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Article

Seed Germination and Plant Growth under Drought Stress of Herbicide-Resistant and Herbicide-Susceptible Biotypes of *Conyza* Species and Smart Farming Approaches

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Abstract: Horseweed (*C. canadensis*) and fleabane (*C. sumatrensis*) are two annual or perennial herbaceous weeds present with high frequency and density in many parts of the world. Their response to water deficit was studied by means of seed germination tests and pot experiments. Seed germination was tested in solutions with different concentrations of polyethylene glycol (PEG). Two biotypes of each species were examined, one glyphosate resistant and the other susceptible. Growth responses were similar in the two species, both being more affected by lower (−1 MPa) than higher water potential (−0.2 MPa). The results revealed a significant effect of the biotype and the weed species on the drought stress response and adaptation. When high PEG concentrations were applied (−0.6 MPa), both *C. sumatrensis* biotypes had higher germination percentages (up to 88%) than the *C. canadensis* biotypes, while in most cases the seeds of the resistant biotypes germinated more (up to 72%) compared to the susceptible ones. These findings were confirmed by means of *NDVI* values, indicating that remote sensing can be used for a quick evaluation of the drought stress response of these weeds. The results obtained highlight the significant effect of species, biotypes and drought stress level on the germination, survival and growth of the weeds.

Keywords: *C. canadensis*; *C. sumatrensis*; drought stress; herbicide resistance; germination; remote sensing

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1. Introduction

Horseweed (*C. canadensis* L. Cronq. or *Erigeron canadensis*) and fleabane (*C. albida* Willd. ex Spreng or *C. sumatrenis* or *Erigeron sumatrensis*) are both genera of flowering plants in the family Asteraceae, which contains about 23,600 species distributed in 1620 genera and 12 subfamilies [1]. Moreover, they are two out of the three main species of *Conyza* in Greece and are mainly found in perennial crops (orchards, vineyards etc.) and noncrop areas. The most common and abundant of the two species is horseweed, while *C. sumatrensis* was lately introduced in the country and is considered to be an invasive species [2]. In general, they prefer tropical and warm temperate regions, but they can also adapt in cold temperate regions such as North America. They are annual species that can grow up to 1–2 m tall having branched stems, alternate leaves and flowers comprised of several inflorescences that are gathered closely on each stem. These cosmopolitan species have recently become much more common and major weeds for many crops and arable lands [2,3]. Horseweed, also known as Canada fleabane, is mostly a winter annual and an individual plant is able to produce more than 200,000 seeds [3,4]. Although its seeds usually germinate in early fall or in spring, germination can take place throughout the year [5]. Fleabane is an annual-biennial herb originating from South America. In Greece it grows in urban habitats, orchards, vineyards and vegetables. Since it can tolerate a variety of habitats and environmental conditions, it is a noxious weed, difficult to control [2,6].

Drought tolerance, avoidance and resistance strategies in several plants include notable features important for their survival [7,8]. In view of climate change, crop varieties that combine improved drought resistance with high yield under suitable conditions and quality characteristics are needed. On the contrary, many weeds also show such ability, allowing us even to predict future shifts of specific species, dispersal of invasive species and changes in weed communities [9]. This is also the case for *Conyza* (*Erigeron*) species, since they are very often exposed to severe drought and other extreme environmental conditions and consequently any plasticity and adaptation would be beneficial for them [10].

