at preharvest stage in La Traversagna

Image 42 and Image 43 show the intraplot and interplot variability in the postharvest phenological stage. In this phenological period, the vegetative development on the farm has increased and homogenized, except in the T4 treatment of block B7 due to the management commented on the previous analyzed date. Except for this case, concrete patterns are no longer observed in the vegetation indexes

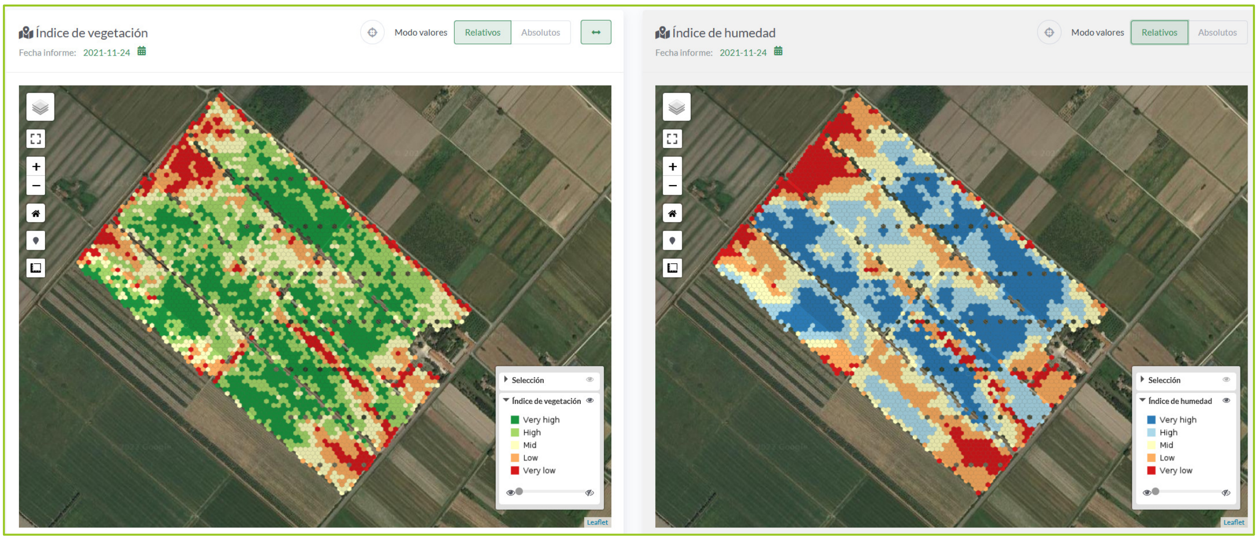


Image 42 Vegetative development absolute spatial variability at postharvest stage in La Traversagna

Image 43 Vegetative development relative spatial variability at postharvest stage in La Traversagna

II.1. Available Water Capacity (AWC)

The AWC was measured at the beginning of the project, before treatments application and at the end of the project in order to evaluate treatments effects. The analytical results of organic matter in 2021 season at the La Traversagna farm have also been reduced compared to the results obtained in 2018. Only treatment T0 increased its organic matter content, with an average increase of 0.23%. The rest of the treatments (T1, T3 and T4) show an average reduction of 1.5% in the organic matter content.

The results obtained have caused the T3 treatment to reduce its AWC by 10 mm/m, that is, 10 liters of water for each cubic meter of soil.

Table 25 Evolution of AWC La Traversagna Farm (2018-2021)

Treatment	T0	T1	T3	T4
Mean AWC (mm/m) 2018	140	140	150	140
Mean Organic Matter (%) 2018	5.27	4.5	6.08	4.79
Mean AWC (mm/m) 2021	140	140	**140**	140
Mean Organic Matter (%) 2021	5.5	3.9	**3.5**	3.5

II.2. Soil microbiological activity

Soil samples was taken from georeferenced points (Image 44) and analyzed in laboratory to determinate fertility biological index and enzymatic activity such as, ß-glucosidase, dehydrogenase, catalase, phosphodiesterase (phosphatase) and urease activities.

