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Water limitation affects weed competitive ability for light. A demonstration using a model-based approach combined with an automated watering platform

Delphine Moreau, Hugues Busset, Annick Matejicek, Marion Prudent, Nathalie Colbach
Agroécologie, INRAE, Institut Agro, Univ. Bourgogne, Univ. Bourgogne Franche-Comté, 21000 Dijon, France

Author for correspondence:
Delphine Moreau
Telephone: 33380693669
Fax: 33380693262
delphine.moreau@inrae.fr

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1. Summary

Because of climate change, investigating how morphological traits involved in competition for light (main resource for which crops and weeds compete in conventional cropping systems and temperate and tropical climate) respond to water limitation is crucial to better understand crop-weed dynamics in the field. Our objective was to develop an innovative approach to quantify the response of weed species to water limitation, and test it with three species. This approach combined (1) key morphological traits involved in competition for light (taken from a mechanistic crop-weed model) as criteria to analyze response to water limitation and (2) a pot/greenhouse platform allowing automated precision-watering and daily quantification of soil water availability in each pot. Response to water limitation differed among species/stages. For all species/stages, increased plant height per unit of aboveground biomass and production of smaller/thicker leaves were the most noteworthy responses. Plants with a strong increase of plant height per unit of aboveground biomass in response to water limitation were able to maintain high specific leaf area, even at low soil water availability. Increase in biomass allocation to roots (vs. aboveground parts) and to leaves (vs. stems and reproductive organs) were also observed, but not for all species/stages. Overall, these effects of water limitation on morphological traits, suggest strong interactions between competition for light and water.

2. Introduction

Weeds can greatly reduce yield and harvest quality, mainly by competing with crops for resources (Oerke, 2006). That is the reason (together with a high efficiency) why herbicides generally play a key role in ensuring crop production efficiency in conventional cropping systems. However, reducing the excessive use of herbicides has become necessary in view of harmfulness for the environment and public health (Hasanuzzaman et al., 2020). In this context, promoting weed ecological regulation by shifting resource availability and use from weed to crop may provide a more sustainable weed management (Petit et al., 2018). One promising option could be to use competitive crop plants, especially for light which is the main resource for which crops and weeds compete in conventional cropping systems and in temperate and tropical climates (Wilson and Tilman, 1993; Perry et al., 2003). However, the frequency of water stress events may increase with climate change, especially in Southern Europe (http://discomap.eea.europa.eu/climate). So, one challenge is to identify crops that are competitive for light, even under water stress conditions.

Species competitive ability is directly related to their capacity to adapt to resource availability (Navas and Violle, 2009). Thus, investigating the response of crop and weed species to water limitation is crucial. Especially, we need to better understand how plant traits involved in competition for light respond to water stress. Some studies characterized the response of weed species to water stress. However, most of them focused on the germination phase (e.g. Gardarin et al., 2010; Ruhl et al., 2016; Yuan and Wen, 2018) or on global plant growth (e.g. Chahal et al., 2018; de Oliveira et al., 2018). Moreover, they were conducted on a low number of weed species, which contrasts with the high number of weed species that may be found in arable fields (Fried et al., 2008). To our knowledge, no study has yet quantified the diversity of the response of weed species to water availability with a focus on the key morphological traits involved in competition for light. The reasons for this are two-fold. Carrying out experiments with a range of water treatments requires individual daily pot watering which is very time-consuming when performed manually, especially on a large number of pots. Moreover, until recently, the key morphological traits involved in crop-weed competition for light were not identified. However, two main recent advances should make it possible to lift these limitations. On the one hand, the development of innovative high-throughput platforms with automatic and precise individual pot watering in greenhouse allows to overcome these technical limitations (e.g. Granier et al., 2006; Jeudy et al., 2016; Brichet et al., 2017). Up to now, such platforms have almost entirely been used for crop species or for species used as models in genetic/genomic studies. Even when the objective was to analyze crop competitive ability with weeds, only crop plants were studied (Nguyen et al., 2018; Anandan et al., 2020). On the other hand, a recent study using a mechanistic simulation model allowed to identify key morphological traits involved in competition for light between arable crops and annual weeds (Colbach et al., 2019). Considering competition for light only (i.e. competition for water was neglected), Colbach et al. (2019) identified the key traits explaining weed harmfulness, crop ability to limit weed-caused yield loss and/or crop potential yield. They were specific leaf area (SLA corresponding to the leaf area per unit of leaf biomass), height to biomass ratio (HBR corresponding to plant height per unit of aboveground biomass) and leaf to aboveground biomass ratio (LBR corresponding to leaf biomass per unit of aboveground biomass).

Based on these recent advances, our objective was to quantify the response of weed species to water stress in order to determine which morphological traits are influenced by water stress and whether the response to water stress depends on plant species and stage. Our aim was also technical: to test an innovative approach for weed characterization combining (1) morphological traits derived from a simulation study as criteria to analyze weed response to water limitation and (2) an experimental platform allowing automatic and precise plant watering. The final aim was to determine whether the results from this approach (applied here to a small number of weed species and stages as a proof of concept) could help in the near future for the large-scale characterization of the large panel of weed species potentially present in agricultural fields.

3. Materials and methods

3.1.Experimental treatments

A greenhouse experiment was conducted in Dijon (France) using three annual weed species: *Alopecurus myosuroides* Huds. (monocotyledonous C3 species preferentially germinating in autumn), *Amaranthus hybridus* L. (dicotyledonous C4 species preferentially germinating in spring and summer) and *Abutilon theophrasti* Medik. (dicotyledonous C3 species preferentially germinating in summer). Five water treatments were applied, corresponding to 20%, 30%, 40%, 55% and 75% of field capacity. The five treatments were applied to the three species, except the 40% treatment on *A. hybridus* (due to space limitation in the greenhouse). Eight plants were grown per species × water treatment combination. Plant species and water treatments were randomly arranged. The duration of the experiment was 62 days.

