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After-Ripening and Stratification Improve the Germination of the *Cakile maritima* Scop. (Brassicaceae) from the Apulia Region, Italy

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Abstract: Understanding seed germination is crucial for refining the propagation techniques of *Cakile maritima*, a wild halophyte species with significant potential for biosaline agriculture. However, research on seed germination within intact fruits of this species is limited. Four trials were conducted to study the seed germination of a population from the Apulia region. The focus was on seeds that had undergone after-ripening for 3 years ($20AR_3$) or 2 years ($20AR_2$) (both collected in 2020), or 1 year ($22AR_1$) (collected in 2022), and freshly harvested seeds in 2022 ($22AR_0$) and 2023 ($23AR_0$). The seeds were either incubated as naked or moist-stratified within intact fruits. A portion of 2022 AR_0 siliques was submerged in saline water before stratification. The naked seeds collected in 2022 and 2020 ($22AR_0$ and $20AR_2$) did not germinate, whereas a portion of the $23AR_0$ (67%), $20AR_3$, and $22AR_1$ (45%, irrespective of after-ripening) lots quickly (T_{50} = 3.5 days) germinated, underlining a lower dormancy level for seeds harvested or dry stored in 2023. Seed germination in the intact fruits was lower than the naked seeds, confirming the role of the pericarp in inducing seed dormancy. Stratification of the shelled seeds was much more effective in improving the germination time (140 days) and levels in the $23AR_0$ (81%), $20AR_3$, and $22AR_1$ (66%, irrespective of after-ripening) lots than in the $22AR_0$ (34%) and $20AR_2$ (61%) ones, which required 240 days to germinate. The saline solution imbibition of fruit seems only to delay the occurrence of the maximum emergence. The physiological seed dormancy of this *C. maritima* population has been proven, which may be variable in depth according to the year of fruit collection, ranging from intermediate to non-deep.

Keywords: sea rocket; physiological dormancy; intact fruits; shelled seeds; seedling emergence; Weibull's function; T_{50}; Brassicaceae; after-ripened; moist-stratified

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1. Introduction

Sea rocket (*Cakile maritima* subsp. *maritima* Scop.) is an annual succulent (facultative) halophyte that belongs to the Brassicaceae family. It is commonly found along the sandy coasts of the Atlantic and Mediterranean regions, especially on strandlines and the associated foredunes with plants experiencing salt spray and occasional seawater inundation. This plant has branched, prostrate, or ascending stems, giving it a bush-like appearance. The inflorescences are dense, with many flowered racemes terminating the main stem and branches with lilac-colored or purple petals. The indehiscent distinctive fruits consist of two-jointed siliquas, which are green and fleshy when unripe before maturing to brown, tough, and corky. The upper part of the fruit is deciduous and has adaptive features, such as a floating ability that enable this species to disperse over long distances by sea currents. Conversely, the lower part remains attached to the parent plant, facilitating in situ dispersal of the plant. Typically, each segment contains one seed [1].

C. maritima is a highly valuable species due to its wide geographical and ecological range as well as its potential economic importance [2,3]. The tender leaves, green stems, flowers, and fruits of *C. maritima* may not only be significant for human food consumption but may also have applications in medicine due to their ability to produce numerous bioactive compounds. These secondary metabolites have demonstrated potent biological activities, such as antioxidant, antimicrobial, and anti-inflammatory properties, which may offer benefits for various human diseases [4,5]. These properties indicate that this species could be a valuable source for developing new nutritional and functional products, addressing the increasing demand for healthy foods and supplements [4,6]. Additionally, *C. maritima* is recognized as a promising oilseed crop, as its seeds contain 30–40% oil, and it is also considered an energy crop and useful for phytoremediation purposes. This renders the cultivation of this plant on salted marginal soil of practical significance in the context of the necessary development of biosaline agriculture [2,3] likewise with other halophytes [7].

One of the main challenges in domesticating and cultivating wild species is the propagation of these plants [7,8]. The main issue behind seed production is the low and erratic germination often observed in many wild species usually linked to seed dormancy [9,10].

Seed primary dormancy is an inherent suppression of early germination during periods that are only ephemerally favorable, and therefore it increases seedling survival. This phenomenon is due to the morphological, physiological, or morpho-physiological aspects in freshly harvested, mature, and water-permeable seeds. Dormancy can be overcome by after-ripening conditions (dry storage), warm or cold moist stratification, or gibberellic acid (GA) treatment [11]. In some species of Brassicaceae, the dispersal unit is a dry indehiscent fruit with the pericarp preventing or delaying germination and resulting in staggered emergence of seedlings [12]. Depending on the species, a period of dry storage (after-ripening, AR), cold stratification, light, or darkness may increase the growth potential of the embryo enough for the radicle to push through the fruit wall and thus for the seeds to germinate [13–15].

Currently, there are limited studies available on the germination of *C. maritima* seeds in intact fruits. The seeds within the indehiscent fruit of British and French *C. maritima* populations are reported to be dormant due to the protective outer layer (testa) and the mechanical properties of the pericarp. This dormancy can be overcome by extended cold stratification and/or treatment with GA_3 [16]. A study on shelled and unshelled seeds from an Italian (Tuscany) population of *C. maritima* found that the extracted seeds had a much higher germination percentage (85%) compared to the shelled seeds (16%) [17]. On the other hand, numerous studies have been conducted on the germination performance of seeds without a pericarp to assess the effect of salinity. Under non-saline conditions, high germination percentages (75–100%) were found, indicating the role of the pericarp in the dormancy of *C. maritima* [18–22].