Herbicide resistance of weeds is one of the major challenges of modern agriculture [11]. The repeated and extended use of the same herbicides or herbicides with the same mode of action are among the main ways of resistance development, even in the absence of herbicide-resistant crops [12,13]. Globally, *Conyza* spp. are among the species most prone to herbicide resistance, while in Greece, glyphosate resistance has already been confirmed for *C. canadensis*, *C. bonariensis* and *C. sumatrensis* [2,14]. Lately, due to the reduced number of registered herbicides and the overreliance on specific active ingredients, there have been many reports from Greek farmers and advisors that *Conyza* spp. have become progressively difficult to control with several herbicides, especially in no-tillage or minimum-tillage systems [2,4]. Evolutionary speaking, fitness cost studies of herbicide-resistant and herbicide-susceptible populations are very important for their potential dispersal and future dominance [15]. Particularly for *Conyza* spp. and glyphosate, little or no fitness penalty has been reported in horseweed and hairy fleabane with similar biomass and seed production for the glyphosate-resistant (GR) and glyphosate-susceptible (GS) populations [4,16]. Nonetheless, in other cases, the GS biotype was less competitive than the GR biotype, particularly when grown at high densities and under moisture-deficit stress [17].

Consequently, it seems rather risky to predict the relative fitness of the several biotypes, since negative, positive, or no impacts of herbicide resistance on weed populations have been reported. Moreover, studies in a limited resources environment are necessary, while climate change and dry conditions in many regions can also affect the dispersal and potential dominance of biotypes with different susceptibility to herbicides. In that context, new tools and technologies, sensor-based techniques and indices like *NDVI* can be used to estimate crop and weed growth and response to stress [18,19]. Specifically, *NDVI* is estimated as the normalized differences between Near-Infrared Red (*NIR*) and red reflectance [$NDVI = (RNIR - Rred)/(RNIR + Rred)$], where RNIR and Rred are the reflectance values measured in the Near-Infrared Red (770 nm) region and the Red region (660 nm), respectively [20]. NDVI is non-destructive tool for evaluating several growth parameters not only for crops but also for weeds.

The objective of the present study was to evaluate the response of resistant and susceptible biotypes of horseweed and fleabane to drought stress by means of seed germination assessment and precision agriculture approaches. This information could be reliable for the overall development of resistance management strategies.

2. Materials and Methods

2.1. Experimental Setup

Horseweed and fleabane seeds were collected from two regions, the Domokos region in Central Greece and the Pelion region in east Central Greece (Table 1). From both regions, we have collected an already-confirmed GR population and a known GS population, where no glyphosate had been used in the last 10 or more years. Seeds were placed to germinate outdoors at Agricultural University of Athens under the same conditions (26/15 °C day/night and a 12–12.30 h photoperiod. This was necessary in order to avoid any effect of maternal environment on seed germination. The soil was a clay loam, whose physicochemical characteristics (0 to 15 cm depth increment) were clay 285 g kg^{-1}, silt 325 g kg^{-1}, sand 392 g kg^{-1}, organic C content 14.5 g kg^{-1}, pH (1:2 H_2O) 7.6, $CaCO_3$ 10.5 g kg^{-1} and organic matter content of 23 g kg^{-1}. Mature seeds were collected and dried. Glass jars were used for the seeds to be placed and afterwards were kept at 4 °C in the dark until their use.

Table 1. Region, geographical position, crop and number of horseweed (*C. canadensis*) and fleabane (*C. sumatrensis*) accessions included in the study.

Region	Positions	Crops	*C. canadensis*	*C. sumatrensis*
Pelion	39°26′19′′ N, 23°2′47′′ E	Orchards	2 (S and R)	-
Domokos	39°08′ N, 22°18′ E	Orchards, vineyards	-	1 (S and R)

To assess the adaptability to osmotic stress, five water potential levels were imposed (−0.2, −0.4, −0.6, −0.8 and −1 MPa) by means of polyethylene solutions (PEG 6000) (Table 2). In the case of the control (untreated), distilled water was used, and referred to as a water potential (WP) of 0 MPa. One hundred viable seeds of each biotype were placed in Petri dishes on two sheets of paper filter disk (Whatman Ltd., Maidstone, UK) saturated with 4 mL distilled water. The Petri dishes were kept at a 15/25 °C temperature regime in incubation chambers (Conviron T 38/Lb/AP) under a 12/12 h day/night light photoperiod. The light was supplied by fluorescent light tubes providing 50 µmol m^{-2} s^{-1}. Seed germination was expressed as a percentage of the total number of tested seeds (germination percentage) after an incubation of 10 days. Seeds were considered germinated when the healthy, white radicle emerged through the integument and reached more than 1 mm in length [21]. After each measurement (every day from 1 to 10), germinated seeds were removed. The experiment was conducted in a completely randomized design and four replications for each treatment and was repeated twice.

