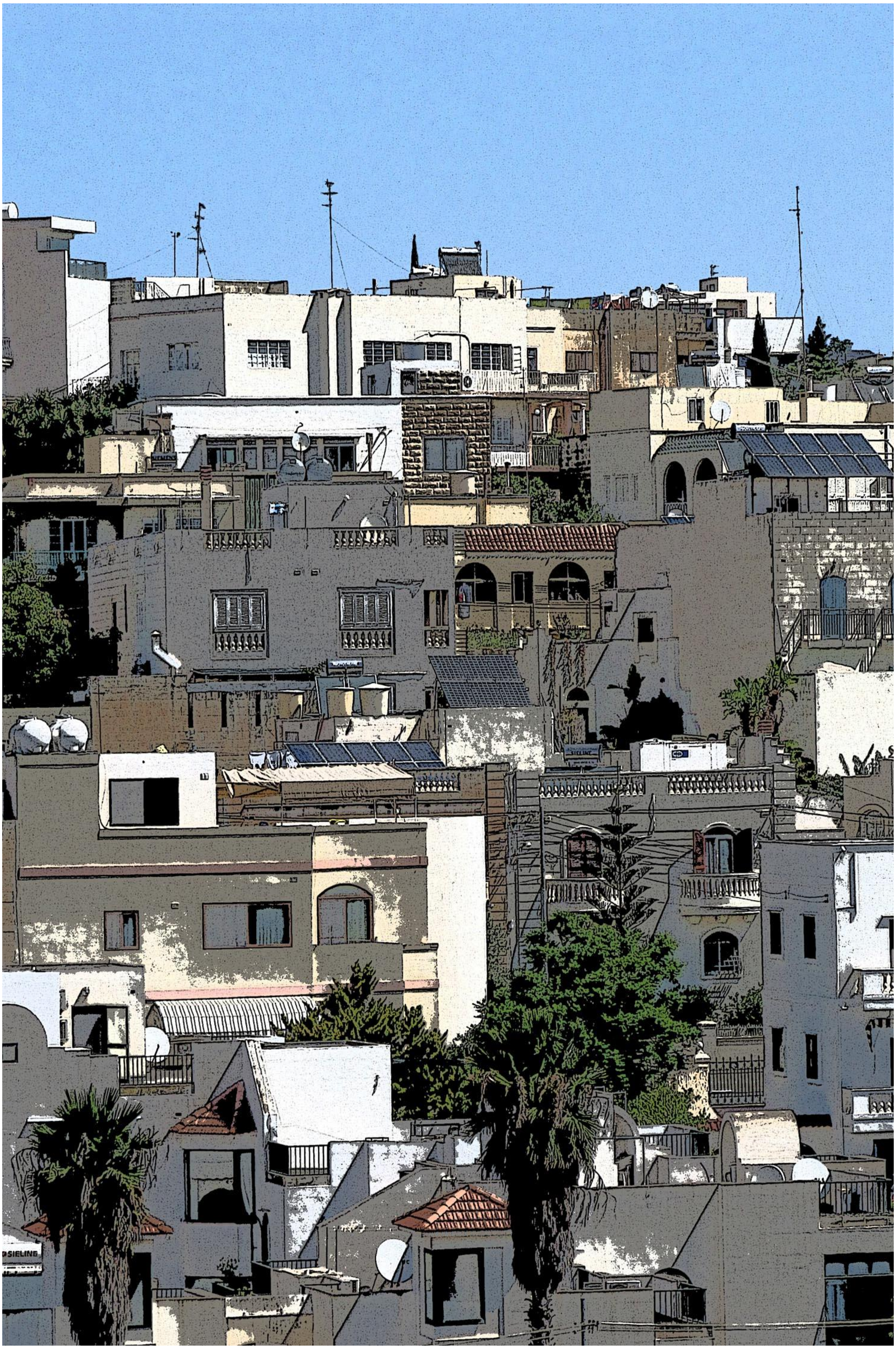

LifeMedGreenRoof Project

Plant hydrological stress and weed monitoring

LIFE12ENV/MT/000732

This project is partially funded through LIFE+ which is the EU's financial instrument supporting environmental and nature protection project throughout the EU.

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Executive Summary

This document has been drafted as part of the LifeMedGreenRoof project. It is partially funded through Life+ which is the EU's financial instrument supporting environmental and nature conservation projects throughout the EU. The LifeMedGreenRoof Project is managed by the Faculty for the Built Environment of the University of Malta with Fondazione Minoprio, Minoprio Analisi e Certificazione and the Malta Competition and Consumer Affairs Authority acting as partners. This report was drafted by Fondazione Minoprio as part of Action A2.

This study covers issues related to the drought tolerance of green roof plants and the monitoring of weeds within the green roof constructed at Fondazione Minoprio. The studies were conducted in 2016.

The tests concluded that there was variability among different species in terms of response to drought. Most of the species tested did not die and survived even severe drought conditions however this effected the water content within the plants as well as reduced their growth rate. Such survival rate was associated with the ability for such plants to have a high wilting point.

In terms of weed problems, the type of substrate composition did influence the degree of infestation. Plants able to resist weed infestation showed a high growth index when compared to plants which had high susceptibility to weeds. Climate and weather patterns also influence the presence and growth of weeds.

1. Background and Introduction

This document forms part of the deliverables required of the LifeMedGreenRoof project, which is an EU funded project under the Life+ Programme, to encourage the widespread use of green roof technology throughout the Maltese territory and Italy. The scope of this document is to highlight issues pertaining to the cultivation of plants on a green roof when using native species. The benefits of green roofs are well documented and utilising native vegetation will render green roofs more efficient in mitigating urban related projects and ameliorating the quality of life of urban dwellers. Using native species will also render the roofs more sustainable when considering the benefits to biodiversity.

Indeed, green roofs were developed in the North of Europe, where generally water availability occurs all year round and weed dissemination is fairly contained thanks to low temperatures and other meteorological conditions. It is worth stressing that generally in the North of Europe people are more sensitive to environmental and climatic issues.

In the last years within the Mediterranean region people's awareness to these problems has, to some extent, increased, not only at political level but also amongst the public in general.

Since their reintroduction in the 1980s, green roof technology became more reliable leading to the development of different types of roof greening. Systems have become lighter and cheaper which can even be utilised on weak structures and requiring less maintenance.

With experience and research, the benefits of roof greening became more apparent. Initially green roofs were installed for their aesthetic appeal, insulation properties and the protection of roof membranes from the natural elements. Over the years, additional benefits became apparent. Green roofs are known to provide important environmental benefits such as storm water retention, summer cooling, improvement of urban biodiversity, economical, sociological and ecological advantages (Provenzano M.E., 2004). It is of no surprise that green roofs have become such an important addition to urban environments in practically all continents.

Today green roofs are considered an important element in the creation of sustainable urban settlements. Unlike grey infrastructure, green roofs provide a number of beneficial services which target both the owner, the community and the natural environment at different levels.

Performance and benefits of a green roof can vary significantly depending on the type and depth of the substrate used, on the vegetation density (Compton and Whitlow, 2006) and on climatic and geographical factors (Getter and Rowe, 2008).

One aspect that remains unchanged is the fact that the roof environment is hostile to plants. Roofs are indeed exposed to weather phenomena such as heavy rain, hail, high winds, high solar radiation and intense shading, water stress, low nutrient availability and so on.

Tolerance to drought is an important aspect to consider in the choice of plants for a green roof. Whereas this might not be an issue in northern European countries because of the precipitation cycle, which is generally spread evenly on an annual basis, in the Mediterranean the picture is very different. Winters are mild and wet, however summers are generally hot and very dry impacting on the survival of plants. Extensive green roofs are made to be of low maintenance and self-regenerating. Thus the water regime is quite an important aspect to consider. On the other hand, weeds can pose a threat to cultivated species as they can be more aggressive weakening the desired plant populations and distorting the original planting design concept.

2. Water stress trial – Methodology and results

In green roofs, especially in Mediterranean environments, water availability is often limited. So, many experiments conducted on green roof plant selection frequently concern tests for drought stress (Durhman et al., 2004).

Water stress negatively affects new growth, and often causes physiological responses such as stomatal closure and reduction in photosynthetic rates (Taiz and Zeiger, 2002). The analyses of plants' physiological responses during growth have been widely used to measure the plants water status and their reaction to drought (Provenzano M.E., 2004).

During the project, water stress trials were conducted on the species which had the best ornamental and coverage response in the simulated green roof.

To detect the drought response of different plant species, many tests were carried out. These include analysing the Leaf Relative Water Content, Growth Index, leaf chlorophylls and carotenoids content, the ratio carotenoids/ chlorophylls, and proline content.

The species tested were cultivated in a greenhouse environment at Fondazione Minoprio, in Vertemate con Minoprio, (CO), at latitude of 45°43'31"08 N and longitude 9°4'26"40 E, at 342m m.s.l.

Plants were placed in plastic pots (Φ 15 cm), filled with substrate consisting of 35% pumice (Ø 3-8 mm), 40% lapillus (Ø 5-10 mm), 10% compost and 10% peat by volume. Additionally 4 g/L of a slow release fertilizer (Osmocote Start) was added in order to induce plants to develop their root system (Fig.1)

Plants were watered daily for at least 15 days before the beginning of the trial. After 15 days they were irrigated daily using gravimetric method according to three different levels of pF (refer to Fig.2):

•pF 1: water volume corresponding to 46% of total volume of substrate (-0,01 bar);

•pF 4,5: 9,89% of water from the volume of substrate (-50 bar);

•pF 5,3: 6,77% of water from the volume of substrate (-100 bar).

Figure 1 Plants undergoing tests in a controlled environment

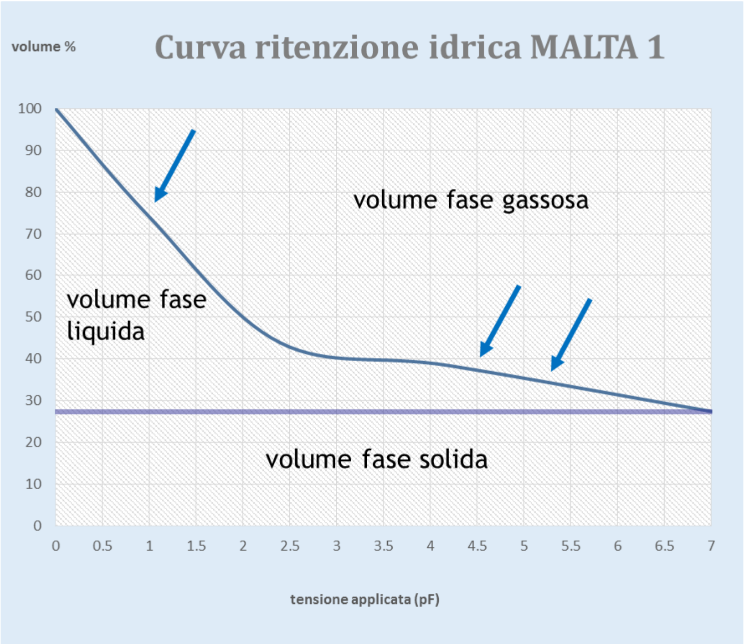


Figure 2 Water retention capacity curve of substrate called "Malta1"

The average daily evapotranspiration recorded per species is reported in the following diagrams (Fig. 3-4-5). As expected, succulent species (*Sedum album, S. acre, S. sediformis*) registered the least evapotranspiration. Species such as *Teucrium chamaedris* had a high water consumption (110ml at pF1). For each species, it transpired that evapotranspiration was decrease the more the pF increased.