3.2.Growing conditions

Seeds were germinated in an incubator with a 16-h photoperiod and day/night temperatures adapted to each species (25/20°C for *A. myosuroides* and 30/25°C for *A. hybridus* and *A. theophrasti*). On 14 March 2017, germinated seeds were sown in the greenhouse into 1-L pots filled with a solid and inert substrate made up of 80 % of expanded clay and 20 % of attapulgite (volume proportions), with a bulk density at 0.67 +/- 0.01 g cm^{-3}. The retention curve of the substrate is given in Supplementary Material Section A online. One seed was sown in each pot. The pots were placed on fixed tables and watered automatically with a complete nutrient-rich solution made up of N-P-K (10-10-10) and oligo-elements via tubes at a frequency allowing non-limiting watering. Twenty-three days later (on 6 April, when plants were between six and nine leaves), plants were transferred into an automated platform where the five water treatments were imposed. This platform (described in Jeudy et al., 2016) is based on conveyors that automatically transport pots towards a watering unit (consisting of a weighing terminal and a high-precision pump-watering station) (Supplementary Material Section B online). In our experiment, plants were conveyed three times per day to be weighed. When actual weight was lower than target weight, they were watered (Supplementary Material Section C online). There was no drain because irrigation did not exceed field capacity. Over all irrigation events, irrigation allowed to reach the pot target weight ± 0.13 % (calculated as the absolute difference between the target and the actual weight just after the irrigation event, divided by the target weight). The elapsed time between the irrigation of the first and the last pot (over 130 pots) was 34 ± 9 minutes.

Throughout the experiment, the same complete nutrient-rich solution was used in order to avoid strong plant-nitrogen-limitation even when water supply was low. In the discussion (Section 5.4), the comparison of our results to the literature suggests that this methodological approach allowed to minimize a potential nitrogen limitation.

Target weight was determined as:

$$\frac{[(sw_{100\%} - sw_{0\%}) \times \%\text{WaterCapacity}]}{100} + sw_{0\%} + \text{EPotW} + \text{PlantW} \qquad \text{[Equation 1]}$$

$SW_{100\%}$ and $SW_{0\%}$ are substrate weight (in g). $SW_{100\%}$ is measured at field capacity, and $SW_{0\%}$ is the weight of dry substrate. *%WaterCapacity* is the target proportion of field capacity (in %, ranging from 20 to 75 %). *EPotW* is empty pot weight (in g). *PlantW* is fresh plant weight (in g). Plant weight was considered as negligible at the beginning of the experiment. It was adjusted during the experiment for *A. theophrasti* (using plant weight data from the first sampling date; see Section 3.3).

At each weighing session, the weight of each pot was recorded before and after watering. These data were used to calculate the daily fraction of evapotranspirable soil water, currently named 'Fraction of Transpirable Soil Water' (FTSWd in %), for each species × water treatment combination. The FTSWd relates the actual plant-available soil water content to the total plant-available soil water content for day d (Lebon et al., 2006). It was calculated as:

$$\text{FTSWd} = \frac{(sw_{100\%} - sw_{0\%}) + \sum_{d=1}^{n}(\text{WaterInput\_d}) - \sum_{\text{d}=1}^{\text{n}-1}(\text{Evapotranspiration\_d})}{(sw_{100\%} - sw_{0\%})} \qquad \text{[Equation 2]}$$

WaterInput_d (in g) is the daily amount of water provided by automatic watering at day d, and *Evapotranspiration_d* (in g) is the daily amount of water lost by evapotranspiration at day d. Note that the day the pots were transferred into the platform, FTSW was close to 100% for all pots, and then differentiated water treatments started (Supplementary Material D). So, during the first days, water input was nil. Water input and evapotranspiration were the same only when the target weight was reached.

Throughout the experiment, artificial light was provided using 400 W lamps (HPS Plantastar, OSRAM, Munich, Germany), in addition to natural light. Mean photoperiod was 14.5 ± 0.7 h. Incident photosynthetically active radiation was 17.2 ± 2.1 mol m^{-2} day^{-1} (silicon sensors; Solems, Palaiseau, France). Air temperature was 21.2 ± 1.7°C (PT100 sensors; Pyro-Contrôle, Vaulx-en-Velin, France). Air relative humidity was 57.7 ± 3.0 % (AIDC HIH-4000-001; Honeywell, Minneapolis, USA).

3.3. Plant measurement and trait calculation

Plant measurements were made at two sampling dates, and four plants were sampled per species × water treatment × sampling date combination. At the first sampling date, all the species × water treatment combinations were sampled. *Abutilon theophrasti* and *A. myosuroides* were at the vegetative stage, and *A. hybridus* was at the flowering stage. At the second date, only one species (*A. theophrasti*) was sampled at the flowering stage. The first sampling was performed 21 days after beginning of water treatments (i.e. 45 days after sowing), leaving enough time for the water treatments to stabilize (Supplementary Material Section D online) and plants to adapt. The second sampling was performed 38 days after beginning of water treatments (i.e. 62 days after sowing), when *A. theophrasti* reached the flowering stage.

At each sampling date, the following plant growth traits were measured. Plant height and plant leaf area (LI-3100 Area Meter; LI-COR, Lincoln, NE, USA) were measured. Leaf, stem and root biomass were independently determined after 48 h at 80°C. These plant growth traits were used to calculate plant morphological traits that play a key role in competition for light (Colbach et al., 2019): (1) leaf to aboveground biomass ratio (LBR) is the ratio of the leaf to aboveground biomass. It reflects the efficiency for producing leaves for a given aboveground plant biomass, with a higher value for leafier plants. (2) Specific leaf area (SLA) is the ratio of the leaf area to the leaf biomass at the plant level. It is the efficiency for producing leaf area from a given leaf biomass. High values indicate thin leaves, while low values mean thicker leaves. (3) Height to biomass ratio (HBR) is plant height relatively to aboveground biomass. The higher height to biomass ratio, the taller the plants are for a given biomass. We also calculated (4) Root to total plant biomass ratio (RBR), i.e. root biomass relative to total plant biomass, reflecting plant efficiency for producing root biomass from total plant biomass, with a higher value for plants favoring root vs. aboveground growth. This trait is often considered to play a key role in the interaction between competition for light and water (McCarthy and Enquist, 2007; Craine and Dybzinski, 2013).

3.4. Statistical analyses

Soil-water availability was expressed in fraction of transpirable soil water, with FTSWmean the mean over FTSWd values from the beginning of water treatment to sampling date. FTSWmean was compared between plant species and plant stages by analysis of variance (lm function of R). As the three weed species were not sampled at both phenological stages, the relative effects of species and stage could not be dissociated. So, the global effect of both species and stage was analyzed.

