Article

"Active" Weed Seed Bank: Soil Texture and Seed Weight as Key Factors of Burial-Depth Inhibition

Stefano Benvenuti * and Marco Mazzoncini

Department of Agriculture, Food and Environment, Via del Borghetto, 80, 56124 Pisa, Italy; marco.mazzoncini@unipi.it
* Correspondence: stefano.benvenuti@unipi.it

Abstract: The ability of weeds to survive over time is highly dependent on an ecological strategy that ensures a high level of viable seed remains in the soil. Seed bank persistence occurs because of the specific characteristics of seed dormancy and longevity and the hypoxic microenvironment, which surrounds the buried seeds. These experiments investigate the role of soil texture, burial depth, and seed weight in seed bank dynamics. Seeds of twelve weed species are sown at increasing depths in various soil textures, and emergence data are used to detect the burial depth at which 50% and 95% inhibition is induced, using appropriate regressions. Clay soil is found to increase the depth-mediated inhibition, while it is reduced by sandy particles. In each soil texture, the highest level of inhibition is found for the smallest seeds. Seed weight is found to be closely related to the maximum hypocotyl elongation measured in vitro, and consequently, the seedlings are unable to reach the soil surface beyond a certain depth threshold. However, the threshold of emergence depth is always lower than the potential hypocotyl elongation. The depth-mediated inhibition of buried seeds is even more pronounced in clay soil, highlighting that the small size of clay particles constitutes a greater obstacle during pre-emergence growth. Finally, the role of soil texture and weed seed size are discussed not only in terms of evaluating the layer of "active" seed bank (soil surface thickness capable of giving rise to germination and emergence), but also in terms of developing a consistent and persistent seed bank. Finally, the role of soil texture and weed seed size are discussed, and the layer of "active" seed bank (the soil surface thickness that enables germination and emergence) is assessed with the aim of developing a consistent and persistent seed bank. Assessing seed bank performance when buried under different soil textures can help increase the reliability of the forecast models of emergence dynamics, thus ensuring more rational and sustainable weed management.

Keywords: seedling emergence; weed management; seed dormancy; seed germination

1. Introduction

An important strategy for weeds is the ability to accumulate long-term viable seeds in the soil. The seed is the plant organ best equipped to tolerate high levels of agronomic disturbances. Most weed species are defined as ephemeral, short-lived plants [1], as their life cycles are mainly annual [2], thus implying a dynamic agroecosystem recolonization through the de-synchronized germination of buried weed seeds. Seed bank size may indicate the degree of invasiveness in a certain field—both in terms of weed density and floristic composition. Except for the potential entry of external weed seeds, due to anemochory [3], buried seeds exclusively constitute the "latent flora" that annually colonizes cultivated fields. The seed bank size usually ranges from a few thousand [4] to about 100,000 seeds per m^2 [5], as a function of crop rotation and cropping systems applied. Seed bank remains generally viable in the agroecosystem for a long time, due to the longevity [6] and dormancy [7] of the seeds. Dormancy prevents synchronized germination, and repeats weed seedling field emergence, despite the cyclical agronomic disturbances. In addition to the "primary dormancy" of the seeds during their ripening on the mother plant [8],

there is a "secondary" dormancy induced by the soil environment during burial [9]. While primary dormancy decreases or disappears a few weeks or months after seed ripening [10], secondary dormancy is induced and then cyclically removed throughout the seasons [11]. When either type of dormancy is reduced or disappears, it is not clear why most of the buried seeds do not germinate, despite favorable ecological conditions, such as optimum temperature and humidity. The lack of light in the microenvironment surrounding the buried seeds is not the main cause of the germination failure. The extreme light sensitivity acquired during burial, or the "very-low-fluence response," (VLFR) [12] usually is not sufficient to effectively trigger germination, as demonstrated by seeds experimentally exposed to light in the soil [13]. However, studies that propose the explanation of soil-mediated germination inhibition have shown that the soil matrix surrounding the seed acts as an obstacle, not only in terms of reduced oxygen availability, which typically inhibits germination [14], but also as a form of physiological inhibition, as the effectiveness of the toxic fermentative metabolites is reduced by hypoxia [15]. This soil-depth mediated inhibition mechanism [16] convincingly explains why the buried seeds are inhibited to the extent that is directly proportional to their degree of burial [17]. The evidence also suggests that sandy soils result in a lower degree of germination-inhibition as they are characterized by greater gas diffusion [18]. Clay soils, however, result in the highest degree, particularly if compacted following the demolition of the structure of colloidal soil particles [19]. In all soils, the degree of burial plays a crucial role in mediating the seed bank germination dynamics. These dynamics are, therefore, closely related to the previous level of soil tillage as the seed bank arrangement through the various soil layers depends on the soil inversion of the annually produced weed seeds, with the exception of a very low self-burial capacity [20]. In cropping systems characterized by reduced or even no-till, the seed bank will obviously accumulate in the shallowest soil horizons, and thus, the degree of depth-mediated soil will be minimized, leading to high emergence rates and crop interference [21].

The seed traits of buried seeds also make the predictability of the emergence dynamics more difficult. Heavier seeds have greater energy reserves in their endosperms, and thus, are able to germinate from greater depths. The heterotrophic growth during pre-emergence hypocotyl elongation exclusively depends on the seed energy reserves acquired from the mother plant during seed ripening [22]. The extremely complex factors involved make it very difficult to establish the real extent of the "active" seed bank, which is the width of the shallowest soil layer capable of giving rise to weed germination [23]. Such knowledge also determines the accuracy of forecast models of weed seedling emergence [24].

The aim of this study, carried out in 2018–2019, was to identify the relationships among the three main factors that determine the active seed bank layer, which are burial depth, soil texture, and seed weight.

2. Materials and Methods
2.1. Plant Material

Twelve weed species were selected for the experiments. The choice of the species was based on (i) their prevalence in common agroecosystems and (ii) the appreciable difference in their relative seed weight, which is a primary focus of the experiment. The study was set off in 2018. During the summer season, seeds of twelve weed species were collected from abundant plant populations in different agro-environments in Tuscany (autumn-winter cereals and spring-summer crops in the province of Pisa 43°42′ N, 10°24′ E) where they were very abundant. The species selected were: *Abutilon theophrasti Medicus*, *Amaranthus retroflexus L.*, *Datura stramonium* L., *Erigeron canadensis* L., *Euphorbia helioscopia* L., *Polygonum convolvulus* L., *Polygonum persicaria* L., *Rumex crispus* L., *Setaria viridis* (L.) Beauv., *Sinapis arvensis* L., *Solanum nigrum* L., *Sorghum halepense* (L.) Pers.