Since seed extraction is a time-consuming process, studying seed germination in intact fruits is crucial to improving propagation techniques for *C. maritima*, making it accessible and attractive for growers.

A recent study on the ecophysiological and nutritional traits of the *C. maritima* population from the Apulia region (Italy) has explored its potential for cultivation as a new vegetable crop in marginal areas and intensive cropping systems [1]. Therefore, as a domestication process, the present research focused on the germination performance of this population. With this aim, four trials were conducted on intact fruits and extracted seeds to assess the impact of dormancy release treatments, such as after-ripening and stratification.

2. Materials and Methods

2.1. Fruit Collection Sites and Climatic Features

Fruits of *C. maritima* were collected from the following two sites: one situated between the Saltworks of Margherita di Savoia (Special Protected Area) and the Adriatic Sea, near the village Margherita di Savoia (BT) (latitude, 41.4° N; longitude, 16.0° E; altitude, 0 m a.s.l.) (MS), and the other situated on the coast of Lagoon of Lesina (Lesina, FG) (latitude, 41.9° N; longitude, 15.3° E; altitude, 0 m a.s.l.) (LL), both north of the Apulia region (Italy). Long-term (last 30 years) monthly temperatures and rainfall (Table S1) show that both sites have a typically Mediterranean climate, characterized by hot and dry summers and mild and rainy winters.

Mature fruits (both upper and lower segments) were collected in September 2020, 2022, and 2023 in MS, and 2023 in LL, from approximately 40 plants within the same retro-dunal zone (5 m × 5 m). This area was shielded from sea tides, ensuring no saline conditions were present [1]. The weekly mean air temperatures and rainfall during the plant growth and flowering-fruit ripening period (May–September) in 2020, 2022 (MS site), and 2023 (MS and LL sites) are reported in Figure 1A,B.

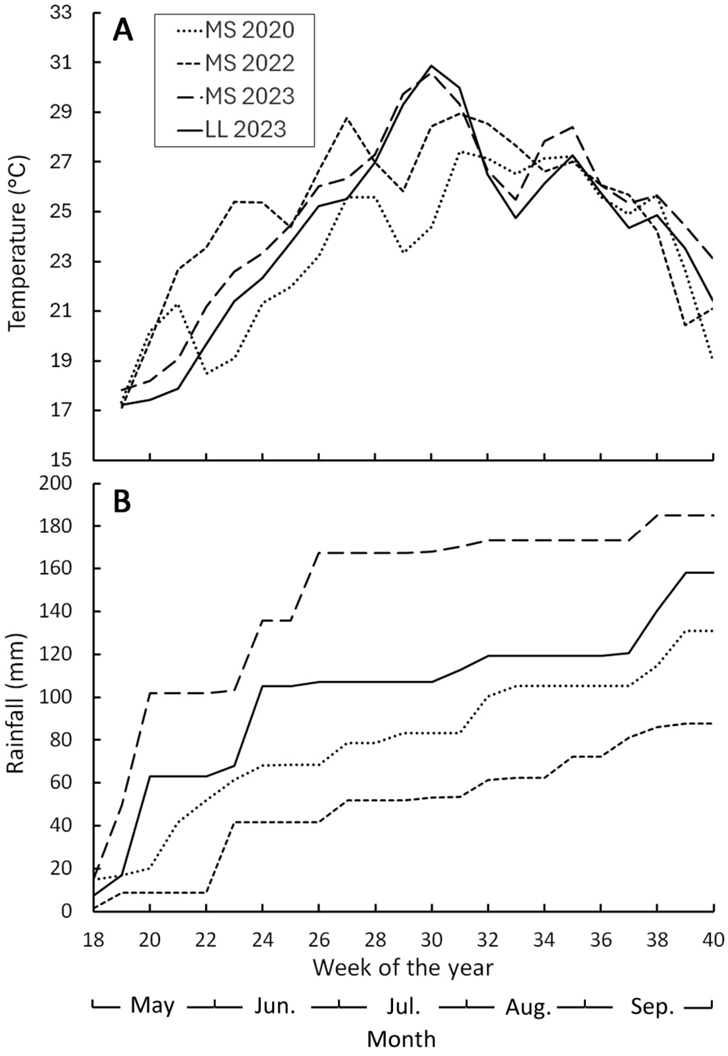

Figure 1. Weekly mean temperature (moving average) (**A**) and cumulative rainfall (**B**) during the 22 weeks (May–September) preceding fruit collection at the Margherita di Savoia (MS) in 2020, 2022, and 2023 and Lagoon of Lesina (LL) in 2023.

The collected material was treated with a 2% sodium hypochlorite solution for 10 min, then washed under running tap water, air-dried, and stored in paper bags at room temperature and humidity until the experiments started. In trials 1, 2, 3, and 4, the MS materials were used, whereas in trials 2 and 4, we also used the fruits and seeds of *C. maritima* from Lagoon of Lesina (LL). A large bulk of upper and lower segments was obtained to provide the materials for the experiments.

A summary of the trial materials is provided in Table 1. All the germination trials were conducted in the Vegetable Crops Lab of the Department of Agriculture, Food, Natural Resources, and Engineering (DAFNE) of the University of Foggia.