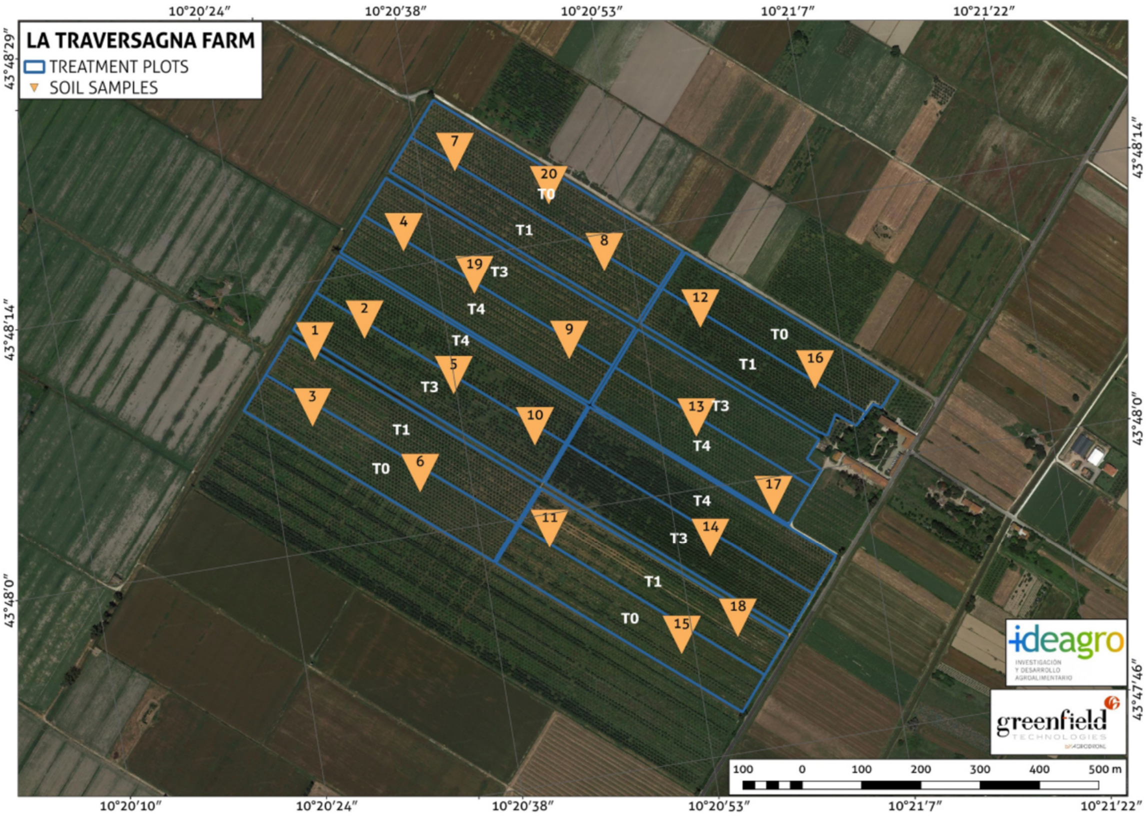


Image 44 La Traversagna soil simple points

Table 26 Soil Chemical Analysis La Traversagna Farm 2021

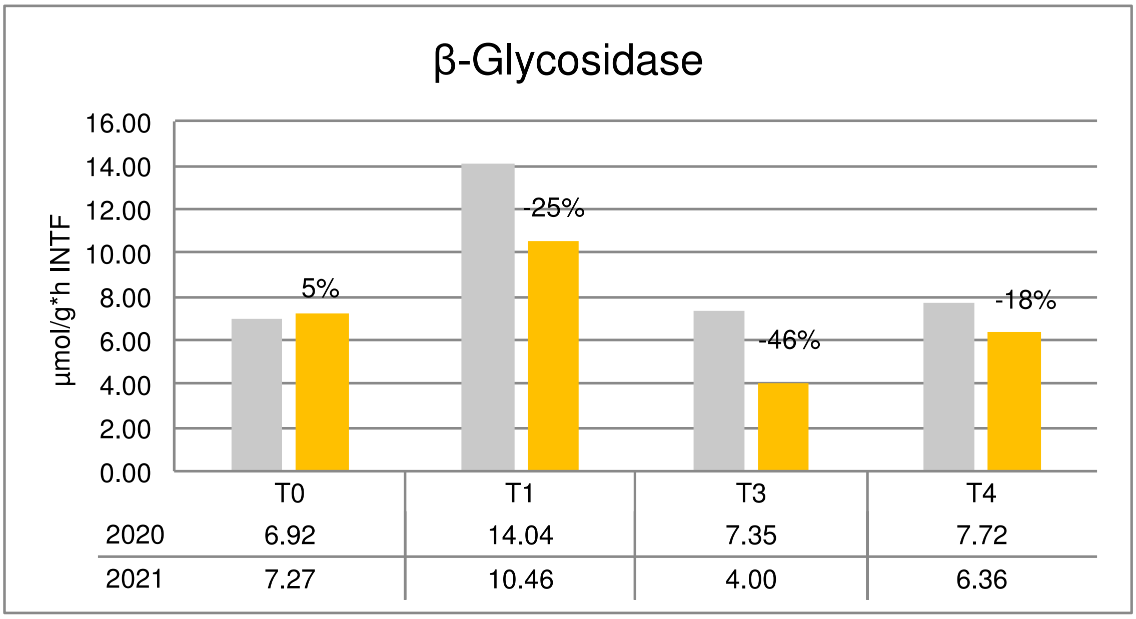
	T0	T1	T3	T4
E.C. (dS/m) (extract 1:5)	0.3	0.2	0.2	0.2
Macronutrients				
%N Total	0.3	0.3	0.2	0.2
NO_3^- (mg/kg)	126.4	42.0	32.8	27.1
P (ppm)	25.3	20.7	13.5	19.4
K (meq/100 g)	1.0	0.9	0.7	0.9
Ca (meq/100 g)	29.9	28.1	26.6	26.6
Mg (meq/100 g)	3.3	1.9	2.1	2.1
SO_4^{-2} (meq/100g)	0.3	0.1	0.1	0.1
Micronutrients				
Fe (ppm)	0.3	0.1	0.1	0.1
Mn (ppm)	3.4	4.0	3.3	3.8
B (ppm)	1.7	0.8	0.7	0.9
Cu (ppm)	15.3	9.7	7.1	8.9
Zn (ppm)	0.7	0.5	0.4	0.4
Cl^- (meq/100 g)	0.4	0.4	0.2	0.4

