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# Potential subterranean interference of *Solanum elaeagnifolium*, commonly known as silverleaf nightshade, on durum wheat

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#### Abstract

*Aim of study:* Silverleaf nightshade (*Solanum elaeagnifolium*) has evolved as an invasive weed in Greece, affecting many farmers' fields. The potential subterranean interference of silverleaf nightshade on durum wheat growth and yield, as well as its aqueous extracts phytotoxic activity on four winter cereals were investigated.

Area of study: Northern Greece.

*Material and methods:* The phytotoxic activity of silverleaf nightshade was determined in the laboratory using a perlite-based bioassay. The interference of silverleaf nightshade on durum wheat yield components was investigated by a 3-year field experiment.

*Main results:* The silverleaf nightshade leaves/stems or the flowers/fruits extracts were in most cases more phytotoxic than those of roots. Durum wheat and oats were more sensitive than winter wheat and barley. In the field, the incorporated summer biomass of 14-18 plants  $m^{-2}$  (about 4.5-4.8 t  $ha^{-1}$ ) of silver nightshade caused 14.5, 23.1, 23.3, 15.9, 17.3 and 16.9% reduction (averaged across years) in durum wheat plant number, tiller number, total fresh weight, total dry weight, ear number and seed yield, respectively, compared with the silver nightshade-free plots (control). The corresponding reductions caused by 30-34 plants  $m^{-2}$  (about 8.3-8.6 t  $ha^{-1}$ ) were 28.5, 41.2, 45.9, 31.5, 27.0 and 31.2%.

*Research highlights:* The presence of aboveground silver nightshade biomass, incorporated by tillage before seeding significantly reduced the emergence, growth and yield of durum wheat. This fact could be attributed to the allelopathic effect of the weed and demonstrates the need for its control before wheat establishment to enhance wheat productivity.

Additional key words: alien species; allelochemicals; competition; weed density; yield components.

Abbreviation used: GY (grain yield); TDW (total dry weight); TGW (1000-grain weight); WAS (weeks after seeding). Citation: Antoniadis, C; Vasilakoglou, I; Dimos, E; Adamou, V; Dhima, K (2024). Potential subterranean interference of *Solanum elaeagnifolium*, commonly known as silverleaf nightshade, on durum wheat. Spanish Journal of Agricultural Research, Volume 22, Issue 2, e1002. https://doi.org/10.5424/sjar/2024222-20723

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# Introduction

Silverleaf nightshade (*Solanum elaeagnifolium* Cav.) is a noxious weed in the United States. It has been introduced in other 42 countries, becoming there an invasive species (Brunel et al., 2010; Roberts & Florentine, 2022). In particular, it has been introduced unintentionally from Texas in 1930 to the area of Thessaloniki (Northern Greece), the starting point of its invasion in the Mediterranean Basin and Europe (Uludag et al., 2016). Today, it is considered

one of the most important invasive weed species in Mediterranean Basin countries (in particular Greece, Portugal and Morocco) where it is in its rapid expansion phase. It has been observed in great densities in farmers' fields and on roadsides, while its successful establishment in new environments could be attributed to its high adaptability to different conditions, as well as to its dispersal ability, its detrimental impacts and its resistance to control (Mekki, 2007; Uludag et al., 2016; Singleton et al., 2020; Krigas et al., 2021; Tataridas et al., 2022). This species is very difficult to control and it can cause serious damage to cultivated fields and pastures by reducing yield and quality (Brunel et al., 2010; Tataridas et al., 2022). This species has also been associated with a variety of negative impacts on forestry, orchards, managed grasslands and other cultivated lands (e.g. cereals, potato, cotton), posing a serious threat to all Mediterranean Basin countries (Brunel et al., 2010; Brunel, 2011). Similarly, in Australia, it has been a noxious weed in cultivated wheat (Feuerherdt, 2010). It has been listed as a European Plant Protection Organization (EPPO) A2 pest recommended for regulation (EPPO Global Database, 2015). However, its full potential range has not yet been studied (Uludag et al., 2016).

Silverleaf nightshade creates an extensive root system that can reach up to 3 m deep and 2 m horizontally. Because of its aggressive vegetative growth from deep rootstocks both chemical and mechanical control methods are ineffective (Feuerherdt, 2010; Roberts & Florentine, 2022).