Table 1. *Cakile maritima* seed lots used in the four germination trials: collection details and lot identification codes.

		Trial 1	**Trial 2**	**Trial 3**	**Trial 4**
		Year of the Germination Trial			
		2022	**2023**	**2022**	**2023**
Collecting site	Collecting year	Lot code (*)			
MS	2020	20S_AR$_2$	20S_AR$_3$	20F_AR$_2$	20F_AR$_3$
MS	2022	22S_AR$_0$	22S_AR$_1$	22F_AR$_0$	22F_AR$_1$
MS	2022			22F_AR$_0$S	
MS	2020	20F_AR$_2$	-	-	-
MS	2022	22F_AR$_0$	-	-	-
MS	2023	-	23S_AR$_0$	-	23F_AR$_0$
LL	2023	-	LL-23S_AR$_0$	-	LL-23F_AR$_0$

(*) In the coding system, the first two characters indicate the year of collection (e.g., 20 = 2020, 22 = 2022, 23 = 2023). The third character represents the type of propagation material used (S = naked seed, F = seed within an intact fruit segment). The fourth to seventh characters specify the after-ripening years: _AR$_0$, _AR$_1$, _AR$_2$, and _AR$_3$ denote seeds or siliques after-ripened for zero (freshly harvested), one, two, or three years, respectively. Specifically, _22F_AR$_0$S refers to 2022 freshly harvested fruits submerged in saline water (EC 36.3 mS cm^{-1}) for 72 h before being subjected to stratification. Margherita di Savoia (MS) was consistently used as a collection site across all sampling years, so the characters 'MS' were omitted from the code, while in 2023, the Lagoon of Lesina was also included and thus, for this instance, the characters 'LL-' were prefixed to the code.

2.2. Germination of the Extracted Seeds of C. maritima

Trial 1

This trial started on 24 September 2022, using seeds manually extracted from the following: (a) freshly harvested, not after-ripened fruits (22S_AR$_0$) and (b) dry-stored fruits collected in 2020 which had experienced a two-year after-ripening (20S_AR$_2$).

The germination test was conducted in 9 cm diameter Petri dishes by first placing 25 seeds on moist Whatman No. 1 filter paper (experimental unit). The seeds were previously treated with a 2% sodium hypochlorite solution for 10 min, then washed under running tap water. The Petri dishes were arranged randomly in a growth chamber at 22 °C ± 2 °C (within the optimal range for the species [22]) in the dark. Each treatment had four replicates. Once germination began, the germinated seeds (young radicles over 3 mm in length) were counted and removed from each Petri dish every day for 30 days (the incubation period). The final germination percentage was calculated as the number of germinated seeds/25 × 100. This trial was also performed using intact fruits.

Trial 2

This trial started on 6 November 2023, using seeds manually extracted from the following: (a) freshly harvested fruits both in MS (23S_AR$_0$) and LL (LL-23S_AR$_0$) sites and (b) dry-stored fruits that had experienced three years of after-ripening (20S_AR$_3$) (collected in 2020) or one year of after-ripening (22S_AR$_1$) (collected in 2022). The germination test was conducted as described in trial 1.

2.3. Seedling Emergence of the Stratified Fruits of C. maritima

Trial 3

The trial began on 26 September 2022. Intact fruit segments not after-ripened (22F_AR$_0$) (200) and dry-stored for a two-year after-ripening (20F_AR$_2$) (200) were stratified in flat aluminum containers (6 cm × 10 cm × 20 cm) filled with a 5 cm layer of washed sand collected at the MS site and saturated with distilled water. The containers (four for each treatment) were placed in plastic bags to prevent the substrate from drying out and stored in a growth chamber at 18/20 °C in the dark for 65 days. After that, they were kept, from 21 December 2022 to 15 May 2023, in the open air at ambient air temperature and under a light cover for rain protection. Additionally, to verify the salinity effect, 200 siliques of the 22F_AR$_0$ lot were submerged in saline water (EC 36.3 mS cm^{-1}) for 72 h (22F_AR$_0$S), mimicking sea water, and washed under running water before being subjected to stratification. The emergence of seedlings was monitored until it remained unchanged for at least 10 consecutive days.

Trial 4

The trial began on 13 November 2023, using intact fruits both freshly harvested in (a) MS (23F_AR$_0$) or (b) LL (LL-23F_AR$_0$), as well as dry-stored that had undergone (c) a three-year after-ripening (harvested in 2020) (20F_AR$_3$) or (d) one-year after-ripening (harvested in 2022) (22F_AR$_1$). These materials were stratified according to the trial 3 specifications. Containers were kept at ambient air temperature with protection from the rain throughout the trial period (13 November 2023–4 April 2024).

At the end of trials 3 and 4, ungerminated seeds were extracted from siliques and were classified as imbibed or dead by the cut test.

Monthly average maximum and minimum temperatures during the open-air phase of trials 3 and 4 are reported in Table S2.

