«VALorization of Mediterranean small-scale FARMs by cropping wild UnExploited species»

Project Number: 1436
Project Acronym: Valuefarm

Deliverable D.2.3

Selection of the most environment sustainable WEP for each country conditions

Περιεχόμενα

Document Information

Deliverable Number	D.2.3
Deliverable name	Selection of the most sustainable WEP for each country conditions.
Contributing WP	WP2: Evaluation of WEPs under innovative farming systems
Contractual delivery date	M24, August 2022
Actual delivery date	M36, August 2023
Dissemination level	Public
Responsible partner	CSIC
Reviewers	All partners
Version	1

1. Selection of the most environment sustainable WEP for Cyprus

The lab of Vegetables, Medicinal and Aromatic Plants (VEGEMAP) at the Cyprus University of Technology (CUT) has expertise on soilless cultivation and worked with two of the species studied in the Valuefarm project, namely *Sonchus oleraceus* and *Portulaca oleracea.*

Initially, the hydroponic infrastructures for relevant experiments of the tested species were prepared. Several experiments were conducted at the experimental farm of the Cyprus University of Technology, Limassol, Cyprus, in a plastic multi-span greenhouse. The greenhouse is oriented North-South, and the cover material is made of transparent polyethylene sheets (anti-drip, resistance to UV radiation, 88% light transmission). The greenhouse is equipped with an automated climate control system (ventilation, shading, and cooling premises) and a hydroponic installation, constructed according to the principles of NFT (Nutrient Film Technique). Twin white plastic NFT channels (each of 4 m long, 8 cm wide, 6 cm deep) were aligned with a relevant catchment (60 L) to form an independent hydroponic unit. Each unit consisted of a twin plastic channel set and was supported with an individual water replenishment tank (60 L). The nutrient solution (NS) absorbed by the plants was replenished through automatic refill of water from the replenishment tank and the adjustment of the electric conductivity (EC) and pH took place on a daily basis by adding appropriate amounts of stock solutions of fertilisers. Each hydroponic unit included 14 plants giving a final plant density of 25 plants m^{-2}.

Seeds from commercial sowthistle and purslane stocks were purchased from Greece and were sown in peat-perlite (90:10 v/v) based growing media (Professional peat, Gebr. Brill Substrate GmbH & Co.KG, Georgsdorf, Germany) into black plastic trays, under nursery conditions. Seedlings at the stage of the 1st true leaf were then transplanted into netted pots (11 days after sowing), filled with perlite and placed into the pot positions of the NFT channels, and were kept for one week under standard nutrient solution, to allow recovery from the transplanting stress.

Experimental studies:

Several experimental studies took place.

Plants were monitored during the growing period as well as sampled at the end of the experiment. Several measurements were made as follows:

- Plant height, leaf number, fresh-dry biomass (leaves, stems & roots)
- Chlorophyll content (SPAD), leaf fluorescence, stomatal conductance, Electrolyte leakage, Chlorophyll a, Chlorophyll b, total Chlorophylls, and total carotenoids
- Total phenols, total flavonoids, antioxidant activity (DPPH, FRAP, ABTS), ascorbic acid, total soluble solids (TSS),
- Antioxidant enzymes, e.g. MDA, H_2O_2, CAT, POD, SOD,
- Mineral content in leaves, stems, roots (N, K, P, Ca, Mg, Na, NO_3) and in the nutrient solution, water use efficiency, nutrient use efficiency, N bioaccumulation coefficient, N translocation factor, N tolerance indices
- Electrical conductivity, pH, nutrient and water consumption was monitored throughout the plant growth.

1.1. Effect of N levels on sowthistle (*Sonchus oleraceus* L.) and purslane (*Portulaca oleracea* L.)

Four concentrations of N (including both NO_3^--N and NH_4^+-N) at a constant NH_4/total-N ratio of 0.055 in all treatments were applied, e.g. 50 mg N L^{-1} (or NO_3^--N 3.37 mmol L^{-1} + NH_4^+-N 0.21 mmol L^{-1}), 100 mg N L^{-1} (or NO_3^--N 6.73 mmol L^{-1} + NH_4^+-N 0.42 mmol L^{-1}), 200 mg N L^{-1} (or NO_3^--N 13.46 mmol L^{-1} + NH_4^+-N 0.83 mmol L^{-1}), and 300 mg N L^{-1} (or NO_3^--N 20.20 mmol L^{-1} + NH_4^+-N 1.23 mmol L^{-1}). The levels of K and P into the nutrient solution were kept constant, at 350 mg L^{-1} (or 8.95 mmol L^{-1}) and 70 mg L^{-1} (or 2.26 mmol L^{-1}), respectively, based on preliminary studies and previous reports (Chrysargyris et al., 2016).