The response of each morphological trait to FTSWmean was analyzed by covariance analysis (lm function of R). The following model was used:

$$\text{plant\_trait}_{ps} = \mathit{constant} + c_s + a \times \text{FTSWmean}_{ps} + b_s \times \text{FTSWmean}_{ps} + \text{error}_{ps} \qquad \text{[Equation 3]}$$

where plant_trait_{ps} was the morphological trait measured on plant p for a given species × stage combination s, and *constant*, a, c_s and b_s were regression slopes, with the latter two depending on the species × stage combination s. This model was applied to the four morphological traits. For HBR, a $\log_n$ transformation was applied to normalize residue distribution. For SLA, a piecewise regression was performed using the segmented package in R (Muggeo, 2008), in order to account for its two-phase response pattern (Figure 1). So, for HBR, RBR and LBR, the response of species × stage combinations to water availability was compared on one slope value, while for SLA it was compared on two slope values and breakpoint (i.e. $\text{FTSW}_{\text{mean}}$ at which slope values change).

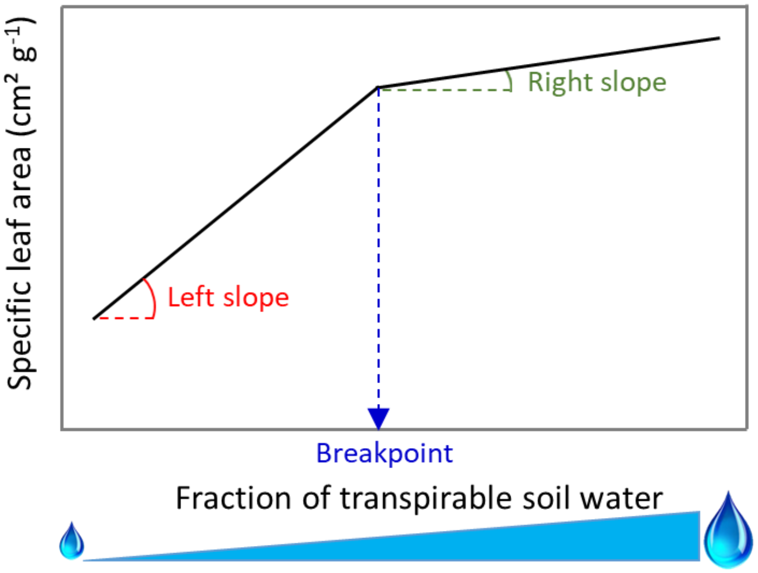

Figure 1: Theoretical example of the two-phase-response-pattern of specific leaf area (SLA) to water availability (characterized by the fraction of transpirable soil water, FTSW). It shows three parameters: breakpoint (i.e. FTSW at which slope value changes) and left and right slopes (i.e. slope of the regression when FTSW is lower and higher, respectively, than breakpoint value).

4. Results

4.1. Dynamics of soil-water availability

The five water treatments resulted in different soil-water status characterized by the mean fraction of transpirable soil water (FTSW) (Figure 2). For most water treatments (defined by the target proportion of field capacity), the mean daily FTSW (averaged over the period running from the beginning of water treatments to sampling date) varied with the plant species/stage (Figure 2). In general, FTSW was lower for *A. theophrasti* at the reproductive stage. This was due to the dynamics of FTSW that progressively decreased over time until stabilization (Supplementary Material Section D online) and to the later sampling date (39 instead of 22 days after the beginning of water treatments) for *A. theophrasti* at the reproductive stage, resulting in more days at low FTSW values. As a given water treatment mostly resulted in different FTSW values depending on species/stage, plant morphology was analyzed in response to FTSW in the following sections.

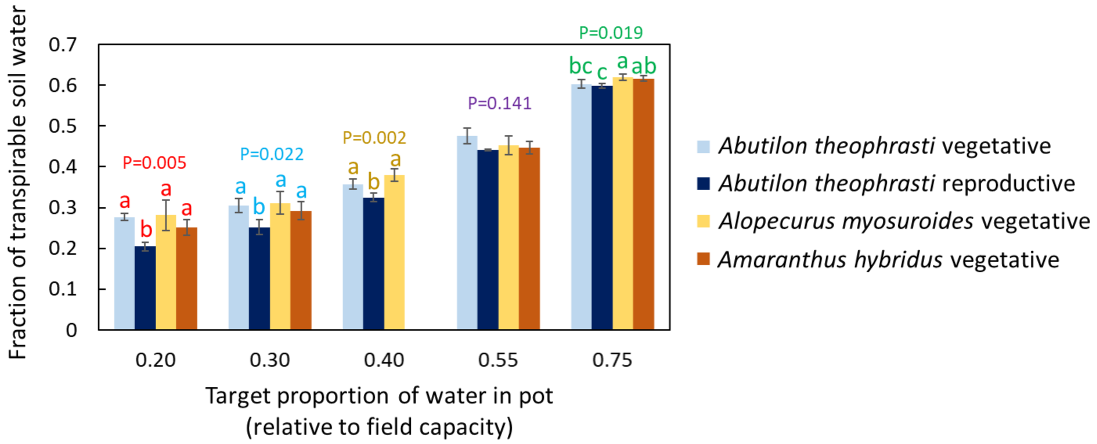

Figure 2: Fraction of transpirable soil water (FTSW) averaged over the period running from the beginning of water treatments to sampling date, for each species/stage and water treatment combination. P-values refer to variance analysis performed independently for each water treatment (defined by the target proportion of water in pot). Within each treatment, bars with the same letter show values that are not significantly different (least significant difference test).

4.2.Response of plant growth to water availability

Water availability strongly influenced all the plant growth traits, i.e. biomass, leaf area and height (Table 1A and Supplementary Material Section E online). The response of all these traits to water availability varied with the plant species/stage (Table 1A). For all species × stage combinations, plant leaf area was the most responsive variable (Table 1B).