2.4. Statistical Analysis

The curves of the cumulative proportion of germinated seeds/emerged seedlings for each treatment were constructed using Weibull's function with three parameters (Conversa and Elia, 2009) [23] as follows:

$$Y = a\left[1 - 100^{-(X/q)^{b}}\right]$$

where Y is the cumulative proportion of germinated seeds at the time X, a is the maximum germination that may be reached, b is a shape parameter, and q is the time required to achieve 99% of a from the beginning of the germination period. The three parameters of Weibull's function for each treatment were estimated through PROC NLIN (SAS Institute, 1999) [24]. Data were subjected to ANOVA, and mean separation was carried out using LSD 0.05 test. Time to 50% cumulative germination (T_{50}) was calculated using the following formula derived from Weibull's function as follows:

$$T_{50} = \left\{log_{10}\left[\frac{-a/(Y-a)}{2}\right]\right\}^{(1/b)} q$$

The values expressed as percentages were transformed by arcsin $\sqrt{x}$ before data analysis.

3. Results

3.1. Germination of the Extracted Seed of *C. maritima*

Trial 1 was conducted in autumn 2022, focusing on the shelled and unshelled seeds collected from the Margherita di Savoia (MS) site. These seeds were harvested in 2020, and after-ripened for 2 years (20S_AR$_2$), as well as in 2022 (freshly harvested, 22S_AR$_0$) (Table 1).

However, in this trial, no germination was observed despite all the seeds being viable at the end of the incubation period. Trial 2, performed in autumn 2023, involved the extracted seeds of MS fruits harvested in 2020 (20S_AR$_3$), 2022 (22S_AR$_1$), and 2023 (23S_AR$_0$) (after-ripened for 3 years, 1 year, and not after-ripened, respectively). In 2023, the *C. maritima* fruits harvested in the Lagoon of Lesina (LL) site (LL-23S_AR$_0$) were also included. The germination percentage was higher in the freshly harvested seeds (23S_AR$_0$ and LL-23S_AR$_0$) compared to the after-ripened seeds for 3 years (20S_AR$_3$) or 1 year (22S_AR$_1$) with a lower T_{50} in the AR$_0$ lots (Table 2).

Table 2. Cumulative germination, T_{50}, and Weibull's function parameters of *Cakile maritima* after-ripened or freshly harvested seeds (Trial 2, Autumn 2023).

Seed Lot [1]	Germination	T_{50}	Weibull's Function Parameters		
	(%)	(Days)	*a*	*b*	*q*
20S_AR$_3$	41.0 b [2]	4.0 a	41.3 d	1.7 a	12.2 a
22S_AR$_1$	50.0 b	4.3 a	50.3 bc	1.9 a	12.1 a
23S_AR$_0$	64.0 a	3.0 b	63.1 ab	4.0 a	6.0 b
LL-23S_AR$_0$	66.9 a	2.9 b	66.1 a	2.5 a	7.4 b
Significance [3]	**	*	*	ns	**

[1] See Table 1 for seed lot identification. [2] Means ($n = 4$) in columns not sharing the same letters are significantly different according to the LSD test ($a = 0.05$). [3] ns, * and ** not significant or significant at $p \leq 0.05$, $p \leq 0.01$, respectively.

The germination pattern showed a lower *q* parameter (the time required to achieve 99% of *a*) of Weibull's function in 23S_AR$_0$ and LL-23S_AR$_0$ compared to the after-ripened seeds (20S_AR$_3$ and 22S_AR$_1$), irrespective of the after-ripening duration (3 or 1 year). Parameter *a*, representing the maximum germination that may be reached, substantially aligns with final germination values (Table 2; Figure 2). All the seeds were imbibed at the end of the incubation period.

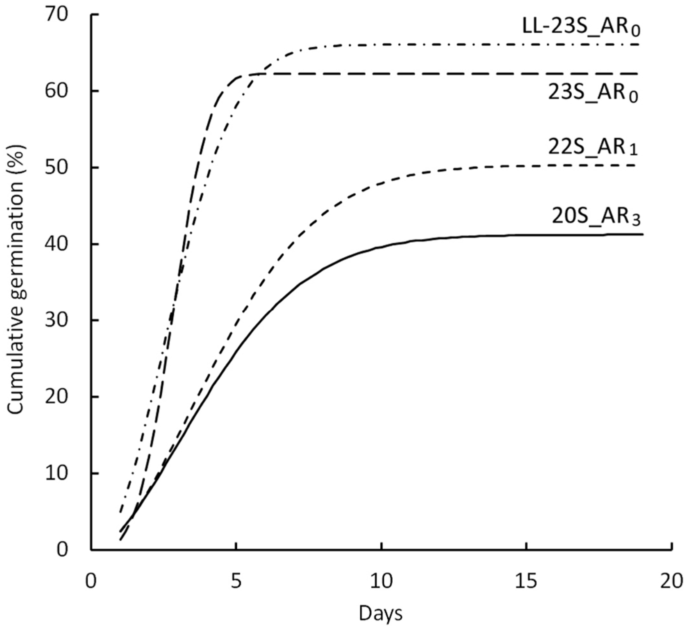

Figure 2. Cumulative germination over the first 20 days of the incubation period of naked seeds of *Cakile maritima* after-ripened for 3 years (20S_AR$_3$, 2020 harvest), one year (22S_AR$_1$, 2022 harvest), and not after-ripened (23S_AR$_0$, 2023 harvest). In all the years, fruits were collected at the Margherita di Savoia (MS) site, whereas in 2023, fruits were also collected at the Lagoon of Lesina (LL) site (LL-23S_AR$_0$) (Trial 2).

3.2. Seedling Emergence from Stratified Fruits of *C. maritima*

In trial 3 (started in September 2022), the intact fruits harvested in the MS site in 2020 (20F_AR$_2$) and 2022 (22F_AR$_0$) were used, along with the 22F_AR$_0$ siliques treated with saline water before stratification treatment (22F_AR$_0$S) (Table 1).

