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Seed age changes the germination response of weed species to cereal rye allelopathy

Aliyah W. Jackson[1], Virginia M. Moore[2], Chris Reberg-Horton[3], Steven B. Mirsky[4] and Ramon G. Leon[5]

[1]Graduate Research Assistant, Department of Crop and Soil Sciences, North Carolina State University, Raleigh, NC, USA;
[2]Assistant Professor, School of Integrative Plant Science, Cornell University, Ithaca, NY, USA;
[3]Blue Cross and Blue Shield of North Carolina/W.K. Kellogg Distinguished Professor, Department of Crop and Soil Sciences, North Carolina State University, Raleigh, NC, USA;
[4]Research Ecologist, Sustainable Agricultural Systems Laboratory, U.S. Department of Agriculture, Agricultural Research Service, Beltsville, MD, USA and
[5]William Neal Reynolds Distinguished Professor and University Faculty Scholar, Department of Crop and Soil Sciences, North Carolina State University, Raleigh, NC, USA

Corresponding author: Ramon G. Leon; Email: rleon@ncsu.edu

Abstract

Cereal rye (*Secale cereale* L.) is grown as a cover crop due to its ability to enhance soil health and suppress weeds through allelopathy, but germination responses of different weed species to allelochemicals have not been studied for seeds varying in age and consequently vigor. This study investigated the allelopathic effects of cereal rye on the germination of Palmer amaranth (*Amaranthus palmeri* S. Watson), large crabgrass [*Digitaria sanguinalis* (L.) Scop.], giant foxtail (*Setaria faberi* Herrm.), and lettuce (*Lactuca sativa* L.). Seeds were germinated in vitro in media with allelochemicals secreted by roots of cereal rye lines varying in allelopathic activity. Seeds were subjected to an accelerated aging treatment to modify their vigor. Results showed that aged seeds exhibited 31% lower germination than non-aged seeds. The magnitude of the germination reduction due to the presence of allelochemicals was species dependent. In the absence of allelochemicals, *L. sativa* exhibited a 20% reduction in germination due to aging, while the reduction was greater than 60% when allelopathy and aging were combined. Non-aged *S. faberi* seeds increased germination under low allelopathy conditions, with 20% greater germination compared with the non-treated control. *Digitaria sanguinalis* did not respond to seed aging, and the high allelopathy treatment reduced germination less than 10%. *Amaranthus palmeri* exhibited the highest germination and was the only species that was not affected by the seed aging and allelopathy treatments. Germination rate was reduced by seed aging and to a lesser extent by allelochemicals. Results suggest that incorporating allelopathic *S. cereale* varieties in cover crop rotations can reduce weed populations. However, the age structure of the seedbank might determine the importance of allelochemicals for emergence at the species level, likely causing weed community shifts.

Keywords: Cover crop; integrated weed management; viability; vigor

Introduction

Weeds have demonstrated potential for rapid evolution of traits that make them more resilient and challenging to control, such as herbicide resistance, resistance to environmental stress, changes in emergence patterns, and increased competitive ability (Clements et al. 2005; Ethridge et al. 2023a, 2023b; Harker 2013; Owen et al. 2015). The reliance on herbicides in conventional and reduced-tillage systems is changing to more diverse approaches because of their value to control weeds in a more robust and sustainable manner (Gage and Schwartz-Lazaro 2019; Riemens et al. 2022). These approaches frequently include the concerted use of herbicides; tillage and cultivation; and cultural practices such as crop rotation, seeding patterns, and cover cropping (Norsworthy et al. 2012). Among cover crops, cereal rye (*Secale cereale* L.) use has increased among farmers as part of their efforts to supplement soil organic matter, improve water infiltration, control erosion, and reduce nitrate leaching (Brandi-Dohrn et al. 1997; Mirsky et al. 2013; Snapp and Surapur 2018; Teasdale and Mohler 1993). Cereal rye is also known to produce benzoxazinoids, which are allelopathic secondary metabolites that act as defenses against insect pests (Barnes and Putnam 1987; Wu et al. 2001) and suppress seedling emergence and growth of nearby plants (Carlsen et al. 2009; Hickman et al. 2021; Nikus and Jonsson 1999; Rebong et al. 2024).