Concluding remarks: To increase yield, nutritional value and efficiency of water and nitrogen use in sowthistle grown in closed hydroponic systems, a N level of 200 mg L^{-1} of N and a Nr of 0.05 are suggested.

1.2. Effect of season and the ammonium to total nitrogen ratio (Nr) on sowthistle (*Sonchus oleraceus* L.) and purslane (*Portulaca oleracea* L.).

The effects of different Nr ratios were examined, considering four Nr ratios of 0.01, 0.05, 0.10, and 0.15. The levels of 200 mg L^{-1} N, 350 mg L^{-1} K and 70 mg L^{-1} P, were kept constant, based on preliminary studies and previous

reports (Tzortzakis et al., 2022). The study took place in autumn and early spring, in order to examine the impacts of the season on crops under different Nr levels.

Concluding remarks: The recommended ammonium/total nitrogen ratio for purslane production of increased yield, improved nutritional value and the efficient use of water and nitrogen sources is to employ Nr of 0.05, while additional care should be addressed during autumn periods as plants are subjected to greater impacts of the Nr ratio.

1.3. Effect of different nitrogen, potassium, and phosphorous levels on sowthistle (*Sonchus oleraceus* L.) and purslane (*Portulaca oleracea* L.).

Six nutrient solution recipes were used, by utilizing two N levels of 100 and 200 ppm with the 350 ppm K and 70 ppm P, as well as their decreased ratios at 0.66 times or increased rations at 1.5 times, as described below:

- N-P-K → 100-47-250
- N-P-K → 200-47-250

0.66 x

- N-P-K → 100-70-350
- N-P-K → 200-70-350

1.0 x

- N-P-K → 100-105-525
- N-P-K → 200-105-525

1.5 x

Results are still analysed before the final concluding remarks.

Representative images of the experiments are listed below.

Figure 1.1. Preparation of seedlings and hydroponic pots

Figure 1.2. Preparation of hydroponic infrastructures and studies implementation

2 Selection of the most environment sustainable WEPs for Spain

The **CSIC** partner has great expertise in the evaluation of farming systems in various crops and was working with *Sonchus oleraceus* and *Portulaca oleracea*, using seeds obtained from Greece and Spain.

Several experiments were conducted at the CEBAS-CSIC Experimental Farm (Santomera, Murcia, Spain), under greenhouse conditions. Seeds of *Sonchus oleraceus* L. and *Portulaca oleracea* L. (with two different genotypes, one obtained from Greece and one from Spain) were sown in 1 L pots containing soil, sand and vermiculite (1/1/1; *w*/*w*/*w*). The soil was obtained from the CEBAS-CSIC Experimental Farm (Santomera, Murcia, Spain 38°06′14.0″ N 1°02′00.1″ W), corresponding to a semiarid climate with annual precipitation lower than 300 mm and a mean temperature of 18 °C.

Experimental studies:

Three experimental studies took place as described below.

Several measurements were taken:

- Purslane fresh and dry weight.
- Total mineral composition in the aerial tissues (Total N, P, K, P, Fe, S).
- Soil pH and electrical conductivity.
- Soil mineral composition (Total N, P, K, P, Fe, S).
- Soil available P, Total carbon and organic carbon content.
- Soil enzymatic activities: β-glucosidase activity, dehydrogenase activity, alkaline phosphomonoesterase activity and urease activity.
- Soil DNA extraction, Illumina sequencing (bacterial 16S V3-V4 and fungal ITS2 region), bioinformatics and biostatistical analyses.

Experiments

2.1- **Effect of fertilization regime on growth parameters of *Sonchus oleraceus* and two genotypes of *Portulaca oleracea.***

The experimental design consisted of 10 fertilization treatments with 7 repetitions for each plant species (*Sonchus oleraceus, Portulaca oleracea* from Greece and Spain): N-P-K treatments were prepared to reach a specific final concentration, namely: IT1, 100-100-100 mg L^{-1}; IT2, 300-100-100 mg L^{-1}; IT3, 600-100-100 mg L^{-1}; IT4, 300-200-100 mg L^{-1}; IT5, 300-300-100 mg L^{-1}; IT6, 300-200-200 mg L^{-1}; IT7, 300-200-300 mg L^{-1}; OT1, organic compost extracts (equivalent to 300 mg L^{-1}), OT2, organic compost extract + P (equivalent to 300 and 200 mg L^{-1}) and the control treatment where no fertilizers were added.
The treatments were applied via nutrient solution to the pots, starting when plants developed 3-4 true leaves. All the plants received 100 mL of nutrient solution per pot by manual irrigation once a week. Harvest took place after 12 weeks.