Seeds were collected from mother plants grown in normal weed-crop interference conditions, and at the time of seed harvesting, they had any symptoms of biotic or abiotic stress. The seeds were extracted from the respective fruits in the laboratory, then cleaned, dried (max 12% humidity), and kept in darkness in glass containers (50% of air relative

humidity) at 20 °C. The below explained laboratory and greenhouse tests were carried out in the Rottaia (PI) experimental center of the Department of Agriculture, Food and Environment of Pisa University.

2.2. Measurement of 1000 Seed Weight, Maximum Hypocotyl Elongation and Their Relationships

Seed weight was determined by the weight of 1000 seeds chosen randomly according to the ISTA rules for seed testing [25].

To evaluate the potential degree of seedling elongation, the seeds of each weed species were sandwiched between two rectangular transparent glasses (12 × 25 cm, held together by rubber bands) suitably equipped with moistened filter paper on one side only, to make it possible to inspect the hypocotyl elongation from the opposite side. Ten seeds (spaced about 1 cm apart) were placed at a distance of 5 cm from the shorter side, so they had enough space along the longest side to ensure hypocotyl elongation. Five packs of each species were vertically arranged in plastic basins (15 × 10 cm per side, 20 high), and kept humid by managing the water level at one-third of the height of the basins, as preliminary tests have shown that this ensures an optimal water capillary rise throughout the filter paper. The basins containing the packages were placed in dark conditions in a climatic cabinet set at a temperature of 25 °C. During seedling growth, hypocotyls elongation was measured every two days. These operations were conducted in the presence of a very dim green safelight with sufficiently low irradiance (less than 10-3 μmol m^{-2} s^{-1}) to avoid any photomorphogenetic alterations. The measurement was prolonged until the end of their elongation, which is characterized by evident tissue browning.

Finally, data of the 1000 seed weights for the 12 weed species were plotted with the corresponding maximum hypocotyl elongation using the equations that best fit these two parameters.

2.3. Soil Texture and Emergence Test

This test was carried out in March–April 2019. Fifty seeds of each of the twelve weed species were sown in plastic pots (15 × 15 cm sides, 25 cm in height), each filled with one of the three types of soils already used in a previous study carried out by the authors [19] characterized by diversified texture and referred to as sand (93, 5, and 2% of sand, lime, and clay, respectively), loam (65, 19, and 16% of sand, lime, and clay, respectively) or clay (30, 25, and 35% of sand, loam, and clay, respectively). The pots were filled gravimetrically with the respective soil texture and packed at a uniform strength to avoid differential resistance to seedling emergence. The selected sowing depths were: 0, 2, 4, 6, 8, 10, or 12 cm. The experiments were conducted using a fully randomized design with three replicates for each seeding depth in the three different soil textures. The pots were placed in a greenhouse. During this period (average max–min temperatures around 18–30 °C), the pots were moistened by sub-irrigation (about 0.3 L pot-1 day-1) through appropriate pot saucers, to keep the soil moisture at an optimal condition to trigger germination even on the soil surface (often suboptimal in field conditions). The emerged seedlings were counted daily at the point of cotyledon appearance and removed. The seedling counting was stopped 3 to 5 days (according to the germination speed of each species) after no further emergence was recorded.

2.4. Calculation of Soil Depth Capable of 50% and 95% Emergence Inhibition

The soil depth-inhibition data of each weed species (calculated as the percentage of soil surface germination) were fitted by the corresponding sigmoid Boltzmann function, which adequately describes the biological response of weed seed germination and emergence. These equations gave the soil depths at which emergence rates reached 50% by using a modified "x-intercept" method [26]. The intercept between the Boltzmann function and the translated *x*-axis on the selected *y* for 50% or 95% depth inhibition provides the relative soil depth inhibition for each weed species.

2.5. Evaluation of the Relationships between Soil Depth-Inhibition and Seed Weight or Soil Texture

The values of the soil depths that resulted in halving the seedling emergence of the twelve weed species were plotted for each soil texture, with the relative weight of 1000 seeds using the equations that best fit these two parameters. Similarly, the 50% inhibition data were related, by means of linear regressions, to the sand or clay content of the soils used for the emergence tests.

2.6. Statistical Analyses

The experimental design for the seedling emergence tests, consisted of a randomized block with three replications (12 weed species, 3 soil texture, 7 seeding depths, 3 replications). The same randomized block with five replications was adopted in the in vitro experiments to measure the maximum hypocotyl elongation. Following the homogeneity test of variance, an arcsine transformation of the percentage data (soil depth of 50% and 95% inhibition) was necessary. Angular values were subjected to analysis of variance (ANOVA) using the Student–Newman–Keuls test ($p < 0.05$ and $p < 0.01$) for mean separations (least-significant difference). Commercial software (CoStat, CoHort Software, Minneapolis, MN, USA) was used for each statistical analysis.

3. Results

The twelve selected weed species demonstrated a very wide range of weights for 1000 seeds (Table 1). This parameter ranged from 0.07 g for the very small seeds of Erigeron canadensis to 9.23 g for the larger Abutilon theophrasti seeds. These various seed weights were also diverse in terms of the degree of achieved maximum hypocotyl elongation, as measured through the in vitro experiments.

Table 1. Botanic family, 1000 seed weight and "in vitro" maximum hypocotyl elongation of the twelve tested species. Means are followed by the relative ± standard error.

Weed Species	Botanic Family	1000 Seed Weight (mg)	Maximum Hypocotyl Elongation (cm)
Abutilon theophrasti Medicus	Malvaceae	9.23 ± 0.12	14.34 ± 1.3
Amaranthus retroflexus L.	Amaranthaceae	0.48 ± 0.06	6.05 ± 1.0
Datura stramonium L.	Solanaceae	8.21 ± 0.11	13.45 ± 1.7
Erigeron canadensis L.	Asteraceae	0.07 ± 0.01	5.23 ± 1.0
Euphorbia helioscopia L.	Euphorbiaceae	4.02 ± 0.05	11.27 ± 1.1
Polygonum convolvulus L.	Polygonaceae	6.23 ± 0.12	14.03 ± 1.3
Polygonum persicaria L.	Polygonaceae	3.13 ± 0.09	11.95 ± 1.6
Rumex crispus L.	Polygonaceae	3.55 ± 0.02	12.48 ± 1.3
Setaria viridis (L.) Beauv.	Poaceae	2.41 ± 0.08	8.52 ± 1.4
Sinapis arvensis L.	Brassicaceae	1.82 ± 0.07	7.15 ± 1.2
Solanum nigrum L.	Solanaceae	0.84 ± 0.08	6.62 ± 1.1
Sorghum halepense (L.) Pers.	Poaceae	5.58 ± 0.14	12.55 ± 1.2
Average		3.57 ± 0.35	10.30 ± 1.14

The extreme values, lower and upper, of hypocotyl elongation (5.23 and 14.34 cm, respectively), were shown by the same already mentioned weed species characterized by the respective lower and upper 1000 seed weight. All the others fluctuated around the average of 10.30 cm.