Table 1: Effects of water availability and species/stage on plant growth traits. A. Partial R^2 (calculated from the type III sum of square of Anova function of R) indicates the proportion of variance explained by each factor for each trait. **B.** Variation factor (calculated as the ratio of the maximum to minimum value) shows the variability for each trait and each species/stage. FTSW for fraction of transpirable soil water. *** for P<0.001

Plant growth traits	**Total biomass**	**Aboveground biomass**	**Leaf biomass**	**Root biomass**	**Leaf area**	**Height**
A. Partial R^2						
FTSW	0.393***	0.374***	0.459***	0.448***	0.482***	0.480***
Species/stage	0.374***	0.378***	0.329***	0.351***	0.343***	0.308***
Interaction	0.212***	0.229***	0.182***	0.141***	0.150***	0.111***

Total R²	**0.980**	**0.982**	**0.970**	**0.940**	**0.974**	**0.899**
B. Variation factors for each species/stage						
Abutilon theophrasti vegetative	7.2	8.0	7.8	5.8	14.9	3.2
Abutilon theophrasti reproductive	15.1	18.4	12.3	9.8	23.0	5.9
Alopecurus myosuroides vegetative	4.9	4.9	4.8	4.9	12.9	2.6
Amaranthus hybridus reproductive	4.9	5.4	5.7	4.0	11.0	5.6

4.3.Response of plant morphology to water availability

The four studied morphological traits varied with soil water availability and, for all of them, the response to water availability varied with the plant species/stage (Table 2A). Height to biomass ratio HBR and specific leaf area SLA were the traits that responded the most to soil-water availability for each species /stage (Table 2B).

Table 2: Effects of water availability and species/stage on morphological traits. A. Partial R² (calculated from the type III sum of square of Anova function of R) indicates the proportion of variance explained by each factor for each trait. **B.** Variation factor (calculated as the ratio of the maximum to minimum value) shows the variability for each trait and each species/stage. FTSW for fraction of transpirable soil water. As the specific leaf area shows a two-phase response pattern, the analysis was performed separately for FTSW values lower vs. higher than breakpoint (i.e. FTSW at which slope value changes). Grey cells indicate when FTSW had non-significant effects on the morphological trait. HBR was $\log_n$-transformed for statistical analysis. ns for P>0.05; ** for P<0.01; *** for P<0.001.

Morphological traits	**Leaf to aboveground biomass ratio LBR**	**Height to biomass ratio HBR**	**Root to total plant biomass ratio RBR**	**Specific leaf area SLA**	
				For FTSW >breakpoint	For FTSW <breakpoint
A. Partial R²					
FTSW	0.05**	0.32***	0.13***	<0.01ns	0.22***
Species/stage	0.40***	0.48***	0.38***	0.47***	0.28***
Interaction	0.12***	0.05***	0.10**	0.10ns	0.21**
Total R²	**0.57**	**0.85**	**0.61**	**0.57**	**0.72**
B. Variation factors for each species/stage					
Abutilon theophrasti vegetative	1.17	3.37	1.37	2.21	
Abutilon theophrasti reproductive	1.64	3.75	1.87	1.97	

Alopecurus myosuroides vegetative	1.33	3.69	1.71	3.42
Amaranthus hybridus reproductive	1.54	2.40	1.72	2.48

4.3.1. *Height to biomass ratio*

For each species × stage combination, height to biomass ratio HBR increased exponentially with decreasing soil water availability (Figure 3). In response to water limitation, both aboveground biomass and plant height decreased, but the effect was larger for aboveground biomass than for plant height (Table 1; Supplementary Material Section E online). *A. theophrasti* was the most responsive species, followed by *A. myosuroides* and *A. hybridus* (see slope values on Figure 3). For A. *theophrasti* (the only species with data at both vegetative and reproductive stages), the phenological stage did not significantly affect the response slope.

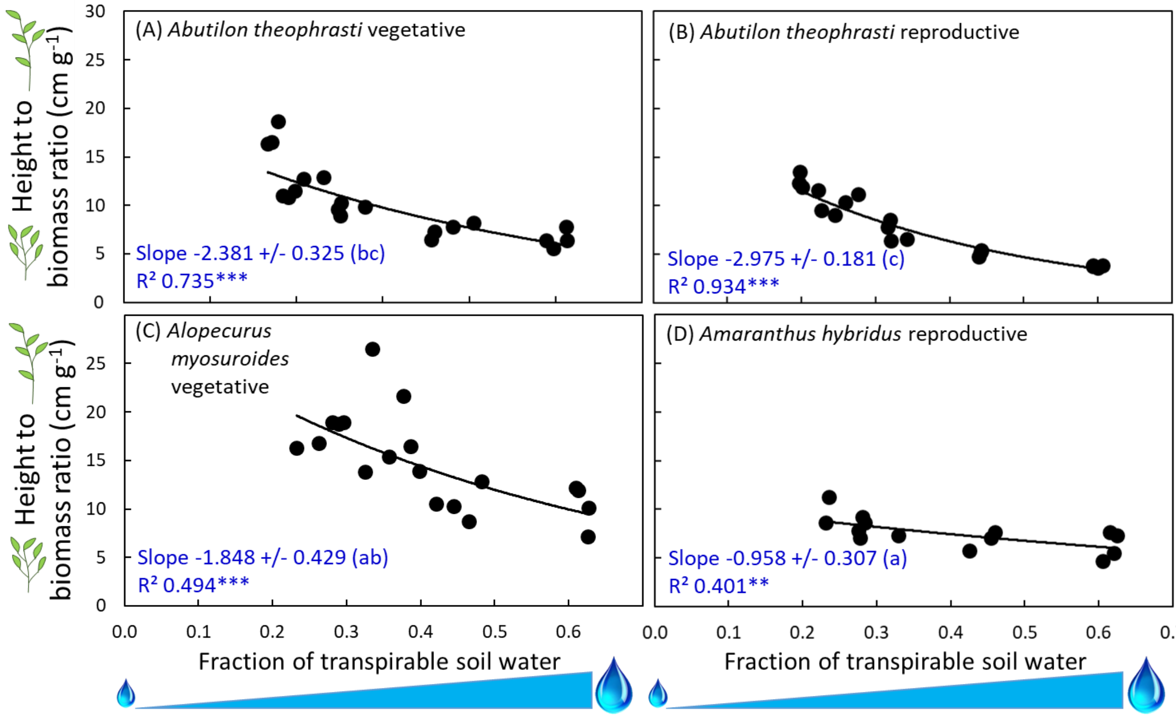

Figure 3: Response of height to biomass ratio ($\log_n$-transformed) to soil-water availability (fraction of transpirable soil water) for each species × stage combination. The slope of the relationships (+/- standard error) and R² are shown (** for P<0.01 and *** for P<0.001). Slopes with the same letter show non-significant differences. Each symbol represents one plant.