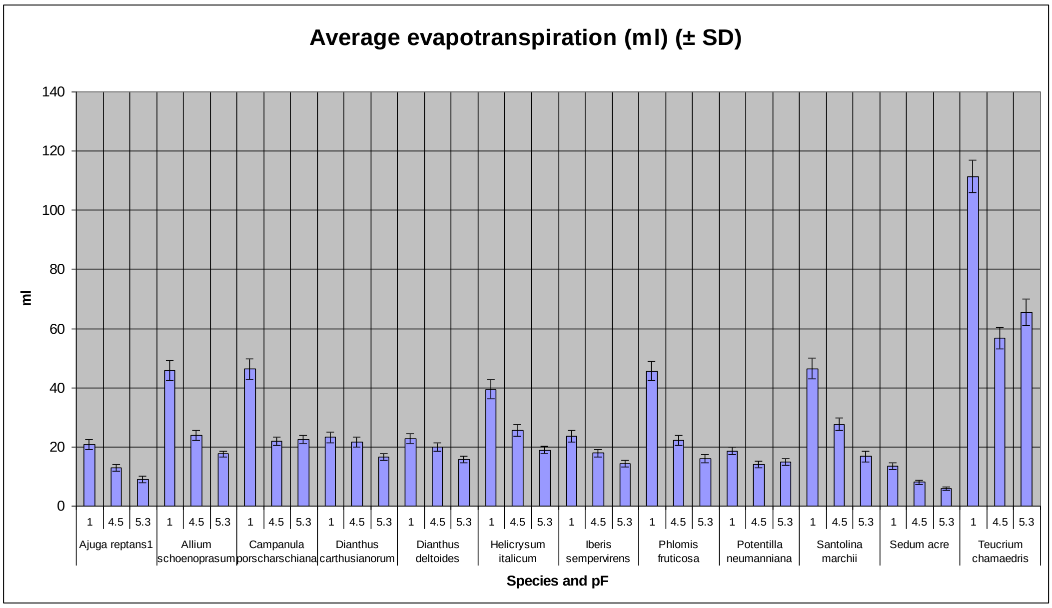


Figure 3 Average daily evapotranspiration of plants

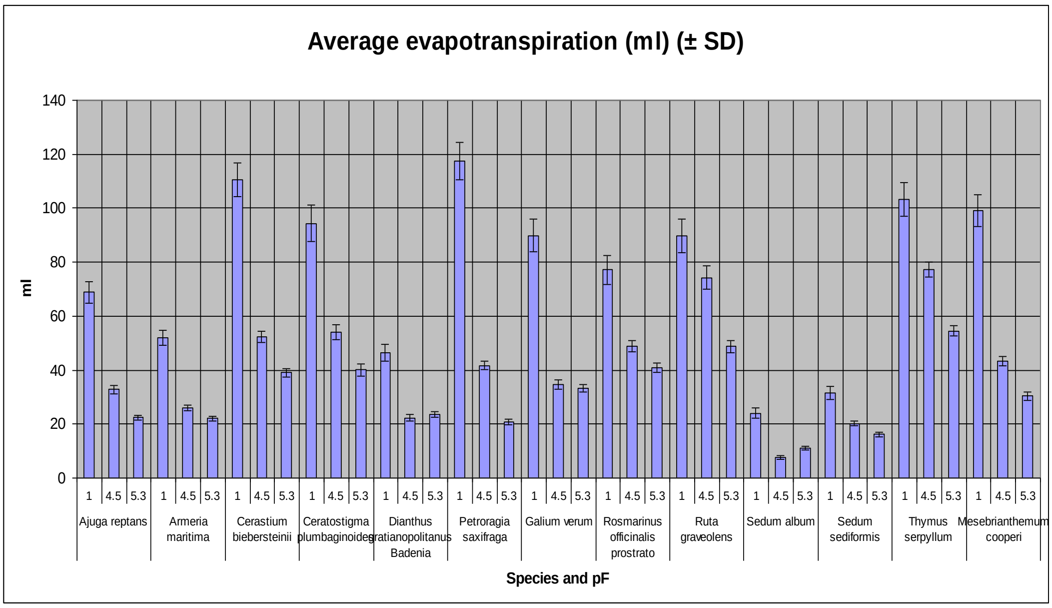


Figure 4 Average daily evapotranspiration of plants

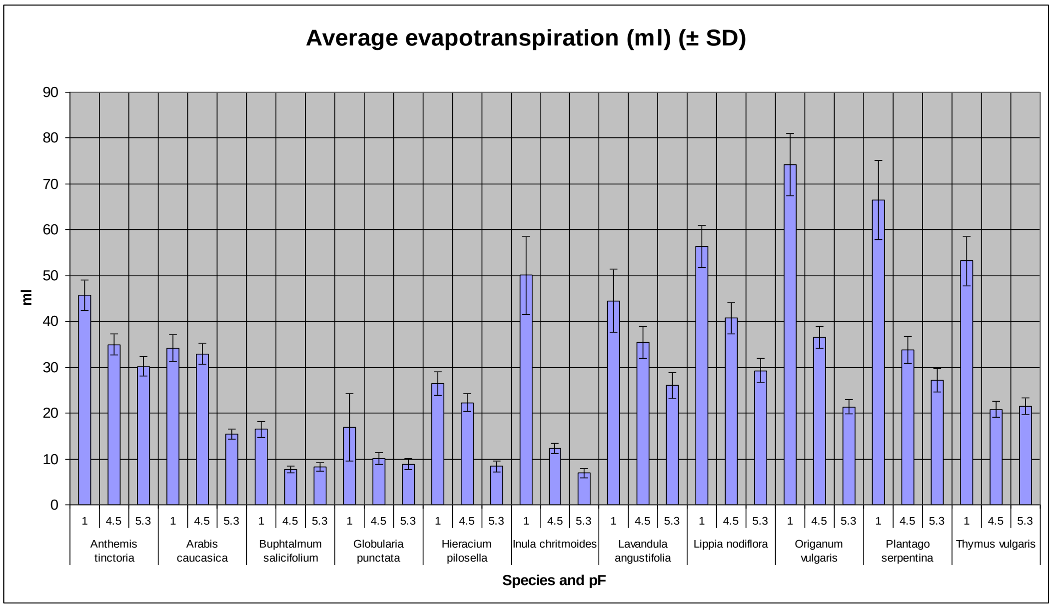


Figure 5 Average daily evapotranspiration of plants

At the beginning and at the end of the experiment the plants were weighed in order to calculate their growth. The growth index was established according to the following formula:

$$GI = \pi \left(\frac{W}{2} \right)^2 H$$

From this experiment, it resulted that generally plants were able to grow throughout the whole trial even in high water stress situation. It was established that at high pF levels, growth rate was reduced. The following graphs illustrate the development of the most significant results achieved.

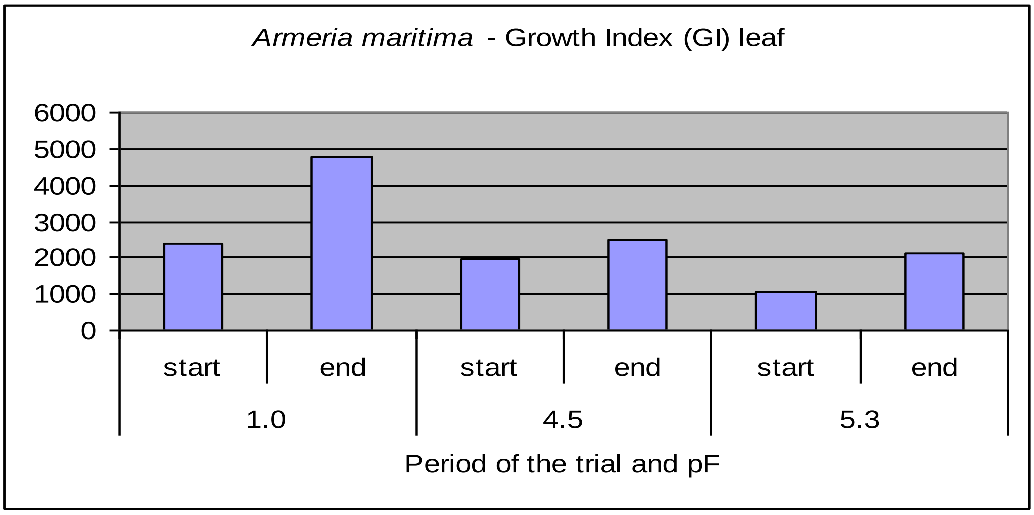


Figure 6 Growth index for Armeria maritima at pF 1.0, 4.5, 5.3

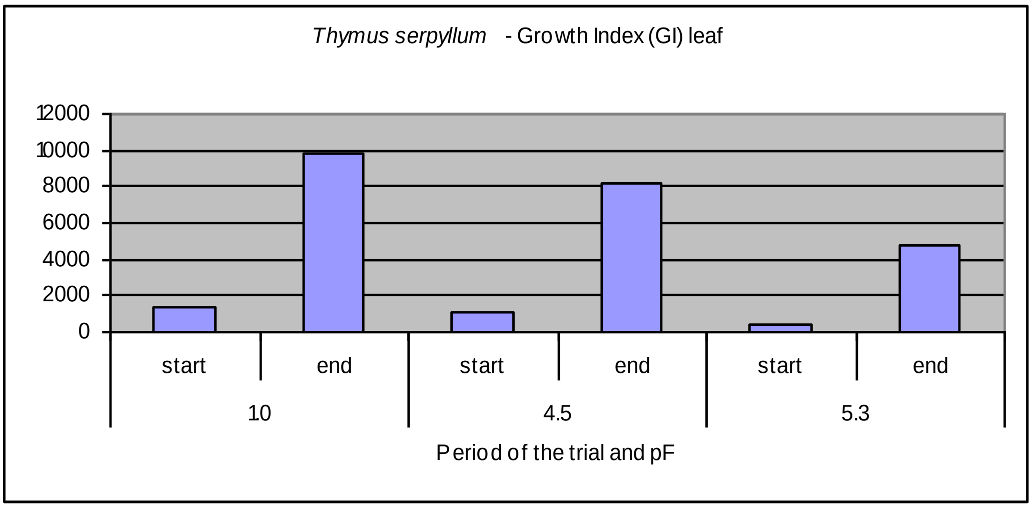


Figure 7 Growth index for Thymus serpyllum at pF 1.0, 4.5, 5.3

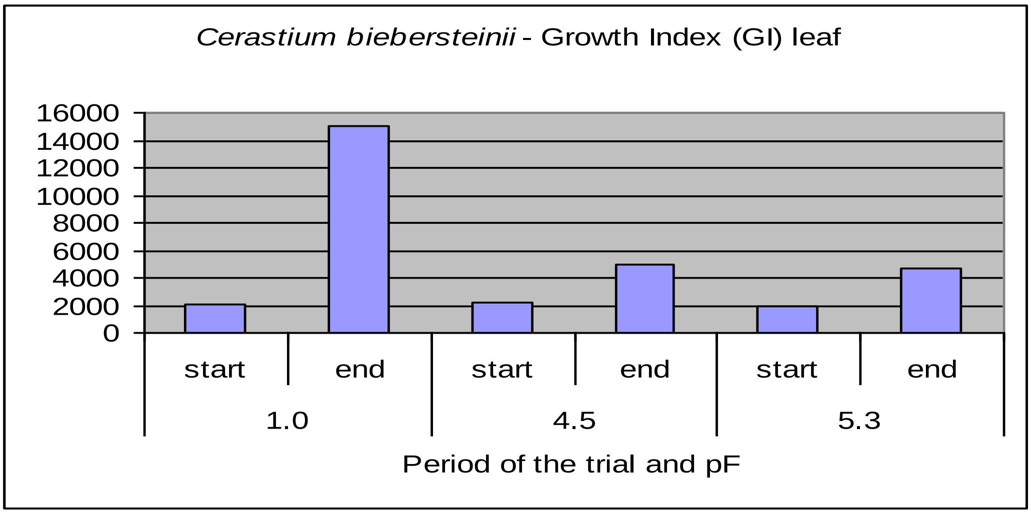


Figure 8 Growth index for Cerastium biebersteinii at pF 1.0, 4.5, 5.3

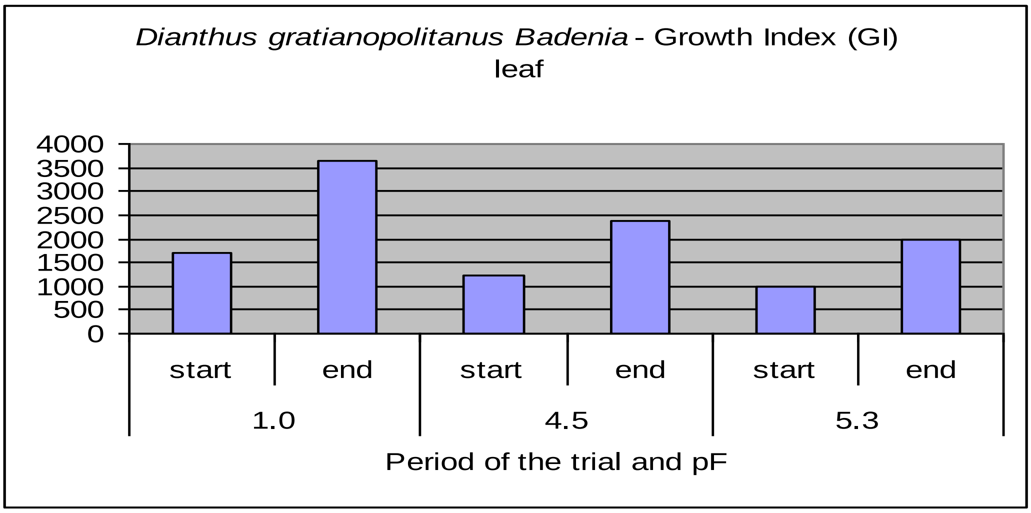


Figure 9 Growth index for Dianthus gratianopolitanus Badenia at pF 1.0, 4.5, 5.3

Sedum species had different behaviour during the period of trail: growth index remained stable in **S. album**, increased in *S. sediformis* and reduced in *S. acre* (Fig. 10-11-12).

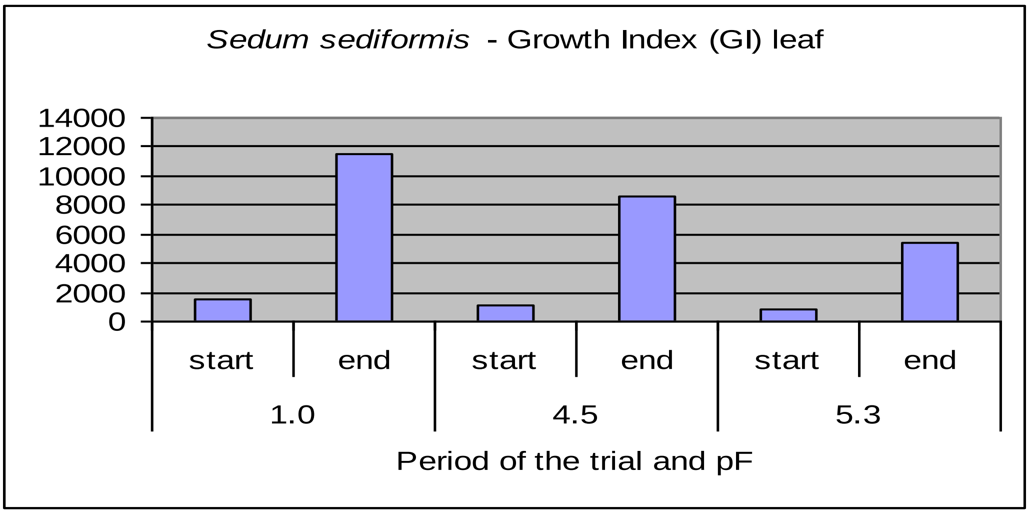


Figure 10 Growth index for Sedum sediformis at pF 1.0, 4.5, 5.3

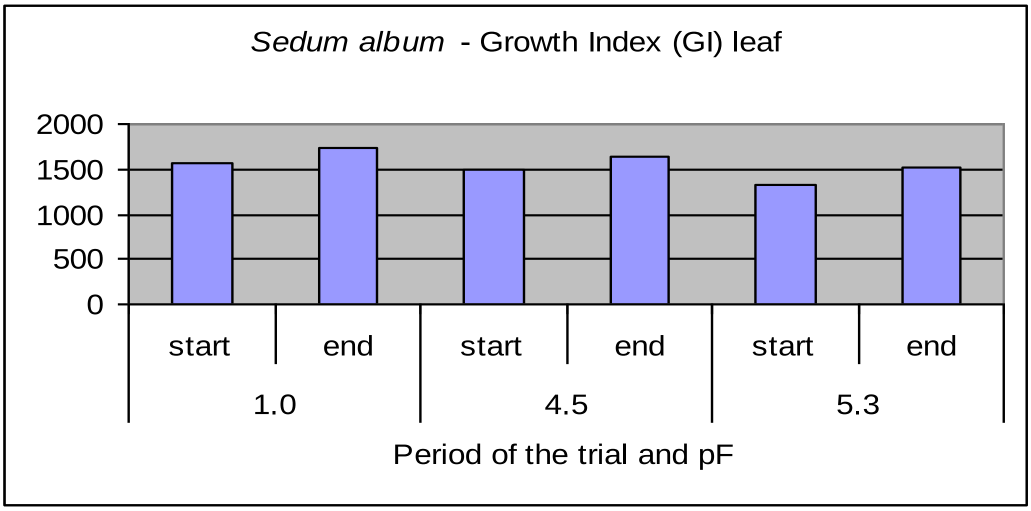


Figure 11 Growth index for Sedum album at pF 1.0, 4.5, 5.3

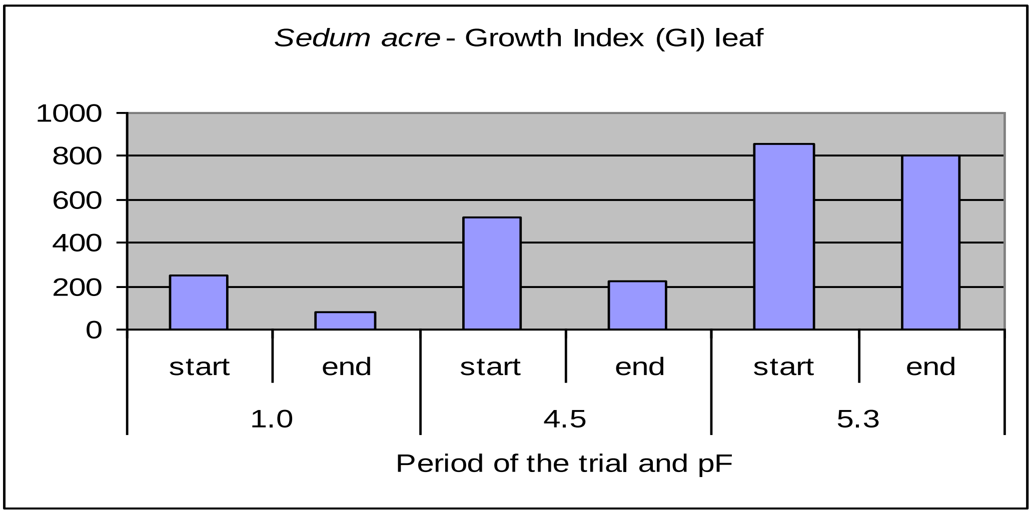


Figure 12 Growth index for Sedum acre at pF 1.0, 4.5, 5.3

Relative Water Content describes the water status of the plant during the period of testing. This is established according to the following formula:

$$RWC = \frac{(Fresh\ weight - Dry\ weight)}{(Turgid\ weight - Dry\ weight)} * 100$$

Relative water content (RWC) is the appropriate measure of plant water status in terms of the physiological consequence of cellular water deficit.