A "one site" binding hyperbola (significant at $p < 0.01$) showed a relationship between these two parameters (Figure 1). In the first phase (up to values of around 4), the increase in the 1000-seed weight showed an almost linear relationship with the maximum epicotyl elongation attained, and after this value was reached, this relationship appeared to decrease in terms of the slope.
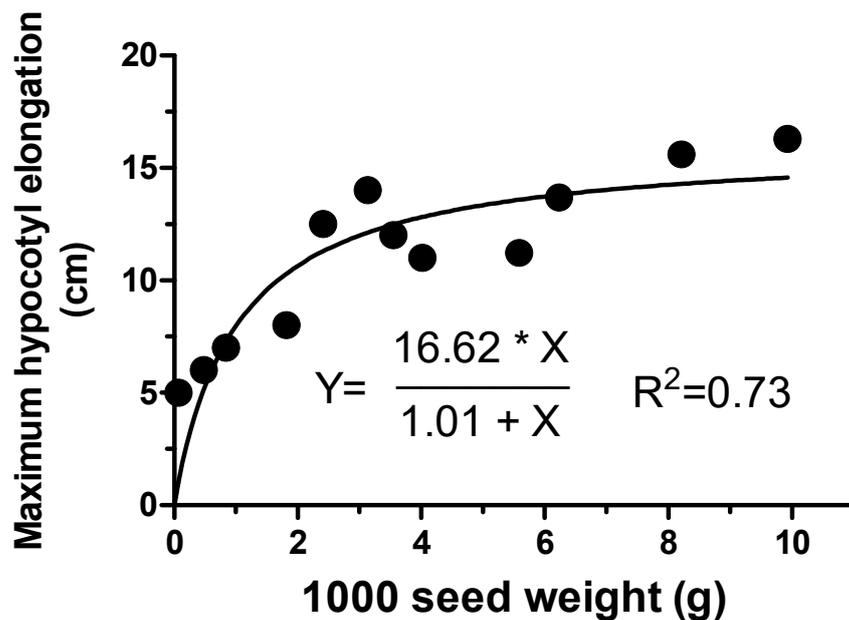
Figure 1. Maximum hypocotyl elongation in the twelve weed species as a function of the relative 1000 seed weight. The equations of "one site" binding hyperbola (significant for $p < 0.01$) and the corresponding R^2 values are reported.

To ascertain if and how seed weight and soil texture can imply a diversified emergence capacity in increasing soil depths, "in pot" tests were conducted. To reduce the number of graphs in this paper, we only illustrate the degree of soil-depth inhibition for a single exemplary species (Figure 2) and report the overall results of all twelve species in Table 2.

As expected, burial depth decreases the A. theophrasti seedling emergence in each soil type decreases the seedling emergence, (Figure 2), similar to all other species (data not shown). Soil texture was also found to influence the degree inhibition. The soil depth-mediated inhibition was found to be at the maximum in the clay soil, intermediate in the loam soil, and low in the sand soil. These inhibition levels were calculated for each of the three experimental soil textures: Sand (Figure 2A), loam (Figure 2B), and clay (Figure 2C). Boltzmann sigmoid functions (significant for $p > 0.01$) were found to fit the soil depth-mediated inhibition well for each soil texture. In the case of *A. thephrasti*, the degree of burial depth capable of halving the emergence was related to the texture of the soil. In the clay soil, 5.22 cm of seed burial is sufficient to halve the relative seedling emergence, while in the loam soil, this value rises to 6.05 cm, and increases further to 6.62 in the sand soil. Similarly, the burial depth capable of reducing the seedling emergence to 95% was found to increase from clay (7.35 cm), to loam (8.38 cm) and to sand soil (9.98 cm). The results regarding this weed species illustrate in detail the methodology used for the calculations in all twelve species, and the two important parameters used for further data processing were identified as the 50% or 95% inhibition resulting from burial depth (Table 2).

For each species, the depths that produced 50% or 95% seedling emergence inhibition showed statistically significant differences ($p < 0.05$) for the three soil textures (Table 2). These ranged from only 1.52 cm for the small seeds of E. canadensis to the aforementioned depth of A. theophrasti in sand soil. The emergence performance of each species also showed a close and significant ($p < 0.05$) relationship with the soil texture. The clay soil confirmed the inhibition activity. Most of the weed species could emerge to at least 5% (95% inhibition) from a depth of about 5 cm in sand soil and 7 cm in clay soil. The most burial-intolerant species was *E. canadensis*, as a 2.26 cm burial depth in clay soil was sufficient to inhibit 95% of its emergence. No species was able to attain the calculated 95% depth inhibition deeper than 10 cm in any of the soil textures. The species belonging to the botanical family of polygonaceae (*P. convolvulus*, *P. persicaria*, and *R. crispus*) showed a

good burial tolerance since the depth of 95% inhibition, in the most difficult conditions of clay soil, reached values of 6.88, 6.21, and 6.62 cm, respectively. Slightly lower values were shown by the two poaceae (*S. viridis* and *S. halepense*), which showed 95% inhibition (in clay soil) at depths of 6.01 and 6.02 cm, respectively. Different values were definitely shown of the two solanaceae (*D. stramonium* and *S. nigrum*), which showed, under the same above-mentioned soil texture, values of 6.62 and 5.82, respectively.
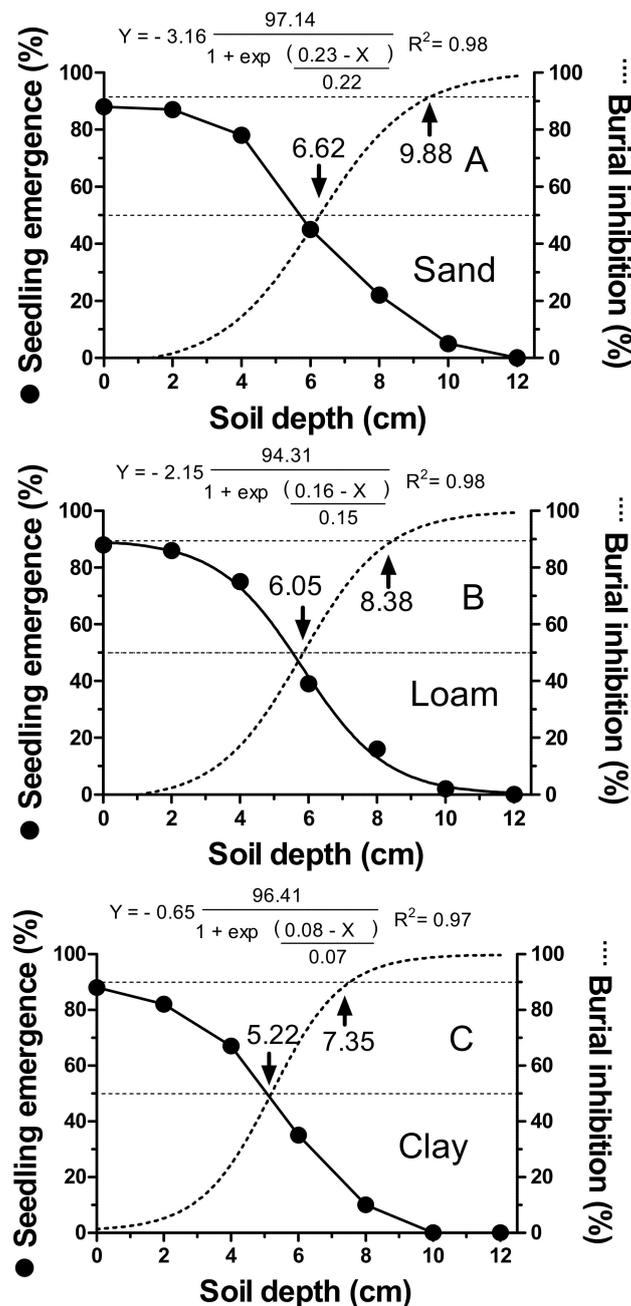