The scientific literature has abundant information about the effect of allelochemicals on germination and seedling growth, but the specificity of allelopathic activity between genotypes of cereal rye and different weed species has not been studied. In fact, such specificity studies are rare, and they have been done only in a few species. For example, Bouhaouel et al. (2015) reported that root exudates of six barley (*Hordeum vulgare* L. *vulgare*) genotypes caused different levels of root and seedling growth inhibition on rigid ryegrass (*Lolium rigidum* Gaudin) and ripgut brome (*Bromus diandrus* Roth), and the sensitivity to each barley genotype was species dependent. This type of interaction between allelopathic activity and weed species sensitivity may impact management, because the suppression may not be uniform across the weed community. Therefore, characterizing those interactions is necessary to determine the value of allelopathy as a component of the suppressive ability of cereal rye.

A factor that has been ignored in allelopathic studies is the fact that weed seedbanks are heterogenous not only in species composition but also in the age and vigor of seeds (Kalisz 1991). Most studies have evaluated the effect of allelopathy testing seeds recently produced and exhibiting high vigor. However, this approach may result in underestimations of the importance of allelopathy for reductions in weed emergence. Considering that older seeds tend to exhibit lower viability and vigor (Reed et al. 2022), one can hypothesize that they would be more sensitive to allelochemicals than new seeds. Testing this hypothesis in situ is challenging, due to the multiple factors that affect seed viability in soil. However, a practical alternative is to artificially modify seed vigor with methods such as accelerated aging (Baker 1974; Reed et al. 2022; Turner et al. 2014). In an accelerated aging procedure, seeds are subjected to controlled high temperature and humidity conditions for a short time (e.g., a few hours to less than a week, depending on the species), which simulates an aging process in which energy reserves, DNA and RNA stability, and cell membrane integrity are reduced (Fleming et al. 2017; Reed et al. 2022; Yi et al. 2025). After aging of seeds, germination tests are conducted to assess the impact of reductions in tissue integrity on germinability and vigor and thus provide insight into seed germination potential under less than optimal conditions (Fenollosa et al. 2020; Marcos-Filho 1998; McDonald 1993; TeKrony 1983, 2005).

In the present study, we hypothesized that older seeds (i.e., aged) are more sensitive to allelochemicals than newer seeds and that the effect of allelochemicals would depend on unique interactions between weed species and distinct cereal rye genotypes characterized by either high or low allelopathy. Thus, the objectives of the present research were (1) to characterize differences in germination of several weed species when exposed to allelochemicals of different cereal rye genotypes and (2) to determine whether weed seed age influences susceptibility to allelochemicals.

Materials and Methods

Plant Material

Laboratory and field screenings quantifying allelopathy levels in various cereal rye genotypes have been performed successfully using different species such as lettuce (*Lactuca sativa* L.), *L. rigidum*, and white mustard (*Sinapis alba* L.) as bioindicators (Belz and Hurle 2004; Rebong et al. 2024; Wu et al. 2000). In the present study, Palmer amaranth (*Amaranthus palmeri* S. Watson), large crabgrass [*Digitaria sanguinalis* (L.) Scop.], and giant foxtail (*Setaria faberi* Herrm.), which are weeds of economic importance in many crops in the United States (Ethridge et al. 2023a; Oreja et al. 2022; Ward et al. 2013), and *L. sativa* (positive control) were used as bioindicators to compare the allelopathic activity of cereal rye genotypes and to quantify variation in sensitivity across species. Weed seeds, derived from parental populations originally collected from an agricultural field in Ames, IA, and maintained in the Iowa State University Weed Science Program germplasm bank, were produced under greenhouse conditions, harvested, dried to 8% moisture content, and stored at 4 C under dry conditions for approximately a year when the present study was initiated (Ethridge et al. 2023a). ‘Salad Bowl’ lettuce seeds were purchased commercially (Wyatt-Quarles Seed Company, Garner, NC).

Allelopathic Media

Cereal rye seedlings (i.e., unique genotypes) were grown in water-agar to freely release allelochemicals for 17 d, and *L. sativa* seedlings were used as bioindicators of injury due to allelopathy. Agar media were chosen and classified with high or low allelopathic activity based on the injury caused to the bioindicator. Thus, the criteria were ≥70% injury and/or ≥50% root growth reduction for high allelopathy classification and ≤45% injury and/or ≤35% root growth reduction for low allelopathy. Agar media with cereal rye seedlings that caused injury between those thresholds were not included in the study to ensure having two distinct non-overlapping categories.

Seed Accelerated Aging and Germination

Seeds were artificially aged following the accelerated aging test for seeds outlined in Tekrony (2005) to change their vigor. Seeds were placed on a filter paper in a sealed, glass container with a paper towel saturated with water to maintain a high relative humidity environment. The containers were then maintained for 48 h in a germinator (Achieva Console Germinator, model A3920/B, Seedburo Equipment Company) set to provide 45 C with no light.

