Effect of field light stress on the sesquiterpene lactone content and phytotoxicity of cultivated cardoon leaf extracts

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Abstract

Intensive agriculture needs new sustainable tools for weed management. Allelopathy offered a valid alternative. Sesquiterpene lactones (STLs) are the most important cultivated cardoon (Cynara cardunculus L. var. altilis DC.) allelochemicals involved in weed toxicity. The present study aims to investigate the effect of field light stress (by 60% of plant shading) on the qualitative and quantitative composition of STLs in cultivated cardoon leaf extracts harvested in January and April. The phytotoxicity of leaf extracts was evaluated on the wheat coleoptile elongation, seed germination, root and shoot length of the weeds Amaranthus retroflexus L. and Portulaca oleracea L. Light stress significantly increased the concentrations of total and single STLs, and this increase was more marked if combined with harvest time (+338, 102 and 94 of cynaratriol, desacylcynaropicrin and 11,13-dihydro-deacylcynaropicrin, respectively) in April. The concentration of STLs and the phytotoxic activity were correlated (light stressed extracts belonging from April reduced by 91, 86 and 93% the wheat coleoptile elongation and the root and shoot length of A. retroflexus, respectively, at 800 ppm). Therefore, the induction of light stress could be used to increase the concentrations of STLs in cultivated cardoon leaves for industrial applications and to improve their phytotoxic potential.

INTRODUCTION

The ability of some plants to produce and release into the environment harmful or beneficial secondary metabolites for target species is called Allelopathy (Rice 1984). These chemical phytotoxic compounds, commonly known as allelochemicals, belong to different chemical classes such as phenols and terpenoids, and play a defensive role for the donor plant (Scavo, Restuccia & Mauromicale 2018a). In recent years, the increasing concerns about the environmental impact of syntyhetic herbicides and the dramatically development of weed-resistant ecotypes, has led to a greater attention by the scientific community on the application of allelopathy as a chemical-free tool for weed management. Indeed, Macias et al. (Macias, Molinillo, Varela & Galindo 2007) suggested how plant allelochemicals could represent a good candidate for the future production of bioherbicides.

The Asteraceae is one of the most studied botanical families comprising allelopathic species, both weeds and crops (Chon & Nelson 2010). Cultivated cardoon (*Cynara cardunculus* L. var. *altilis*DC.) is a C_3 herbaceous perennial species, originated from the Mediterranean basin together with the globe artichoke [var.*scolymus* (L.) Fiori], which evolved separately as a result of different selection criteria from their common ancestor wild cardoon [var. *sylvestris* (Lamk) Fiori] (Rottenberg & Zohary 1996). Cultivated cardoon is known since ancient times as a vegetable for its enlarged bleached petiole. However, in the rainfed farmlands of Mediterranean climates, it is cultivated mainly as an industrial crop for the production of energy (biomass for direct combustion, biomethane, bio-ethanol, oil from seeds) and paper pulp thanks to its high lignocellulosic biomass, as well as for the extraction of bioactive (Pesce, Negri, Bacenetti & Mauromicale 2017; Mauromicale *et al.*2019). The allelopathic activity of cultivated cardoon has been investigated in deep on seed germination (Scavo, Restuccia, Pandino, Onofri & Mauromicale 2018b) and seedling growth of several weed species (Scavo *et al.* 2019b), as well as against bacterial species of agricultural and food interest (Mazzaglia *et al.* 2018; Scavo*et al.* 2019c). It was also found that *C. cardunculus*cropping significantly reduced the number of seeds in the soil seed bank, while showing a positive effect on some bacteria involved in the soil N-cycle (Scavo, Restuccia, Abbate & Mauromicale 2019d). Allelochemicals involved in *C. cardunculus* phytotoxicity were discussed in previous researches and indicated as sesquiterpene lactones (STLs) and polyphenols (Rial, Novaes, Varela, G. Molinillo & Macias 2014; Rial *et al.* 2016; Scavo, Pandino, Restuccia & Mauromicale 2020). STLs (i.e. cynaropicrin, deacylcynaropicrin, 11,13-dihydro-deacylcynaropicrin, aguerin B, grosheimin, 11,13-dihydroxi-8-deoxygrosheimin and cynaratriol), the most abundant terpenic compounds of *C. cardunculus* leaves, possess a very wide range of biological activities (Picman 1986; Zhang, Won, Ong & Shen 2005; Chaturvedi 2011), being widely reported as phytotoxic compounds (Rial *et al.* 2014; Scavo *et al.* 2019f). For instance, cynaropicrin, aguerin B and grosheimin have shown phytotoxic activity on two weed species of economic importance, i.e., barnyardgrass (*Echinochloa crus-galli* L.) and brachiaria [*Urochloa decumbens* (Stapf) R.D. Webster] (Rial *et al.* 2014). Moreover, the phytotoxic activity of *C. cardunculus* leaves have been correlated with the joint action of this STLs, being identified synergistic and antagonistic interaction between them (Rial *et al.* 2016). Also cynaropicrin, the major compound found in *Cynara*species, has been reported as anti-inflammatory (Mizuno & Usuki 2018; Hayata *et al.* 2019), cytotoxic against several types of cancer cells (Shimoda *et al.* 2003; Cho *et al.* 2004; Yasukawa, Matsubara & Sano 2010) or antispasmodic (Emendörfer *et al.*2005). Scavo et al. (Scavo *et al.* 2019e) developed the best procedure for extracting the secondary metabolites from cultivated cardoon leaves based on costs and phytotoxic activity. Nevertheless, studying the influence of genotype and harvest time on the *C. cardunculus* STL profile and phytotoxicity, Scavo et al. (Scavo *et al.* 2019f) indicated that wild cardoon had the highest amount of STLs among the three *C. cardunculus* botanical varieties, with globe artichoke showing the lowest concentration. The amount of STLs and the inhibitory activity of the six *C. cardunculus* genotypes under study was closely affected by climatic conditions, which were favorable in April.