Table 2. Amount of PEG 6000 soluble substance (g L^{-1}) for the preparation of the five osmotic solutions.

Water Potential (MPa)	g L^{-1}
−0.2	112.2
−0.4	169.4
−0.6	213.6
−0.8	251.0
−1	284.0

Furthermore, a pot experiment was conducted twice during the summer of 2020 with all the 4 biotypes mentioned above. In both runs, the average minimum/maximum air temperature and relative humidity in the glasshouse during the experiments were 20/40 °C and 40/60%, respectively. The soil was the same as the one used in the previous pot experiments. At the beginning and until 15 days after sowing (DAS) of 50 seeds on the soil surface of each pot, pots were irrigated with ample distilled water up to 50% of the water-holding capacity. Then, by withholding irrigation, soil water content of all pots fell to 25% of the water-holding capacity and the differentiation of the water treatments started from 20 days after sowing (no drought stress: 200 mL three times a week, moderate drought stress: 200 mL once a week and high drought stress: 200 mL every 10 days). At 20 days after the differentiation of water regime, canopy reflectance was measured using a Trimble® GreenSeeker® handheld crop sensor (Trimble Agriculture Division, Westminster, CO, USA). The sensor unit has self-contained illumination in both red and near-infrared bands and measures reflectance in the red and near-infrared (*NIR*) regions of the electromagnetic spectrum [20] according to Equation (1):

$$NDVI = \frac{NIR - Red}{NIR + Red} \quad (1)$$

The sensor was held stable at approximately 20–25 cm above weeds' leaf area for 5 s and *NDVI* measurements were taken between 13:00 pm and 15:00 pm in the way that was previously described by Travlos et al. [22].

2.2. Statistical Analysis

Data obtained from the laboratory germination test experiment and the pot trial were analyzed by ANOVAs at a significance level of $p < 0.05$ by means of the Statistica 9.0 software package (StatSoft, Inc. 2300 East 14th Street, Tulsa, OK, USA). All data were tested for normality and variance before further analyses. The seed germination percentages were angular transformed and then subjected to ANOVAs.

3. Results

The ANOVAs revealed the significant effect of the species, the biotype and the concentration of PEG on seed germinability. Furthermore, there was a significant interaction ($p < 0.001$) between species and biotype and also species and water potential (Table 3).

Table 3. Analysis of variance (ANOVA) for the effects and the interactions of *Conyza* species (SP), biotype (BI) and concentration (CO).

Source of Variation	Df	Sum of Squares	Mean of Squares	F Ratio	*p* Value
SP	1	2161.972	2161.972	18.844	<0.001 *
BI	1	2585.891	2585.891	22.539	<0.001 *
CO	5	85,158.082	17,031.616	148.45	<0.001 *
SP × BI	1	2462.83	2462.83	21.466	<0.001 *
SP × CO	5	5141.643	1028.329	8.963	<0.001 *
BI × CO	5	1161.694	232.339	2.025	0.085
SP × BI × CO	5	1157.709	231.542	2.018	0.086

* significant at $p < 0.001$.

3.1. *C. sumatrensis*

Comparing the two biotypes of *C. sumatrensis*, a significant difference can be noted. The GR biotype was less affected by drought stress than the GS biotype (Figure 1). For instance, 10 days after treatment with PEG of −0.2 MPa, the resistant biotype had a germination percentage of 84%, while the corresponding value for the susceptible biotype was 36% lower. Moreover, a significant difference ($p < 0.001$) appeared on the −0.8 MPa treatment. From 5 to 10 days after placement, some GR seeds managed to germinate, while there was not any germination of the GS seeds. Furthermore, the susceptible biotype seemed more stressed than the resistant biotype on the first days of germination (days 2–4) on the majority of the treatments. Also, the highest drought stress level (−1 MPa) had a full inhibiting effect on both biotypes during the entire experimental period (Figure 1).