4.3.2. *Specific leaf area*

Contrary to height to biomass ratio, specific leaf area SLA decreased with decreasing soil water availability (Figure 4), meaning that plants produced smaller and thicker leaves in response to water limitation. This was due to a stronger effect of water deficit on leaf area than on leaf biomass (Table 1; Supplementary Material Section E online). Broken lines were used to account for the two-phase response pattern of SLA to FTSW, providing three parameters on which the four plant species × stage combinations were compared: breakpoint (i.e. FTSW at which slope value changes) and left and right slopes (i.e. slope of the regression when FTSW is lower and higher, respectively than breakpoint value)(Section 3.4). The SLA was constant during the first phase for three out of the four combinations (right slope on Figure 4). For *A. theophrasti* at the vegetative stage only, SLA decreased slightly with decreasing soil water availability. Soil water level from which SLA started to decrease sharply and the intensity of this SLA decrease (determined by the breakpoint and the left slope, respectively, on Figure 4) were used to discriminate species. As the breakpoint and the left slope were negatively correlated (r=-0.99; P=0.012; n=4), two contrasted patterns were identified. On the one hand, *A. theophrasti* at the reproductive stage maintained its SLA constant (non-significant right slope) until a FTSW value as low as 0.23, but below this threshold, SLA dropped very sharply (high left slope value). On the other hand, *A. hybridus* with SLA starting to decrease while soil water availability was much higher (FTSW at 0.50) but, below this threshold, SLA decrease was less steep (low left slope value).

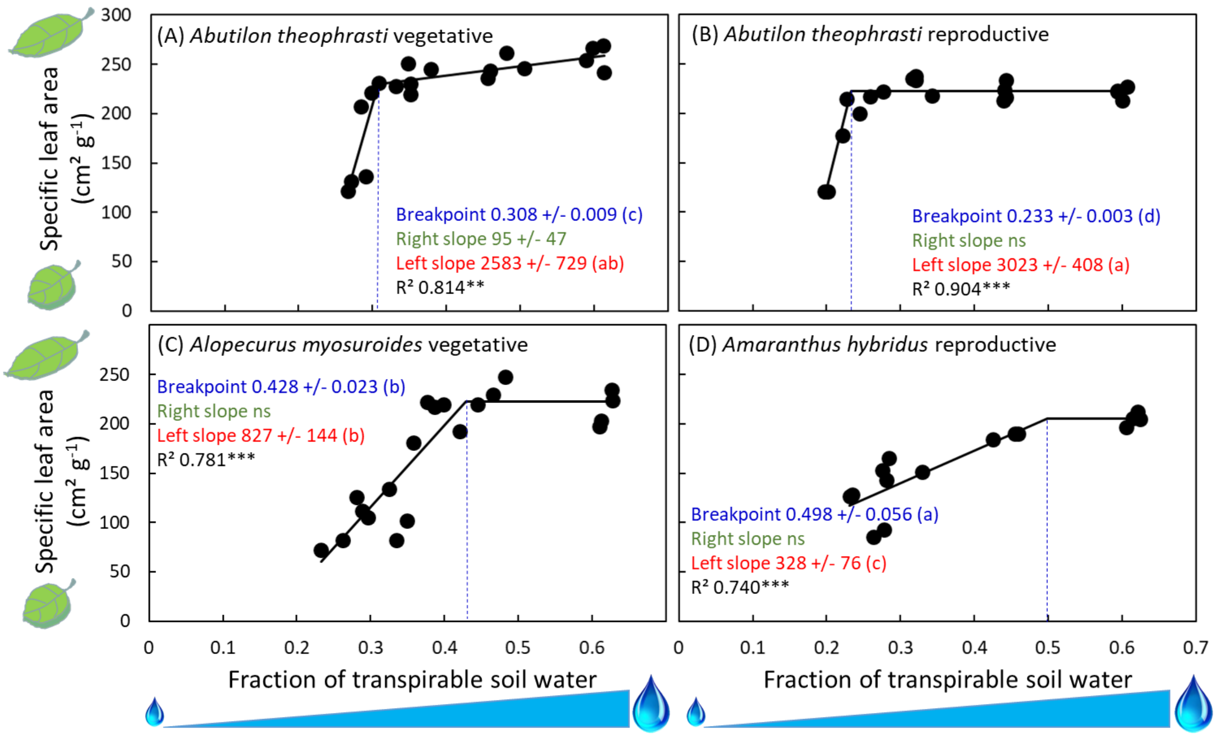

Figure 4: Response of specific leaf area to soil-water availability (fraction of transpirable soil water) for each species/stage combination. The slopes and breakpoint of the relationships

(+/- standard error) and R² (** for P<0.01 and *** for P<0.001) are shown. Values with the same letter show non-significant differences. Each symbol represents one plant.

4.3.3. *Root to total plant biomass ratio*

For two out of the four combinations of species × stage (*A. theophrasti* at the vegetative stage and *A. myosuroides*), root to total plant biomass ratio RBR was independent of soil water availability (average value at 0.25 g g^{-1} on Figure 5). Only for *A. hybridus* and *A. theophrasti* at the reproductive stage, RBR increased linearly with increasing soil water limitation. This indicated that proportionally more biomass was invested to root vs. aboveground plant parts in response to water limitation. The intensity of this RBR increase (reflected by the slopes on Figure 5) was similar for both species.