Oligoelements				
Na (meq/100 g)	0.5	0.3	0.3	0.4
Organics				
% O.M. Oxidable	5.5	3.9	3.5	3.5
%C Total	4.2	3.0	2.7	2.7
C/N	12.1	11.5	11.6	11.1
Carbonates				
% $CaCO_3$ Total	6.7	8.7	7.6	8.8
% $CaCO_3$ active	7.9	6.2	6.6	6.7

ß-glycosidase is linked to the organic carbon content of the soil and organic matter degradation. What it is mean a contribution of micronutrients to the crop. About ß-glycosidase activity a slight decrement was observed in all the treatments respect to 2020.

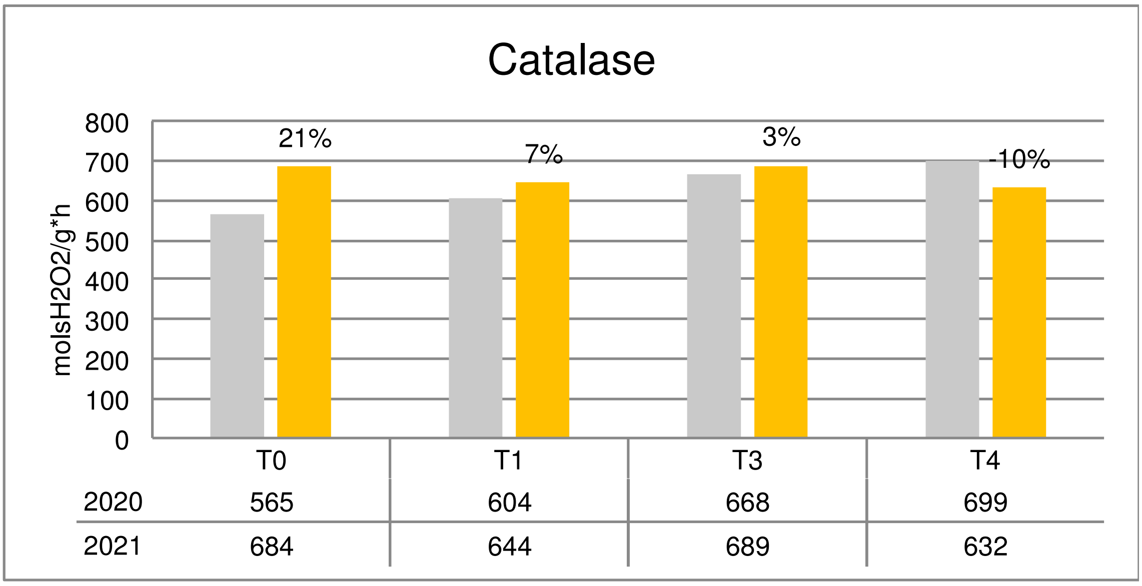
Catalase activity indicates a great soil capacity to degrade organic debris. Although an increment by 21% was observed in T0 respect to 7% observed in T1 respect to 2020, T1 values are still superior. On the contrary a decrease in the activity of this enzyme was observed in the T4 treatment. While in the T3 treatment there is an activity very similar to that observed in 2020.