In Europe, silverleaf nightshade emerges in spring and its life cycle fits with those of spring crops. In late autumn and winter, the plant is less vigorous or its above-ground part is dried by the frost and consequently does not compete strongly with autumnal crops. Its interference is greater in spring crops, especially in dryland and non-irrigated areas, probably due to its dense, deep and extensive root system. Production losses have been reported at levels of up to 64% in maize (Morocco), 5-75% in cotton and 4-10% in sorghum (USA), as well as 12-50% in wheat (Australia) (Heap & Carter, 1999; Utah & Rico, 2007).

Secondary metabolites, known for their insecticidal activity and toxicity, are present in silverleaf nightshade tissues (Jonasson & Olsson, 1994; Sanford et al., 1997; Mkula, 2006). This plant also exhibits allelopathic effects, including the root exudation of various substances that prevent the germination and growth of various crops (Balah et al., 2022). In particular, silverleaf nightshade contains a high proportion of known allelochemicals such as the steroid alkaloid glycosides solamargin, solasonine, solanine  $\alpha$ -,  $\beta$ -solanine, as well as chlorogenic acid, cinnamic acid, apigenin, quercetin and kaempferol (Balah, 2015; Balah et al., 2022).

Silverleaf nightshade competition with spring crops and the allelopathic effects of its extracts on some (not cereals) species have been studied (Travlos et al., 2013). On the contrary, published data regarding the indirect interference of silverleaf nightshade on durum wheat or other autumnal crops under field conditions are limited in the literature (Feuerherdt, 2010). Therefore, studies regarding the interference under field conditions between silverleaf nightshade and durum wheat can provide greater insight into the optimal management strategies in this crop. Thus, the objectives of this research were: (i) to assess under field conditions the effect of incorporated biomass (aboveground parts and creeping roots) of silverleaf nightshade on durum wheat emergence, growth and yield, and (ii) to determine under laboratory conditions the possible phytotoxic activity of its aqueous extracts on germination and root growth of four winter cereals (durum wheat, winter wheat, barley and oats), a fact which could strengthen the hypothesis of the interference in the field.

## Material and methods

### Allelopathic potential experiment

#### Plant material

Whole plants of silverleaf nightshade at the maturity stage (BBCH code 71-79; Meier, 2001) were harvested from a durum wheat field in 2019 and 2020. Weed plants were divided into three parts: (i) creeping roots, (ii) leaves + stems, and (iii) flowers + fruits. Thereafter, parts were air-dried under shadow at 28±4°C for 72 h and then, the dry parts were grounded in a Wiley mill through a 1-mm screen.

#### Extract preparation

Regarding the possible allelopathic potential of silverleaf nightshade on winter cereals, aqueous extracts (w/v) were prepared in 400-mL glass jars by adding 1.5, 3.00, 6.0, 12.0 or 24.0 g from each plant part in 200-mL of deionized water and shaken in an even shaker (4 h at 200 rpm). Four layers of cheesecloth and then a layer of filter paper (Whatman no. 42) were used to expel fibrous debris from the solutions. The solutions were then centrifuged (at 1750 g by a rotator for 30 min) and the supernatants were placed for 1 day in a refrigerator (about 5°C) in order for the bioassays to be prepared. For each combined treatment [weed part × extract concentration (0.75, 1.50, 3.00, 6.00 and 12.00 g per 100 mL)], three replicates (glass jars) were used.

#### Bioassay procedure

The possible effects of the three silverleaf nightshade part extracts on the germination and root elongation of durum wheat (Triticum durum L., var. 'Cannavaro'), winter wheat (Triticum aestivum L., var. 'Zanzibar'), barley (Hordeum vulgare L., var. 'Olympic') and oats (Avena sativa L., var. 'Kassandra') were evaluated in perlite Petri dish bioassays. In these bioassays, 12 seeds of durum wheat, winter wheat, barley or oats were placed in the bottom of 8.5-cm diam plastic Petri dishes. The seeds were covered with perlite (5 g per Petri dish) and moistened with 12 mL per Petri dish of the previously prepared silverleaf nightshade part extract. However, deionized water was used for the control. Each silverleaf nightshade part extract × concentration treatment was replicated three times and two Petri dishes for each replicate extract were used. Petri dishes were randomized and placed on shallow trays of 40 cm diameter. Then, the trays were covered with plastic bags to retain moisture and placed in an unilluminated (so long as the shoot development was not evaluated) growth chamber at 19±2°C for 12 days. At the end of the incubation period, the average germination (mean of the two Petri dishes used for each replicate glass jar extract) and root elongation (of the germinated seeds only) were measured. All data were expressed as a percentage of

treated).

the control. The bioassay was conducted twice using the weed samples collected in 2019 and 2020. The electrical conductivity of all extracts was also measured to exclude any salinity effect on the cereals.