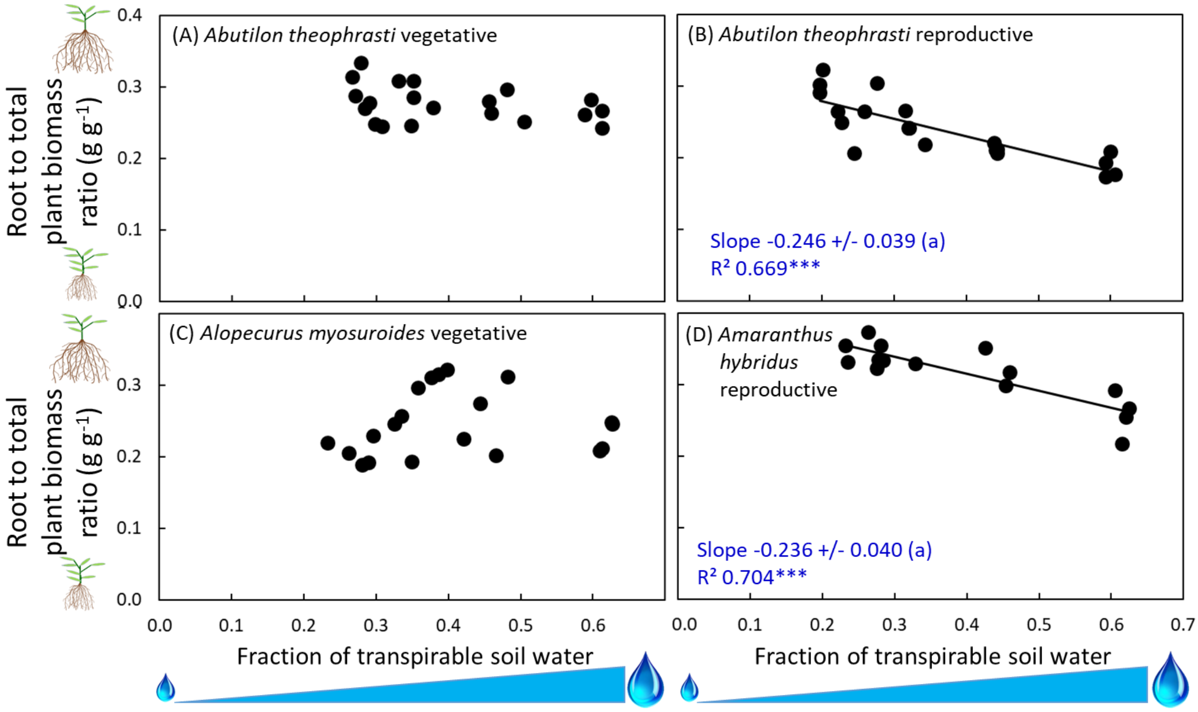

Figure 5: Response of root to total plant biomass ratio to soil-water availability (fraction of transpirable soil water) for each species/stage combination. The slopes (+/- standard error) and R² (*** for P<0.001) are shown when significant. Values with the same letter show non-significant differences. Each symbol represents one plant.

4.3.4. *Leaf to aboveground biomass ratio*

Leaf to aboveground biomass ratio LBR (i.e. ratio of leaf to aboveground biomass) was independent of soil water availability in most situations (Figure 6). Only for *A. theophrasti* at the reproductive stage, LBR increased in response to water limitation, meaning that proportionally more biomass was invested to leaf vs. stem and reproductive organs in response to water limitation.

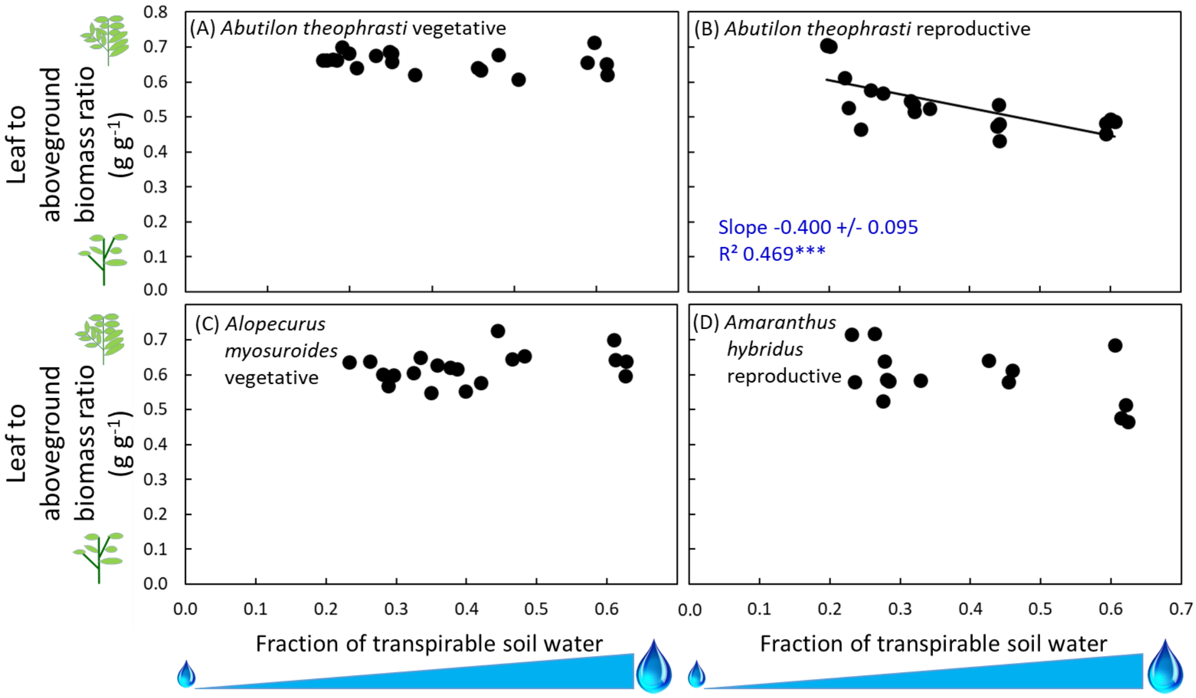

Figure 6: Response of leaf to biomass ratio to soil-water availability (fraction of transpirable soil water) for each species/stage combination. The slope (+/- standard error) and R^2 (*** for P<0.001) are shown when significant. Each symbol represents one plant.

4.3.5. Correlations among morphological parameters

To identify potential synergies/antagonisms among processes involved in plant morphological response to water limitation, correlations among parameters were analyzed. We focused on the three parameters for which we had values for the four species × stage combinations (SLA breakpoint, SLA left slope and HBR slope). Interestingly, the more height to biomass ratio increased in response to water limitation (low HBR slope), the more they were able to maintain high SLA even at low FTSW (low SLA breakpoint), and the stronger the decrease of SLA to water limitation (high SLA left slope) (**Figure 7**).