Figure 2. Seedling emergence and the relative degree of depth inhibition (as a percentage of soil surface germination) of *Abutilon theophrasti* as a function of increasing burial depth in the sand (**A**), loam (**B**), and clay (**C**) soil. The equations (Boltzmann sigmoid function, significant for *p* < 0.01) of the soil depth-mediated inhibition and the corresponding R^2 values are reported. Arrows indicate the burial depth of 50% (lower horizontal dotted line) and 95% inhibition (upper horizontal dotted line) evidenced by their intersection with the function of inhibition.

Table 2. Calculated depth of 50% and 95% inhibition (using the method that is shown in Figure 2) of the twelve studied weed species after sowing in the three different soil textures. Different letters within each species indicate significant differences for $p < 0.05$).

| Weed Species	Unburied Seed Germination (%)	Soil Texture	Depth of 50% Emergence Inhibition [1] (cm)	Depth of 95% Emergence Inhibition [1] (cm)
Abutilon theophrasti	86.2 ± 3.3	Sand	6.62 a	9.88 a
		Loam	6.05 b	8.28 b
		Clay	5.22 c	7.35 c
Amaranthus retroflexus	88.5 ± 2.2	Sand	5.66 a	6.28 a
		Loam	3.67 b	6.52 b
		Clay	3.73 c	5.94 c
Datura stramonium	65.6 ± 3.3	Sand	6.04 a	8.45 a
		Loam	6.35 b	8.12 b
		Clay	5.01 c	6.62 c
Erigeron canadensis	78.2 ± 3.1	Sand	2.44 a	4.24 a
		Loam	2.57 b	3.01 b
		Clay	1.52 c	2.26 c
Euphorbia helioscopia	73.4 ± 3.5	Sand	4.52 a	6.62 a
		Loam	4.14 b	6.73 b
		Clay	3.24 c	6.04 c
Polygonum convolvulus	65.8 ± 3.5	Sand	5.53 a	8.32 a
		Loam	5.85 b	7.92 b
		Clay	5.15 c	6.88 c
Polygonum persicaria	63.2 ± 2.8	Sand	4.18 a	6.55 a
		Loam	4.88 b	7.05 b
		Clay	3.28 c	6.21 c
Rumex crispus	82.6 ± 4.1	Sand	5.08 a	7.23 a
		Loam	4.42 b	6.95 b
		Clay	4.23 c	6.62 c
Setaria viridis	72.8 ± 3.5	Sand	5.22 a	7.02 a
		Loam	3.98 b	6.26 b
		Clay	3.85 c	6.01 c
Sinapis arvensis	85.2 ± 3.5	Sand	4.98 a	7.25 a
		Loam	4.56 b	6.73 b
		Clay	4.02 c	6.02 c
Solanum nigrum	70.2 ± 2.6	Sand	5.04 a	6.12 a
		Loam	4.16 b	6.56 b
		Clay	3.15 c	5.82 c
Sorghum halepense	73.4 ± 3.5	Sand	5.23 a	7.56 a
		Loam	4.55 b	7.03 b
		Clay	4.04 c	6.02 c

[1] to respect of unburied seed germination.