The final emergence was two-fold higher from the fruits which were harvested in 2020 and after-ripened for 2 years (20F_AR$_2$) compared to the freshly harvested ones, while the T_{50} was, on average, 25 days (Table 3).

Table 3. Seedling emergence, T_{50}, and Weibull's function parameter from moist-stratified, after-ripened, or freshly harvested *Cakile maritima* fruits (Trial 3, Autumn 2022–Spring 2023).

Seed Lot (1)	**Emergence**	$\mathbf{T_{50}}$	**Weibull's Function Parameters**		
	(%)	**(Days)**	***a***	***b***	***q***
20F_AR$_2$	61.1 a (2)	22.2 a	69.3 a	2.0 a	62.5 a
22F_AR$_0$	34.0 b	24.4 a	32.6 b	3.0 a	44.1 b
22F_AR$_0$S	23.9 b	28.4 a	20.8 b	2.0 a	64.7 a
Significance (3)	*	ns	*	ns	**

(1) See Table 1 for seed lot identification. (2) Means (n = 4) in columns not sharing the same letters are significantly different according to the LSD test (a = 0.05). (3) ns, * and ** not significant or significant at $p \leq 0.05$, $p \leq 0.01$, respectively.

The emergence of the seedlings started 175/180 days after the beginning of stratification (DAS) and lasted 64 days (5 March–15 May 2023), including the last 10 days when no further emergences were observed. The emergence pattern was affected by a higher q parameter for 20AR$_2$ and 22AR$_0$S compared to the 22AR$_0$ siliques (Table 3; Figure 3).

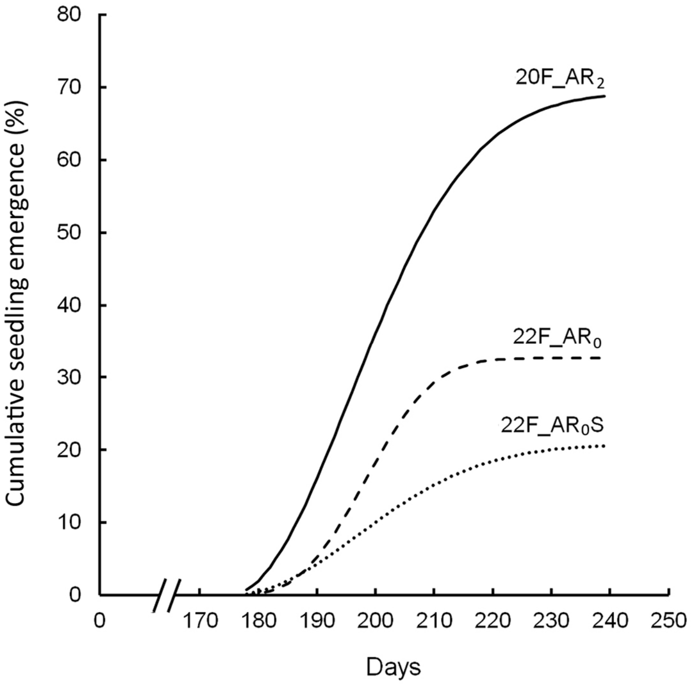

Figure 3. Cumulative seedling emergence during the 8 months following the beginning of the moist stratification of *Cakile maritima* fruits collected from Margherita di Savoia site and after-ripened for 2 years (20F_AR$_2$, 2020 harvest) and not after-ripened (22F_AR$_0$, 2022 harvest). 22F_AR$_0$S: not after-ripened siliques submerged in saline water (EC 36.3 mS cm^{-1}) for 72 h before being subjected to stratification (Trial 3).

In trial 4 (started in November 2023), we used the intact fruits harvested in the MS site in 2020 (20F_AR$_3$), 2022 (22F_AR$_1$), and 2023 (23F_AR$_0$), and in the LL site in 2023 (LL-23F_AR$_0$) (Table 1). The emergence of seedlings occurred in two phases. The first one (phase 1) started within a few DAS, and the second phase (phase 2) began at close to 80 DAS (February 2024) (Figure 4), with the fruits experiencing at least 2 months of cold stratification (Table S2).

The cumulative emergence in phase 1 was higher in the 2023 freshly harvested siliques (23F_AR$_0$ and LL-23F_AR$_0$) compared to the after-ripened ones (20F_AR$_3$ and 22F_AR$_1$). Mean T_{50} was 10.3 days, except for the 20F_AR$_3$ fruit lot (close to 17 days) (Table 4).