The dehydrogenase activity is linked to the metabolic activity of the soil. A decrease in the activity of this enzyme is linked to the activity of the enzyme catalase and the mobilization of certain soil bacteria. In this sense a slight decreases in the activity of this enzyme was observed in 2021 in all treatments.

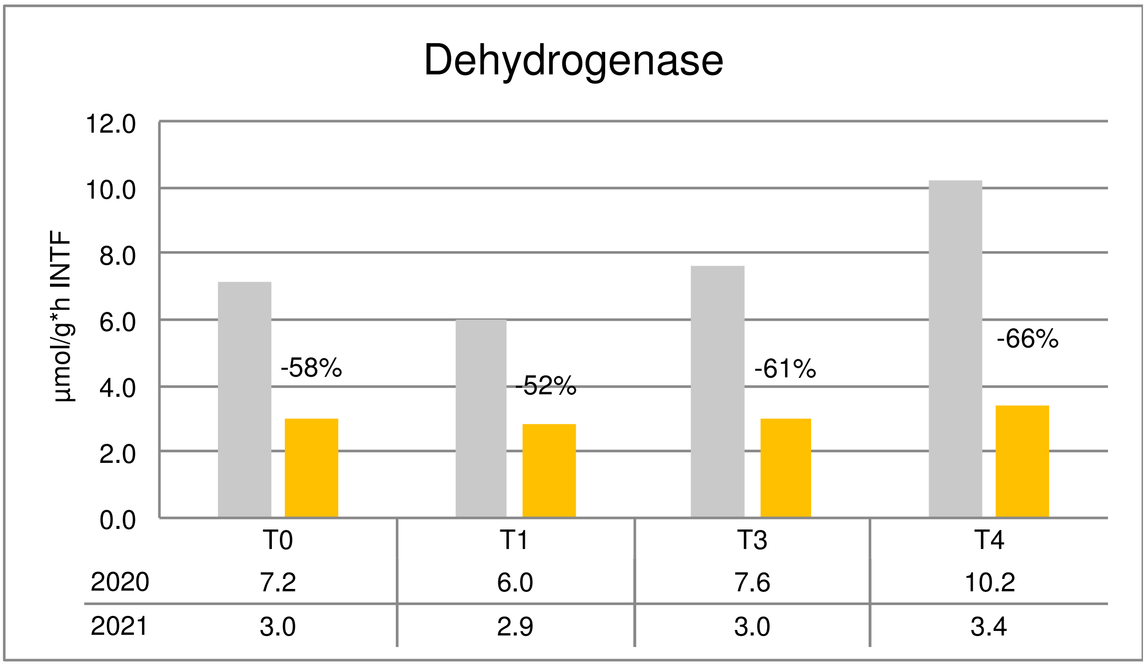


Graph 26 La Traversagna farm ß-Glycosidase activity

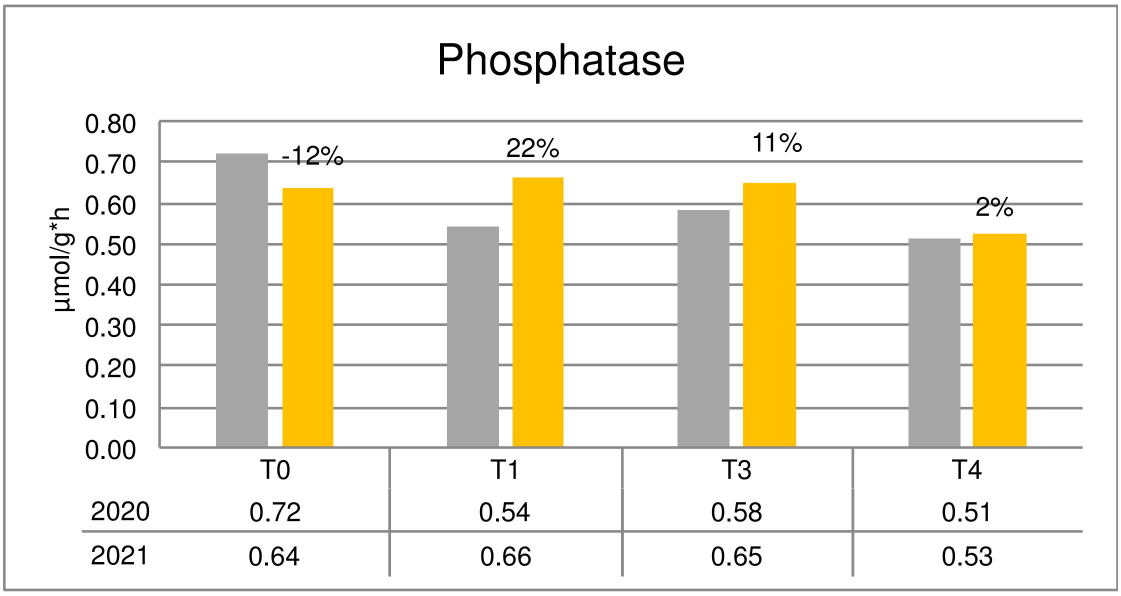
With regards to phosphatase activity a higher activity of this enzyme indicates a greater mobilization of phosphorus in inorganic form in the soil and a greater availability of this element to the plant. In this sense a slightly increase in phosphatase activity was observed in all the treatments in 2021 in comparison to 2020, this is probably linked to phosphate mobilization in soil. Urease activity indicates urea conversion to ammonia by soil microorganism. In 2021, the activity of the urease enzyme increases in all treatments respect to 2020.