To identify the relationship between depth inhibition and soil texture, the data of the soil depth capable of halving seedling emergence were plotted with the sand or clay (Figure 3B) soil content of the two of the three soils characterized by extreme texture (markedly sandy or clayey). A linear regression (statistically significant at $p < 0.05$) shows that the sand content reduces the inhibition (Figure 3A). Conversely, the clay content increases the degree of soil depth-inhibition, as shown by a further linear regression (Figure 3B). The greater slope of this regression, compared to the previous instance, demonstrates the greater influence of clay particles in mediating inhibition than that of the sand.
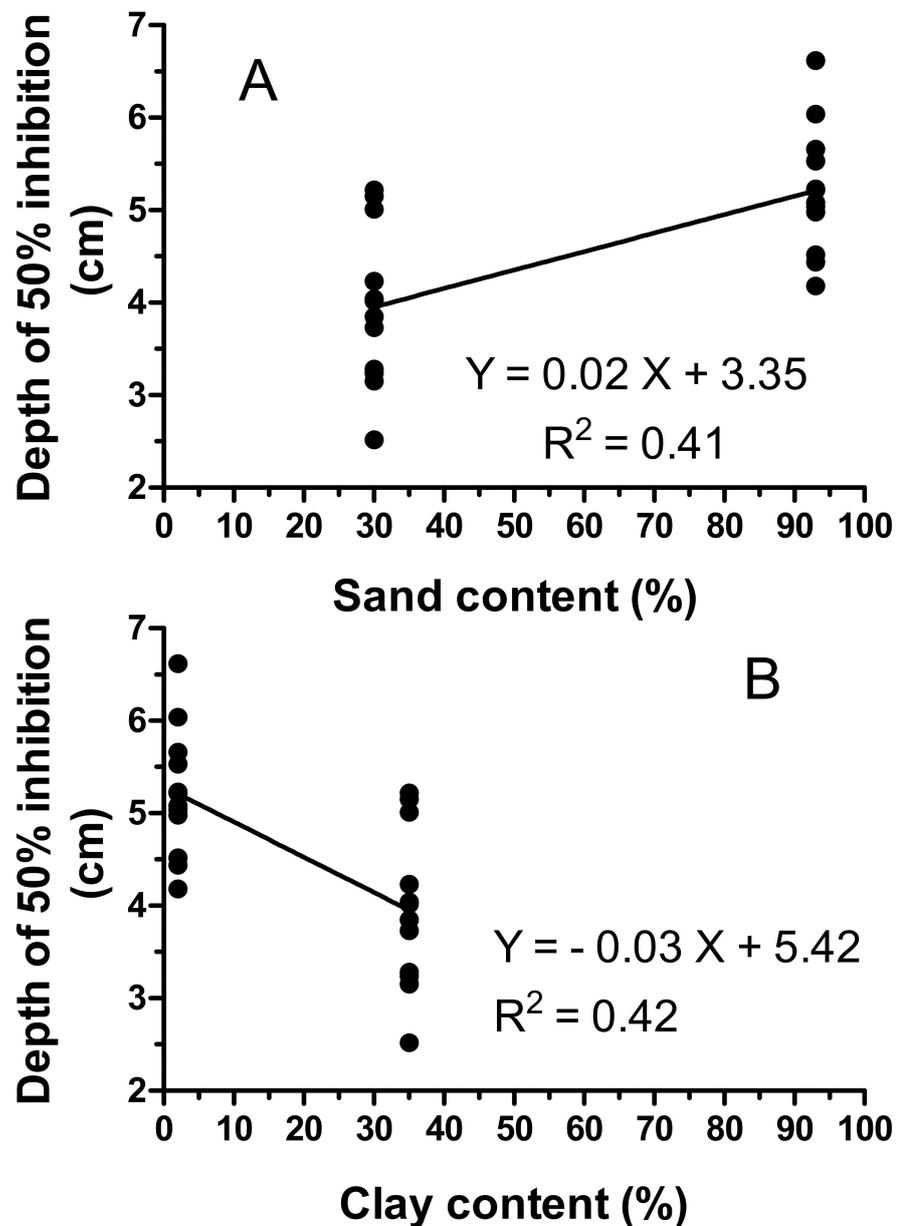
Figure 3. Linear regressions between the depth capable of halving the seedling emergence of the twelve weed species and sand (**A**) or clay (**B**) percentage of soil texture. The equation of the two linear regressions (statistically significant for *p* < 0.05) and the R^2 values are reported.

However, the degree of soil depth inhibition is not only mediated by the texture, but also by the weed seed traits in terms of seed weight. Linear regressions (statistically significant for *p* < 0.05) show that in all types of soil texture, the higher weight values of 1000 seeds reduce the depth-mediated inhibition (Figure 4). The main difference between these three linear regressions is not so much their slopes, which are similar, but rather their interceptions on the *y* axis: These are above a value of 4 in sand (Figure 4A), below this value in loam (Figure 4B), and about 3 in clay soil (Figure 4C). These linear regressions appear substantially transformed along the *y*-axis, highlighting the significant role of soil texture in addition to that of seed weight.
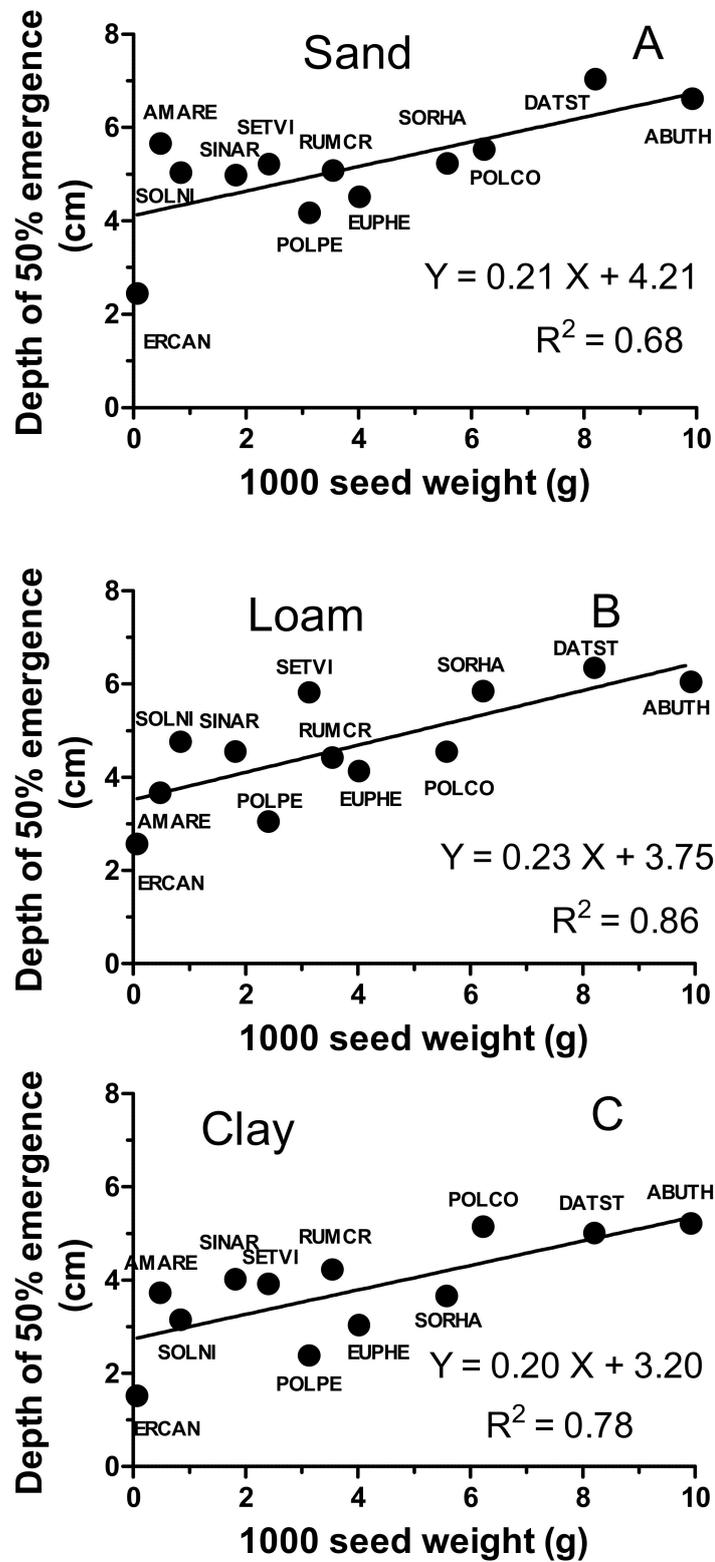
Figure 4. Linear regressions (statistically significant for *p* < 0.05) between the depth capable of halving the seedling emergence of the twelve weed species after sowing in different soil texture (sand (**A**), loam (**B**), and (**C**) clay) and 1000 weed seed weight (g). Each point shows the twelve weeds indicated with the respective Bayer code. The equation and the R^2 values are reported.