Concluding remarks: *Portulaca oleracea* was more dependant on nitrogen input than *Sonchus oleraceus*. Nitrogen had a main role improving plants growth parameters and yield in both wild plant species being the beneficial effect depending on the fertilizer doses and origin (inorganic or organic) applied.

2.2 **Effects of inorganic and compost tea fertilizers application on the taxonomic and functional microbial diversity of the *Portulaca oleracea* rhizosphere**

The experiment consisted of a mesocosm assay with four inorganic fertilization treatments which differed in the amount of N:P:K namely IT1: 300:100:100; IT2: 300:200:100; IT3: 300:200:200; IT4: 600:100:100 (ppm N:P:K

ratio); one treatment of compost tea which reached at the end of the assay the dose of 300 ppm N; and one non-fertilizered control treatment.
The experiment was maintained for three months (from sowing of seeds) under greenhouse conditions at CEBAS-CSIC Experimental Farm (Santomera, Murcia, Spain). Day/night temperature was 35 ºC/25 ºC. Plants were irrigated regularly with water to keep growth substrate at 60% of field capacity.

Concluding remarks: The application of compost tea and inorganic fertilizers significantly increased the plant biomass, and some soil chemical properties such as pH and soil enzymatic activities related to C, N and P biogeochemical cycles. The majority of inorganic fertilization treatments decreased the microbial diversity as well as some predictive bacterial functional pathways, suggesting that the inorganic fertilization might lead to a loss of microbial functioning.

2.3 **Different Functional and Taxonomic Composition of the Microbiome in the Rhizosphere of Two Purslane Genotypes.**

Two kilograms of soil mixed with sand and vermiculite (1:1:1; w:w:w) were put in 2 L pots (15.7-cm diameter, 12.5-cm height). Seeds of purslane (*Portulaca oleracea* L.; Hortus Sementi Srl., Budrio, Italy; 2020 production lot) were provided by the Department of Agriculture Crop Production and Rural Environment (University of Thessaly, Greece) and seeds of wild purslane plants collected from semiarid areas of the Southeast of Spain were directly sown in the pots in order to obtain one plant per pot after thinning. The experiment consisted of a mesocosm assay with seven pots (n=7) for each plant genotype. Each plant was fertilized with 100 mL of nutrient solution (300:100:100 ppm N:P:K ratio) per pot by manual irrigation once a week. The experiment was maintained for three months (from sowing of seeds) under greenhouse conditions at CEBAS-CSIC Experimental Farm (Santomera, Murcia, Spain).

Concluding remarks: A clear effect of purslane genotype microbial community composition and functional profiles was observed. It is necessary considering differences in the functional characteristics of plant genotypes of *Portulaca oleracea* when selecting the beneficial microorganisms to be used as biofertilizers aiming to maximize plant growth and resistance to environmental stressors.

Representative images of the experiments are listed below.

Figure 2.1. *Sonchus oleraceus* and *Portulaca oleracea* plants growing under different fertilization treatments

Figure 2.2. *Portulaca oleracea* plants from Spain (€) and Greece (G) growing under different fertilization treatments

3 Selection of the most environment sustainable WEPs for Greece

The lab of Vegetable Production at the University of Thessally, Greece (UTH) has great expertise in cultivation of WEPs under filed and greenhouse conditions and worked with all the species studied in the Valuefarm project, namely *Cichorium spinosum*, *Crithmum maritimum*, *Scolymus hispanicus*, *Sonchus oleraceus* and *Portulaca oleracea.*

Several experiments were conducted at Experimental Farm of University of Thessaly (Velestino, Greece), under field greenhouse conditions. Seeds of *Sonchus oleraceus* L., and *Portulaca oleracea* L. were purchased from supply stores, while *Cichorium spinosum*, *Crithmum maritimum* and *Scolymus hispanicus* were collected from the wild. Seeds were sown in seed trays and transplanted at the stage of 3-4 true leaves either in pots containing peat and perlite (1:1; v/v) or directly in soil.

A. Pot experiments regarding the nutrient requirements of WEPs.
B. Field experiments regarding the use of mulching with plastic films in the cultivation of WEPs.
C. Field experiments regarding the evaluation of irrigation requirements of *Cichorium spinosum* and *Scolymus hispanicus*
D. Field experiments regarding the use of soil amendments in WEPs cultivation

Description of experiments

A) Experiments for the evaluation of nutrient requirements

1. The effect of fertilization regimes on the crop performance of *Cichorium spinosum*

The experiment took place during October 2020-April 2021. For the conduction of the experiment they were used 7 treatment in total which differed in the amounts of N:P:K, namely 100:100:100, 200:100:100, 200:200:200, 300:100:100, 300:200:200 and 300:300:300 ppm ratio of N:P:K and the control treatment where no fertilizers were added. The treatments were applied via nutrient solution in the *C. spinosum* plants. Each treatment contained fifteen pots (n=15) and in total they were used 105 pots, according to the Completely Randomized Design (CRD). All the treatments received the same amount of nutrient solution in which the plants were fertigated manually once a week comprising of 150 mL of nutrient solution per plastic plot. Before harvest, it was recorded the chlorophyll content of leaves (SPAD values). Harvest took place on 26th of April when the rosette reached the marketable size in order to examine the effect of different fertilization regimes on crop performance.