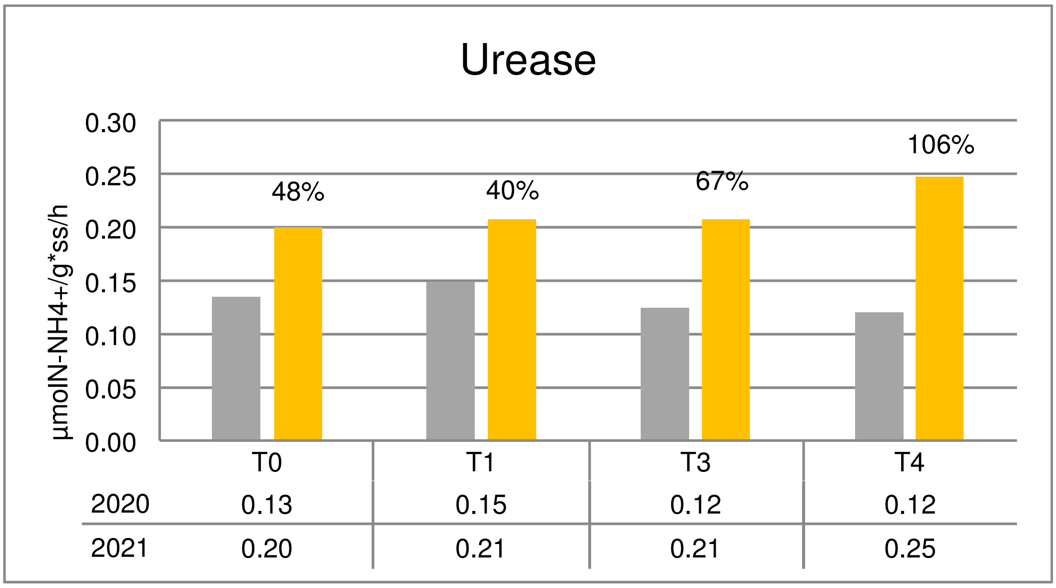
Graph 27 La Traversagna farm Catalase activity