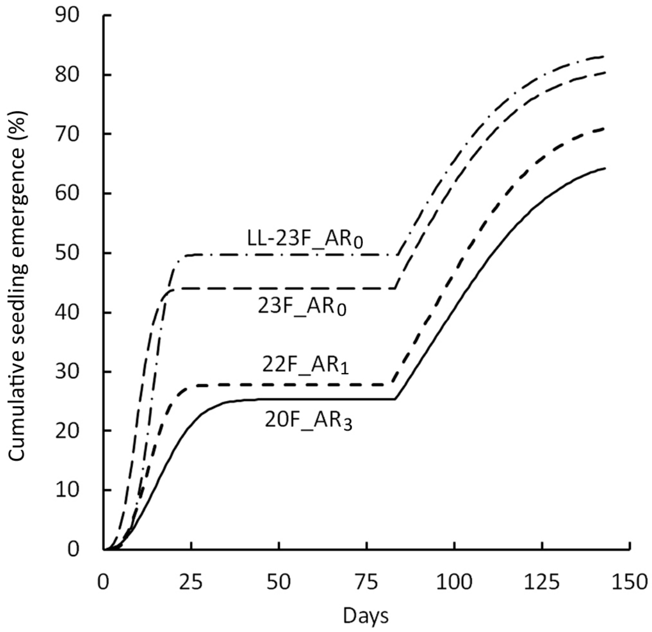

Figure 4. Cumulative seedling emergence during the 5 months following the beginning of the moist stratification of fruits of *Cakile maritima* after-ripened for 3 years (20F_AR$_3$, 2020 harvest), one year (22F_AR$_1$, 2022 harvest), and not after-ripened (23F_AR$_0$, 2023 harvest). In all the years, fruits were collected at the Margherita di Savoia (MS) site, whereas in 2023, fruits were also collected at the Lagoon of Lesina (LL) site (LL-23F_AR$_0$) (Trial 4).

Table 4. Seedling emergence, T$_{50}$, and Weibull's function parameter from moist-stratified, after-ripened, or freshly harvested *Cakile maritima* fruits (Trial 4, Autumn 2023–Spring 2024).

Seed Lot (1)	**Emergence**		**T$_{50}$**		**Weibull's Function Parameters**					
	(%)		**(Days)**		**Phase 1**			**Phase 2**		
	Phase1	**Phase2**	**Phase1**	**Phase2**	***a***	***b***	***q***	***a***	***b***	***q***
20AR$_3$	25.3 b (2)	62.7 c	16.9 a	90.0 a	25.3 c	2.3 a	37.9 a	65.8 b	3.9 a	149.9a
22AR$_1$	28.5 b	69.0 bc	11.6 b	89.7 a	27.7 bc	2.9 a	25.3 ab	72.1 ab	4.1 a	143.6a
23AR$_0$	44.0 ab	80.0 ab	10.9 b	79.5a	44.1 ab	2.8 a	19.2 b	81.3 a	3.3 a	143.8a
LL-23AR$_0$	50.0 a	82.5 a	8.3 b	76.3 a	48.7 a	2.9 a	22.4 ab	83.8 a	3.3 a	141.7a
Significance (3)	*	*	*	ns	*	ns	*	*	ns	ns

(1) See Table 1 for seed lot identification. (2) Means (n = 4) in columns not sharing the same letters are significantly different according to the LSD test (a = 0.05). (3) ns and * not significant or significant at $p \leq 0.05$ respectively.

In phase 1, the emergence patterns (Figure 4) differed for a lower q parameter registered for 23F_AR$_0$ fruits, especially compared to the 20F_AR$_3$ ones, while parameter a confirmed the values of the final germination (Table 4). In phase 2, emergence significantly increased up to 81% in 23F_AR$_0$ and LL-23F_AR$_0$, while the lowest value was observed for 20F_AR$_3$, with a T$_{50}$ of 84 DAS on average (Table 4). Phase 2 lasted almost 60 days, and the emergence pattern was affected by the higher a parameter for the 23F_AR$_0$ and LL-23F_AR$_0$ fruit lots, especially compared to 23F_AR$_3$ (Figure 4). The observation of fruits at the end of trials 3 and 4 highlighted that almost all (95–97%) of the ungerminated seeds were viable.

4. Discussion

In dormant seeds, the radicle protrusion does not occur even under favorable environmental conditions for germination [13]. Dormancy is caused by endogenous (characteristics of the embryo) and/or exogenous (e.g., seed coats, fruit walls) aspects [25]. Seeds with morphologic dormancy have underdeveloped embryos that prevent immediate germination. They can be induced to germinate quickly when exposed to specific conditions [11], such as warm or cold (moist) stratification depending on the species, or when treated with gibberellic acid [26]. Physiological dormancy in seeds can be due to an embryo and/or coat (testa, endosperm, pericarp) component, and the combination and interaction of these factors determine the level of physiological dormancy [13]. Previous studies for Brassicaceae species have reported a physiological dormancy (PD) occurring at variable intensity, namely non-deep, intermediate, and deep PD [11,15]. For PD dormancy breaking, the after-ripening during the dry storage and moist stratification, through the simulation of some natural events, can induce metabolic and physiological changes in seeds, affecting both the embryo and its covering layers [27,28].

In the following discussion, we will explore the significance of the pericarp on seed germination in *C. maritima* and will consider the benefits of after-ripening and stratification in enhancing and synchronizing seedling emergence from intact fruits. We will also consider the yearly variations in dormancy depth.