#### Field underground interference experiment

A 3-year field experiment was conducted in 2019 through 2022 at Agios Athanassios (northern Greece; longitude 22°23'58'' E, latitude 40°43'32'' N; 45 m above sea level) to investigate the possible interference (allelopathic effect) of silverleaf nightshade decomposed leaves/stems/fruits on durum wheat growth and yield components. The soil was a calcareous sandy loam (Typic xerorthents) with sand 55%, silt 30%, clay 15%, organic matter 1.2% and pH 7.9. During the experiment, the mean monthly temperature and the total monthly rainfall data were recorded and are presented in Fig. 1. No irrigation was applied during the experiment according to the practice of cereal farmers in the area.

A natural silverleaf nightshade infestation of 14 to 34 plants  $m^{-2}$  was present as when evaluated at early summer in 2019. During this summer three treatments (A, B and C) were established in a complete randomized design with four replicates:

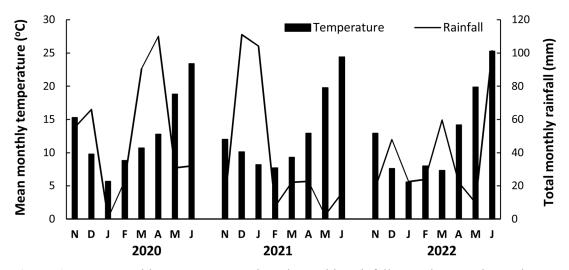
- A: without silverleaf nightshade plants {in these plots, the herbicide glyphosate [N-(phosphonomethyl) glycine] was applied four times during the summer (from late June to early September) at the vegetative propagation stage (BBCH code 40-41), at a rate of 3960 g a.i. ha<sup>-1</sup> (maximum registered single rate for difficult-to-control perennial weeds) to successfully control weed regrowth}. In this treatment, the total glyphosate rate was 15840 g a.i. ha<sup>-1</sup>, necessary to suppress the continued silverleaf nightshade summer regrowth,

B: with 14-18 plants m<sup>-2</sup> of silverleaf nightshade (in these plots the herbicide glyphosate was applied once at rate of 3960 g a.i. ha<sup>-1</sup> to reduce weed regrowth), and
C: with 30-34 plants m<sup>-2</sup> of silverleaf nightshade (not

Plot size was  $20 \times 10$  m. The biomass of the survived silverleaf nightshade {about 4.5-4.8 t ha<sup>-1</sup> (which could be compared with the concentration of 6% of bioassays) or 8.3-8.6 t ha<sup>-1</sup> (which could be compared with the concentration of 12% of bioassays) at 14-18 or 30-34 plants m<sup>-2</sup>, respectively} was incorporated into the soil during the seedbed preparation in November. In particular, before the durum wheat seeding, the soil was prepared by a four-bottom moldboard plow to a depth of ~ 22 cm and a cultivator to a depth of ~ 12 cm. At the same time, nitrogen and phosphorus at 50 and 25 kg ha<sup>-1</sup>, respectively, were incorporated.

Durum wheat var. 'Cannavaro' was seeded by mechanical seeder at 190 kg ha<sup>-1</sup> in late November of the three growing seasons. Nitrogen at 30 kg N ha<sup>-1</sup> was also applied post-emergence in early March. Broadleaved weeds and grasses were controlled by iodosulfuron-methyl-sodium {methyl 4-iodo-2-[3-(4-methoxy-6-methyl-1,3,5-triazin-2-yl)ureidosulfonyl] benzoate sodium salt} + mesosulfuron-methyl {methyl 2-[(4,6-dimethoxypyrimidin-2-yl)carbamoylsulfamoyl]-4-(methanesulfonamidomethyl) benzoate} at 5.6 + 5.8 g a.i. ha<sup>-1</sup> when durum wheat was at the middle of tillering (BBCH code 24-28; Meier, 2001) growth stage. After the destruction of silverleaf nightshade plants during the soil cultivation in November, weed regrowth was not observed during the durum wheat growing period. However, weed plants emerged at crop maturity.