Concluding remarks: Most of the studied treatments did not increase crop performance compared to the control treatment, whereas treatments 200:100:100, 200:200:200 and 300:100:100 decreased the number of leaves per plant and treatment 200:200:200 decreased the fresh weight of plants. Leaf areα increased for the treatment of 200:100:100, while the highest specific leaf area was recorded for the treatment of 200:200:200.

2. The effect of fertilization regime on the crop performance of *Cichorium spinosum*

The same experiment was conducted during the period September-December 2021. Apart from the parameters measured in the previous experiment, net photosynthesis was also recorded.

Concluding remarks: In this experiment crop performance as expressed by the number of leaves was significantly increased for the treatment of 200:200:200, without being significantly different from 100:100:100 and 300:200:200; while leaves weight increased for the 100:100:100 treatment being significantly different from the control and the 300:100:1 and 300:300:300 treatments. Moreover, chlorophyll content increased for all the fertilization treatments compared to control, while leaf area was the highest for the 10:100:100 treatment. Regarding net photosynthesis, the highest values were recorded for the control treatment for all the tested light

intensities. These results indicate that the growing season has an impact on crop performance since growing condition may affect nutrient uptake and the overall performance of plants.

3. The effect of fertilization regime on the crop performance of *Cichorium spinosum*

The same experiment was conducted during the period February-May 2023. Apart from the parameters measured in the previous experiment, net photosynthesis was also recorded.

Concluding remarks: for this growing period, the number and weight of leaves increased at the highest fertilization rates, especially for the treatment 300:100:100 and 300:300:300 where the highest values were recorded. Moreover, chlorophyll content was higher for all the fertilization treatments compared to control, while leaf area increased for all the treatments compared to control and the 100:100:100 treatment. Therefore, it could be concluded that *C. spinosum* can be cultivated under commercial growing conditions in Greece, although agronomic practices such as fertigation have to be fine-tuned depending on the growing period.

4. The effect of fertilization regimes on the crop performance of *Scolymus hispanicus*

The trial was carried out at the experimental field of the University of Thessaly in Velestino during the period of October 2020-August 2021. The experimental setup was similar to experiment A.

Concluding remarks: The application of 300:100:100 resulted in the highest number of leaves, while treatments 200:200;200 and 300:200:200 recorded the highest yield (weight of leaves/plant and weight of roots/plant).

5. The effect of fertilization regimes on the crop performance of *Scolymus hispanicus*

The same trial was also carried out during the period September 2021 and February 2022. Apart from the previously recorded parameters, the Photosynthesis Active Radiation (Pn) was also recorded at different light intensities (PAR: 0-1300 $\mu mol \cdot s^{-1} \cdot m\text{-}^{2}$).

Concluding remarks: The application of 300:100:100 also resulted in the highest number of leaves, while the highest weight of leaves was recorded for the 300:100:100 and 300:300:300 treatments. Chlorophyll content was increased for the abovementioned treatments, as well as for 200:200:200. The weight of roots increased for the treatment 200:200:200, while all the fertilization treatments increased leaf area compared to the control treatment. Moreover, Pn was the highest for the control treatment at the intensity of 0 $\mu mol \cdot s^{-1} \cdot m^{-2}$ compared to the 300:300:300 treatment, whereas no significant differences were recorded among the fertilization treatments for the rest of light intensities.

6. The effect of fertilization regimes on the crop performance of *Scolymus hispanicus*

The same trial was also carried out during the period December 2022 and April 2023.

Concluding remarks: in this growing period, the treatment of 300:300:300 resulted in the highest values for rosette diameter, weight of plants, number and weight of leaves per plant, while the control treatment recorded the lowest values for the same parameters, except for dry matter of leaves where the opposite trend was recorded. On the other hand, chlorophyll content was the highest for the 200:200:200 treatment, while all the fertilization treatments recorded higher root weight and leaf area compared to the control.

7. The effect of fertilization regimes on the crop performance on *Sonchus oleraceus*

The trial was conducted at the experimental farm of the University of Thessaly in Velestino during the growing period of March-July 2021. The experimental setup was similar to experiment A.