RWC is an appropriate estimate of plant water status in terms of cellular hydration under the possible effect of both leaf water potential and osmotic adjustment. It estimates the water content of the sampled leaf tissue relative to the maximal water content it can hold at full turgidity. RWC is a measure of water deficit in the leaf.

Normal values of RWC range between 98% in fully turgid transpiring leaves to about 30-40% in severely desiccated and dying leaves, depending on plant species. In most crop species the typical leaf RWC at around initial wilting is about 60% to 70%, with exceptions.

During the tests carried out and as expected, RWC decreased in water stressed plants, but often the value did not decrease below 70-75% (Fig.13, 14, 15).

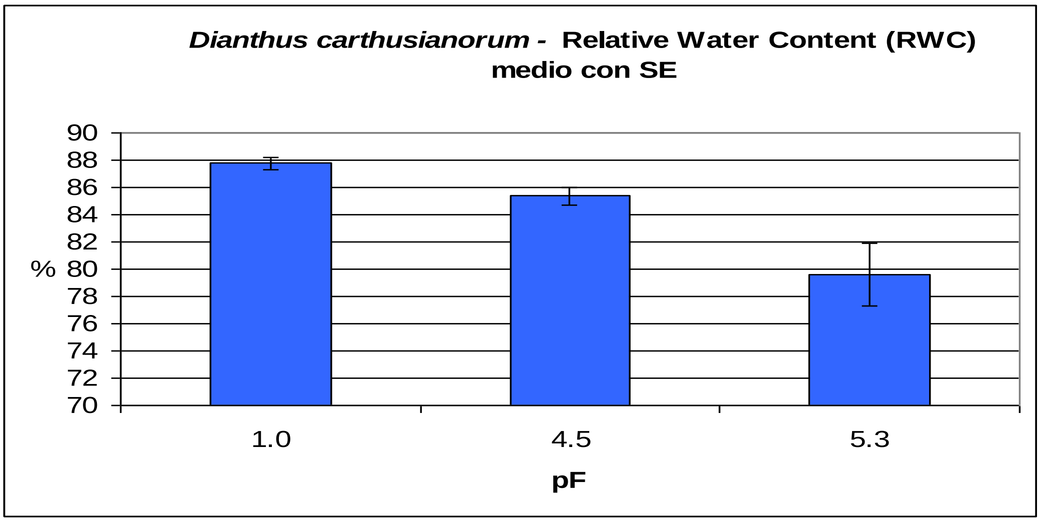


Figure 13 Relative Water Content of Dianthus carthusianorum at pF 1.0, 4.5, 5.3

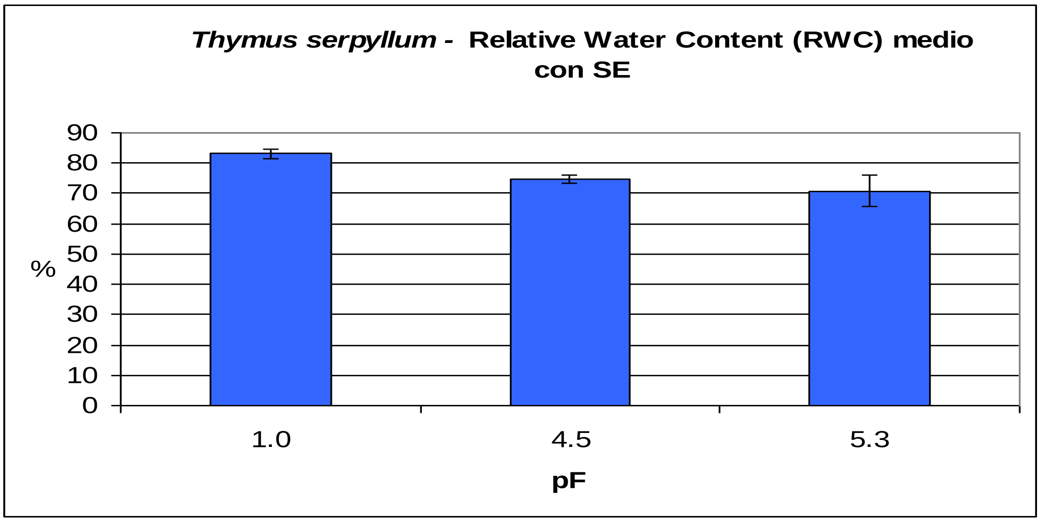


Figure 14 Relative Water Content of Thymus serpyllum at pF 1.0, 4.5, 5.3

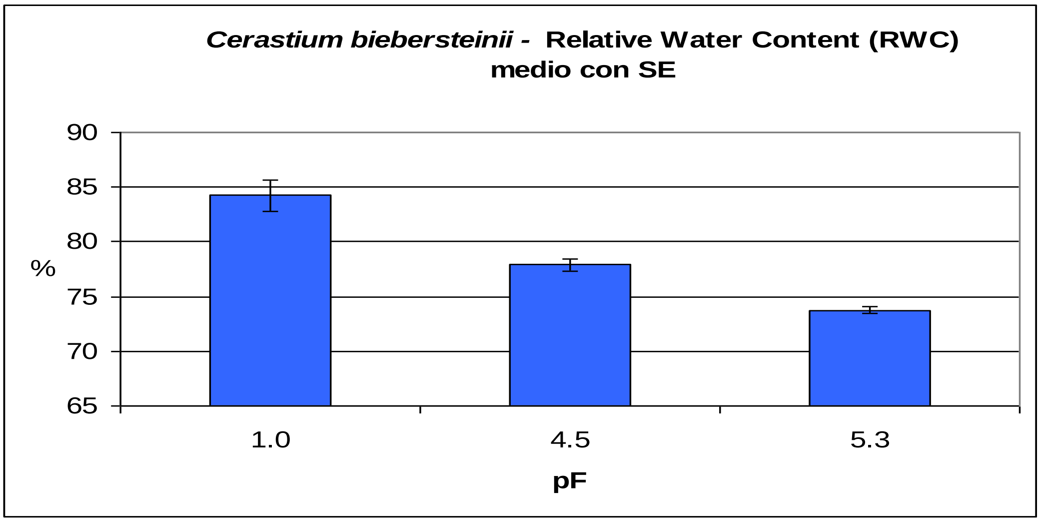


Figure 15 Relative Water Content of Cerastium biebersteinii at pF 1.0, 4.5, 5.3

Chlorophylls and carotenoids contents were evaluated at the beginning and the end of the trials, according to the Arnon method (1949).

Variations in pigment content may provide valuable information concerning the physiological status of the plants. Chlorophylls and carotenoids decline when plants are under stress or during leaf senescence (Gitelson et al., 2003). However, other authors (Monterusso et al., 2005) observed that native species do not necessarily perform as expected in stressful conditions.

The two species which reduced chlorophylls and carotenoids content during hydrological stress situations were *Santolina marchii* and *Buphtalmum salicifolium* (Fig. 16, 17, 18, 19). This in line with reported studies. Tests conducted during the project period confirm previous observations (Gitelson et al., 2003).

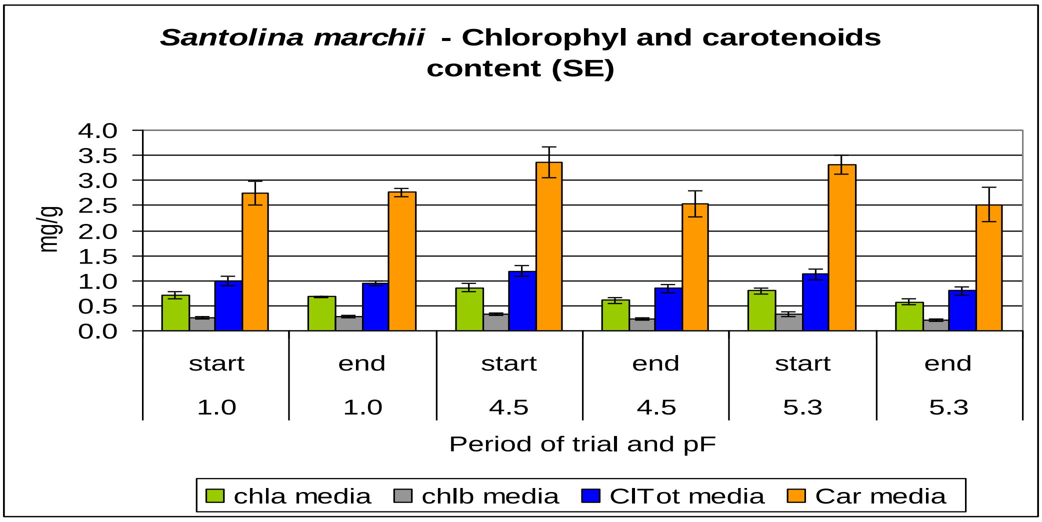


Figure 16 Chlorophyl and carotenoid content in Santolina marchii

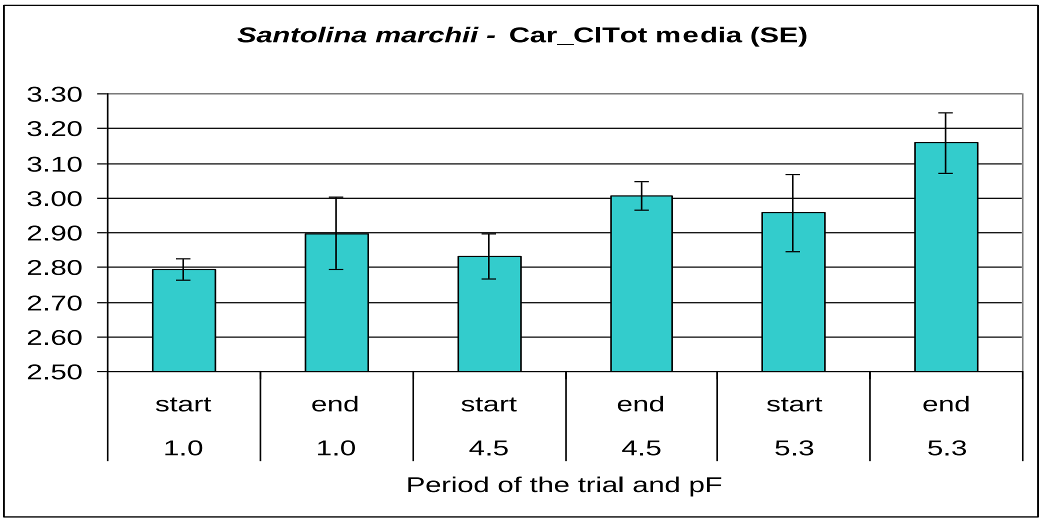


Figure 17 Ratio between carotenoid and chlorophyll in Santolina marchii

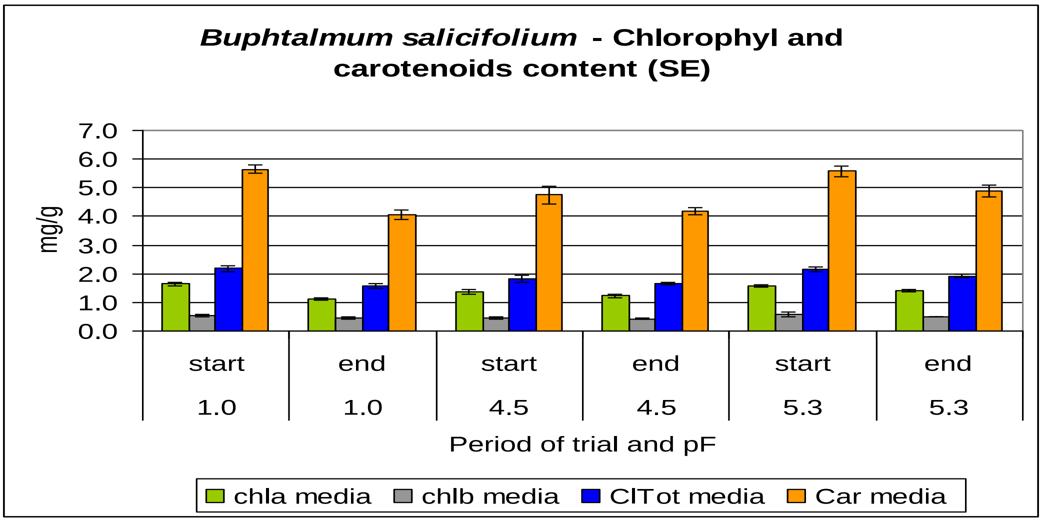


Figure 18 Chlorophyl and carotenoid content in Buphtalmum salicifolium

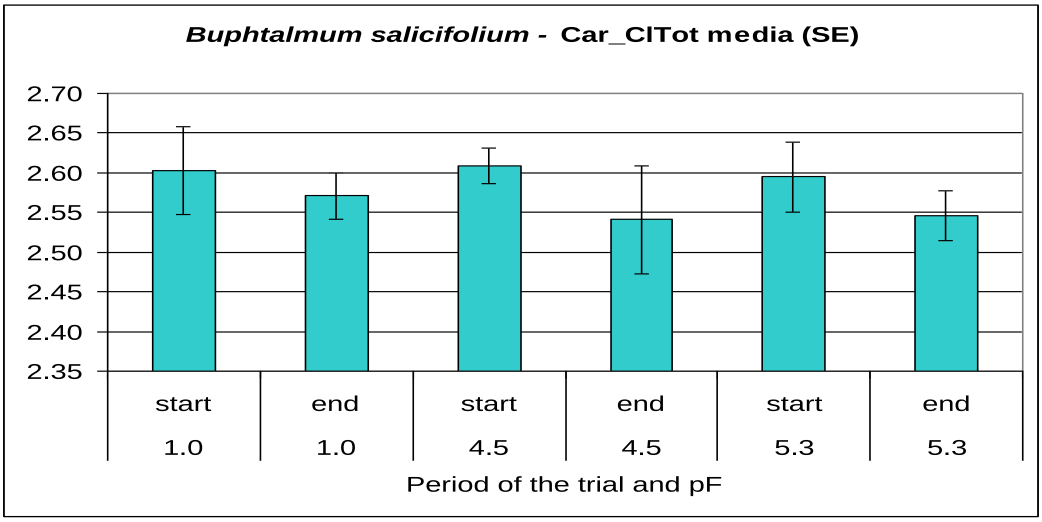


Figure 19 Ratio between carotenoid and chlorophyll in Buphtalmum salicifolium

Different behaviour was observed in many of the species tested. For example, *Armeria maritima* increased chlorophylls and carotenoids content (Fig. 20, 21), as reported by Provenzano (2004). Water status and pigment content at higher pF verified the high drought tolerance of *Armeria maritima*.