On about 12 weeks after seeding (WAS) each year, durum wheat stand (plant number) was assessed at the 2-to 3 leaves growth stage (BBCH code 12-13; Meier, 2001) in a 1 m<sup>2</sup> in the centre of each plot. Also, at 16 WAS, durum wheat shoot number (tillering) and fresh weight were determined at the 2 nodes growth stage (BBCH code 32)



**Figure 1.** Mean monthly temperature and total monthly rainfall near the experimental area during the three years.

by hand-cutting crop plants from a 1 m<sup>2</sup> area located in the centre of each plot. In late June each year, durum wheat plants from 1 m<sup>2</sup> in the centre of each plot were harvested by hand. Durum wheat yield components [total dry weight (TDW), ear number, grain yield (GY), grain number per 10 ears and 1000-grain weight (TGW)] were recorded.

#### Statistical analyses

The data collected from the four bioassays (one for each of the crop species) were analysed across years but separately for each crop species. In particular, a  $3 \times 5$  (3 weed parts  $\times 5$  extract concentrations) factorial approach in a completely randomized design with three replicates was used for the four bioassay experiments.

The phytotoxic dose-response effects of silverleaf nightshade extracts on the germination and root length of the four cereals were evaluated by the use of the whole-range assessment method reported by An et al. (2005). In particular, the equation:

## $I = \int_{D_0} \int_{D_0} [R(0) - f(D)/dD / \int_0 \int_{D_0} R(0) dD$

was used for the calculation of the inhibition indices (*I*). In particular, 0 to  $D_n$  was the range of the concentrations;  $D_c$ was the threshold concentration threshold which equaled the value of the control and above that the responses were inhibitory; *R*(0) was the response at 0 g 100 mL<sup>-1</sup> (control); and the *f*(D) represented the response function. For the calculation of the inhibition indices (*I*) (across the whole range of concentrations of the silverleaf nightshade extracts) the WESIA (Whole-range Evaluation of the Strength of Inhibition in Allelopathic-bioassay) software was used (Liu et al., 2007). Furthermore, for both germination and root length *I* value means, the differences between the cereal pairs (durum wheat and winter wheat, durum wheat and barley, durum wheat and oats, winter wheat and barley, winter wheat and oats or barley and oats) were examined using the paired student's t-test ( $\alpha = 0.05$ ).

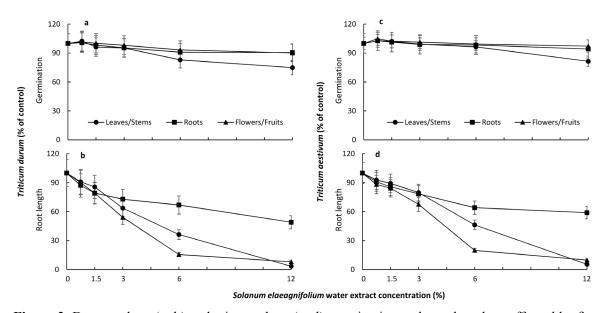
The field data of durum wheat were analysed across years using the one-factor randomised complete block design. Also, the linear regression equation was tested for its suitability to describe the relationship between the durum wheat parameters and silverleaf nightshade density, and also to compare wheat yield differences amongst years.

SPSS (version 17.0) was used to conduct t-tests (SPSS, 1998), Microsoft Excel was used for the linear regressions, while the ANOVAs were conducted by MSTAT (MSTAT-C, 1988). The Bartlett's test was used to examine homogeneity of variances, while the Fisher's protected least significant difference test procedure at p = 0.05 was used to detect and separate the mean treatment differences.