Concluding remarks: The application of 300:200:200 resulted in the highest weight of plants, while the control treatment and 300:100:100 resulted in the highest number of leaves and weight of leaves per plant, respectively.

8. The effect of fertilization regime on the crop performance on *Sonchus oleraceus*

The same trial was repeated in the growing period of September-December 2021. Apart from the previously mentioned parameters, Photosynthesis Active Radiation (Pn) at different light intensities (PAR: 0-1200 μmol·s^{-1}·m^{-2}) was also estimated.

Concluding remarks: The treatment 300:100:100 resulted in the highest weight of plants and leaves/plant, while the highest number of leaves was recorded for the treatment of 200:100:100. Significant differences were also recorded in Pn values which varied for the tested light intensities although in all the cases the control treatment recorded the lowest overall values.

9. The effect of fertilization regime on the crop performance on *Sonchus oleraceus*

The same trial was repeated in the growing period of September 2021 and April 2022.

Concluding remarks: The treatment 300:100:100 resulted in the highest weight of plants and leaves/plant, although weight of leaves did not differ significantly from the 300:200:200 treatment. On the other hand, the control treatment recorded the highest dry matter of leaves. Chlorophyll content was also the highest for the 300:100:100 treatment, while the control treatment recorded the lowest leaf area. No differences were recorded among the tested treatments for specific leaf area. A varied response to fertilization regime was recorded for Pn depending on the tested light intensity, although the lowest values were recorded for the control treatment for 400, 800 and 1200 μmol·s^{-1}·m^{-2}.

10. The effect of fertilization regime on the crop performance on *Sonchus oleraceus*

The experiment was also carried out during the period December 2022 and March 2023, whereas apart from the studied morphological traits (cm) diameter of the plant was also evaluated.

Concluding remarks: in this growing period, the treatment 300:200:200 resulted in the highest rosette diameter, weight of plants and weight of leaves/plant, whereas the number of leaves significantly increased for the 300:300:300 treatment. On the other hand, the control treatment recorded the lowest values for all the parameters. Chlorophyll content was the highest for the 300:300:300 treatment, while the highest leaf area was measured for the 300:200:200 and 300:300:300 treatments. Specific leaf area was the highest for the control treatment.

11. The effect of fertilization regimes on the crop performance on *Portulaca oleraceae*

The current trial was conducted at the experimental farm of the University of Thessaly during the period of July-August 2021. The layout was similar to experiment A.

Concluding remarks: the fertigation treatment of 300:300:300 resulted in significantly higher growth parameters compared to the control and the rest of the fertigation treatments, while chlorophyll content and dry matter of leaves increased for both 30:200:200and 300:300:300 treatments. On the other hand, dry matter of shoots was

the highest for the control treatment, whereas dry matter of plants was not affected by the tested treatments. Regarding the photosynthetic activity, a varied response was recorded with treatments 300:100:100, 300:200:200 and 300:300:300 resulting in the highest values for the light intensities of 400, 800 and 1200 $\mu mol \cdot s^{-1} \cdot m^{-2}$).

12. The effect of fertilization regimes on the crop performance of *Crithmum maritimum* L. grown in pots

The trial was conducted at the experimental farm of the University of Thessaly in Velestino during the growing period of September 2020-October 2021. The experimental setup was similar to experiment A. Two harvests were applied.

Concluding remarks: The application of 300:300:300 resulted in the highest weight of leaves/plant for both harvests. Moreover, Pn values were significantly higher for treatment 300:300:300 at light intensities 400, 800, 1200 $\mu mol \cdot s^{-1} \cdot m^{-2}$.

13. The effect of fertilization regimes on the crop performance of *Crithmum maritimum* L. grown in pots

The same trial was conducted during the period of April and August of 2022. The experimental setup was similar to experiment A, while two harvest were performed.

Concluding remarks: the results showed that the high amount of nutrients (300:200:200 and 300:300:300 treatments) significantly increased crop performance (weight of leaves/plant), while chlorophyll content was not affected. By fertigation treatments. Similar results were obtained for the second harvest, although only treatment 300:200:200 increased the weight of leaves/plant, without being significantly different from treatment 300:100:100. In contrast to the first harvest, treatments 100:100:100 and 200:100:100 resulted in higher Pn values.

14. The effect of fertilization regimes on the crop performance of *Crithmum maritimum* L. grown in pots

The same trial was conducted during the period of April and July of 2023 with the same plants. The harvest of the plants took place at 18th of July 2023

Concluding remarks: the fertigation regime of 200:100:100 resulted in the highest weight of plants which could be attributed to the build-up of nutrients throughout the experimental periods.