However, the allelopathic behaviour of a plant is the result of different abiotic and biotic stress factors, which influence the quantity of allelochemicals released by the donor plant and the effect of an allelochemical on the target plant (Inderjit & del Moral 1997; Scavo *et al.* 2018a). According to the "stress hypothesis of Allelopathy" formulated by Reigosa et al. (Reigosa, Souto & González 1999), a stress condition generally increases the production of allelochemicals in the donor plant, as well as the sensitivity of the target plant. Quantity and quality of light are reported to affect the allelopathic potential in plants (Kato-Noguchi 1999; Li, Pan, Liu & Wang 2009; Scavo *et al.* 2018a). While long photoperiods have been commonly indicated to increase the production of secondary metabolites, including allelochemicals, in several plants (Pramanik, Nagai, Asao & Matsui 2000; Chaves Lobón, Alias Gallego, Sosa Diaz & Escudero Garcia 2002), there is no evidence in literature about the increasing of allelochemicals biosynthesis in response to short days.

Given these findings, we hypothesised that cultivated cardoon plants under light stress increased both their STL leaf concentrations and phytotoxic activity. Therefore, the aim of the present study was to evaluate the effect of light stress (by plant shading) in field conditions on the quali-quantitative composition of STLs in cultivated cardoon leaf extracts and their phytotoxic potential expressed as inhibition of wheat coleoptile elongation, weed germination and growth.

MATERIAL AND METHODS

Experimental site design, crop management and leaf sampling.

Plants of 'Bianco gigante', an Italian cultivated cardoon commercial cultivar, were grown in the experimental station of Catania University [South Italy, 37° 25I N; 15° 30IE; 10 m a.s.l.]. The area is characterised by a typical semi-arid Mediterranean clime comprising mild-wet winters and warm-dry summers. The soil of the experimental field was typical vertical and/or xerochrept (Staff 1999) with 27% sand, 45% clay, 28% silt, 1%

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organic matter, $0.1P_2O_5$, 210 ppm exchangeable K_2O, and pH 7.2.