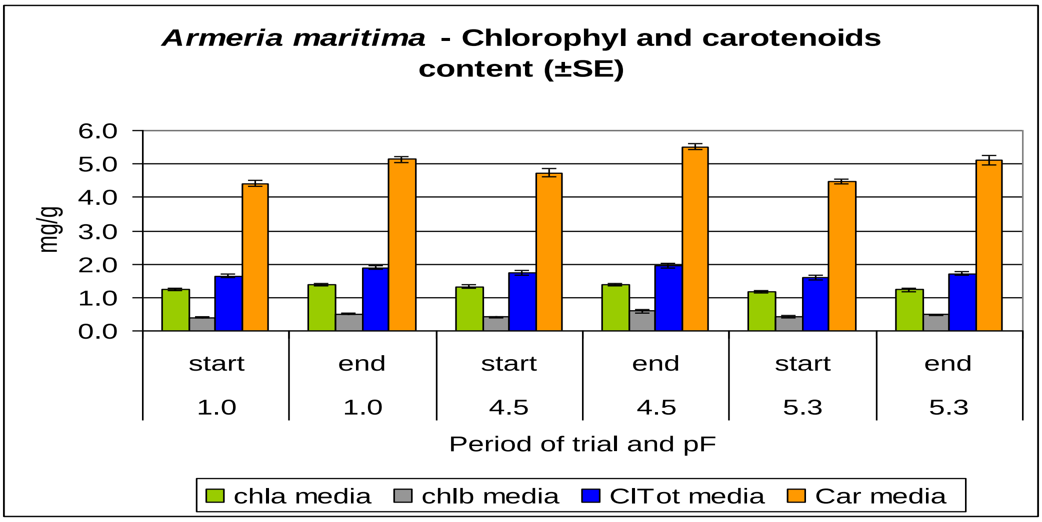


Figure 20 Chlorophylls and carotenoids content in Armeria maritima

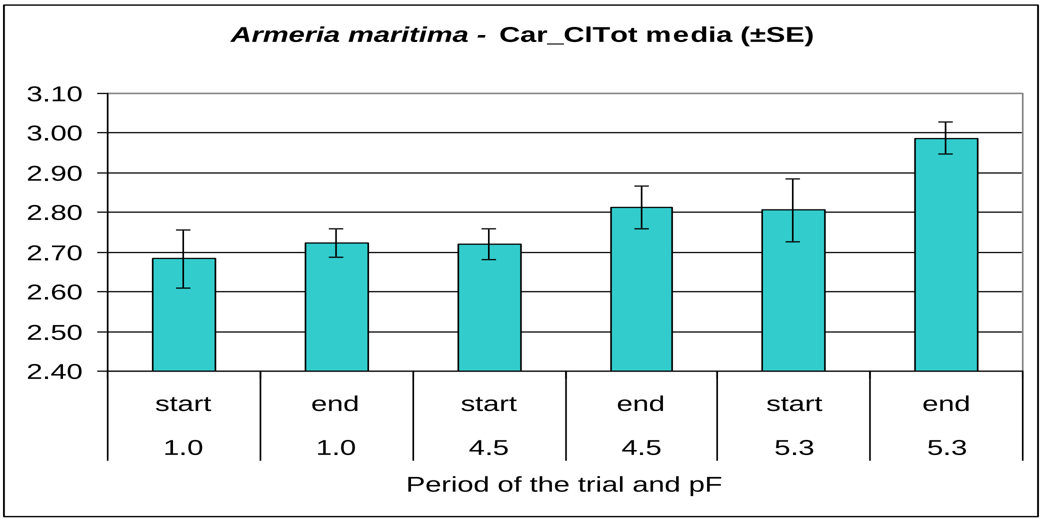


Figure 21 Total carotenoids and chlorophylls in Armeria maritima

This attitude was also observed in *Allium schoenoprasum* (Fig.22, 23). According to a study by Egret & Tevini (2002), pigments content in stressed plants does not differ when compared to well-watered plants.

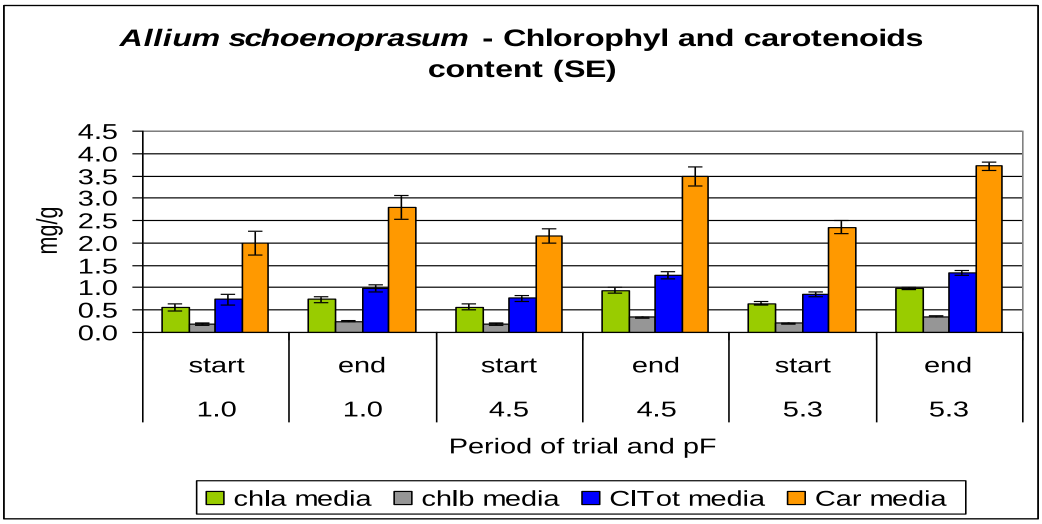


Figure 22 Chlorophylls and carotenoids content in Allium schoenoprasum

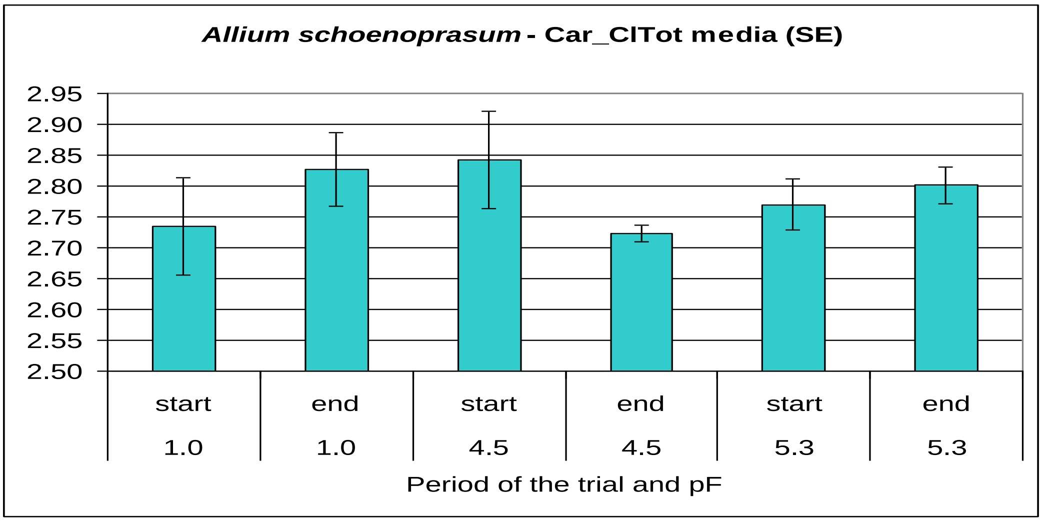


Figure 23 Total carotenoids and chlorophylls in Allium schoenoprasum

Proline is an α-amino acid that is used in the biosynthesis of proteins. Many studies reported an increase in free proline in water stressed plants (Oraki et al., 2011).

In fact, recent studies demonstrated that biosynthesis of low-molecular-weight metabolites, such as proline improved plant tolerance to drought and salinity in a number of crops (Molinari et al., 2004). Proline accumulation in plant cells exposed to water stress or salt is a widespread phenomenon and is often considered to be involved in stress resistance mechanisms, although its precise role continues to be controversial (Hare et al., 1999). Its role in stress tolerance is due to protein structure maintenance by free proline.

Proline testing was evaluated according to Bates (1972). At the end of the trial many species increased their proline content at high pF values, in accordance to the above mentioned studies (Fig. 24, 25, 26).

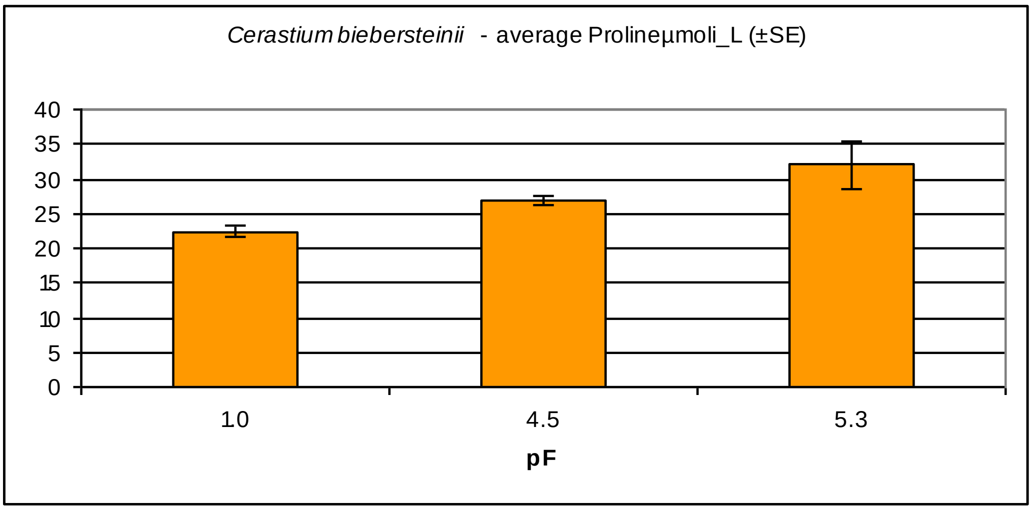


Figure 24 Increase in Proline content in Cerastium biebrestenii at pF 1.0, 4.5 and 5.3

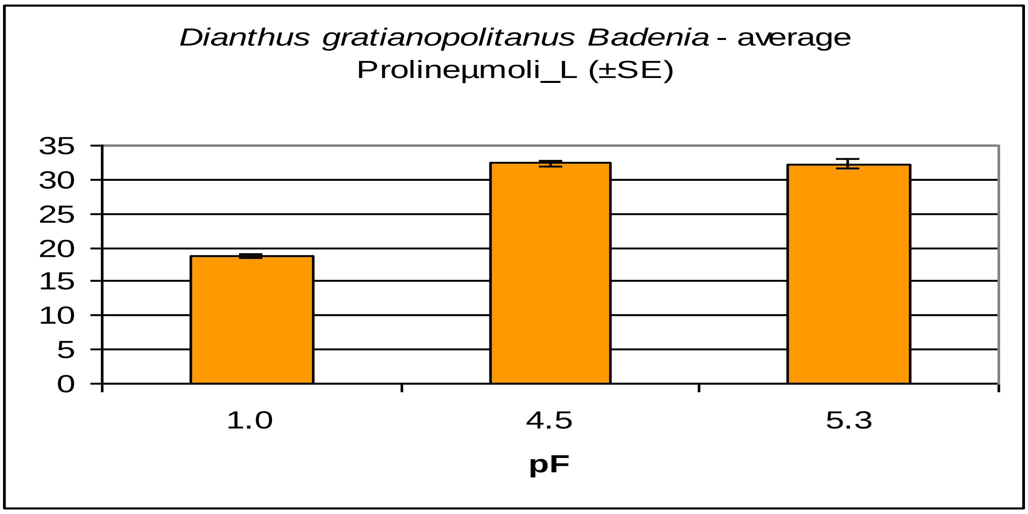


Figure 25 Increase in proline content in Dianthus gratianopolitanus Badenia at pF 1.0, 4.5 and 5.3

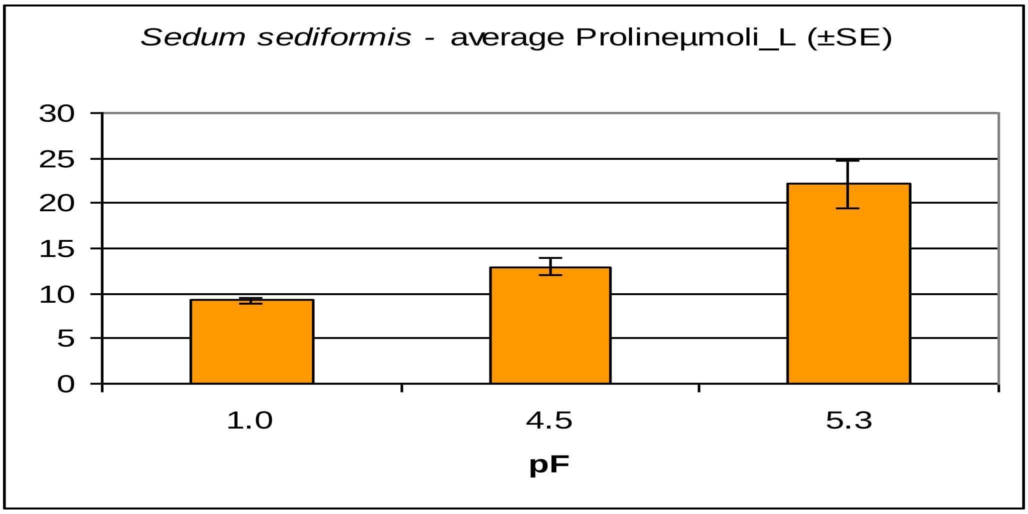


Figure 26 Increase in proline content in Sedum sediformis at pF 1.0, 4.5 and 5.3

3. Weed monitoring

During the project, areas of the Italian green roof were monitored in order to detect the different types of weeds, their spread during the vegetative season and the growth of plants cultivated during the period of observation. Weed and plant monitoring occurred during 2016 and are still on going. Fig. 27, 28, 29, 30 illustrate the weather conditions of the period of testing.