B. Field experiments regarding the use of mulching with plastic films in WEPs cultivation

1. The effect of mulching with plastic film and deficit irrigation on the crop performance of *Cichorium spinosum*

The trial was conducted on the experimental farm of the University of Thessaly during the period March 2022 and August 2022. The goal of this study was to evaluated the effect of mulching with plastic film and water deficit stress on the morphological traits and crop development. Four treatments were tested, namely Deficit irrigation with Plastic film, Full Irrigation with Plastic film, Deficit Irrigation without Plastic film and Full Irrigation without Plastic film.

Concluding remarks: no significant differences in yield parameters, whereas dry matter of leaves increased when plants were grown without mulching with plastic film. The same trend was recorded for the other parameters tested, namely chlorophyll content, leaf area and specific leaf area.

2. The effect of mulching with plastic film and deficit irrigation on the crop performance of *Cichorium spinosum*

The same trial was conducted during the growing period of March 2023 and June 2023.

Concluding remarks: in the second experiment, the effects were more profound on parameters related to crop performance. Rosette diameter increased when plants were grown without mulching, regardless of the irrigation regime, as well as when plants were subjected to full irrigation, regardless of mulching. On the other hand, the combination of full irrigation and no plastic film resulted in a decreased number of leaves per plant, as well as in a decreased weight of leaves compared to the respective deficit irrigation treatment (deficit irrigation x no plastic film). Moreover, chlorophyll content and specific leaf area were the lowest for the combination of full irrigation and no plastic film.

3. The effect of mulching with plastic film and deficit irrigation on the crop performance of *Crithmum maritimum*

The trial was similar to experiment F. The harvest took place on 19th of October, 2022.

Concluding remarks: contrasting results were recorded for the effect of plastic mulching and deficit irrigation on chlorophyll content. Deficit irrigation increased chlorophyll content was plastic film was implemented, whereas the opposite trend was recorded for plants where soil was not covered with mulching. The weight of plants was the highest for plants treated with full irrigation and no mulching, followed by deficit irrigation and no mulching, indicating that mulching is not recommended for *C. maritimum* cultivation.

4. The effect of mulching with plastic film and deficit irrigation on the crop performance of *Crithmum maritimum*

The trial was continued for a consecutive growing period and the harvest took place on 11th of July 2023.

Concluding remarks: chlorophyll content was the highest for plants treated with full irrigation and no mulching, while no differences were recorded among the rest of the treatments. In contrast to the previous experiment, plants subjected to deficit irrigation and no mulching recorded the lowest weight of leaves.

C. Field experiments regarding the irrigation requirements of WEPs

1. The effect of water deficit stress on the crop performance of *Cichorium spinosum* grown in the greenhouse

The trial took place at the greenhouse of the experimental greenhouse of University of Thessaly during the growing period September 2022 and March 2023. The plants were subjected to the different irrigations regimes i) Deficit irrigation 1 (I1= 30% of field capacity) ii) Deficit Irrigation 2 (I2=70% of field capacity) and Full Irrigation (I3=100% of field capacity).

Concluding remarks: deficit irrigation did not affect any of crop performance parameters tested, except for dry matter of leaves which decreased at the full irrigation regime. These findings highlight that *C. spinosum* is a drought tolerant species that could be cultivated under deficit irrigation conditions.

2. The effect of water deficit stress on the crop performance of *Scolymus hispanicus* grown in the field

The trial was carried out at the experimental field of the University of Thessaly in Velestino during the period of October 2020-August 2021. Three treatments were applied in total, namely the treatment of water deficit stress (50% of field capacity), the second treatment where the plants were fully irrigated (100% of field capacity) and the control treatment in which the plants were not irrigated (rain-fed).

Concluding remarks: Deficit and full irrigation resulted in higher weight of leaves/plant, while the number of leaves was the highest for both full irrigation and deficit irrigation. On the other hand, deficit irrigation decreased the weight of roots/plant.

3. The effect of water deficit stress on the crop performance of *Scolymus hispanicus* grown in the greenhouse

The trial took place at the experimental greenhouse of the field of University of Thessaly during the growing period September 2022 and March 2023. The layout was similar to experiment D.

Concluding remarks: the most sever water shortage (I1) treatment decreased the rosette diameter, while the number and weight of leaves were also lower for this treatment compared to the full irrigation. On the other hand, the weight of roots and leaf area was negatively affected by I1 treatment.

4. The effect of water deficit stress on the crop performance of *Sonchus oleraceus* grown in the greenhouse

The trial took place at the greenhouse of the experimental field of University of Thessaly during the growing period November 2022 and February 2023. The layout was similar to experiment D.

Concluding remarks: the highest level of deficit irrigation (I1) decreased all the growth parameters and leaf area, whereas no significant differences were recorded for chlorophyll content and specific leaf area. On the other hand, dry matter content of leaves increased for this particular treatment (I1).