A completely randomized design with four replications, each of which comprised 100 plants spaced 0.80 m apart, in rows separated apart by 1.25 m (1.0 plant m^{-2}), was adopted to study two imposed shading levels, removal of 0% (**control**) and 60% (**light stress**) of sunlight. Shading was imposed by erection of a black polyethylene net ("Ombra 60") at ˜1.60 m aboveground level from middle October to April. The effectiveness of the shading was tested on a weekly basis, both inside and outside the field experiment, using a solarimeter (Licor Line Quantum LQA, One Meter Sensing Length; LI-Cor Inc., Lincoln, NE, USA). The netting was extended down 0.30 m aboveground level at each edge, in order to avoid lateral irradiations and to minimize the development of microclimate differences among main plots. Before planting, the experimental field soil was ploughed up to 30 cm and harrowed. The fertilisation program was: 50 kg ha^{-1} of urea (N), 80 kg $ha^{-1}$of double perphosphate (P_2O_5) and 80 kg ha^{-1} of potassium sulphate (K_2O) prior to awakening with two furthers 80 kg ha^{-1} of ammonium nitrate. Irrigation was supplied. Weed and pest control was performed by spraying oxyfluorfen and imidacloprid, respectively, when required. The effect of light stress was evaluated on two different harvest times: January 9th and April 14th, 2018. January harvest corresponds to 30% of the maximum leaf mass reached, code 43 according to the BBCH scale proposed by Archontoulis et al. (Archontoulis, Struik, Vos & Danalatos 2010), while in April 50% of the maximum leaf mass is reached (code 45). The choice of these harvest times is owed on the fact that they intercept very different meteorological conditions affecting STL concentration in *C. cardunculus* leaves, as suggested by Scavo et al. (Scavo *et al.*2019f). Fifty full-expanded leaves were randomly sampled in the central part of each plot at the two phenological stages described.

Meteorological conditions.

A meteorological station (Mod. Multirecorder 2.40; EGT, Florence, Italy) sited within 250 m of the experimental field was used to measure global radiation and photoperiod data from October to April. Data were recorded every hour. Global radiation fell from October to February (6.8 MJ m^{-2} in the first decade of February) and then rose up to 23.3 MJ m^{-2} in the third decade of April (Fig. 1). Similarly, the hours of radiation decreased in November and December (up to 10 h in the latter month) and increased from January up to 14 h in the first decade of April. Mean air temperatures and rainfall were found to comply with the 30-years weather conditions of the zone.

Leaf extract preparation.

Leaf extract preparation followed Scavo et al. (Scavo *et al.*2019f). Here again, chlorophylls were removed from the ethanolic extracts through solid-phase extraction (SPE) to facilitate the ultra high-performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) analysis.

Reagents and UHPLC-MS/MS analysis.

Seven STLs were detected in cultivated cardoon leaves: cynaropicrin, cynaratriol, grosheimin, aguerin B, deacylcynaropicrin, 11,13-dihydro-deacylcynaropicrin and 11,13-dihydroxi-8-deoxygrosheimin using santamarine as internal standard (IS). They were obtained following Rial et al. (Rial *et al.* 2014) and Scavo et al. (Scavo*et al.* 2019e), while santamarin was synthesized according to Lu and Fischer (Lu & Fischer 1996). Methanol, ethanol, dichloromethane and formic acid were purchased from Fischer Chemicals (Geel, Belgium), ammonium formate was obtained from Sigma-Aldrich (St. Louis, MO, USA) and water for LC-MS/MS analysis was type I obtained from an Ultramatic system from Wasserlab (Barbatain, Spain). All the solvents were UHPLC-grade. The UHPLC-MS/MS analysis was carried out in accordance to Scavo et al. (Scavo *et al.* 2019f).

Wheat coleoptile and weed phytotoxicity bioassays.

The wheat coleoptile and weed phytotoxicity bioassays were performed as reported by Scavo et al. (Scavo *et al.* 2019f e). Tested extract concentrations were 800, 400 and 200 ppm. A negative control (0 ppm), represented by a phosphate-citrate buffer containing 2% sucrose at pH 5.6, and a positive control (the commercial herbicide Logran(r)) were used. The allelopathic activity on seed germination, root and shoot

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length of the weeds *Amaranthus retroflexus* L. and *Portulaca oleracea* L. was evaluated in a completely randomized block design with four replicates of 20 seeds for each species. Petri dishes of both weeds were placed inside a Memmert ICE 700 controlled environment chamber at 25 degC in continuous darkness.