Finally, we examined whether and how the capacity of hypocotyl elongation is related to seedling emergence ability at increasing burial depths.

Linear regressions (statistically significant for $p < 0.05$) show (Figure 5) that the maximum epicotyl elongation is directly proportional to the maximum depth from which only 5% of the seedlings can reach the soil surface (the depth for 95% of inhibition). This relationship is also linked to the soil texture. While the sandy soil demonstrates most of the maximum degree of hypocotyl elongation during the pre-emergence growth, the clay soil limits such potential of hypocotyl elongation in all of the tested species. The inverse relationship between the inhibition of hypocotyl elongation and soil particle size was confirmed by the intermediate performances of loam soil, which is typically characterized by a balanced mix of small and larger particles.
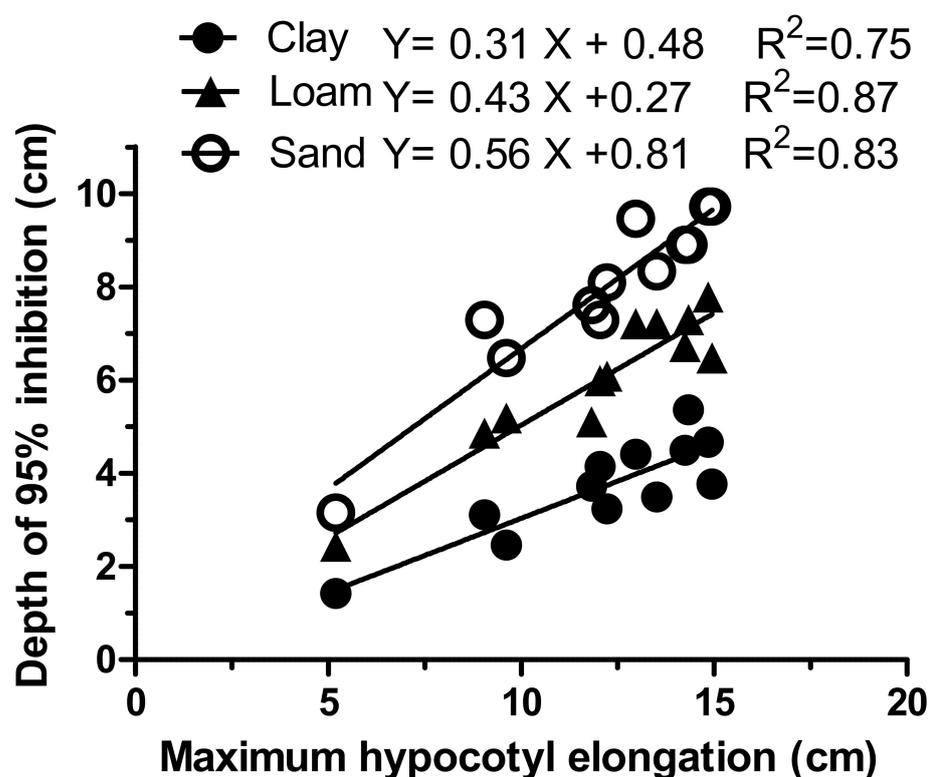
Figure 5. Linear regressions between the depth capable of giving rise to 95% of emergence inhibition of the twelve weed species in the three soil texture (pots experiments) and their maximum hypocotyl elongation measured in "in vitro" experiment. The equations (significant for $p < 0.05$) and the corresponding R^2 values are reported for each soil texture.

4. Discussion

Weed seed weight was extremely variable but generally low in the different species, especially in the case of *E. Canadensis*, *A. retroflexus*, *S. nigrum*, *S. arvensis*, and *S. viridis* (Table 1). However, weed ephemerality, which has evolved as a survival strategy in erratic environments, almost always implies a high seed number [27], as this is necessary for the cyclical field recolonization of the agroecosystem after the frequent agronomic disturbances. This high quantity implies a significant limitation in terms of their weight [28]. The seed weight evolved by each weed species appears to be a trade-off between their quantitative potentiality (light seeds enable the plants to be more prolific [29]) and their competitiveness in the early growth stages during seedling establishment, due to the higher energy reserves of heavier seeds [30]. In addition to the increased competitiveness after seedling emergence, larger seeds are also characterized by the greater potential for elongation during the heterotrophic phase of pre-emergence growth. As the weight of the 1000 seeds increases,

the degree of hypocotyl elongation increases. However, they are not correlated in a linear way, but according to the equation of "one site" binding hyperbola. When the 1000-seed weight is above about 5 g, the maximum hypocotyl elongation increases slightly. Why the maximum hypocotyl elongation does not increase beyond this weight, as it does in the lighter seeds, is not clear. This may be linked to the diversified nature of seed energy reserves as they are usually more concentrated as fatty acids in the smaller seeds and less concentrated as carbohydrates in the larger seeds. Supporting this hypothesis, an inverse relationship between weed seed weight and their oil content was found [31]. In practice, the smaller seeds need to concentrate energy substances, and this partially compensates for their potential heterotrophic hypocotyl elongation during the pre-emergence growth stage.

To verify if and how seed weight can mediate the emergence performance, the degree of inhibition, due to increased burial depth in diversified soil textures was calculated for each weed species.