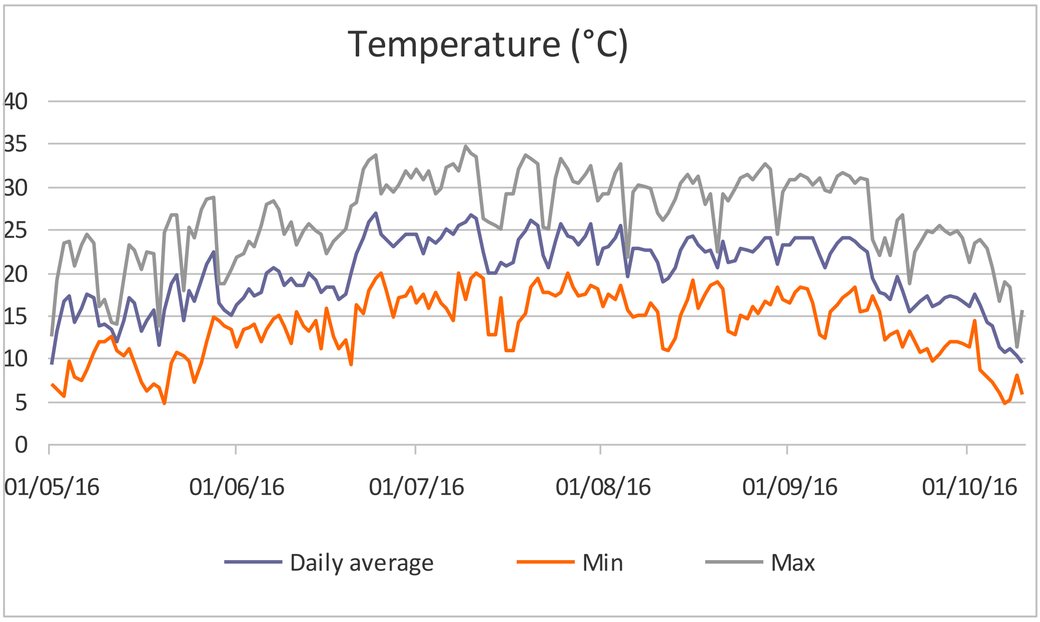


Figure 27 Daily Min, Ave, and Max temperatures for 2016

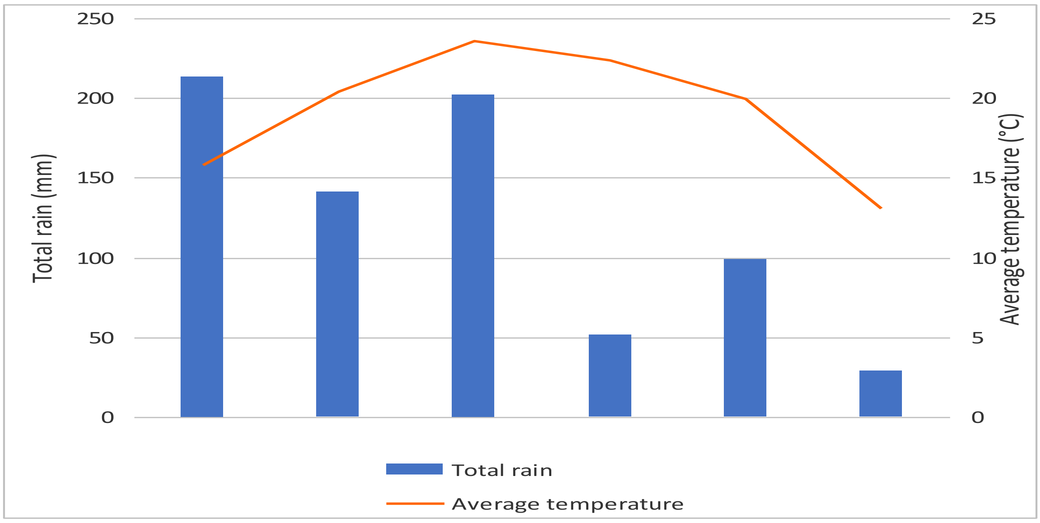


Figure 28 Average temperature and total rainfall at FM

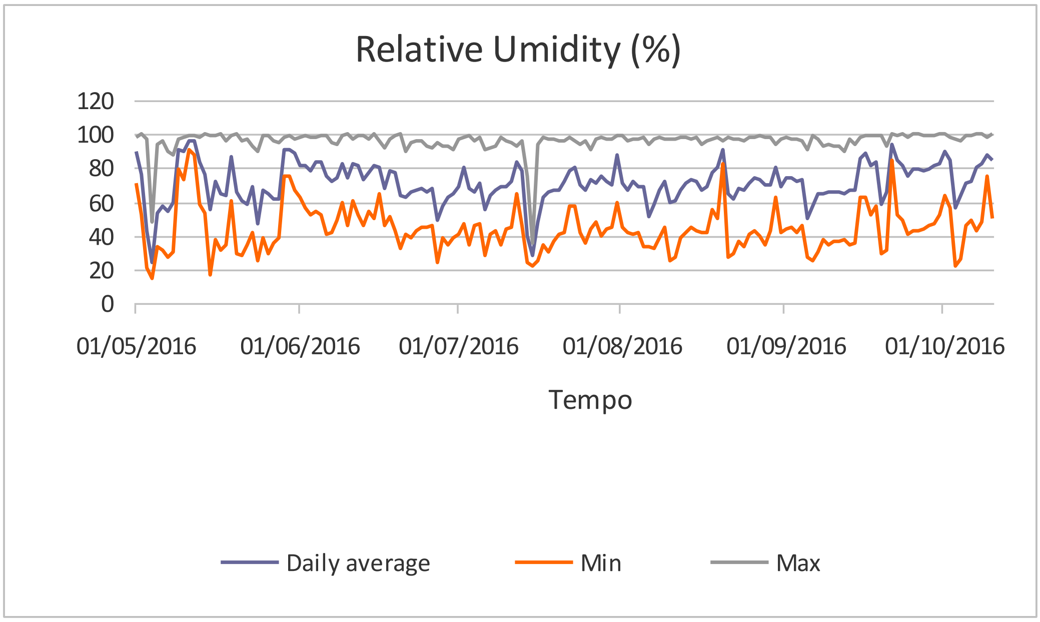


Figure 29 Relative humidity % (Min, Ave and Max)

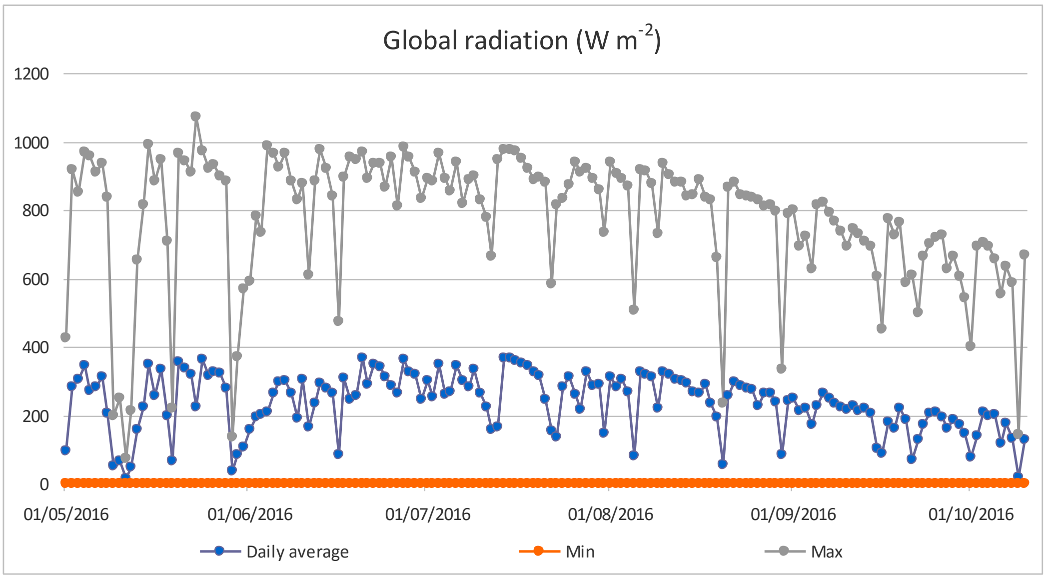


Figure 30 Global radiation (Wm")

The Italian green roof has a total area of 217 m^2 and is divided into 6 sectors one of which is the reference gravel roof (Fig. 31). The cultivated species are *Armeria maritima* (Mill.)Willd, *Cerastium biebersteinii* DC., *Dianthus carthusianorum* L., *Dianthus gratianopolitanus* L.(two varieties ,'Badenia' and 'Stafa'), *Potentilla neaumanniana* Rchb, *Thymus serpyllum* L., and *Sedum album* L.. The latter was planted vegetatively interspersed with the other species to act as weed suppressant.

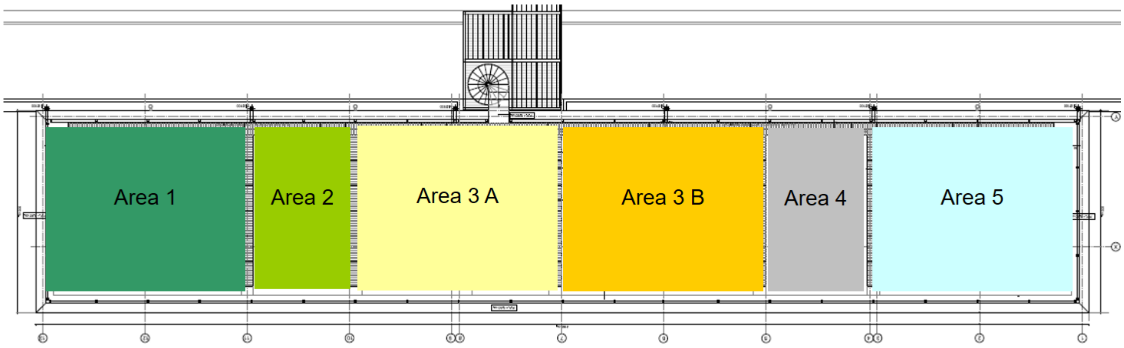


Figure 31 Diagram of the Italian green roof at Fondazione Minoprio

Table 1 Substrate types used on the green roof at Fondazione Minoprio

	MAC 7 –	MAC 7 no biochar –	MAC 7 no biochar no peat –	Gravel	TA - 6 –
	AREA 1 + 2	AREA 3a	AREA 3b	AREA 4	AREA 5
	(Substrate 1)	(Substrate 3A)	(Substrate 3)		(Substrate 5)
COMPONENTS	%	%	%	%	%
Pumice 3-8	-	-	-	-	45
Pumice 6-14	30	30	30	-	-
Green compost	5	10	10	-	15
Biochar	10	-	-	-	-
Peat	15	20	-	-	10
Coconut fibre	-	-	20	-	-
Expanded clay 2-8	40	40	40	-	30
	100	100	100	-	100
Gravel	-	-	-	100	-

Because of the lack of significant differences between the five areas, weeds analysis was evaluated based on the 6 species used.

In 2016, four sets of data were collected. These took place in May, early July, August and October. Data analysis were carried out by means of a wooden quadrat with an area of 0.25 m^2 (Fig.32) for each species and repeated twice per each of the 4 plots. Density data were then presented as number per square meter.
For every species, the Growth Index (GI) was calculated, in order to verify their development.
Weed density measured abundance of plants in a plot and was calculated as the number of species contained in each quadrat divided by 2 (the number of quadrats per plot) and by its surface (0.25 m^2).
Weeds were classified as total weeds, dycotiledon and graminaceous, anemocore and non-anemocore. Fig. 32. Illustrates the wooden quadrat used to evaluate plants.

Figure 32 Wooden quadrat used for evaluation

Plants growth of the selected species

Thymus serpyllum has shown the highest growth, which was homogeneous in all substrates (Fig. 33). *Potentilla neumanniana* was not effected much by the weeds however it registered a Growth Index lower than *Thymus sp*.

Cerastium biebersteinii, *Dianthus carthusianorum*, *Dianthus gratianopolitanus* and *Potentilla neumanniana* exhibited similar growth.

The species with the lowest growth was *Armeria maritima*, probably due to its susceptibility to weeds.