Statistical analysis.

The Bartlett's test was used to test for homoscedasticity, following which data were subjected to analysis of variance (ANOVA). A two-way ANOVA with "shading level" and "harvest time" as fixed factors was used for STL profile, while a three-way ANOVA model "2 shading level x 2 harvest time x 4 extract concentration" was employed to evaluate the allelopathic activity in both wheat coleoptile and weed phytotoxicity bioassays. Means of STL data were separated by the Student–Neuman–Keuls test only when the F -test was significant at the 0.05 probability level. Wheat elongation data are expressed as percentage difference from the control. Zero ppm was also included in the statistical analysis.

RESULTS

STL concentration and composition.

Yields of extracts, calculated as [(g extract/ g dried leaves)*100], were 14.8 and 24.1% for Control ('Bianco gigante' without stress), and 13.3 and 25.0 % for stressed 'Bianco gigante' in January and April harvest, respectively. The interaction "light stress x harvest time" was highly significant for all STLs and the latter factor, in particular, more contributed than stress condition to the analysis of variance for the total concentration (F =637) and for all the STLs (Table 1). Light stress significantly affected STL concentrations, with an increase of 338, 102, 94 and 35% in the April harvest of cynaratriol, deacylcynaropicrin, 11,13-dihydro-deacylcynaropicrin and grosheimin, respectively, compared to control (Figs. 2 and 3). A higher concentration in the control was registered only in aguerin B. April harvest showed the highest concentration for both total and single STLs, except for grosheimin, deacylcynaropicrin and 11,13-dihydro-deacylcynaropicrin controls. The effect of harvest time was more marked if combined with the light stress. For instance, an increase of 221, 190 and 703% was registered from January to April in the total, cynaropicrin and cynaratriol concentrations, respectively (Fig. 2). Overall, cynaropicrin was the predominant STL of cultivated cardoon leaf extracts (70 and 73 mg L^{-1} in April harvest of control and light stress, respectively), followed by cynaratriol (14.9 mg L^{-1} in April harvest of light stress). Among minor STLs, aguerin B was the most abundant even if with higher values in the control (3.6 mg L^{-1} in April harvest).

Wheat coleoptile bioassay.

The light stress did not affect the wheat coleoptile elongation, while the interaction "stress x harvest time x concentration" was significant at the 0.05 probability level (Table 2). Extract concentration explained much of the inhibitory activity, contributing for 55.5% on variance.

Wheat coleoptile's inhibition was marked for all the extracts (Fig. 4), with a high contribution of extract concentration. On the average of all treatments, in fact, 800 ppm reduced by 69% coleoptile's elongation, compared to 46 and 31% of 400 and 200 ppm, respectively. Moreover, April harvest exerted a higher allelopathic activity compared to January, inhibiting by 64% *vs* . 33% on the average of all treatments. The light stress condition in the April harvest showed the best results, with values of 91, 77 and 46% at 800, 400 and 200 ppm, respectively, compared to 85, 54 and 33% of Control.

Weed phytotoxicity bioassay.

The light stress and the interaction "stress x harvest time x concentration" were not significant for all parameters under study in both species (Table 3). Germination of both species was not affected by light stress and harvest time, while the extract concentration was highly significant. In particular, all the concentrations were significantly lower than to the negative control (0 ppm) (Fig. 5). In both *A. retroflexus* and *P. oleracea* , 800 ppm resulted the most efficient in reducing seed germination, even if not statistical differences were observed with respect to the other concentrations.

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Regarding root length, the interaction "harvest time x concentration" was significant in both weed species, with a greater contribution to variance of concentration. In both weeds the allelopathic activity significantly increases with increasing concentrations (Fig. 5). Moreover, regardless of extract concentration, April confirmed to be the most appropriate period for the harvest time of cultivated cardoon leaf extracts since it significantly stimulates the production of STLs and their phytotoxicity. A reduction of 86 and 62% in *A. retroflexus*root length was observed going from January to April at 800 and 400 ppm, respectively. A similar trend was observed for *P. oleracea* .