4.1. Effect of Fruit Pericarp on Seed Germination of C. maritima

In this study, the seeds 20S_AR$_3$, 22S_AR$_1$, 23S_AR$_0$, and LL-23S_AR$_0$ (trial 2), irrespective of the after-ripening treatments, germinated quickly and abundantly (Table 2; Figure 2). In trial 4, the same batches of seeds in the intact fruits (20F_AR$_3$, 22F_AR$_1$, 23F_AR$_0$ and LL-23F_AR$_0$) in phase 1, when cold stratification did not still occur, exhibited lower and slower germination (Table 4; Figure 4). These findings confirm the significance of the pericarp in inducing seed dormancy reported in *C. maritima* and other species of Brassicaceae (*Matthiola tricuspidata*) by Benvenuti et al. [17]. Moreover, the germination of *Bunias erucago* from intact silicles was very low (<5%) compared to the naked seeds (78–98%) [29]; in *Raphanus raphanistrum*, it increased from 114% registered for seeds inside the pod to 88–99% for the extracted seeds [12], and similar results were reported in *Isatis violascens* [15]. Previous studies have suggested that the pericarp may delay or inhibit the initiation of germination [12,30,31]. One potential reason is that the pericarp physically constrains the growth of the embryo because it lacks sufficient capability to break the fruit wall [12,15,32,33]. Furthermore, the pericarp may decrease the speed of water absorption and/or hinder complete water uptake (physical dormancy, [9]) necessary for seed germination [34,35], and/or it may contain substances that inhibit germination [12,32]. No specific experiments were included in this study to determine the mechanisms of pericarp-inducing dormancy; however, previous research highlighted that, in *C. maritima*, the mechanical properties of the pericarp are involved in the dormancy without any evidence for existing inhibitory substances [16].

4.2. Effect of Harvest Year on Unshelled Seed Germination of C. maritima

In *C. maritima*, the mechanisms that regulate seed dormancy have not been widely studied, except for the response to the salinity of naked seeds [18,20,21,36], showing high germinability without salinity stress. However, some studies have underlined that seeds require cold stratification, and the mechanical properties of testa have been reported to be involved in dormancy [16].

In this study, the lack of germination observed in trial 1 for both the after-ripened (20S_AR$_2$, 20F_AR$_2$) and not after-ripened (22S_AR$_0$, 22F_AR$_0$) shelled and unshelled seeds collected in 2020 and 2022 deserves further investigation as this result pointed out a deeper dormancy compared to 2023 lots (trial 2).

The characteristics of the 2020, 2022, and 2023 seed lots were likely significantly influenced by variations in the climatic conditions. To minimize the impact of changing soil characteristics (nutrient and water availability) on the mother plant's nutritional status, fruit collection was indeed restricted to the same sampling area within the MS site each year.

In trial 2, the 2023 freshly harvested seeds (23S_AR$_0$ and LL-23S_AR$_0$) showed a fast and high germination percentage, irrespective of the collecting site (Table 2; Figure 2). This is likely because the mother plants, during fruit ripening (July 2023), grew for two or three weeks under very high temperatures and aridity in both sites. In 2023, the weekly mean temperatures at MS and LL sites were the highest in this period, surpassing those of 2020 and 2022 by approximately 4–6 °C (Figure 1A) and, in the same year, rainfall was substantially absent in the July–September period (Figure 1B). It seems like the 2023 climatic conditions during the fruit ripening period may result in the production of a significant portion (65%, Table 2) of non-dormant seeds capable of rapid germination, while the not germinated seeds were dormant. Supporting this hypothesis, numerous studies conducted on populations from the semi-arid Mediterranean climate of the Tunisian coasts have reported rapid and high germination rates of *C. maritima* seeds [18,19,21]. Moreover, a study carried out on the *C. maritima* populations from Mallorca (Spain) and Central Italy showed variability in the germination percentage according to site-specific climatic features [22]. Similarly, studies have shown that dormancy depth can vary among different harvest years for various other plant species [23,37]. Dormancy is a complex genetic trait controlled by multiple genes and influenced by environmental factors during seed development. This interaction leads to a continuous (non-discrete) spectrum of phenotypes [13].

On the other hand, in the model species *Arabidopsis* (Brassicaceae), the dormancy depth has been affected by parental environmental conditions including temperature, nitrogen availability, light intensity, and photoperiod [38]. Responses of the $20S\_AR_3$ and $22S\_AR_1$ seeds in trial 2 were unexpected since no germination was observed in trial 1 for the after-ripened seeds ($20S\_AR_2$). However, they could have experienced high temperatures during the dry storage (in paper bags) in the summer of 2023, resulting in a deeper after-ripening involving the physiological changes breaking seed dormancy. Therefore, a portion of the $20S\_AR_3$ and $22S\_AR_1$ seed lots became non-dormant and quickly germinated (Table 2; Figure 2).

4.3. Effect of Moist Stratification on Shelled Seeds

Dormancy release on seeds inside the indehiscent siliques is still scarcely studied in *C. maritima*, despite its potential impact for improving the cultivation and use of this species. To our knowledge, this is the first comprehensive research focused on germination treatments for intact *C. maritima* fruits.

According to Baskin and Baskin [11], non-deep PD is most frequently recognized for seeds with PD, including *Arabidopsis* and various model organisms. Species with non-deep PD may require cold or warm stratification, or their germination can be promoted by gibberellic acid (GA) treatment, and the dry storage can result in seed after-ripening, whereas dry storage may reduce the requirement in cold stratification for intermediate PD seeds.

Examining the pattern of emergences from the intact fruits of trials 3 and 4 (Figures 3 and 4), it can be confirmed that different PD levels have characterized these fruit lots (as argued for naked seeds in paragraph 4.2) which showed different responses to stratification and after-ripening.

In trial 3, both the after-ripened ($20F\_AR_2$) and not-after-ripened fruits ($22F\_AR_0$, $22F\_AR_0S$) underwent close to 6 months of stratification including three months of cold temperatures (December 2022–March 2023, Table S2) before seedling emergence started. The latter occurred within a further 2 months (March-May 2023).