## Results

#### Allelopathic potential experiment

The germination and root length of the four cereals were not significantly affected by year (the two collection years of the silverleaf nightshade plants), but they were significantly (p < 0.001) affected in most cases by the silverleaf nightshade plant part (roots, leaves/stems, flowers/fruits) utilized to produce the aqueous extract, by the concentrations of the aqueous extract, and by the interaction between the plant part and the aqueous extract concentration. Thus, the plant part × aqueous extract concentration interaction means are presented. In general, the



**Figure 2.** Durum wheat (a, b) and winter wheat (c, d) germination and root length as affected by five concentrations of silverleaf nightshade aqueous extracts. The bars indicate the standard errors.

germination of the durum wheat, winter wheat and barley was not significantly affected by the extracts of the roots or the flowers/fruits (Figs. 2a, 2c and 3a). In contrast, the highest concentration of leaves/stems extracts reduced the germination of all cereals. However, the germination and the root length inhibition of all cereals increased with increasing extract concentration of the three weed parts, but this increase was not proportionally similar (Figs. 2 and 3).

The durum wheat germination inhibition caused by the leaves/stems extract concentrations of 6% and 12% was 17.0% and 25.1%, respectively (Fig. 2a). The lower concentrations did not cause any significant germination inhibition. The durum wheat root length inhibition caused by the leaves/ stems extract concentrations of 6% and 12% was 63.7% and 96.6%, respectively (Fig. 2b). The corresponding reductions caused by the roots or flowers/fruits extracts were 33.1% and 51.0% or 84.4% and 92.0%, respectively. The root length

reduction caused by the lower extract concentrations (0.75, 1.5 and 3%) ranged from 0% to 45.9% (Fig. 2b).

The winter wheat germination was significantly reduced (18.5%) only by the highest extract concentration (12%) of leaves/stems (Fig. 2c). However, winter wheat root length was reduced by all silverleaf nightshade extracts (Fig. 2d). In particular, the two greatest concentrations (6% and 12%) caused a 35.9% to 95.6% root length reduction, while the reduction caused by the lower concentrations ranged from 9.1% to 32.4%.

Barley germination was not significantly affected by silverleaf nightshade extracts (Fig. 3a). On the contrary, root length was reduced by 35.2% to 91.7% by the greatest extract concentrations (6 and 12%), while the root length reduction caused by the lower concentrations ranged from 8.8% to 38.3% (Fig. 3b). Similarly to the responses of durum wheat and winter wheat, the leaves/stems and flowers/fruits extracts were more phytotoxic to barley than the ones from the roots.

**Table 1.** Allelopathic effects of silverleaf nightshade water extracts on durum wheat, winter wheat, barley or oats germination and root length assessed by inhibition index  $(I)^1$ .

| Silverleaf nightshade parts | Inhibition index   |             |
|-----------------------------|--------------------|-------------|
|                             | Germination        | Root length |
| Durum wheat                 |                    |             |
| Leaves/stems                | 11.66 <sup>2</sup> | 55.64       |
| Roots                       | 5.81               | 30.65       |
| Flowers/fruits              | 0.00               | 69.14       |
| Mean                        | 5.82 ab            | 51.81 a     |
| Winter wheat                |                    |             |
| Leaves/stems                | 4.30               | 41.84       |
| Roots                       | 0.21               | 17.71       |
| Flowers/fruits              | 0.00               | 54.32       |
| Mean                        | 1.50 b             | 37.96 b     |
| Barley                      |                    |             |
| Leaves/stems                | 4.26               | 47.98       |
| Roots                       | 4.05               | 25.09       |
| Flowers/fruits              | 2.85               | 49.80       |
| Mean                        | 3.72 b             | 40.96 ab    |
| Oats                        |                    |             |
| Leaves/stems                | 14.41              | 35.35       |
| Roots                       | 12.24              | 44.68       |
| Flowers/fruits              | 10.10              | 42.39       |
| Mean                        | 12.25 a            | 40.81 ab    |
| Mean of 4 cereals           |                    |             |
| Leaves/stems                | 8.66               | 45.20       |
| Roots                       | 5.53               | 29.53       |
| Flowers/fruits              | 3.24               | 53.91       |

<sup>1</sup>A inhibition index (*I*) calculation is specified in Materials and Methods. <sup>2</sup>In each group, means were compared by the paired student's t-test ( $\alpha = 0.05$ ).

The extract concentrations of 0.75%, 1.5% and 3.0% did not significantly reduce the germination of oats. However, the germination reduction caused by the 6.0% and 12.0% concentrations ranged from 9.0% to 27.0% (Fig. 3c). Regarding oat root length, the highest extract concentrations (6.0% and 12.0%) reduced oat root length by 41.6% to 61.7%, while the lower concentrations caused root length reductions that ranged from 0.7% to 32.5% (Fig. 3d). The leaves/stems extracts were slightly less phytotoxic than the ones from roots or flowers/fruits.