Harvest time was the major factor affecting the analysis of variance for*A. retroflexus* and *P. oleracea* shoot length (Table 3). As observed for root length, the interaction "harvest time x concentration" was highly significant in both species. Contrary to the April harvest, no statistical differences were observed among concentrations for the January one (Fig. 5). April harvest exerted a greater allelopathic activity compared to January. In particular, the highest inhibitory activity was observed in 800 ppm of *A. retroflexus* with a reduction of 93% between the two harvest periods, followed by 64% in 800 ppm of *P. oleracea* .

DISCUSSION

In order to better understand the phytotoxic potential of *C. cardunculus* and increase the production of secondary metabolites, the effect of light stress (shading) in field conditions was studied on the STL profile of cultivated cardoon leaf extracts. The influence of light stress on the production of plant STLs has never been studied. Given the importance of these chemical compounds for their numerous biological activities(Chaturvedi 2011), the knowledge of the factors affecting their biosynthesis and production represents an important aspect that should be considered for their future application.

In this study, the yield extract was greater in the April harvest than January, with the stressed treatment showing the highest yield. These yields are similar to those reported by Ramos et al. (Ramos *et al.* 2013) and Scavo et al. (Scavo *et al.* 2019f). The yields of extracts were closely correlated to STL concentrations. In fact, extracts belonging to April harvest generally showed higher concentrations of total and single STLs than January, probably due to the higher levels of global radiation and longer photoperiod. A similar trend was reported by Scavo et al. (Scavo *et al.* 2019f) for STLs on the three *C. cardunculus* taxa and by Pandino et al. (Pandino, Lombardo, Lo Monaco & Mauromicale 2013) on globe artichoke leaf polyphenols. The leaves of many Asteraceae members, including *C. cardunculus* , are known for the high cocentrations of STLs, mainly represented by cynaropicrin, which is responsible of its bitter taste(Seaman 1982). Here, in accordance with Ramos et al. (Ramos*et al.* 2013), cynaropicrin was the most abundant STL detected. However, most of previous studies are focused on the globe artichoke(Rouphael *et al.* 2016), while cultivated cardoon has been little investigated.

While the influence of light quality (e.g. ionizing or ultraviolet radiation, intensity of visible light, red and far-red light) on the allelopathic expression of plants has been reported in previous researches (del Moral 1972; Kato-Noguchi 1999; Li *et al.* 2009), only few works investigated the effect of daylength. Zucker et al. (Zucker, Nitsch & Nitsch 1965) stated that chlorogenic acid concentration in *Nicotiana* spp. species is affected by photoperiodic alteration. Rice (Rice 1984) indicated that long days generally increase the production of inhibitory compounds in donor plants. Similarly, Taylor (Taylor 1965) found that the concentration of different phenolics and cinnamic acid derivatives increase in the leaves of *Xanthium strumarium* L. with increases in daylength. Here we found an opposite trend to those reported in literature. Indeed, the amount and composition of STLs in cultivated cardoon leaf extracts varied in relation to both light stress and harvest time, with a more pronounced effect of the latter factor, suggesting how the meteorological conditions (e.g. global radiation, photoperiod, air temperature and total rainfall) affect their biosynthesis. However, except for aguerin B, the shading significantly increased the concentration of total and single STLs in the April harvest, compared to control, and this increase was more marked if combined with harvest time. It is known that once a stress factor is recognised at cellular level by a plant, a signal transduction begins leading to gene expression and metabolic responses with the aim of helping the plant survive or minimizing the effectiveness of the stress agent (Reigosa *et al.* 1999). Eljounaidi et al. (Eljounaidi *et al.* 2014) elucidated the genes and the enzymes involved in the biosynthetic pathway of STLs in *C. cardunculus* . However,

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the alteration of gene expression in this species is still unknown, but appears reasonable how this plant, as commonly happens in other ones, prevents the oxidative damages induced by ROS (reactive oxygen species such as hydrogen peroxide, superoxide, etc.) through the synthesis of secondary metabolites, STLs and polyphenols in this case. However, in nature plants interact with the complex of abiotic and biotic influences of the environment, hence an allelopathic phenomenon depends on the type and intensity of stress, on the genotype and development stage of the plant, as well as on the seasonal changes and, therefore, may vary through time (Scavo, Abbate & Mauromicale 2019a). The influence of genotype and harvest time have been previously studied (Scavo *et al.* 2019f), while light stress is the first abiotic factor evaluated in *C. cardunculus* .