3.2. *C. canadensis*

Almost the same pattern was followed on the *C. canadensis* biotypes as well. The herbicide-resistant biotype showed significantly higher germination values than the susceptible one for the −0.2 MPa treatment. On the contrary, on the −0.8 MPa treatment, this difference was not significant (Figure 2). On the tenth day, the −0.8 MPa treatment of the resistant *C. canadensis* biotype caused only 5% germination compared with the *C. sumatrensis* resistant biotype that had 27%; thus, it is shown that the effect of the species was significant, as previously reported. Also, at the level of low drought stress (e.g., WP = −0.2 MPa) the seed germination of the *C. canadensis* resistant biotype was equal to the unstressed seed lot of the same biotype (Figure 2).

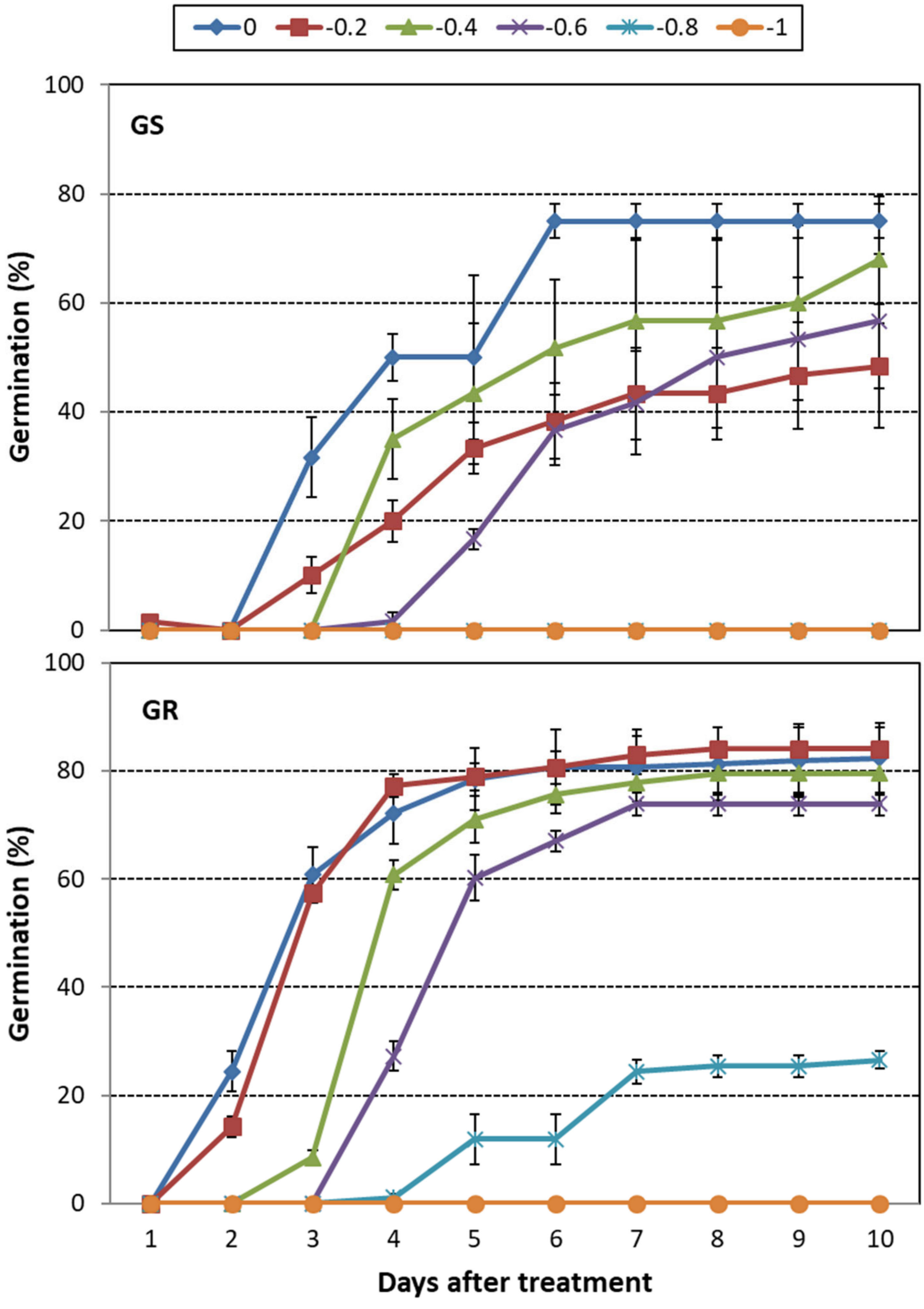

Figure 1. Effect of drought stress (different concentrations of PEG, namely 0, −0.2, −0.4, −0.6, −0.8 and −1 MPa) on germination percentage of the two *C. sumatrensis* biotypes (GS and GR). Vertical bars denote standard errors of the means.