Graph 28 La Traversagna farm Dehydrogenase activity

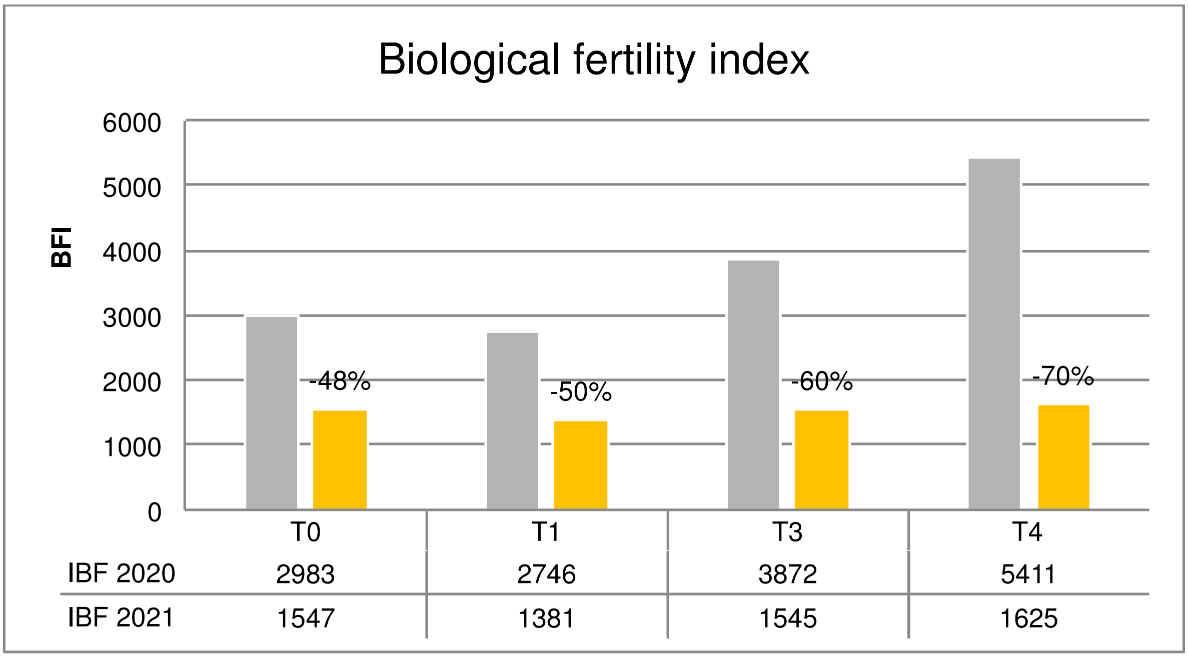


Graph 29 La Traversagna farm phosphatase activity



Graph 30 La Traversagna farm urease activity

About biological fertility index the same pattern was observed in 2021 in all the treatments.



Graph 31 La Traversagna farm Biological fertility index

XI. Disease prevalence

III.1. *Xylella fastidiosa* disease control

The Villa Filippo Berio is located in a *Xylella*-free area (Pisa province), although this pathogen was detected in Tuscany in 2018. Since the risk of a new outbreak of *X. fastidiosa* in Tuscany, plant tissue and vector specimens were sampled in 2020 and 2021 in order to monitor and eventually detect the presence of the bacteria in the experimental olive grove.

The collection of samples was carried out following the protocol established by the European Union and applied by the Tuscany Regional Plant Health Service (EPPO Standard – Diagnostic PM 7/24).

Briefly, sample of mature tissues (from 4 to 10 branches with attached leaves) were collected from different parts around the canopy of four olive plant (per year), during the period of active growth, in order to maximize the likelihood of detection. After the collection, refrigerated samples were brought to Tuscany Regional Plant Health Service laboratory where molecular analysis was conducted.

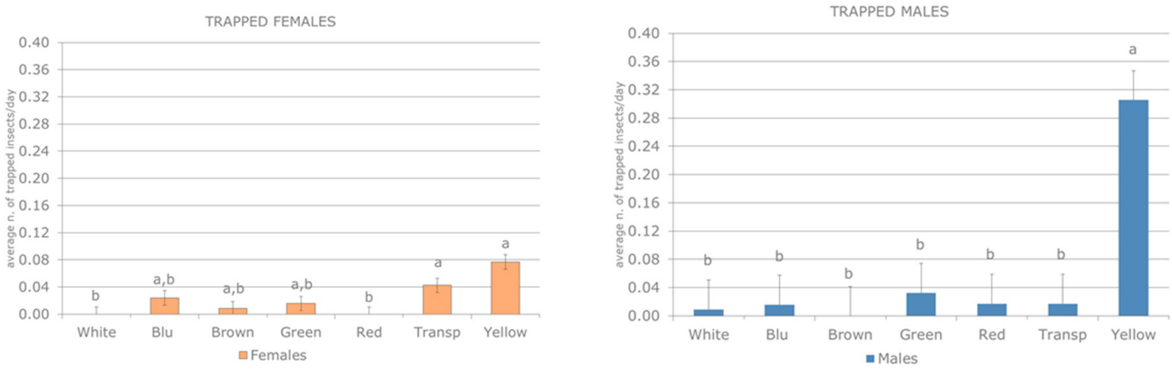
Concerning the sampling of vectors, five adult spittlebugs (or other potential vectors) per years were collected by sweeping net in the olive grove. After the capture, insect were individually stored in a 1,5 ml microvial filled with ethanol 99%. Then, samples were bring to Tuscany Regional Plant Health Service laboratory where molecular analysis were conducted.

All the collected samples of plant and vector were tested negative to *X. fastidiosa* in both years 2020 and 2021.