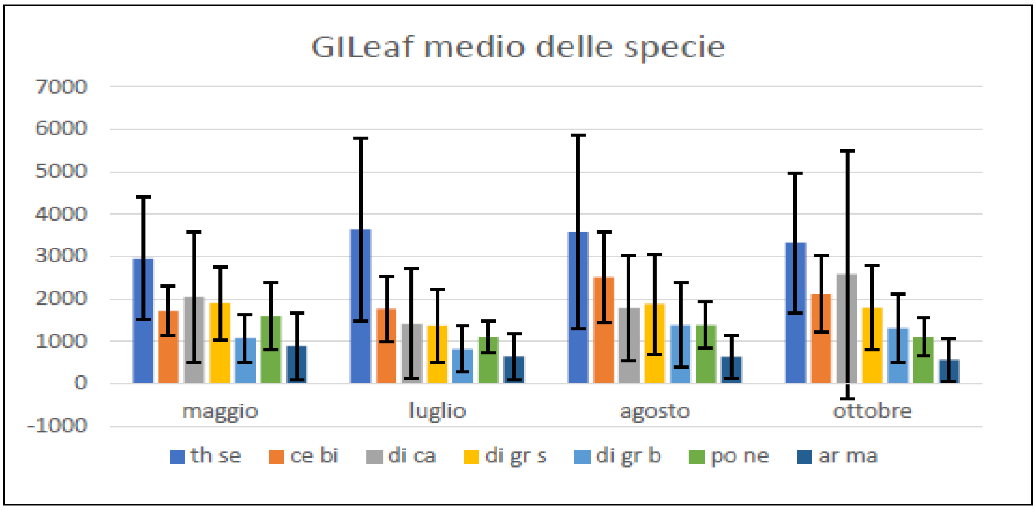


Figure 33 Growth Index of various plant species

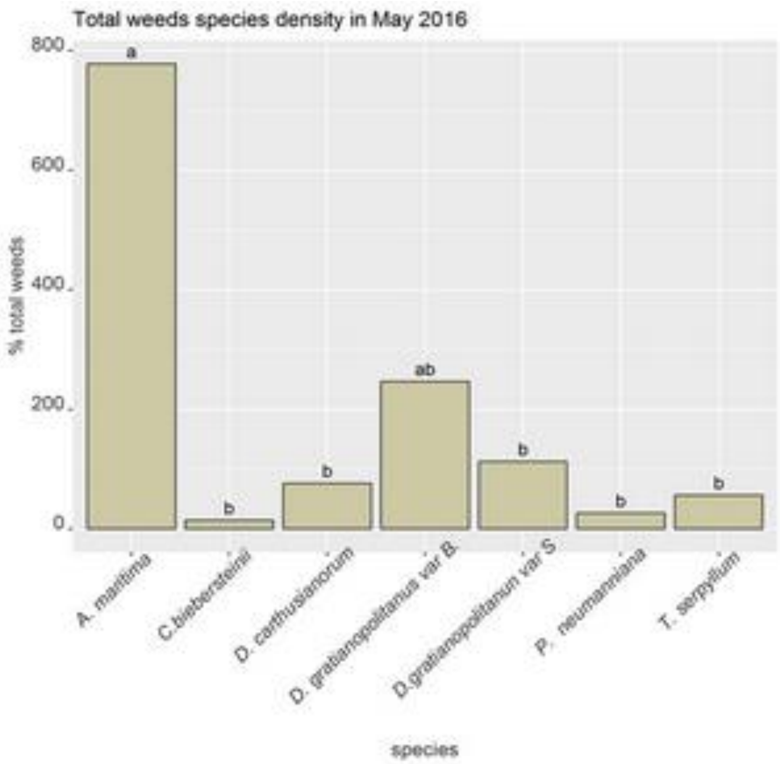
Sedum album, which was interspersed with other plants to suppress weeds because of its ease in establishment and propagation, had highest coverage when growing with *Armeria maritima*, the species with the lowest Growth Index (Fig. 33). Maximum coverage was achieved in October.

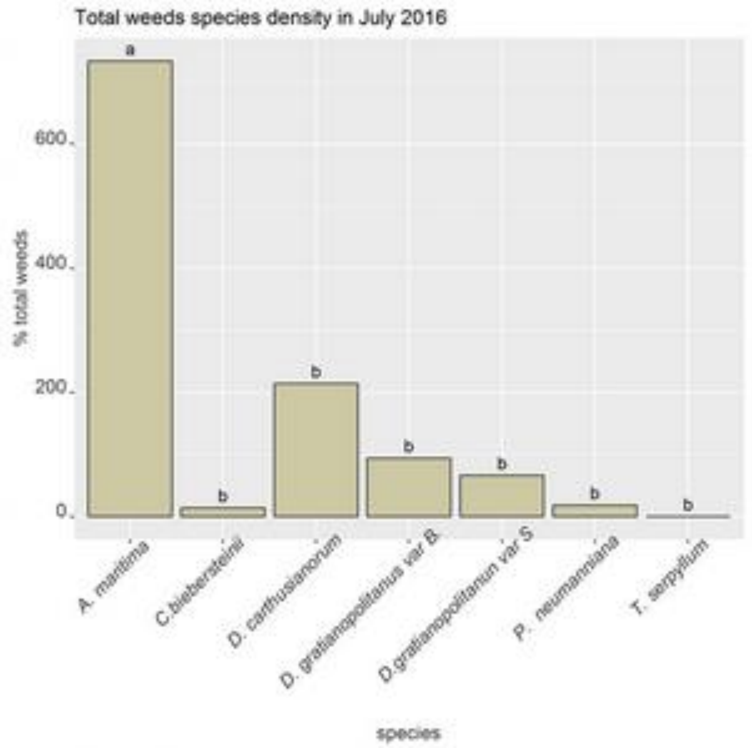
Figure 34 Sedum album coverage in the quadrat with Armeria maritima, in May (to the left) and October (to the right).

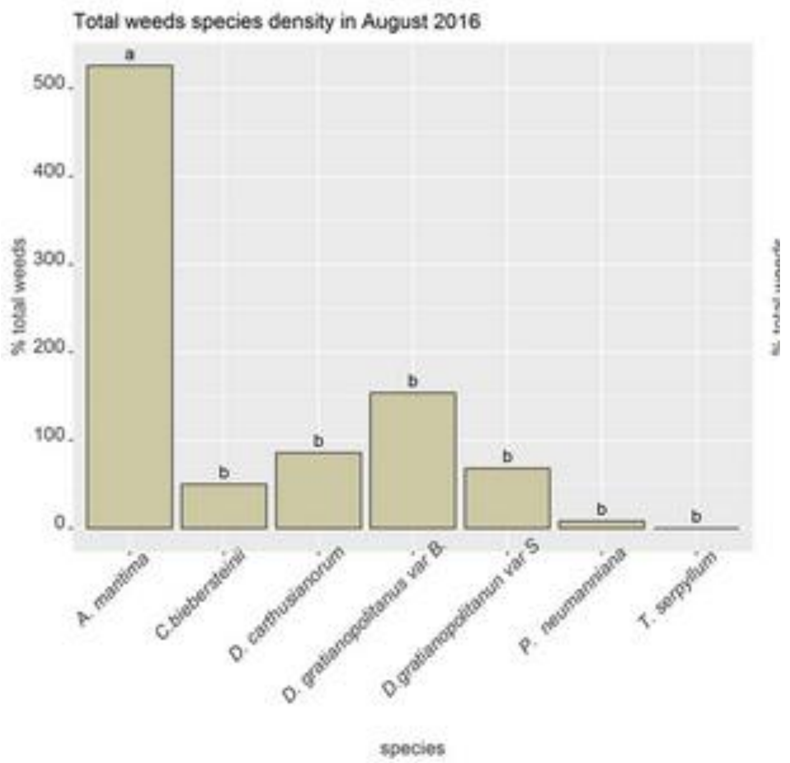
Cultivated specie did not differ for their growth in different substrates.

Weeds monitoring

Total weed density was found to be much higher in *Armeria* during all four sampling events, and density was always above 400 individual plantlets per square meter (Fig.35): the maximum value recorded was of almost 800 plantlets. This happened in May. From then on the numbers recorded decreases every time records were taken. The Dianthus species were the second most infested: their scores were stable in the first two surveys, but increased in the autumn when they reach 150-300 weed plantlets/m^{-2}. This was significantly different from the other species.




Total weeds species density in July 2016
% total weeds
600
400
200
0
a
b
b
b
b
b
b
A. maritima
C.biebersteinii
D. carthusianorum
D. gratianopolitanus var B
D.gratianopolitanun var S
P. neumanniana
T. serpyllum
species


Total weeds species density in August 2016
% total weeds
500
400
300
200
100
0
a
b
b
b
b
b
b
A. maritima
C.biebersteinii
D. carthusianorum
D. gratianopolitanus var B
D.gratianopolitanun var S
P. neumanniana
T. serpyllum
species

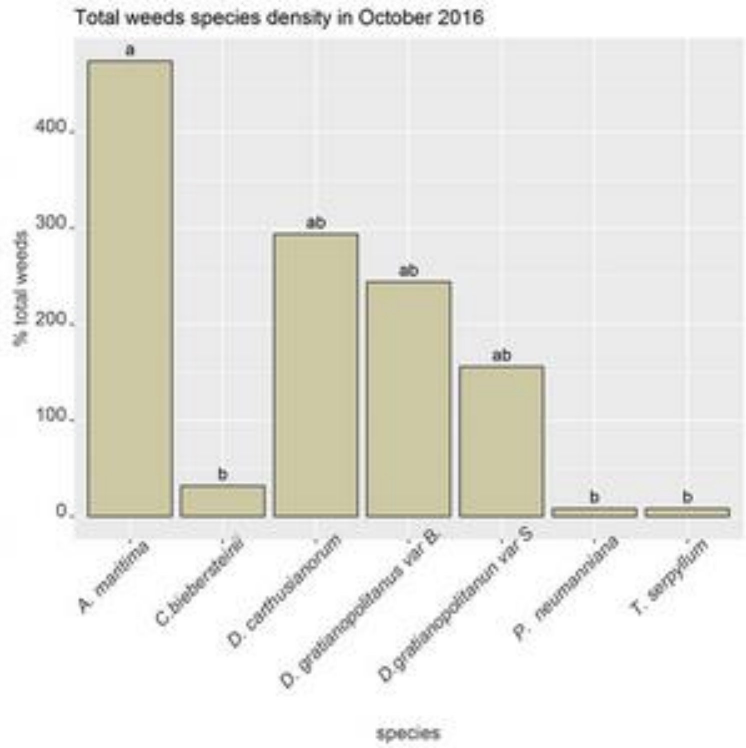
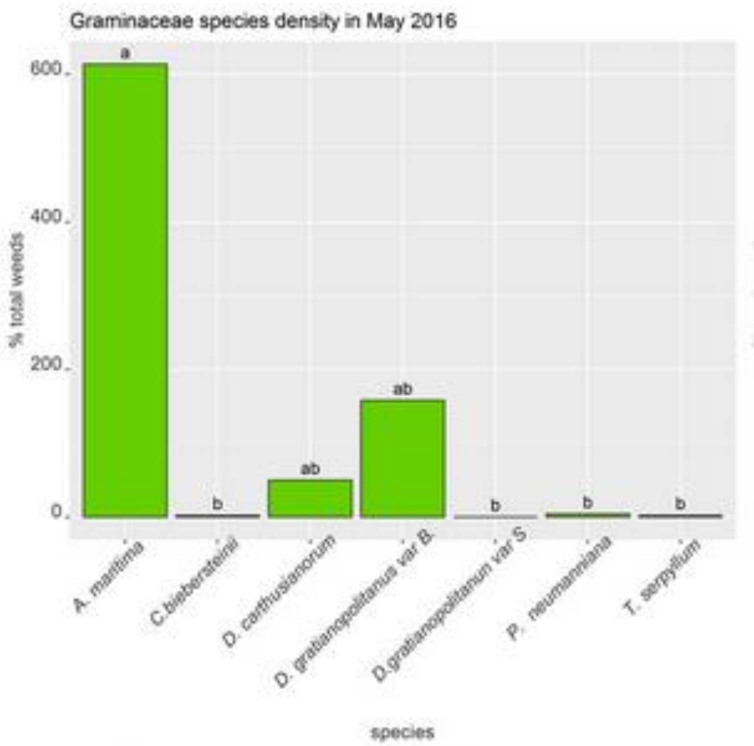
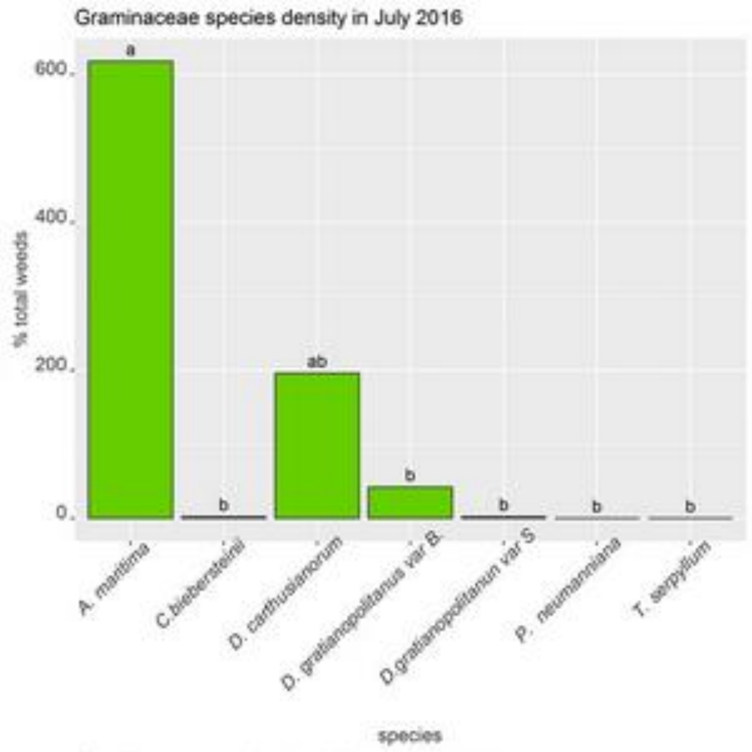
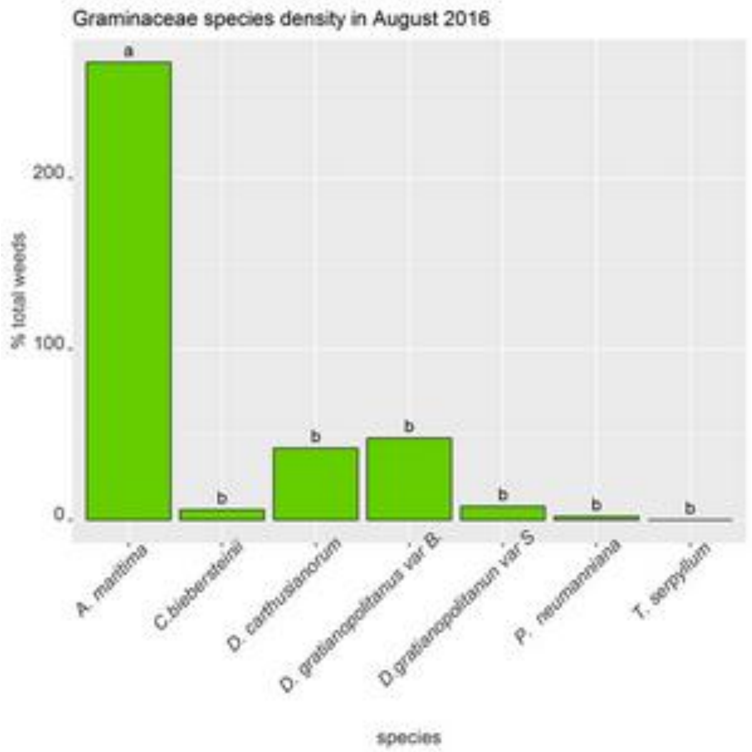


Figure 35 Total weeds density along different months and within the planted species

Cerastium,Thymus and *Potentilla* were always the least infested species. This was likely due to very good plant coverage and allelopathic activities.