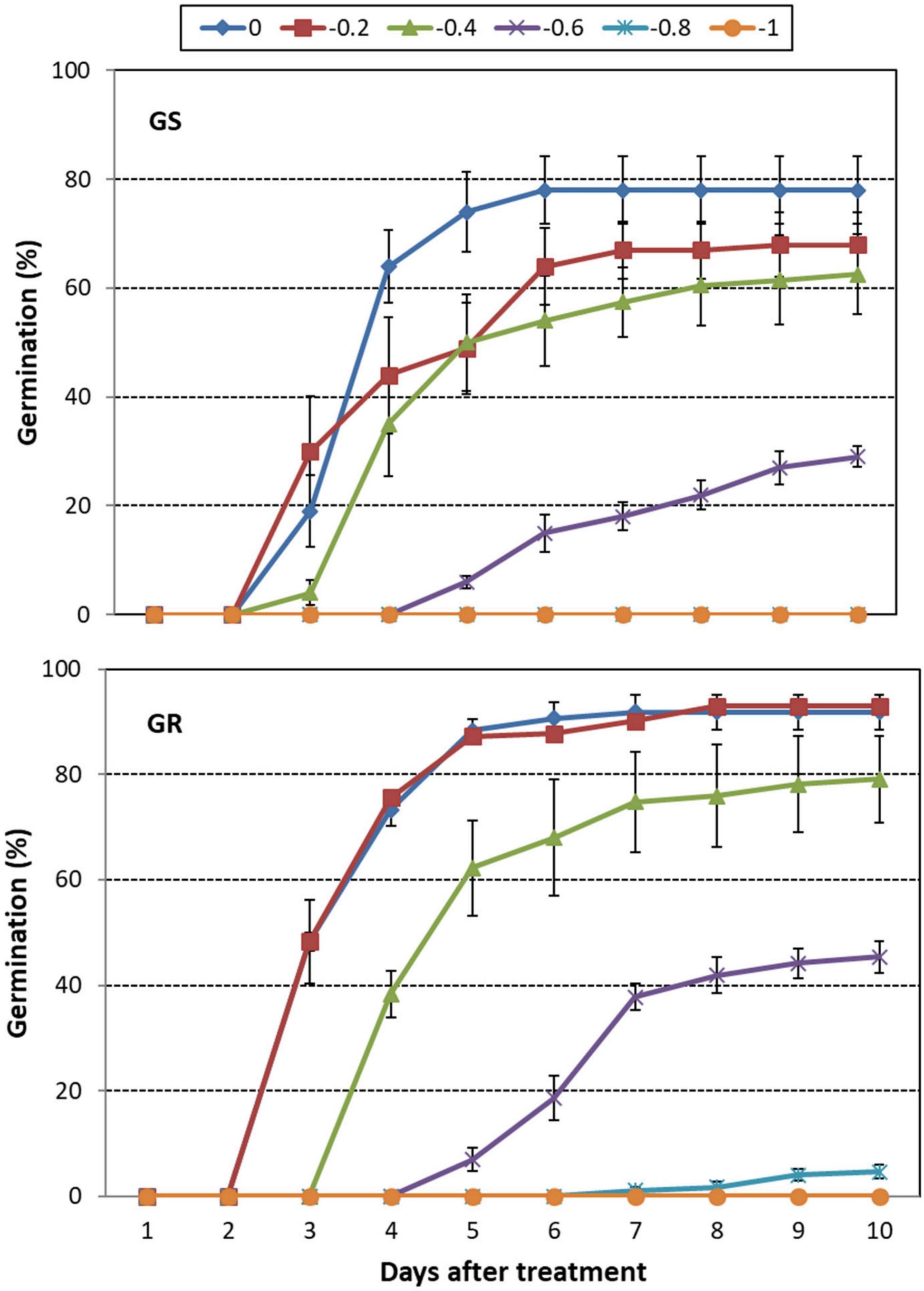

Figure 2. Effect of drought stress (different concentrations of PEG, namely 0, −0.2, −0.4, −0.6, −0.8 and −1 MPa)) on germination percentage of the two *C. canadensis* biotypes (resistant and susceptible). Vertical bars denote standard errors of the means.

3.3. Overall Drought Stress Effect

The −0.2 MPa treatment had a lower effect on the germination percentage for all four examined biotypes compared to the −0.6 MPa treatment (Figure 3). Additionally, a significant difference between the two species was observed. In both resistant and susceptible biotypes, the *C. canadensis* species had higher germination values than the *C. sumatrensis* concerning the −0.2 MPa treatment (68% vs. 48% for the susceptible and 93% vs. 84% for the resistant ones, respectively). On the contrary, under higher drought stress (i.e., WP = −0.6 MPa), *C. sumatrensis* species had a better performance than *C. canadensis* (57% vs. 29% for the susceptible and 74% vs. 45% for the resistant biotypes, respectively). Another observation was that in the −0.2 MPa treatment, germination started very early (2 days after treatment), while under higher drought stress (−0.6 MPa), seed germination was significantly delayed for almost all the biotypes (Figure 3).