D. Field experiments regarding the use of soil amendments in WEPs cultivation

1. The effect of manure on the crop performance of *Cichorium spinosum*

The current trial was carried out at the experimental farm of the University of Thessaly during the period March 2022 and June 2022. The goal of the current study was to estimate the effect of manure on the crop development and morphological traits of the crop. For the conduction of the experiment, there used two treatments in total (control and manure).

Concluding remarks: the application of manure increased the rosette diameter and the fresh and dry weight of leaves and the specific leaf area, whereas the rest of the parameters remained unaffected. Therefore, it could be concluded that manure application has a positive effect on biomass yield.

2. The effect of manure on the crop performance of *Cichorium spinosum*

The same trial was also conducted for the second time in the spring season during the period January 2023 and May 2023.

Concluding remarks: similar results were recorded for the fresh weight of leaves and the rosette diameter, while chlorophyll content and leaf area were also increased after the manure application. The rest of the parameters remained unaffected, whereas specific leaf area decreased for the manure application.

Figure 2.3. The effect of manure on the crop performance of *C. spinosum* (From left to right the treatments namely Control and Manure).

3. The effect of manure on the crop performance of *Scolymus hispanicus* grown in the field

The same trial was carried out at the experimental farm of the University of Thessaly during the period March 2022 and June 2022.

Concluding remarks: Similar results were recorded in this trail, except for the case of specific leaf area which remained unaffected. Therefore, it could be concluded that manure application has a positive effect on biomass yield.

Figure 2.4. The effect of manure on the crop performance of *S. hispanicus* grown in the field (From left to right the treatments namely Control and Manure).

4. The effect of zeolite and manure on the crop performance on *Sonchus oleraceus* grown in pots

The experiment was carried out at the experimental farm of the University of Thessaly during the growing period of March-July 2021. Three treatments were applied, namely manure, zeolite and control with 15 pots used for each treatment.

Concluding remarks: manure recorded the highest values for the weight of plant and leaves of leaves, whereas the number of leaves and the dry matter of leaves were not significantly affected. The same trend was recorded for the chlorophyll content and leaf area, whereas specific leaf area was decreased after the manure application.

5. The effect of growth substrate on the crop performance on *Sonchus oleraceus*

The trial was conducted at the experimental farm of the University of Thessaly in Velestino during the growing period of March-July 2021. Three treatments were applied, namely manure, zeolite and control as described in experiment D.

Concluding remarks: The application of manure had a positive impact on crop performance over the control and the zeolite treatment, resulting in higher weight of plants and weight of leaves/plant, whereas the number of leaves/plant was not significantly affected. Similarly, chlorophyll content and leaf were positively affected by manure, whereas the opposite trend was recorded for specific leaf area.

6. The effect of growth substrate on the crop performance on *Sonchus oleraceus* grown in pots

The current trial took place at the experimental farm of University of Thessaly during the growing period of April 2023 and June 2023. We tested six treatments in which the growth substrate was varied in the amounts of peat, perlite and manure namely i) perlite and peat (1/1; v/v) (GS1) ii) perlite and manure (1:1; v/v) (GS2) iii) perlite, peat and manure (1:0.2:0.8; v/v) (GS3), iv) perlite, peat and manure (1:0.4:0.6; v/v) (GS4), v) perlite, peat and manure (1:0.6:0.4; v/v) (GS5) and vi) perlite, peat and manure (1:0.8:0.2; v/v) (GS6).

Concluding remarks: GS1 treatment resulted in significantly increased rosette diameter and weight of plants, whileGS1 and GS3 treatments performed the best in the case of the number and weight of leaves per plant, chlorophyll content and leaf area. On the other hand, dry matter of leaves and specific leaf area were not affected by the tested treatments. Our results indicate that peat and perlite and for some parameters the use of perlite, peat and manure (GS3) may improve crop performance of *S. oleraceus* plants.

Figure 2.6. The effect of growth substrate on the crop performance on *S. oleraceus* grown in pots (From upper left to right the treatments namely namely i) perlite and peat (1/1; v/v) (GS1) ii) perlite and manure (1:1; v/v) (GS2) iii) perlite, peat and manure (1:0.2:0.8; v/v) (GS3), iv) perlite, peat and manure (1:0.4:0.6; v/v) (GS4), v) perlite, peat and manure (1:0.6:0.4; v/v) (GS5) and vi) perlite, peat and manure (1:0.8:0.2; v/v) (GS6).

7. The effect of manure on the crop performance of *Sonchus oleraceus*

The current trial was carried out at the experimental farm of the University of Thessaly during the period March 2022 and June 2022. The experimental setup was similar to experiment D.

Concluding remarks: manure had a beneficial effect on all the tested parameters compared to the control treatment.