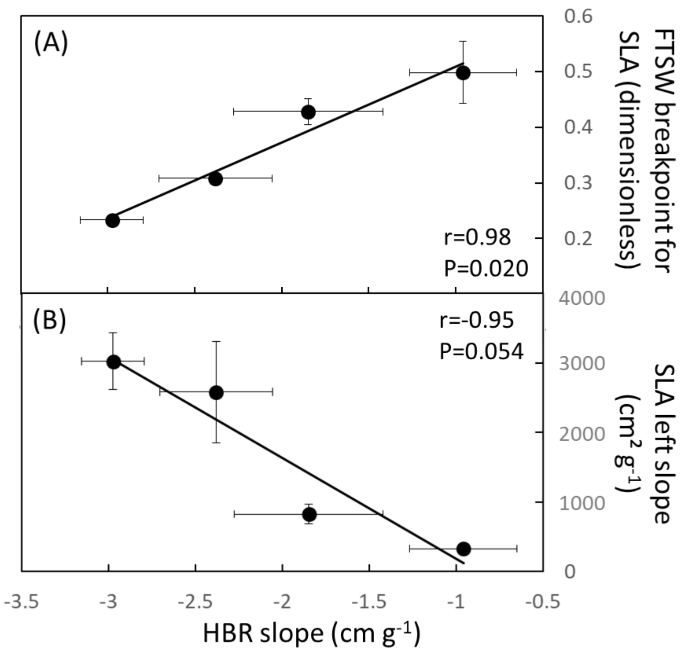

Figure 7: Correlation between the slope of the response of height to biomass ratio (HBR) to soil-water availability (FTSW) and (A) the specific leaf area (SLA) breakpoint and (B) the left slope of the response of SLA to soil-water availability. The parameter values (+/- standard error) come from Figure 3 (for height to biomass ratio) and Figure 4 (for specific leaf area). Each symbol represents a species × stage combination.

5. Discussion

5.1. An efficient methodology to analyse the diversity of weed response to water availability

The response of weed species to water stress has rarely been studied. Here, we used an innovative approach by (1) focusing on morphological traits previously identified in a simulation study as playing a key role in light competition between arable crops and annual weeds (Colbach et al., 2019), and (2) growing weed species on an experimental platform allowing automatic and precise plant watering as well as quantification of soil water availability in each individual pot. Only Jeudy et al. (2016) used such a platform, that they combined with high-throughput phenotyping, in order to compare root distribution among species including both crop and weed species, in response to soil-nitrogen availability. The present study is the first one using an automated watering platform to quantify the response of weed species to soil water availability. The possibility of this platform (1) to automatically weight and water each pot individually with a high precision and several times a day, and (2) to quantify daily soil water availability in each individual pot was crucial. In most previous studies, the response of weed species to different water regimes was based on manual watering. For example, in some studies a constant volume of water was supplied daily (Monaco et al., 2005). Moreover, in these studies, water availability in each individual pot was not quantified. As a consequence, water treatments were most often characterized qualitatively (for example, referring to ‘full’ vs. ‘medium’ vs. ‘low’ water in Zhang and Wen (2009) or to the target proportion of water in pot in Chahal et al. (2018)). Yet, our study shows that a given water treatment may result in different amounts of soil water availability (described here by the fraction of transpirable soil water, FTSW) depending on plant species/stage or sampling date. Moreover, FTSW values may diverge from target proportion of water in pot. Finally, such platforms make it easier to water pots several times a day, allowing a more constant soil water availability than with manual watering (limiting pot watering to a maximum of once per day). Based on this innovative approach, our study provided new insights on how competition for water and light may interact in determining crop-weed dynamics in the field.

5.2. Water availability strongly affected light competition traits

All the studied morphological traits responded to water availability. For the best-documented morphological traits, results agreed well with the literature. Decrease of specific leaf area in response to water limitation is generally observed in annual plant species (Poorter et al., 2009) including weeds (Monaco et al., 2005). This classical physiological adaptation in plants allows them to decrease transpiring leaf area, thereby reducing plant water requirement under dry conditions (Poorter et al., 2009).

Similarly, biomass allocation to roots vs. aboveground plant parts frequently increases with water limitation, both in crop and in wild/weed species (Monaco et al., 2005; Acciaresi and Guiamet, 2010; Eziz et al., 2017; Chahal et al., 2018). This phenomenon refers to the ‘functional equilibrium’ or ‘optimal partitioning’ theories, considering that plants preferentially allocate biomass to the compartment that acquires the most limiting resource (Brouwer, 1962). A proportionally larger investment in root biomass in case of water limitation is often considered as a way for plants to increase soil exploration and access to water (McCarthy and Enquist, 2007).

In our study, the preferential biomass allocation to [leaf] vs. [stem and reproductive organs] in response to water limitation occurred for only *A. theophrasti* at the reproductive stage (not for the other species/stages). Previous studies identified that the leaf to aboveground biomass ratio either increased with or did not respond to water limitation (Lu et al., 2014; Chahal et al., 2018; de Oliveira et al., 2018). Further studies with a larger number of plant species are needed to test the importance of leaf to aboveground biomass ratio in the response of plant morphology to water stress.

For height to biomass ratio, the scarcity of references in the literature made the comparison difficult. As this trait is known to play a key role in competition for light, it is often analyzed in response to light availability (Leishman and Westoby, 1994; Pakeman et al., 2015; Colbach et al., 2020). We did not find any references quantifying its response to water limitation. The observed increase of height to biomass ratio in response to water limitation can simply be interpreted as the result a stronger impact of water limitation on aboveground biomass than on plant height, without necessarily conducting to a physiological/competitive advantage for plants.

Our study allowed ranking plant traits according to the magnitude of their response to water limitation. Increased plant height per unit of aboveground biomass and production of smaller/thicker leaves were the most noteworthy responses to water limitation. Not only these phenomena occurred for all the studied plant species/stages, but also the magnitude of the response of the corresponding morphological traits (height to biomass ratio and specific leaf area, respectively) to water were the strongest. Conversely, the response of root to total plant biomass ratio and leaf to aboveground biomass ratio to water limitation was significant for, respectively, only two and one out of the four plant species × stage combinations under study. Moreover, the order of magnitude of the response of these morphological traits was lower than for height to biomass ratio and specific leaf area. When focusing on the most documented traits in the literature, our ranking results differed from Monaco et al. (2005) showing that root to total plant biomass ratio was more responsive than specific leaf area. The focus of their study on another weed species (*Isatis tinctoria*), other water treatments (50 vs. 100 mL of water supply per day) and other environmental conditions (e.g. pot size, light intensity, photoperiod…) probably explains these discrepancies, as reported by Poorter et al. (2012a) and Poorter et al. (2012b).

5.3. Species and stage-specificities

Our study provides information regarding the interspecies variability of the morphological response to water limitation in a few weed species. To avoid confounding effects with plant stage and sampling date (and therefore duration of water limitation), the species effect could be analyzed only by comparing *A. myosuroides* and *A. theophrasti* at the vegetative stage. Only the response of specific leaf area SLA differed between species, with *A. theophrasti* maintaining high SLA values even when soil water availability was low, which was not the case for *A. myosuroides*.