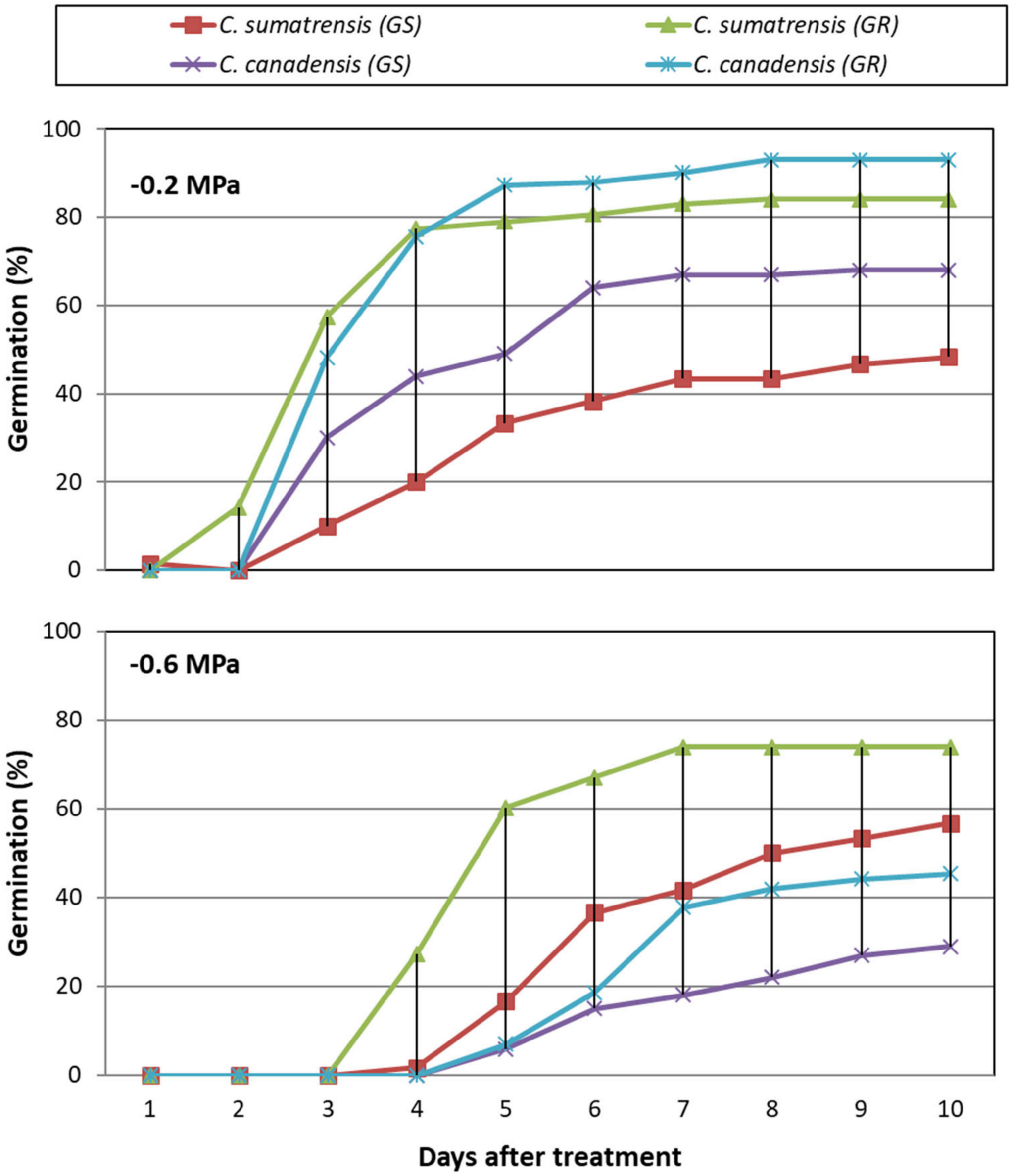

Figure 3. Effect of selected drought stress treatments on the germination percentage of the four different *Conyza* spp. biotypes during a ten days' period. Vertical bars denote standard errors of the means.

3.4. Evaluation of Drought Response by Means of Remote Sensing

NDVI values highlighted significant differences between the biotypes (Table 4). In particular, without any drought stress, all biotypes performed well and had similar growth (NDVI values not significantly different). Under moderate and high drought stress, GR biotypes of both species performed better than the GS biotypes (NDVI values significantly higher). Furthermore, under high stress, *C. sumatrensis* biotypes responded better than the respective biotypes of *C. canadensis*, confirming the above-mentioned results of the seed germination tests.

Table 4. NDVI values for the GS and GR biotypes of *C. sumatrensis* and *C. canadensis* without any drought stress, under moderate drought stress and at high drought stress. Different lowercase letters indicate significant differences between the means for the several biotypes ($p < 0.05$).