The paired student's t-tests conducted showed differences between *I* indices indicating that the extracts of silverleaf nightshade parts inhibited the germination of durum wheat and oats slightly more than that of winter wheat and barley (Table 1). No significant differences were observed amongst winter wheat, barley and oats regarding the root length inhibition, while root growth was more sensitive to the extracts than the germination. In particular, the increasing order of the silverleaf nightshade extract phytotoxicity, according to the estimated germination inhibition indices, was flowers/fruits (3.24) < roots (5.53) < leaves/stems (8.66), while the respective order, according to the estimated root length inhibition indices, was roots (29.53) < leaves/stems (45.20) < flowers/fruits (53.91).

#### Field underground interference experiment

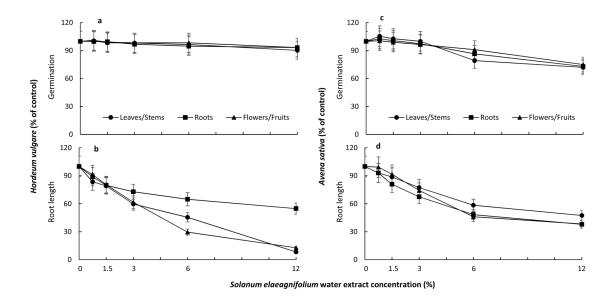
The ANOVAs performed for durum wheat parameters indicated that crop stand (plant number) and fresh weight were significantly (p < 0.001) affected by year, silverleaf nightshade biomass (incorporated in soil) and their interaction, while the crop shoot number (tillering ability) was significantly (p < 0.001) affected by silverleaf nightshade

biomass and year  $\times$  silverleaf nightshade biomass interaction. Thus, the year  $\times$  silverleaf nightshade biomass interaction means are presented (Fig. 4).

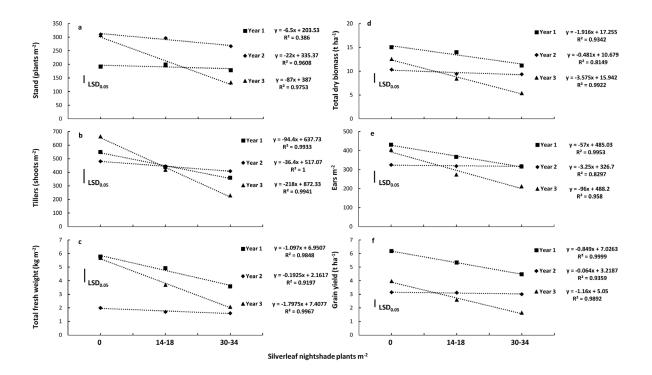
At 12 and 16 WAS, the silverleaf nightshade biomass that was incorporated into the soil reduced the durum wheat crop plant number, shoot number and fresh weight (Figs. 4a, 4b and 4c). The incorporated 30-34 plants m<sup>-2</sup> of silver nightshade caused greater growth reduction than the 14-18 plants m<sup>-2</sup>, while the crop plant number was less affected, except for the year 3, by silverleaf nightshade biomass, compared with those of crop shoot number and fresh weight. In particular, in plots where 14-18 and 30-34 silverleaf nightshade plants m<sup>-2</sup> had been incorporated, durum wheat plant number was reduced by 1.4% and 11.3%, respectively (averaged across year 1 and year 2), compared with silverleaf nightshade mulch-free plots (control) (Fig. 4a). The corresponding reductions in year 3 were 36.0% and 56.5%. Durum wheat shoot number was reduced by 21.4% and 41.2% (averaged across years) by the 14-18 and 30-34 silverleaf nightshade incorporated plants m<sup>-2</sup>, respectively (Fig. 4b). The corresponding reductions in durum wheat total fresh weight were 23.3% and 45.9%, with the lower reductions (14.5% and 19.2%) observed in year 2 (Fig. 4c).

Each year, the plant number (Fig. 4a), shoot number (Fig. 4b) and total fresh weight (Fig. 4c) of durum wheat grown under different densities of silverleaf nightshade indicated, in most cases, a proportional decrease with increasing density. This was confirmed by the high  $R^2$  for the linear regression used to describe the relationship between the durum wheat characteristics and weed density.