8. The effect of manure on the crop performance of *Sonchus oleraceus*

The current trial was carried out at the experimental farm of the University of Thessaly during the period June 2023 and August 2023.

Concluding remarks: similar results to the first experiment was recorded in this trial, except for the case of chlorophyll content where no differences were recorded and the dry matter of leaves which decreased for the manure treatment.

Figure 2.9. The effect of manure on the crop performance of *S. oleraceus* (From left to right the treatments namely Control and Manure).

9. The effect of growth substrate on the crop performance on *Portulaca oleracea* grown in pots

The current trial took place at the experimental farm of University of Thessaly during the growing period of May 2023 and July 2023. The layout was similar to experiment J.

Concluding remarks: weight of plants and weight of leaves per plant increased for GS4 treatment, without being significantly different from GS3 and GS1 treatments, while the weight of shoots increased for treatments GS3 and GS4. Chlorophyll content was negatively affected by GS5 and GS6 treatments.

Figure 2.10. The effect of growth substrate on the crop performance on *P. oleracea* grown in pots (From upper left to right the treatments namely namely i) perlite and peat (1/1; v/v) (GS1) ii) perlite and manure (1:1; v/v) (GS2) iii) perlite, peat and manure (1:0.2:0.8; v/v) (GS3), iv) perlite, peat and manure (1:0.4:0.6; v/v) (GS4), v) perlite, peat and manure (1:0.6:0.4; v/v) (GS5) and vi) perlite, peat and manure (1:0.8:0.2; v/v) (GS6).

10. The effect of zeolite and manure on the crop performance on *Portulaca oleracea* grown in pots

The experiment was carried out at the experimental farm of the University of during the growing period of June-August 2021. The experimental setup was similar to experiment I.

Concluding remarks: a varied response of the tested growth media in growth parameters was recorded with control and manure resulting in higher plant height than zeolite, manure resulting in the highest yield and zeolite having a positive impact on the weight of shoots and leaves per/plant. On the other hand, no effects were recorded on chlorophyll content, control increased the dry matter of shoots and manure resulted in decreased dry matter of leaves and whole plants. Photosynthetic activity was beneficially affected by zeolite for the light intensities of 800and 1200 $\mu mol \cdot s^{-1} \cdot m^{-2}$.

Figure 2.11. The effect of zeolite and manure on the crop performance on *P. oleracea* grown in pots.

11. The effect of manure on the crop performance of *Portulaca oleracea*

The current trial was carried out at the experimental farm of the University of Thessaly during the period May 2022 and June 2022. The experimental setup was similar to experiment D.

Concluding remarks: manure had a beneficial effect on all the tested parameters compared to the control treatment, except for chlorophyll content and dry matter of shoots and leaves where no differences were recorded.

Figure 2.12. The effect of manure on the crop performance of *P. oleracea* (From left to right the treatments namely Control and Manure).

12. The effect of manure on crop performance of *Crithmum maritimum*

The trial was similar to experiment D. The experiment took place during the period June 2022 and August 2022.

Concluding remarks: manure increased the weight of leaves per plant.

13. The effect of manure on crop performance of *Crithmum maritimum*

The same experiment was continued for the period Amy2023 and July 2023. Two harvests were implemented.

Concluding remarks: manure increased the weight of leaves per plant for both harvests.

Figure 2.13. The effect of manure on crop performance of *C. maritimum* (From left to right the treatments namely Control and Manure).

Below are provided some representative photos of the experiments:

Figure 3.1. Field experiment for the evaluation of irrigation requirements of *Scolymus hispanicus*.

Figure 3.2. Field experiment for the evaluation of irrigation requirements of *Scolymus hispanicus*.

Figure 3.3. Pot experiment for the evaluation of fertigation requirements of *Crithmum maritimum*.

Figure 3.4. Pot experiment for the evaluation of fertigation requirements of *Scolymus hispanicus*.

Figure 3.5. Field experiment with *Portulaca oleracea*.

General conclusions

In general, the studied species showed a varied response to fertilization treatments depending on the growing conditions which differ among the countries. Moreover, the species that were studied under water deficit conditions were resilient to water stress which is essential to integrate them in the existing farming systems under the ongoing climate crisis. Manure also was very beneficial for the growth of the tested species The overall inputs requirements were not high therefore they could be suggested in farming systems with low inputs. On the other hand, covering soil with plastic film did not have positive results on the studied perennial species (*Cichorium spinosum* and *Crithmum maritimum*) on plant growth and water saving after testing deficit irrigation in combination with mulching with plastic film. Therefore, although labor for weed management is reduced the benefits in crop performance or in water saving are not visible and this system cannot be suggested in WEPs cultivation.