With the aim to find a correlation between the STL composition and phytotoxicity, cultivated cardoon leaf extracts were bioassayed on the wheat coleoptile elongation, as well as on germination, root and shoot length of *A. retroflexus* and *P. oleracea* . In agreement with previous results with the same bioassay and target species both*C. cardunculus* (Scavo *et al.* 2019e) and other donor plants (da Silva *et al.* 2017), seed germination percentage was the least affected parameter. Contrariwise, wheat coleoptile elongation and root length of both weeds were significantly inhibited. The phytotoxic activity increase at increasing concentrations, as widely reported in literature (Ambika 2013), and in correspondence of the April harvest, as found also by Scavo et al. (Scavo *et al.* 2019f) and Pandino et al. (Pandino *et al.* 2013) for polyphenols. These results are corroborated by STL profile. During April, in fact, the plant accumulated more STLs in the leaves, mainly cynaropicrin. Probably, the combination of high temperatures, low rainfall, high global radiation and long photoperiod stimulates the production of *C. cardunculus*secondary metabolites (caffeoylquinic acids, flavones and STLs) and increases its inhibitory potential. Nevertheless, the imposition of plant shading in April was found to stimulate the production of STLs in the leaves and to enhance, albeit to a lesser extent, the allelopathic activity.

In conclusion, here we demonstrated, for the first time, that plant shading in field conditions enhances the production of STLs in the leaves of cultivated cardoon, especially in the April harvest. This increase was accompanied by a higher phytotoxicity on wheat coleoptile elongation, germination percentage, root and shoot length of *A. retroflexus* and *P. oleracea* . The inhibitory activity of cultivated cardoon extracts caused by shading was weak if taken alone, becoming stronger when combined with the harvest time due to the optimal combination of climatic conditions, development stage of the plant and intensity of light stress. The induction of light stress by plant shading could be a useful tool to stimulate the biosynthesis of STLs for industrial applications and to increase the phytotoxic potential of cultivated cardoon as a sustainable weed control method within an integrated weed management system for the future production of a bioherbicide.

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We declare that we do not have any conflict of interest

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Table 1. F –values of main factors and their interactions resulting from analysis of variance in sesquiterpene lactone (STL) concentration.

	Source of variation	**Source of variation**	**Source of variation**
	Light stress (LS)	**Harvest time (HT)**	**(LS) × (HT)**
degrees of freedom	*1*	*1*	*1*
Total STLs	12^{**}	637^{***}	116^{***}
cynaropicrin	33^{***}	707^{***}	57^{***}
cynaratriol	41^{***}	95^{***}	324^{***}
aguerin B	278^{***}	803^{***}	146^{***}
grosheimin	5248^{***}	21780^{***}	22311^{***}
11,13-dihydroxi-8-deoxygrosheimin	17^{**}	270^{***}	176^{***}
deacylcynaropicrin	24^{**}	193^{***}	301^{***}
11,13-dihydro-deacylcynaropicrin	3^{NS}	0^{NS}	31^{***}

Note: Values are given as F of Fisher. *** and** indicate significant at $P< 0.001$ and $P< 0.01$, respectively, and^{NS} , not significant.

Table 2. F –values as absolute value and sum of squares (in brackets) of main factors and their interactions resulting from analysis of variance in wheat coleoptile elongation.