An example of the calculation of the depth-mediated degree of inhibition is given for *A. theoprasti*, which illustrates for all species (data not shown) how the Boltzmann sigmoid function can be used to describe the seedling emergence following increasing seed burial depths. The translation of the x-axis on this function allowed us to identify, for each soil texture, the degrees of inhibition capable of reducing the emergence of the seedlings by 50% and 95%, indicating a marked influence of the soil texture on this species. This trend was then confirmed in all the other species (Table 2). Although each species demonstrated diverse levels of inhibition, most of the seed bank activity clearly occurs within 5–6 cm of the shallowest soil layer (Table 2). The shallowest 10 cm of soil was found to be virtually the only layer that allowed a transition from "potential" to "real" flora, at least in the tested species. These data fit well with previous studies that have found that the total field emergence dynamics, in both experimental [32] and field conditions [33], are derived from seeds buried within the top 10 cm of soil. These evaluations of "active seed banks" refer exclusively to experiments conducted on agroecosystems in Mediterranean grassland species, and almost all emergences derive from the shallowest first centimeter of the soil [34], due to their extremely low self-burial capacities in the natural environment [20]. The seed bank architecture of conventional agroecosystems, typically in the upper 30–40 cm [35], is almost exclusively derived from soil tillage conducted in the agroecosystem. Here, the soil tillage methods of the different cropping systems [36] play a crucial role in making the respective seed banks more (i.e., minimum tillage) or less (i.e., plowing) "active", due to their diverse soil arrangements. However, burial depth is not the only factor capable of affecting weed seed bank activity. The soil texture also significantly influences the germination and emergence of the buried seeds (Figure 3). While at the sand content increasing, the soil mediated depth-inhibition decreases, conversely at the clay content increasing, the inhibition decreases. Thus, prediction models of seed bank emergence dynamics should consider weed quantity and botanical composition [37] in addition to seed dormancy [38] and climatic data [39], and also soil texture, and the relative degree of compaction [19]. The involvement of soil texture in seed bank germination inhibition suggests that clay soils can accumulate a greater seed bank. Germination and emergence rates decrease when buried seeds are surrounded by very small soil particles, as these can reduce both the supply of oxygen and the removal of toxic fermentative metabolites elicited by hypoxia. In addition to suggesting a lower germination and emergence rate, clay soil appears to provide a more favorable environment for seed longevity, as its typically lower gaseous diffusion [40] can prevent the oxidative stress [41] from hypoxia, and consequently, limits the aging of buried seeds [42]. The seed bank "forced dormancy" in clay soils appears to be even more marked in weed species characterized by small seeds. Seed weight, in addition to the aforementioned pedological factors, plays a crucial role in "activating" a greater or lesser thickness of the shallowest soil layer (Figure 4). The burial depth inhibition increases with the decreasing of the 1000 seeds weight, in any soil texture. This confirms that, as observed in other weed species [17,43], larger seed size and weight can reduce the soil mediated depth-inhibition.

Although seed weight is not equivalent to energy content, as the energy content is also determined by the chemical nature of the endosperm (oils, starch, proteins, etc.), the relationship between them is clear. For example, the large seeds of *Avena fatua* are one of the few weed species that show seedling emergence up to about 20 cm [44].

Despite the non-coincidental relationship between seed weight and maximum hypocotyl elongation (see Table 1), elongation in each soil texture was found to be closely related to the soil depth threshold that could induce almost total seed bank "de-activation" (Figure 5). The fact that the depth-inhibition was found strongly related to the clay content, suggests that the colloidal matrix of the smallest soil particles requires additional effort, and therefore, higher energy consumption during pre-emergence seedling growth.

5. Conclusions

Weed seed weight and soil texture are highly influential in making the shallowest weed seed banks more or less "active." The upper 5–6 cm of the soil layer is primarily involved in germination and emergence, and for the tested weed species, the emergence rate is absent or negligible beyond 10 cm. Although the maximum hypocotyl elongation of common weeds frequently exceeds 10 cm, the soil particles hinder this theoretical potential, and the extent is inversely proportional to the particle size of the soil. Although in sand soil, the layer of the "active" seed bank is more extensive, this soil appeared to be less suitable for accumulating a consistent and long-lived seed bank. More seeds appear to accumulate more readily in clay soils, as they are induced to enter dormancy [19], and particularly almost spherical seeds [45], and these soils also increase their longevity, due to the hypoxic burial conditions resulting from lower gaseous diffusion. The false seedbed agronomic technique, which is a preventative weed management practice [46], becomes less effective, due to the thinner "active" seed bank in clay soils, particularly for small seeds, as they are more easily inhibited by burial in clay particles.

The relationships found between depth-mediated seed bank inhibition, soil texture, and seed weight should be confirmed in other agroecosystems, and may be important in increasing the precision of forecast models of weed emergence dynamics. Agronomic strategies based on sustainability are increasingly required, and thus, expanding our current knowledge through further experiments will enable more rational and environmentally friendly weed management.

References

1. Crawley, M.J. The population dynamics of plants. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **1990**, *330*, 125–140.
2. Gaba, S.; Perronne, R.; Fried, G.; Gardarin, A.; Bretagnolle, F.; Biju-Duval, L.; Colbach, N.; Cordeau, S.; Fernández-Aparicio, M.; Gauvrit, C.; et al. Response and effect traits of arable weeds in agro-ecosystems: A review of current knowledge. *Weed Res.* **2017**, *57*, 123–147. [CrossRef]
3. Benvenuti, S. Weed seed movement and dispersal strategies in the agricultural environment. *Weed Biol. Manag.* **2007**, *7*, 141–157. [CrossRef]
4. Sosnoskie, L.M.; Herms, C.P.; Cardina, J. Weed seedbank community composition in a 35-yr-old tillage and rotation experiment. *Weed Sci.* **2006**, *54*, 263–273. [CrossRef]
5. Benvenuti, S.; Pardossi, A. Weed seedbank dynamics in Mediterranean organic horticulture. *Sci. Hortic.* **2017**, *221*, 53–61. [CrossRef]
6. Burnside, O.C.; Wilson, R.G.; Weisberg, S.; Hubbard, K.G. Seed longevity of 41 weed species buried 17 years in eastern and western Nebraska. *Weed Sci.* **1996**, *44*, 74–86. [CrossRef]