III.2. Insect vectors trap

In 2020, several field trials has been carried out in three olive groves: Azienda Agricola Fabio (Prato), Villa Filippo Berio (Pisa) and Azienda Sperimentale Santa Paolina of CNR (Grosseto) in order to evaluate the attraction of different color sticky traps towards spittlebug adults. In each olive grove seven traps were tested (transparent, white, yellow, green, brown, red and blue). Two traps were installed for each color at two different heights: 50 cm and 100 cm. Each chromotropic trap consists in two faces: one southeast oriented and one northwest oriented. The traps were distributed in a single row, spaced 15m between them. Every week collected specimens were counted and the color order was changed. At the time of the counting, a sampling of adult specimens has been carried out (by a sweep net) to assess the presence and the density of spittlebug population in the olive groves.

For Villa Filippo Berio, average number of females and males of *P. spumarius*, trapped by different color sticky boards, was compared by a univariate ANOVA analysis. Yellow traps captured significantly more males than the other colors and significantly more females than red and white traps. These results are partially consistent with those reported in the only one previous study concerning color attractiveness in *P. spumarius*.



Graph 32 Comparison of average number of trapped females and males per day.

XII. Weather

IV.1. Weather conditions

Annual information on the parameters measured in public or private local climate stations was done including: Temperature Maximum (ºC), Average Wind Speed (m/s), Temperature Time Maximum, Middle Wind Direction (º), Temperature Minimum (ºC), Radiation (MJ/m^2), Temperature Time Minimum, Precipitation (mm), Temperature Average (ºC), ET_0 (mm/day), Maximum Relative Humidity (%), Minimum Relative Humidity (%), Accumulated Precipitation (mm), Relative Medium Humidity (%), and Cumulative ET_0 (mm).

Regarding the climatic data recorded, no extreme meteorological phenomena have been observed that may have affected the treatments applied on La Traversagna farm.

V.1. Organoleptic characteristics (olive oil)

The olive tree fruits and olive oil obtained will be annually evaluated to determine its quality by means of the following parameters: (i) content of fatty acids and volatile compounds present in green olives and their oil; (ii) total phenolic compounds; and (iii) sensory analysis.

The majority fraction of the oil (95%) is constituted mainly by triglycerides and to a lesser extent by diglycerides, monoglycerides and free fatty acids. The fatty acid composition is characterized by being high in monounsaturated fatty acids, oleic being the main fatty acid, whose concentration varies between 60-80%. The table presents the profile of fatty acids identified and quantified in the 4 treatments oil samples, in which it can be seen that, in all the samples, the majority acid was oleic acid, with values higher than 60%, being very similar in all treatments. The second most important acid is palmitic acid, which appeared in very similar concentrations in all the samples. Regarding the groups of fatty acids, the majority were monounsaturated fatty acids, oleic acid being the most important, with values greater than 75% in all the treatments.

Table 27 Olive oil fatty acid content (%)

Fatty acids	T0	T1	T3	T4
<C14:0	nd	nd	nd	nd
C14:0	nd	nd	nd	nd
C16:0 (Palmitic)	12.9	12.7	13.3	13.1
C16:1 (Palmitoleic)	0.9	0.9	0.9	0.9
C18:0 (Stearic)	2.3	2.4	2.3	2.4
C18:1 *cis*-9 (Oleic)	75.3	75.5	75.3	75.6
C18:2 *n6c* (Linoleic)	6.9	6.7	6.4	6.3
C18:3	0.7	0.7	0.7	0.6
C20:0	0.4	0.4	0.4	0.4
C20:1	0.3	0.3	0.3	0.3
C22:0	0.1	0.1	0.1	0.1
C22:1	nd	nd	nd	nd
t-C18:1	0.1	0.1	0.1	0.1
tC18:2:3	nd	nd	nd	nd

All the treatments increased phenolic content in comparison to T0 treatment. However, very similar values was observed between T3 and T4 treatments (Table 28).

Table 28 Total Phenolic content

	T0	T1	T3	T4
Total Polyphenols	203	236	266	268

Pesticides in oil were also analyzed but non of the treatments have pesticide content.

The sensory analysis established by the COI only includes as key attributes to define the commercial category of the oil the total fruity and the presence or absence of defects, in addition to taking into account the basic bitter taste and the pungency of the oil. The last two attributes are attributes that depend on factors such as harvest time and variety. Respect to bitterness and fruity taste, all the treatments increased oil fruity and bitterness in comparison to T0 treatment. About almond and grass taste all treatments have similar values.

Table 29 Sensory analysis

Sensory attribute	T0	T1	T3	T4
Fruity	3.6	4.2	4.2	4.3
Bitter	2.3	3.1	3.2	3.2
Spicy	3.1	3.3	3.4	3.2
Almond	3.5	3.1	3.5	3.3
Grass	3.1	3.3	3.1	3.6

XIV. Carbon footprint

VII.1. CO_2 emitted during the agricultural processes

Annual CO_2 emitted in each farm will be will be described at the end of the project. In order to do that, annual CO_2 emitted in each farm will be estimated by Carbon Footprint Assessment ISO/TS 14067:2013.