	C. sumatrensis		*C. canadensis*	
	GS	**GR**	**GS**	**GR**
No drought stress	0.78 a	0.75 a	0.82 a	0.77 a
Moderate drought stress	0.45 c	0.53 b	0.42 c	0.51 b
High drought stress	0.36 f	0.49 d	0.27 g	0.42 e

4. Discussion

The seeds of the two species germinated at a different rate, with *C. sumatrensis* being the faster one. The highest percentage of seed germination for *C. sumatrensis* was absolutely comparable to the one mentioned by Bellache et al. [10]. Under low drought stress (−0.2 MPa), the resistant biotype of *C. sumatrensis* had significantly higher germination percentage than the susceptible biotype. It is also notable that even under high drought stress (−0.8 MPa), up to 27% of the seeds of the resistant biotypes germinated. This is a finding of huge ecological importance if we take into account that one single plant can produce up to 500,000 seeds. In that case, one plant of an herbicide-resistant biotype can produce more than 100,000 new seedlings. This is a really important information in order to raise the awareness of the farmers regarding the risk of herbicide resistance development. In a recent study, there were no significant differences between the GR and GS biotype of the same species (*C. sumatrensis*), with seed germination reduced by 17 and 85% at water potential of −0.4 and −0.8 MPa, respectively, compared with the unstressed control [23]. However, our results revealed significant differences between the resistant and the susceptible biotypes, and significant interactions between species and biotypes and species and drought stress. For instance, at 7 DAS, seed germination of the resistant and the susceptible biotype was reduced by 6 and 24%, respectively, at drought stress of −0.4 MPa compared to the control. Thus, it could be said that the relative response of the resistant and susceptible biotype is rather case-specific. This hypothesis is further supported by studies in relative species (*C. bonariensis*), revealing that severe drought can result to larger germination reduction, while there were significant differences between the studied biotypes [24]. Other studies in *C. sumatrensis* showed that seeds of arid populations exhibited higher germination under increased drought stress levels indicating that maternal environment affected germination traits of the tested populations [25]. However, this is not the case in our study since we studied biotypes of similar maternal environment (due to their origin from the same region) with the only different parameter being herbicide resistance.

Our results are in accordance with previous studies in other species (e.g., *Echinochloa colona*) showing that the GR biotype was more tolerant to drought stress than the GS biotype of the same species [26]. Additionally, our results revealed that the species also has a significant effect on seed germination. Notably, *C. canadensis* performed better at low drought stress conditions than *C. sumatrensis*, while the latest performed better at high drought stress conditions. Differences on the drought resistance between Asteraceae species were also observed in a previous study, highlighting the higher ability of certain species to withstand and disperse under extreme climatic conditions [27].

Furthermore, *C. sumatrensis* probably has the capacity to adapt better to harsher environmental conditions regarding soil moisture than *C. canadensis*. Understanding of these characteristics associated with germination justifies the invasive nature of this species and is also useful to weed management and control. Hence, knowledge of germination of these species is essential for the development of effective weed management systems both economically and environmentally. Information on the fitness and the dynamics of the several weed species and herbicide-resistant biotypes is clearly needed to prevent potential long-term impacts of these weeds in arable lands [4,15,28,29].

Regarding the pot experiments, the lower NDVI values which were recorded are in accordance with previous studies, confirming the role of this index in evaluating vegetation's health status [18,30–32]. Indeed, the response of the several weed biotypes as recorded by means of NDVI were in close correlation with their response in the seed germination trials. Consequently, our results reveal that remote sensing can be a very important tool for the evaluation of drought adaptation of weeds like *Conyza* spp. as it was used in the past for the evaluation of drought stress effects on crops and weeds [33,34] and herbicide efficacy on several weeds [19]. In addition, despite the limitations and the requirement of specific weather conditions for NDVI measurements, this is of rather minor importance in studies like this one, when the focus is in the comparison of different weed biotypes under the same conditions.

5. Conclusions

In conclusion, the germination percentages of horseweed and fleabane are not only affected by the level of the drought stress but the effect of species and biotype is also significant. As shown in the germination tests and confirmed in the pot experiments, *C. sumatrensis* and herbicide-resistant biotypes can germinate and survive under higher drought stress than *C. canadensis* and herbicide-susceptible biotypes, respectively. Furthermore, remote sensing can be used as an important tool for the quick evaluation of drought adaptation of the weeds and the potential of this method for the specific purpose is going to be further examined in pot and field experiments with a wide range of species and biotypes.

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