7. Batlla, D.; Benech-Arnold, R.L. Predicting changes in dormancy level in natural seed soil banks. *Plant Mol. Biol.* **2010**, *73*, 3–13. [CrossRef]
8. Hilhorst, H.W. A critical update on seed dormancy. I. Primary dormancy. *Seed Sci. Res.* **1995**, *5*, 61–73. [CrossRef]
9. Baskin, J.M.; Baskin, C.C. A classification system for seed dormancy. *Seed Sci. Res.* **2004**, *14*, 1–16. [CrossRef]
10. Chahtane, H.; Kim, W.; Lopez-Molina, L. Primary seed dormancy: A temporally multilayered riddle waiting to be unlocked. *J. Exp. Bot.* **2017**, *68*, 857–869. [CrossRef]
11. Benech-Arnold, R.L.; Sánchez, R.A.; Forcella, F.; Kruk, B.C.; Ghersa, C.M. Environmental control of dormancy in weed seed banks in soil. *Field Crop. Res.* **2000**, *67*, 105–122. [CrossRef]
12. Batlla, D.; Benech-Arnold, R.L. Weed seed germination and the light environment: Implications for weed management. *Weed Biol. Manag.* **2014**, *14*, 77–87. [CrossRef]
13. Scopel, A.L.; Ballaré, C.L.; Sánchez, R.A. Induction of extreme light sensitivity in buried weed seeds and its role in the perception of soil cultivations. *Plant Cell Environ.* **1991**, *14*, 501–508. [CrossRef]
14. Boyd, N.; Van Acker, R. Seed germination of common weed species as affected by oxygen concentration, light, and osmotic potential. *Weed Sci.* **2004**, *52*, 589–596. [CrossRef]
15. Holm, R.E. Volatile metabolites controlling germination in buried weed seeds. *Plant Physiol.* **1972**, *50*, 293–297. [CrossRef]
16. Benvenuti, S.; Macchia, M. Effect of hypoxia on buried weed seed germination. *Weed Res.* **1995**, *35*, 343–351. [CrossRef]
17. Benvenuti, S.; Macchia, M.; Miele, S. Quantitative analysis of emergence of seedlings from buried weed seeds with increasing soil depth. *Weed Sci.* **2001**, *49*, 528–535. [CrossRef]
18. Benvenuti, S. Soil texture involvement in germination and emergence of buried weed seeds. *Agron. J.* **2003**, *95*, 191–198. [CrossRef]
19. Benvenuti, S.; Mazzoncini, M. Soil physics involvement in the germination ecology of buried weed seeds. *Plants* **2019**, *8*, 7. [CrossRef]
20. Benvenuti, S. Natural weed seed burial: Effect of soil texture, rain and seed characteristics. *Seed Sci. Res.* **2007**, *17*, 211–219. [CrossRef]
21. Santín-Montanyá, M.I.; Martín-Lammerding, D.; Zambrana, E.; Tenorio, J.L. Management of weed emergence and weed seed bank in response to different tillage, cropping systems and selected soil properties. *Soil Tillage Res.* **2016**, *161*, 38–46. [CrossRef]
22. Gardarin, A.; Coste, F.; Wagner, M.H.; Dürr, C. How do seed and seedling traits influence germination and emergence parameters in crop species? A comparative analysis. *Seed Sci. Res.* **2016**, *26*, 317–331. [CrossRef]
23. Dekker, J. Soil weed seed banks and weed management. *J. Crop Prod.* **1999**, *2*, 139–166. [CrossRef]
24. Masin, R.; Loddo, D.; Benvenuti, S.; Otto, S.; Zanin, G. Modeling weed emergence in Italian maize fields. *Weed Sci.* **2012**, *60*, 254–259. [CrossRef]
25. ISTA. International rules for seed testing. *Seed Sci. Technol.* **1999**, *27*, 50–52.
26. Wiese, A.M.; Binning, L.K. Calculating the threshold temperature of development for weeds. *Weed Sci.* **1987**, *32*, 177–179. [CrossRef]
27. Norris, R.F. Weed fecundity: Current status and future needs. *Crop Prot.* **2007**, *26*, 182–188. [CrossRef]
28. Rees, M. Evolutionary ecology of seed dormancy and seed size. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **1996**, *351*, 1299–1308.
29. Thompson, B.K.; Weiner, J.; Warwick, S.I. Size-dependent reproductive output in agricultural weeds. *Can. J. Bot.* **1991**, *69*, 442–446. [CrossRef]
30. Geritz, S.A.; Van der Meijden, E.; Metz, J.A. Evolutionary dynamics of seed size and seedling competitive ability. *Theor. Popul. Biol.* **1999**, *55*, 324–343. [CrossRef]
31. Bretagnolle, F.; Matejicek, A.; Grégoire, S.; Reboud, X.; Gaba, S. Determination of fatty acids content, global antioxidant activity and energy value of weed seeds from agricultural fields in France. *Weed Res.* **2016**, *56*, 78–95. [CrossRef]
32. Grundy, A.C.; Mead, A.; Bond, W. Modelling the effect of weed-seed distribution in the soil profile on seedling emergence. *Weed Res.* **1996**, *36*, 375–384. [CrossRef]
33. Forcella, F. Prediction of weed seedling densities from buried seed reserves. *Weed Res.* **1992**, *32*, 29–38. [CrossRef]
34. Traba, J.; Azcárate, F.M.; Peco, B. From what depth do seeds emerge? A soil seed bank experiment with Mediterranean grassland species. *Seed Sci. Res.* **2004**, *14*, 297–303. [CrossRef]
35. Colbach, N.; Roger-Estrade, J.; Chauvel, B.; Caneill, J. Modelling vertical and lateral seed bank movements during mouldboard ploughing. *Eur. J. Agron.* **2000**, *13*, 111–124. [CrossRef]
36. Swanton, C.J.; Shrestha, A.; Knezevic, S.Z.; Roy, R.C.; Ball-Coelho, B.R. Influence of tillage type on vertical weed seedbank distribution in a sandy soil. *Can. J. Plant Sci.* **2000**, *80*, 455–457. [CrossRef]
37. Rahman, A.; James, T.K.; Grbavac, N.I.K. Correlation between the soil seed bank and weed populations in maize fields. *Weed Biol. Manag.* **2006**, *6*, 228–234. [CrossRef]
38. Batlla, D.; Benech-Arnold, R.L. Predicting changes in dormancy level in weed seed soil banks: Implications for weed management. *Crop Prot.* **2007**, *26*, 189–197. [CrossRef]
39. Werle, R.; Sandell, L.D.; Buhler, D.D.; Hartzler, R.G.; Lindquist, J.L. Predicting emergence of 23 summer annual weed species. *Weed Sci.* **2014**, *62*, 267–279. [CrossRef]
40. Moldrup, P.; Olesen, T.; Yoshikawa, S.; Komatsu, T.; Rolston, D.E. Three-porosity model for predicting the gas diffusion coefficient in undisturbed soil. *Soil Sci. Soc. Am. J.* **2004**, *68*, 750–759. [CrossRef]

41. Wiebach, J.; Nagel, M.; Börner, A.; Altmann, T.; Riewe, D. Age-dependent loss of seed viability is associated with increased lipid oxidation and hydrolysis. *Plant Cell Environ.* **2020**, *43*, 303–314. [CrossRef] [PubMed]

42. Bailly, C. Active oxygen species and antioxidants in seed biology. *Seed Sci. Res.* **2004**, *14*, 93–107. [CrossRef]

43. Grundy, A.C.; Mead, A.; Burston, S. Modelling the emergence response of weed seeds to burial depth: Interactions with seed density, weight and shape. *J. Appl. Ecol.* **2003**, *40*, 757–770. [CrossRef]

44. Gardarin, A.; Dürr, C.; Colbach, N. Effects of seed depth and soil aggregates on the emergence of weeds with contrasting seed traits. *Weed Res.* **2010**, *50*, 91–101. [CrossRef]

45. Gardarin, A.; Colbach, N. How much of seed dormancy in weeds can be related to seed traits? *Weed Res.* **2015**, *55*, 14–25. [CrossRef]

46. Travlos, I.; Gazoulis, I.; Kanatas, P.; Tsekoura, A.; Zannopoulos, S.; Papastylianou, P. Key factors affecting weed seeds' germination, weed emergence and their possible role for the efficacy of false seedbed technique as weed management practice. *Front. Agron.* **2020**, *2*, 1. [CrossRef]