Graminaceae species density in July 2016
% total weeds
600
400
200
0
a
ab
b
b
b
b
b
A. maritima
C.biebersteinii
D. carthusianorum
D. gratianopolitanus var B.
D.gratianopolitanun var S
P. neumanniana
T. serpyllum
species


Graminaceae species density in August 2016
% total weeds
200
100
0
a
b
b
b
b
b
b
A. maritima
C.biebersteinii
D. carthusianorum
D. gratianopolitanus var B.
D.gratianopolitanun var S
P. neumanniana
T. serpyllum
species

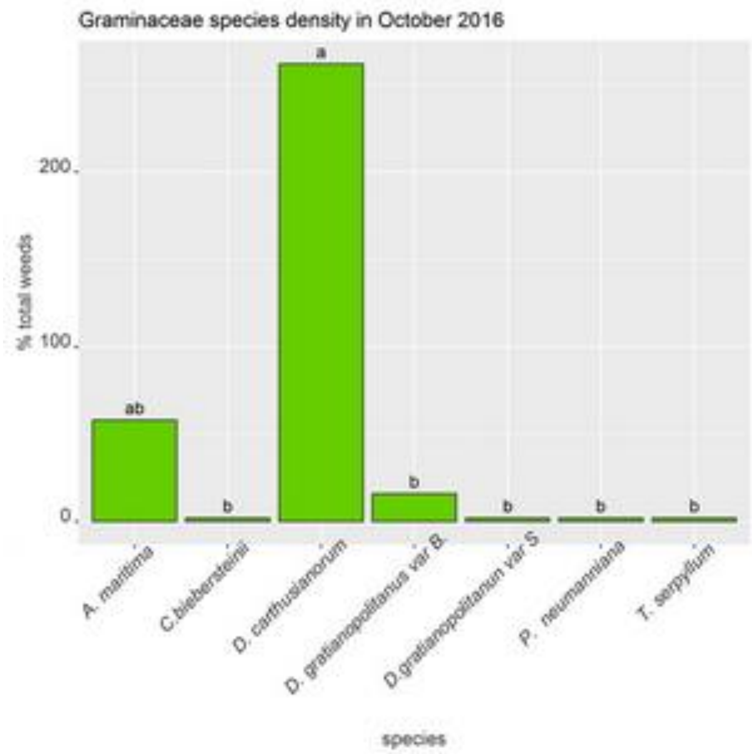
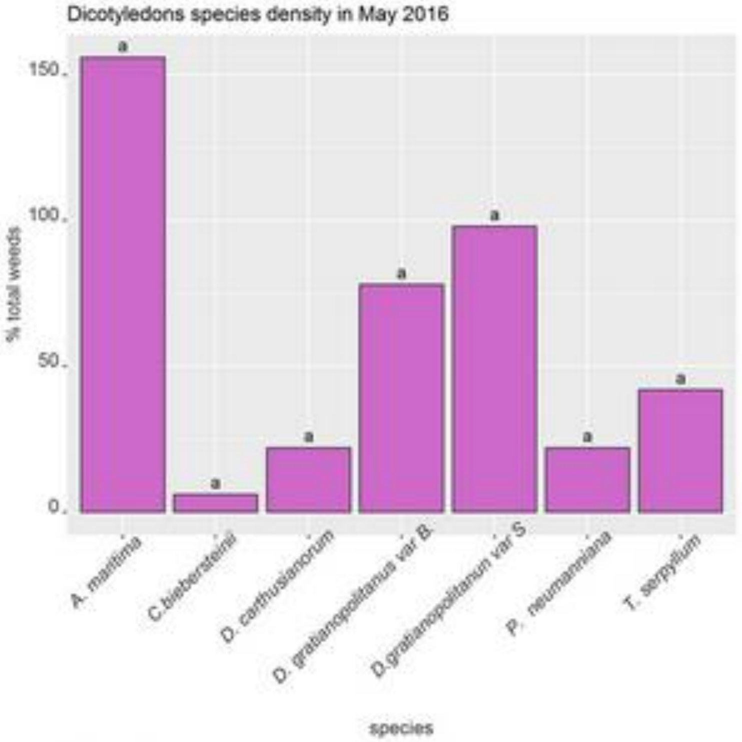


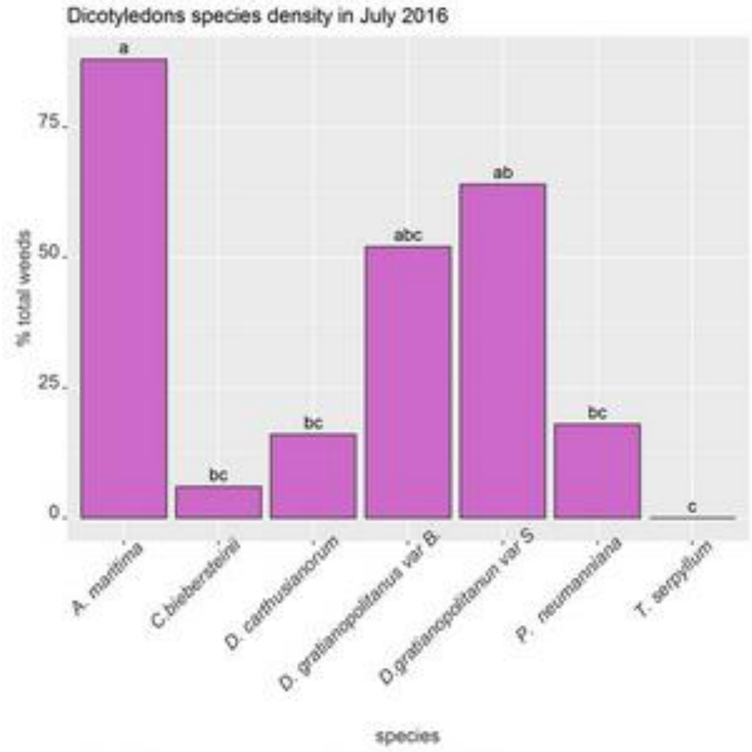
Figure 36 Graminaceous weed density along different months and within the planted species

The graminaceous weed species were the most frequent and their trend was similar to that of the total weeds. The prevalent species was represented by *Poa annua*, but some Poa *pratensis* and summer annuals like *Echinocloa*, *Digitaria* and *Cynodonas* have been observed as well.
Surprisingly, in autumn, *D. carthosianorum* overcame the weeds infestation of *Armeria*.
From spring to autumn there was a decrease in maximum weed density from over 600 weeds/m^2 to 50 weeds/m^2 for the most infested cultivated species, *Armeria*. Remarkable is the low density of weeds (only some individuals) in the least infested species, *Potentilla*, *Thymus* and *Cerastium*. Around the *Dianthus* species weed infestation varied due to the inconsistency in the coverage of the cultivated species.

Among dicotyledons, the most frequent weeds included species from the *Asteraceae* family and Euphorbia *maculata*, Oxalis and *Cardamine*. The latter two having a ballistic mode of dissemination. All three species happen to be escapees from the local nursery which have easily spread.

The high variety of weed species recorded in the spring did not result in any significant differences in statistical terms. This, despite the fact that weed populations were significantly different for each cultivated species (Fig. 37). The two *D. gratianopolitanus* patches showed even populations of dicot weeds. *Cerastium* and *D. carthusianorum*, even if not statistically different from *Thymus* and *Potentilla* resulted in much higher weeds density. Dicots population was remarkably low in the autumn amongst *Thymus* and *Potentilla*.


Dicotyledons species density in May 2016
% total weeds
150
100
50
0
a
a
a
a
a
a
a
A. maritima
C.biebersteinii
D. carthusianorum
D. gratianopolitanus var B.
D.gratianopolitanun var S
P. neumanniana
T. serpyllum
species


Dicotyledons species density in July 2016
% total weeds
75
50
25
0
a
bc
bc
abc
ab
bc
c
A. maritima
C.biebersteinii
D. carthusianorum
D. gratianopolitanus var B.
D.gratianopolitanun var S
P. neumanniana
T. serpyllum
species

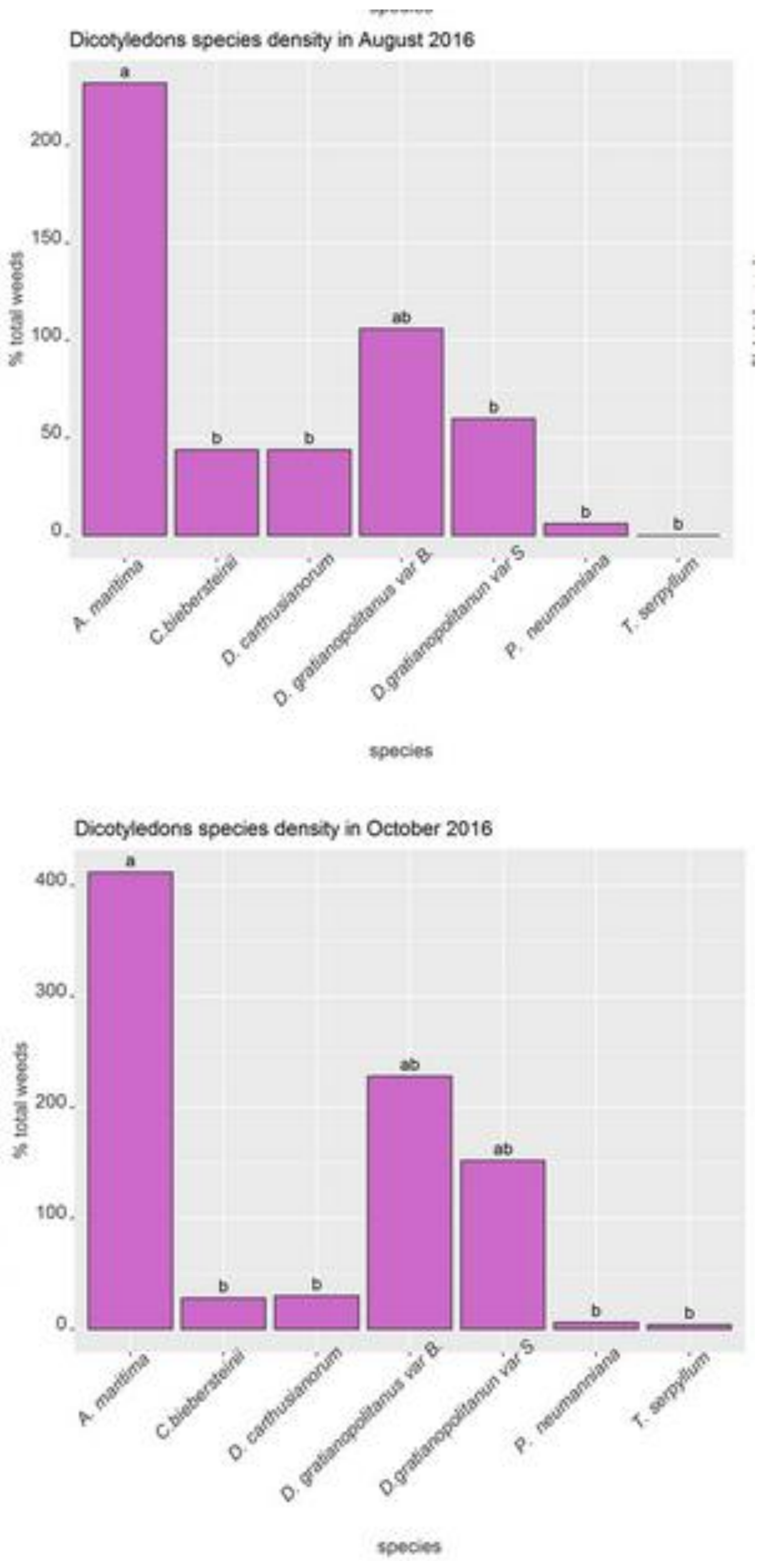
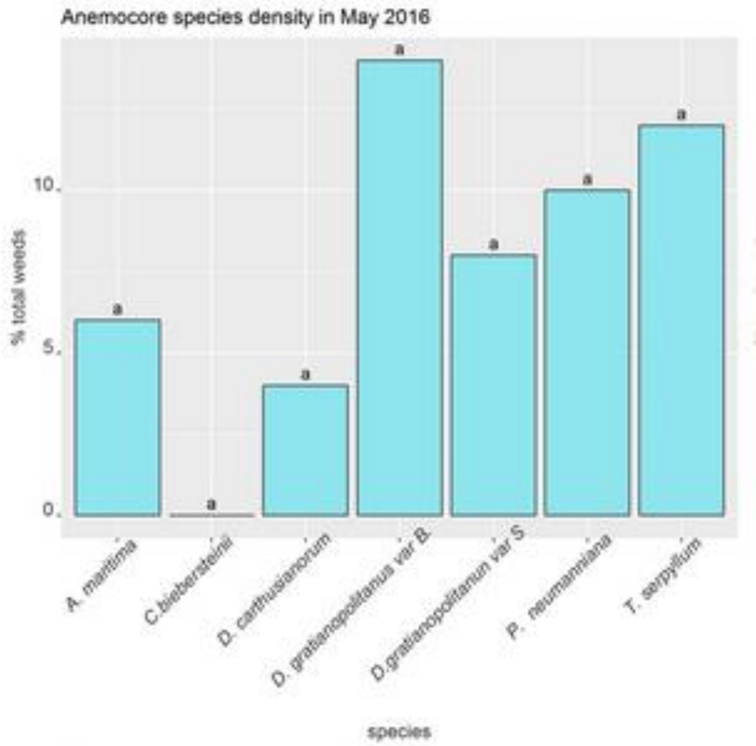
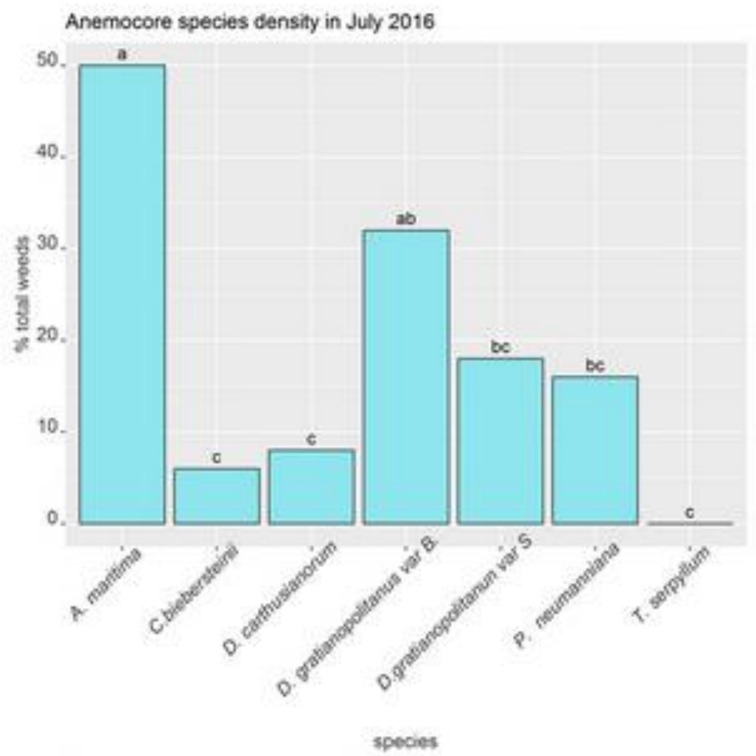