During harvest, durum wheat TDW, ears number, GY and TGW were significantly (p < 0.001) affected by year, silverleaf nightshade biomass (incorporated in soil) and



**Figure 3.** Barley (a, b) and oats (c, d) germination and root length as affected by five concentrations of silverleaf nightshade aqueous extracts. The bars indicate the standard errors.



**Figure 4.** Plant number (a) at 12 weeks after seeding (WAS), shoot number (b) and total fresh weight (c) at 16 WAS, as well as total dry weight (d), ear number (e) and grain yield (f) of durum wheat as affected by silverleaf nightshade presence during three years.

by their interaction, while the grain number per ear was significantly (p < 0.001) affected by year and by year × silverleaf nightshade biomass interaction. Thus, the year × silverleaf nightshade biomass interaction means are presented (Figs. 4 and 5).

Durum wheat TDW, GY and ear number were reduced by the silverleaf nightshade biomass that was incorporated in soil more than the TGW and grain number per 10 ears. In particular, the incorporation of 30-34 silverleaf nightshade plants m<sup>-2</sup> reduced durum wheat TDW, ear number and GY by 31.5%, 27.0% and 31.2% (averaged across years), respectively, when compared with the silverleaf nightshade free plots (Figs. 4d, 4e and 4f). The corresponding reductions caused by the incorporated 14-18 silverleaf nightshade plants m<sup>-2</sup> were 15.9%, 17.3% and 16.9%. Similarly, the durum wheat TGW and grain number per 10 ears in plots where the 30-34 silverleaf nightshade plants m<sup>-2</sup> were incorporated were 17.9% and 15.3% (averaged across years), respectively, lower than those in the silverleaf nightshade free plots (Fig. 5). The corresponding reductions caused by the incorporated 14-18 silverleaf nightshade plants m<sup>-2</sup> were 11.8% and 9.4%.

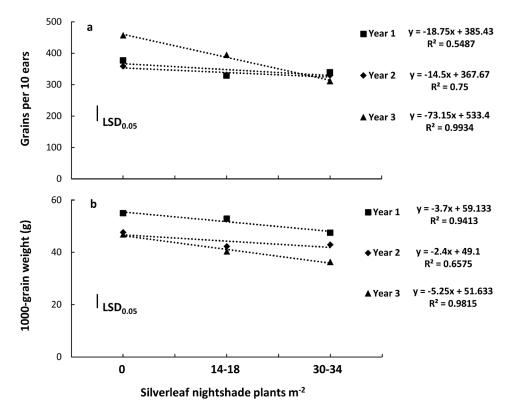
Similarly to 16 WAS in each year, the TDW (Fig. 4d), ears number (Fig. 4e) and GY (Fig. 4f) of durum wheat grown under different densities of silverleaf nightshade indicated a proportional decrease with increasing density. This result was confirmed by the high  $R^2$  for the linear regression used to describe the relationship between the durum wheat characteristics and weed density.

## Discussion

#### Allelopathic potential experiment

The germination and root length inhibition of cereals by the aqueous extracts of the three silverleaf nightshade plant parts are in agreement with results reported by Alhemedy et al. (2016), who found that the powder of silverleaf nightshade had allelopathic impact on durum wheat germination and growth. Similarly, Balah et al. (2022) found that silverleaf nightshade extracts reduced germination, as well as root and shoot length of various plants such as winter wheat, barley, Egyptian clover (*Trifolium alexandrinum* L.), alfalfa (*Medicago sativa* L.), horsebean (*Vicia faba* L.), corn (*Zea mays* L.), common wild oat (*Avena fatua* L.), littleseed canarygrass (*Phalaris minor* Retz.) and common purslane (*Portulaca oleracea* L.).