Source of variation	**df**	**Wheat coleoptile elongation**
Light stress (LS)	1	2.0^{NS} (0.4)

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Source of variation	**df**	**Wheat coleoptile elongation**
Harvest time (HT)	1	165.9^{***} (34.2)
Concentration (C)	3	268.9^{***} (55.5)
(LS) × (HT)	1	17.9^{***} (3.7)
(LS) × (C)	3	1.4^{NS} (0.3)
(HT) × (C)	3	25.0^{***} (5.1)
(LS) × (HT) × (C)	3	3.7^{*} (0.8)

Note: Values are given as F of Fisher. df: degrees of freedrom.***and * indicate significant at$P<$ 0.001 and $P<$ 0.05, respectively, and^{NS} , not significant.

Table 3. F –Values as absolute of main factors and their interactions resulting from analysis of variance in weed species.

Source of variation	**df**	Amaranthus retroflexus	Amaranthus retroflexus	Amaranthus retroflexus	
		Germination	**Root length**	**Shoot length**	**Shoot length**
Light stress (LS)	1	1.5^{NS}	0.4^{NS}	1.4^{NS}	1.4^{NS}
Harvest time (HT)	1	0.02^{NS}	190.5^{***}	228.3^{***}	228.3^{***}
Concentration (C)	3	10.4^{***}	589.1^{***}	219.8^{***}	219.8^{***}
(LS) × (HT)	1	1.3^{NS}	3.8^{NS}	0.3^{NS}	0.3^{NS}
(LS) × C)	3	0.9^{NS}	0.5^{NS}	1.1^{NS}	1.1^{NS}
(HT) × (C)	3	0.9^{NS}	35.0^{***}	116.9^{***}	116.9^{***}
(LS) × (HT) × (C)	3	0.2^{NS}	2.6^{NS}	2.4^{NS}	2.4^{NS}

Note: Values are given as F of Fisher. df: degree of freedom.*** indicates significant at $P<$ 0.001, and ^{NS} , not significant.

FIGURE LEGENDS

Figure 1. Global radiation (up) and photoperiod (down) occurred during the cultivated cardoon growing season (October-April) at the Catania experimental station. 1: from 1st to 10th; 2: from 11th to 20th; 3: from 21st to 28th, 30th or 31st.

Figure 2. Quantification of total sesquiterpene lactone, cynaropycrin and cynaratriol content from cultivated cardoon 'Bianco gigante' leaf extracts in two different harvest times as affected by light stress. Control: 0% of shading; Light stress: 60% of shading. H Jan: harvest time at January 9th; H Apr: harvest time at April 14th. Different letters indicate statistical significance for P [?]0.05.

Figure 3. Quantification of minor sesquiterpene lactones (aguerin B, grosheimin, 11,13-dihydroxi-8-deoxygrosheimin, deacylcynaropicrin and 11,13-dihydro-deacylcynaropicrin) content from cultivated cardoon 'Bianco gigante' leaf extracts in two different harvest times as affected by light stress. Control: 0% of shading; Light stress: 60% of shading. H Jan: harvest time at January 9th; H Apr: harvest time at April 14th. Different letters indicate statistical significance for P [?]0.05.

Figure 4. Effect of light stress, in two different harvest times, on the phytotoxicity of cultivated cardoon 'Bianco gigante' leaf extracts expressed as inhibition of wheat coleoptile elongation. Control: 0% of shading; Light stress: 60% of shading. H Jan: harvest time at January 9th; H Apr: harvest time at April 14th. Values are expressed as percentage difference from control. Each bar means ± standard deviation.

Figure 5. Effect of extract concentration on the phytotoxicity of cultivated cardoon 'Bianco gigante' leaf extracts expressed as inhibition of *Amaranthus retroflexus* and *Portulaca oleracea* seed germination, root

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and shoot length. H Jan: harvest time at January 9th; H Apr: harvest time at April 14th. Different letters indicate statistical significance for P [?]0.05. Each bar means +- standard deviation.

Summary statement

The current work evaluates the effect of light stress by plant shading in field conditions on the sesquiterpene lactone content and phytotoxicity (wheat coleoptile elongation, germination, root and shoot length of the weeds Amaranthus retroflexus L. and Portulaca oleracea L.) of cultivated cardoon.

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