The stage effect was analyzed by focusing on *A. theophrasti* (the only species studied at two different stages). For this species, the phenological stage affected three out of the four morphological traits. From the vegetative to the reproductive stage, specific leaf area became less sensitive to water limitation, while root to total plant biomass ratio and leaf to aboveground biomass ratio became sensitive to water limitation. Poorter et al. (2012c) hypothesized that this delayed change of biomass allocation in response to water limitation could be a mechanism of plant preservation. Indeed, they argued that changing biomass allocation too quickly might result in a suboptimal growth after restoration of the water supply, as water availability often depends on rain events that are unpredictable. Thus, in accordance with this hypothesis, our results suggest that changes in biomass allocation would occur in the long term. Short-term response to water limitation would primarily change plant height per unit of aboveground biomass and specific leaf area.

To be noted that correlations among these short-term responses were identified, with plants strongly increasing height per unit of aboveground biomass in response to water limitation being able to maintain high specific leaf area, even at low soil water availability. Studies on additional plant species would be necessary to confirm this interaction between morphological changes.

5.4. Disentangling the effects of nitrogen from those of water limitation

In our experiment, plants were watered with a nutrient-rich solution in order to limit a possible indirect effect of a nitrogen limitation that could be associated with a lower water supply. Nonetheless, a nitrogen limitation cannot be excluded in our study (Supplementary Material Section F online). A parallel study was conducted on *A. myosuroides* in order to analyse the morphological response to nitrogen limitation, focusing on the same morphological traits except root to total plant biomass ratio (Supplementary Material Section G online) (Perthame et al., 2022). Height to biomass ratio was unresponsive to soil-nitrogen limitation in Perthame et al. (2022), which contrasts with the strong response of this trait to water in the present study. Moreover, leaf to aboveground biomass ratio decreased in response to nitrogen limitation in Perthame et al. (2022), while this trait was unresponsive to water limitation in the present study. Specific leaf area was the only trait responding in the same direction (i.e. decrease) to both nitrogen and water limitation. However, the responsiveness was stronger to water than to nitrogen. Moreover, the two-phase response pattern observed in the present study for water was not identified when analysing the response to nitrogen. Thus, altogether these results strongly suggest that, even if a nitrogen limitation cannot be totally excluded in our study, the water effect was larger than a possible nitrogen effect. It is likely that the lower nitrogen supply in treatments with a strong water limitation was sufficient to fulfil the lower nitrogen requirements of water-stressed plants, thus limiting the putative confounding effects of water and nitrogen deprivations.

5.5. Practical implications

In temperate and non-irrigated field conditions, water availability may vary, depending on rainfall events. Given the strong influence of water on seed germination and emergence (Durr et al., 2015), these events play a key role in determining which weed species emerge and when in a given field during the cropping season. Situations of alternation among conditions of water limitation also result in diverse crop-weed competition situations: either for water mainly, or for light mainly under non-limiting water conditions (provided that nitrogen is not a limiting factor). In this context, the strong effect identified in the present study of water limitation on key morphological traits involved in competition for light, suggests that water limitation/competition could affect light competition in the field. Consequences on crop-weed communities could be expected.

Water limitation affects weed dynamics in the field (Souza et al., 2020). The present study provides insight regarding the underlying mechanisms, with plant morphology possibly playing a key role. Moreover, by showing species- and stage-specificities in the response of morphological traits to water availability, our study provides explanations on why the consequences of water limitation on crop-weed dynamics may depend on the starting date of the water limitation, its intensity and its length, as well as on the weed species (that may respond differently to water limitation). Our results also suggest that promoting weed ecological regulation by competition requires taking into consideration competition not only for light but also for water (Andrew et al., 2015), especially in areas with dry climates and/or subjected to the context of climate change (Iglesias et al., 2012). However, the results from our comparative ecology study are, as such, insufficient to identify management options that would be relevant to regulate weeds in situations of water limitation. First, we studied only three weed species. Second, interactions among traits should not be neglected. For example, plant species A with a high ability to increase height to biomass ratio could be assumed to have a competitive advantage over a neighboring plant species B with a lower ability. However, a better tolerance of photosynthesis (per unit leaf area) to water stress of species B could counterbalance the shading effect of species A. Third, many and diverse components interact in the agroecosystem (e.g. management techniques, pedoclimate, biophysical processes, diversity of the weed flora, long time-step resulting from the persistence of the weed seed bank in the soil).

Using a simulation model quantifying the effects of cropping systems on weed and crop dynamics is essential to cope with this complexity. In particular, process-based models are useful to synthesize existing knowledge, identify knowledge gaps, explore prospective scenarios in different contexts in the long term, and design new cropping systems (Colbach, 2010; Renton and Chauhan, 2017). Among the existing models simulating weed dynamics (Holst et al., 2007), FLORSYS is, to our knowledge, the only individual-based model quantifying the effects of cropping systems on the dynamics of multispecies weed floras, in interaction with pedoclimate (Colbach et al., 2014; Colbach et al., 2021). This model currently only considers crop-weed competition for light (Munier-Jolain et al., 2013) and nitrogen (Moreau et al., 2021), assuming that water resources are sufficient to sustain both crop and weed requirements. So, using the approach developed in the present study, the next step will consist of investigating the large diversity of response to water limitation among the numerous weed and crop species in arable fields. Such information will be useful to (1) introduce competition for water into FLORSYS, and (2) perform simulations with the new model version in order to identify innovative weed management strategies that are both sustainable (e.g. low reliance on herbicide use) and robust to climatic hazards (e.g. the weed management strategies are efficient even in case of climatic hazards).

6. Conclusions

Weed response to water availability was analyzed using an original approach (1) focusing on key species traits involved in competition for light that were derived from a mechanistic simulation model and (2) using an experimental platform allowing automatic and precise watering. Focusing on three weed species, this study allowed ranking traits according to their responsiveness to water limitation and characterizing species- and stage-specificities in responses. The strong effect of water limitation on key morphological traits involved in competition for light suggests that water competition could strongly affect light competition in the field, with consequences on crop-weed communities. In the near future, the methodological approach presented here will be applied to the high-throughput characterization of the large diversity of weed species. Knowledge gained from this approach will feed both comparative ecology approaches on weed species and mechanistic simulation models that will be used to better understand crop-weeds dynamics in different scenarios of water limitation.

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