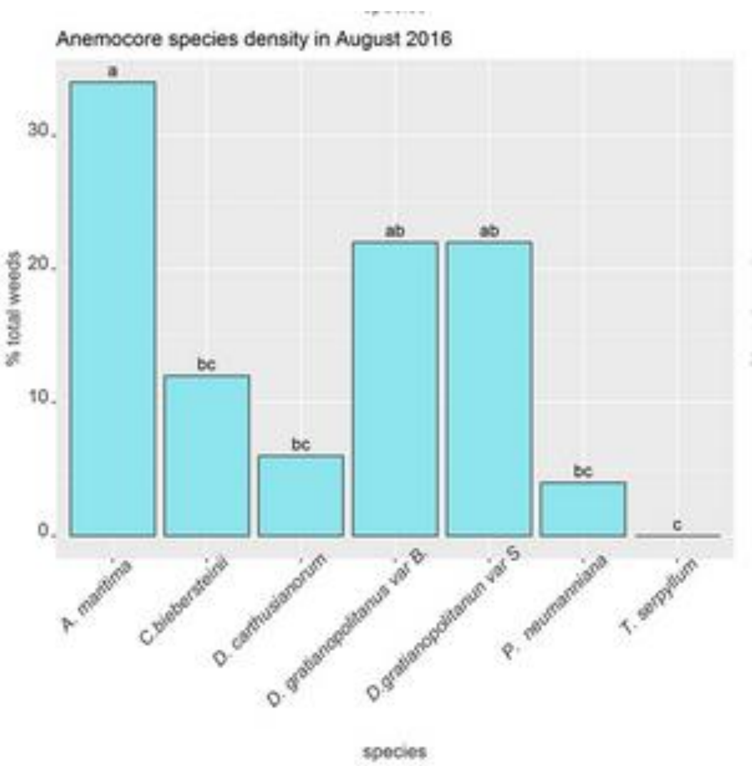
Figure 37 Dicotyledon weeds density along different months and within the planted species

Classifying weeds according to their seed dispersal method can be helpful when studying green roofs. In fact it is accepted amongst green roof academics that anemocores (i.e. species which disperse their seeds by wind) are the most threatening of weeds. This is because of the seeds ability to reach even the highest roof top. The most significant species belong to the *Asteraceae* family. A few *Acer* seedlings have also been observed on the green roof.

Records of a*nemocore* weeds were erratic in spring. Populations were quite small at times below fifteen units resulting in insignificant quantities (refer to fig 38). In summer, *anemocore* especially *Asteraceae*, became more persistent. Amongst the *Armeria* sp 50 plantlets were recorded compared to only one within Thymus. In D. gratianopolitanus their density was always around 20 individuals, while in *Cerastium* and *D. carthusianorum* they reach values not higher than 10 except for Cerastium in August with 12 saplings.







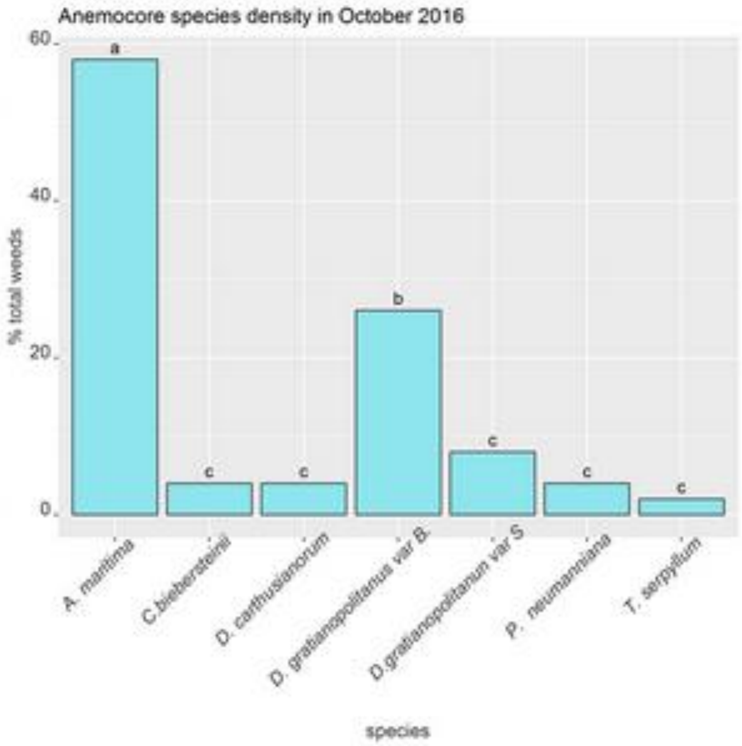
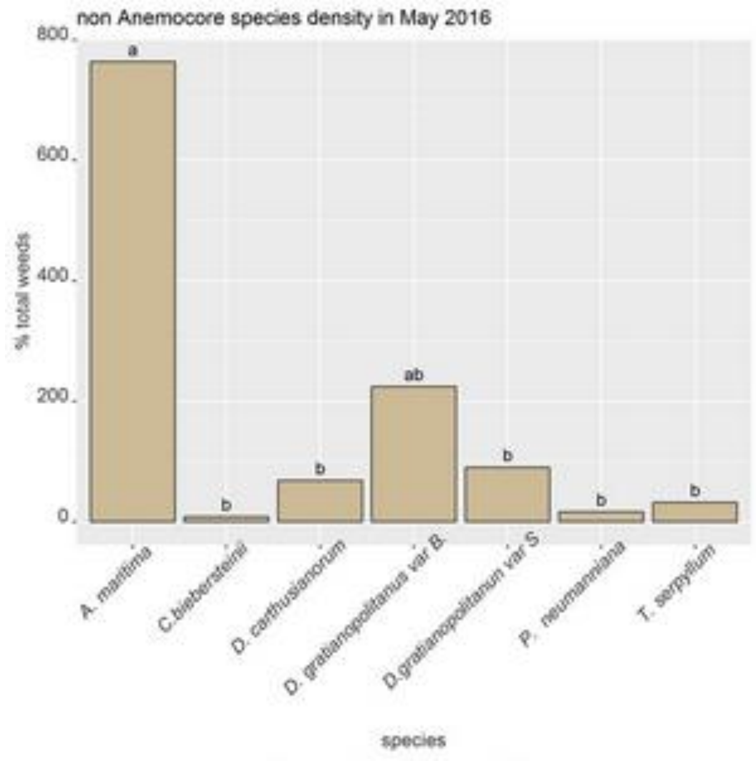
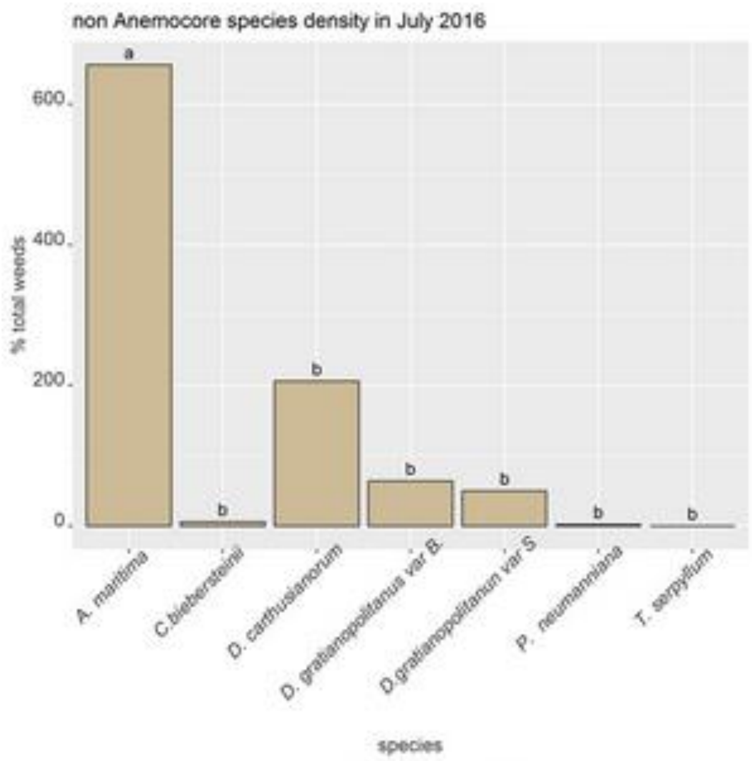


Figure 38 Anemocore weeds density along different months and within the planted species

The anemocore species represented only 10 % of the weeds recorded. The non anemocore species represented the majority and their trend followed those of the total weeds (Fig. 39).
The small amount of anemocore species present may be attributed to the young age of the roof. The major weed species recorded were introduced to the green roof from the plant nursery on site. These weeds were introduced onto the green roof through the substrate of the cultivated plants even though the growing medium of these transplants was substituted fifteen days prior to their planting. The high presence of *Poa annua* can be attributed to the involuntary transportation of seed from the neighbouring park which visitors walk through before entering

the green roof. In addition, the first winter following the planting of the vegetation on the green roof was very warm, allowing annuals and other short lived species like *Poa annua* to complete their reproductive cycle many times over than usual.





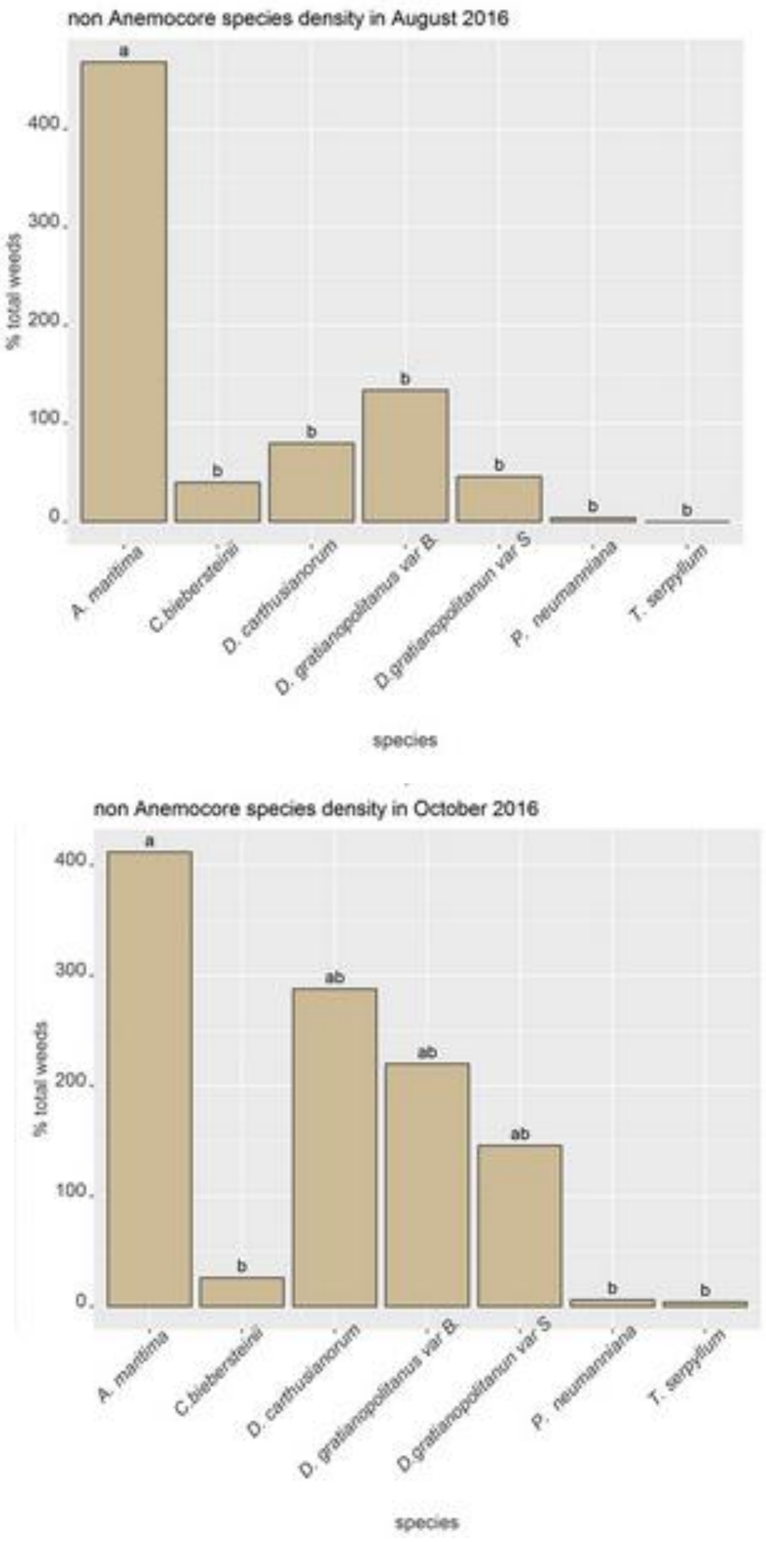


Figure 39 Dicotyledon weeds density along different months and within the planted species


Figure 40 General view of the green roof at Fondazione Minoprio.

4. Conclusion

In conclusion, there was variability among different species in terms of response to drought. It is worth observing that most of species tested did not die and survived even at high values of pF, reducing relative water content and growth index and often increasing chlorophylls, carotenoids and proline content as expected. Their survival capacity is much higher than common plants, whose wilting point is estimated to be around a pF of 4.5.

In terms of weed problems, the behaviour of cultivated species did influence the development of weeds. Planting *Sedum album* as a means of suppressing weeds did not prove effective. As expected, plants least infested (e.g. *Thymus*) showed a high growth index when compared to plants which had high susceptibility to weeds (e.g. *Armeria*). This susceptibility to weeds is attributed to the growth habit of the species which is compact making it inefficient in inhibiting the germination of *Poa* and *Cardamine* even within its clump.

After *Armeria*, *D. grathianopolitanus* was the most susceptible to weeds even if at a fraction of a degree (¼ to ½) less than Armeria. This variability is dependent on seasonal variations.

Of note is the fact that although *D. carthusianorum* did not cover the soil as well as D*. gratianopolitanus*, it often performed better in suppressing weed.

Cerastium was very successful in suppressing weed, and this was due to good surface coverage. *Potentilla* did cover well the surface, suppressing the development of weeds. It often performed as well as *Thymus* with the lowest recorded values. *Thymus* results are due to the very good soil coverage as well as its reported allelopatic properties.

Weed growth is also influenced by climatic and weather conditions: in full summer and winter their presence is reduced.

The choice of the species in a Mediterranean green roof is dependant not only on drought resistance but also on the ability to compete with weeds.

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