The greatest inhibition of root length in durum wheat, winter wheat, barley and oats was caused by the leaves/ stems extracts comparted with those of flowers/fruits or



**Figure 5.** Grain number per 10 ears (a) and 1000-grain weight (b) of durum wheat as affected by silverleaf nightshade presence during three years.

roots. Although allelocamicals composition or concentration were nor determined in silverleaf nightshade extracts tested in this study, the different inhibition effects on cereals caused by the leaves/stems, flowers/fruits and roots extracts could possibly be explained by the differences in amount and characteristics of physicochemicals included in these plant parts. Balah (2015) found that the allelochemicals chlorogenic acid and kaempferol β-D-(6-O-cis-cinnamoyl) glucosode, isolated from silverleaf nightshade seeds, were more phytotoxic to common purslane than coumaroyl glucoside, mangiferin, kaempferol or coumaroyl quince acid. Alhemedy et al. (2016) found that the foliage powder of silverleaf nightshade provided greater durum wheat growth inhibition than the one coming from the root. Balah et al. (2022) also found that the vegetative part extracts of silverleaf nightshade had greater allelopathic potential than the ones from the root. Anantharaju et al. (2017) found that silverleaf nightshade contains a high proportion of steroid alkaloid glycosides, including solamargin, solasonine,  $\alpha$ -solanine and  $\beta$ -solanine. Eleftherohorinos et al. (1993) also found that saponins in the fruits of silverleaf nightshade exhibited allelopathic effects on cucumbers in Greece raising the possibility of allelopathic effects on other crops. In addition, Curvetto et al. (1976) found that the saponin derived from the fruits of the silverleaf nightshade reduced root growth of squirting cucumber (Ecballium elaterium A. Rich.).

## Field underground interference experiment

In Greece, winter cereals are seeded after autumn rainfalls because the cereal fields are no-irrigated; furthermore, nitrogen fertilization is applied before seeding. Thus, the depletion of soil moisture and nitrogen because of the presence of silver nightshade during summer or the change of C/N ratio in soil because of the incorporation of silverleaf nightshade biomass could not significantly affect the durum wheat emergence and its initial growth. Consequently, the significant inhibition of durum wheat emergence and growth observed in the field could be attributed to the allelopathic effect of silverleaf nightshade incorporated biomass. This result is strengthened by the results reported by Balah et al. (2022), who found great amounts of allelochemicals in the rhizosphere of silverleaf nightshade. These allelochemicals caused significant suppression of rhizosphere bacteria. Alhemedy et al. (2016) also found that silverleaf nightshade powder had a negative effect on durum wheat emergence and growth. Similarly, Mkula (2006) found that the silverleaf nightshade extracts and soil-incorporated residues reduced the germination and early growth of cotton (Gossypium hirsutum L.). Travlos et al. (2013) found that the silverleaf nightshade competition caused 8% to 26% annual yield loss in alfalfa. Lemerle & Leys (1991) also reported that when silverleaf nightshade

was chemically controlled (by repeated applications of 2,4-D or glyphosate) prior to wheat seeding, GY was increased by 14% to 69%, with the largest increases in the drought growing season.

The greater reduction in durum wheat emergence observed in year 3, caused by the incorporated biomass of silverleaf nightshade, could be attributed to the observed lower rainfall (about 50%) during November and December (the period of durum wheat emergence) (Fig. 1). This fact may lead to lower leaching of the allelochemicals of silverleaf nightshade and consequently to their greater concentration around the crop rhizosphere (Real et al., 2019; Zhang et al., 2021).

Similarly, the lower reduction of durum wheat growth and yield recorded in Year 2, compared to the reductions in Year 1 and Year 3, could be attributed to greater rainfall and temperature recorded from December to January in Year 2 (Fig. 1). These conditions could favor the durum wheat establishment, while they increased the decomposition and the leaching of the silverleaf nightshade allelochemicals (Latif et al., 2017).

The glyphosate total rate used (15840 g ai ha<sup>-1</sup>) to suppress the continued silverleaf nightshade summer regrowth in treatment A (without weed presence) was at least twice the maximum glyphosate rate allowed in Greece. Considering the probable future limitation of herbicide use in the European Union, combining herbicides with mechanical control methods could be used for silverleaf nightshade suppression (Roberts & Florentine, 2022).

Conclusively, the results of this study indicated that the silverleaf nightshade extracts significantly reduced the root length of four winter cereals but less their germination, with the leaves/stems and flowers/fruits extracts being more phytotoxic than the ones from the roots. In field conditions, residues of the aboveground part of silver nightshade incorporated in soil by tillage before seeding significantly reduced the growth and yield components of durum wheat. This fact could be attributed to the allelopathic effect of the weed. Therefore, the effective management of silver nightshade (maybe by combined herbicide applications and mechanical control) before durum wheat seeding could increase wheat productivity.

Data availability: Not applicable.

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