On the contrary, fruit lots used in the 2023–2024 trial (trial 4) exhibited a two-phase seedling emergence (Table 4; Figure 4). The first one started within a few days after stratification and lasted 20 or 37 DAS (November–December 2023) for the not after-ripened or after-ripened fruits. It involved non-dormant seeds since both lots germinated in a short time without requiring prolonged stratification. No emergence occurred during the subsequent 35–40 days (January–February 2024). However, a second phase of emergence, lasting two months (February–April 2024), was observed. This phase involved the seeds experiencing nearly two months of cold stratification (Table S2). Compared to trial 3, both the whole (mild–cold) and the cold stratification period were shorter in trial 4 (Table S2, Figures 3 and 4). The observation of not germinated seeds at the end of the stratification trials pointed out that almost all of them were viable but still dormant.

These findings corroborate the hypothesis of deeper physiological dormancy in the seeds from trial 3, harvested in 2020 and 2022, as evidenced by the prolonged cold stratification required for shelled seed germination [13]. Conversely, the trial 4 fruit lots likely exhibit non-deep physiological dormancy, with a proportion of non-dormant seeds (Figure 4). To definitively establish the presence and type of non-deep physiological dormancy in *C. maritima*, further investigations are warranted, including germination responses to gibberellic acid treatments and varying temperature conditions.

Irrespective of the variability in the seed depth dormancy, fruit stratification could have mimicked the natural environment where silique burial in moist sand is involved in removing the physiological mechanisms, resulting in the synthesis of gibberellins [11]. Additionally, the stratification could have promoted a slow microbial degradation of the pericarp as also reported for other sand species [17,39].

In this study, moist stratification was conducted under the natural autumn–winter temperature regime of the Foggia area, simulating the field/nursery management of fruits. Cold stratification at a constant low temperature (e.g., 5 °C), previously reported as an effective dormancy breaking treatment [11], warrants testing to improve germination in this species, even though it may be more costly. However, a study on *C. maritima* collected along the Atlantic coast of Northern Spain revealed a limited improvement in germination when fruits were stratified at 3 °C for 12 weeks [40].

4.4. Effect of After-Ripening and Salinity on Shelled Seeds

As regards the after-ripening effect, the AR significantly enhanced the germinability only when combined with the stratification (trial 3, Figure 3; trial 4, Figure 4, phase 2). Given that in intermediate PD, the stratification effectiveness in the dormancy release increased after the fruits were allowed to after-ripen in dry storage, we assumed an intermediate PD for the 2020 and 2022 fruit lots according to the Baskin and Baskin [11] classification. Additionally, our findings agree with the other studies [23,25,37], including the Brassicaceae family [15], highlighting that after-ripening enhanced seed sensitivity to the factors that relieve dormancy and stimulate radicle protrusion.

The intermediate PD dormancy assumed for the intact fruit harvested in 2020 and 2022, requiring prolonged moist stratification, can also explain the lack of germination of the same shelled ($20F\_AR_2$ and $22F\_AR_0$) and naked ($20S\_AR_2$ and $22S\_AR_0$) seed lots without being stratified (trial 1). However, further studies are needed to test the response to gibberellins treatment and confirm the intermediate PD level of seeds of *C. maritima*.

Notably, $20F\_AR_2$ and $22F\_AR_0$ lots were dry stored for a year more (2023) and the high temperatures of the 2023 summer may have triggered a deep after-ripening and the release of dormancy, resulting in a transition from an intermediate to a non-deep physiological dormancy (PD) state. Specifically, these lots, when used in trial 4 ($20F\_AR_3$ and $22F\_AR_1$), included non-deep physiological dormant seeds with seedling emergence occurring within a short stratification period (Figure 4, phase 1). A similar effect of dry storage during 2023 has been highlighted in the speed of germination of the naked seeds (trial 2) (Figure 2).

The effect of saline solution imbibition of fruit freshly harvested in 2022 (trial 3) seems to only delay the occurrence of the maximum emergence compared to $22F\_AR_0$ fruits (q parameter 44.1 vs. 64.7 days) (Table 3, Figure 3), likely because there was residual NaCl in the pericarp [10].

5. Conclusions

The *C. maritima* population of the Northern Apulia area shows seed dormancy, which may be variable in depth according to the year of fruit collection. The depth of physiological dormancy appears to range from intermediate to non-deep and null (non-dormant seeds). The presence of the pericarp exacerbates dormancy, reducing and delaying germination.

For freshly harvested material, germination can occur in the same harvest year at varying levels ranging from 45% for intact fruits to 65% for naked seeds. However, this behavior is unpredictable, therefore further research is needed to understand the conditions promoting the production of freshly harvested non-dormant seeds.

To achieve a rapid and efficient propagation of *C. maritima*, fruit dry storage and moist stratification performed under Mediterranean autumn–spring temperatures can result in high emergence rates (70–80%). For more synchronized emergence, specific treatments such as stratification at constant low temperatures and gibberellic acid (GA) application may prove beneficial and warrant further investigation.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy14092127/s1, Table S1: Poliannual (1993–2023) temperatures and rainfall in the collection area of *Cakile maritima* fruits, in the Northern Apulia region; Table S2: Monthly maximum and minimum temperatures during stratification trials (3 and 4) of *Cakile maritima* fruits.

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