VIII.1. Auxiliary fauna

Nest hotel, insect boxes and bat boxes were installed at Villa Filippo Berio on 2020 autumn. Occupancy rate of these devices was determined by visual counting in 2021 autumn.

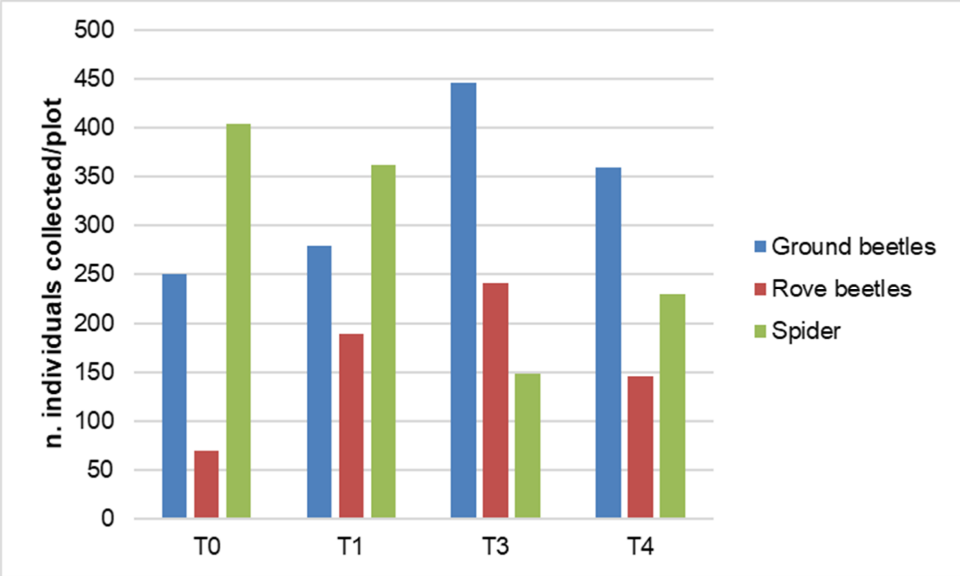
In general, the registered occupancy rate was very low, probably due to the availability of natural refuges in the olive grove and in its surroundings.

Indeed, the Villa Filippo Berio experimental olive grove is a diverse agroecosystem characterized by the presence of an almost perennial soil coverage (composed by spontaneous plant species), as well as several wild trees and shrubs that can offer suitable sites for the establishment and the development of wild fauna.

During the visual inspections of the devices several bird species were observed in the olive grove, feeding and or stationing on soil coverage or on olive tree canopy. For example, *Parus major*, *Cyanistes caeruleus*, *Jynx torquilla*, *Certhia brachydactyla*, *Upupa epops*, *Serinus serinus*, *Coracias garrulus*, *Falco tinnunculus*, *Buteo buteo*, *Tyto alba* were recorded in the olive grove. Since most of these species are insectivorous, their maintenance in the olive agroecosystem could be useful in order enhance the control of some phytophagous insect species, including *X. fastidiosa* vector. For these reason, in 2021, some herbaceous riparian strips were left at the edges of the experimental plots in order to offer natural habitats for nidification and feeding of wild bird species.

Despite the low occupancy rate registered on nest hotels, some of them (2 out of 10) were colonized by specimens of *Parus major.*

Besides the installation of the above mentioned devices, a sampling of soil functional biodiversity was carried out in 2020 using pitfall traps. The aims of this sampling were: a) to investigate the composition of soil generalist predator fauna in olive grove agroecosystem; 2) to assess some eventual difference between areas where different soil management are applied. Indeed plot with natural soil coverage (T0 and T1) were compared with plot were a seed mixture was sown (T3 and T4). Three taxonomic groups of generalist predator have been considered to compare different plots: Carabidae (ground beetles), Staphilinidae (rove beatles) and Spiders.



Graph 33 Number of collected specimens in the four experimental plots.

As show in the graph the three groups are generally abundant in the olive grove, probably due to the perennial soil coverage and the availability of different habitats. Anyway, spiders appear to be more abundant in plots characterized by a majority of grasses in the herbaceous soil coverage, while ground beetles seem to be the more abundant group in plots characterized by a greater presence of dicotyledonous species.

Image 45 Nest hotel and insect boxes installation in La Tarversagna Farm

References

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