For the germination test, all seeds were surface sterilized using an ethanol and bleach protocol outlined in Wu et. al. (2000) and placed onto the surface of the allelopathic agar extracts in a 100 by 15 mm petri dish, with each species being in a separate petri dish. Twenty-five seeds that underwent the accelerated aging were placed on top of the agar in one-half of the petri dish, and 25 non-aged seeds were sown on the other half. Petri dishes were sealed with Parafilm® and placed in the germinator set to provide 22 ± 2/20 ± 2 C and a light/dark cycle of 12/12 h. Germination counts were collected daily for 21 d. Seeds were considered germinated when protruded radicles were at least 2-mm long.

Experimental Design and Statistical Analysis

The experiment was a completely randomized design with five replications per treatment, and it was conducted twice. The treatments were a factorial combination of three allelopathic levels (high, low, and control) and two accelerating aging conditions (aging [AA] and non-aging [NA]). Final germination was analyzed with an ANOVA model including allelopathy level, aging, species, and their interactions as fixed effects, while replication and experiment run were considered random effects. All statistical analyses were performed using the R software 4.4.1 (R Core Team 2024). The GGPLOT2 package was used for visualization of results (Wickham 2016). The EMMEANS and the AGRICOLAE packages were used for ANOVA and for mean separation using Tukey's honestly significant difference (De Mendiburu 2006; Lenth 2016). The DRC package (Ritz et al. 2015) was used to describe cumulative germination over time using log-logistic models. Estimated coefficients and model parameter confidence intervals were inferred with the *coeftest* function of the LMTEST package (Zeileis and Hothorn 2002). The coefficient matrix was determined with the SANDWICH package (Zeileis et al. 2020). The *ED* function was used to estimate the time needed to reach 50% of the total cumulative germination (GR_{50}) observed for each treatment and species.

Results and Discussion

The statistical analysis revealed significant interactions among allelopathy level, seed aging, and weed species ($P < 0.001$; Table 1). *Amaranthus palmeri* was less affected by allelochemicals and aging, reaching 80% regardless of the treatments. Conversely, the germination rate of this weed was reduced by aging, and the time to reach 50% of the final cumulative germination (GR_{50}) ranged between 7.53 to 8.33 d for the aged seeds and 2.48 to 3 d for the non-aged seeds (Figure 1; Table 2). This result is likely due to the small seed size of *A. palmeri* not withstanding the high temperatures and humidity of the accelerated aging process (Walters et al. 2010). With *A. palmeri* being one of the more problematic weed species due to its rapid evolution and resistance to herbicides and current methods of weed management, this result indicates that cereal rye allelopathy alone might not be as effective in inhibiting seedling emergence and growth as other factors such as cover crop biomass (Roberts and Florentine 2022).

Seed aging and allelopathy had minor effects on the germination of *D. sanguinalis* (Figure 2), but both factors reduced the rate of germination (Figure 1), increasing GR_{50} 3 to 6 d compared with Control_NA, which was the fastest treatment (GR_{50} = 4.15 d). Therefore, at least under the conditions of the present study, it seems that cereal rye allelochemicals may not reduce the number of *D. sanguinalis* seeds germinating in a season, but the germination rate may be decreased by presence of allelochemicals. This negative effect on germination rate in addition to the substantial biomass and dense foliage that cereal rye produces may still create a synergism for the suppression of this species. However, the significance of such synergism for detectable changes in weed pressure under field conditions is yet to be determined.

Accelerated aging decreased germination of both *L. sativa* and *S. faberi* more than the other species, with reductions of approximately 50% to 70% when compared with the Control_NA treatment (Figure 2). *Lactuca sativa* was the only species for which there was a clear interaction between aging and allelopathy. Thus, aged seeds maintained in allelopathic media (high or low) suffered up to 70% reduction in germination and almost double GR_{50} compared with germination in agar without allelochemicals (Table 2).

Interestingly, when germinated in media with allelochemicals, non-aged seeds of *S. faberi* were stimulated, and final germination increased compared with Control_NA. This was more evident in the low allelopathy treatment. This positive response to allelochemicals may be due to a reduction in seed dormancy. Several researchers have documented reductions in dormancy when *S. faberi* seeds were exposed to abiotic stresses (Taylorson 1986; Yoshioka et al. 1995).

In the case of final germination, the results of the present study confirmed only part of our original hypothesis. Thus, it was demonstrated that the magnitude of germination reduction in response to allelopathy was species dependent. Nevertheless, seed aging did not always make the seeds more susceptible to allelochemicals, as we originally proposed. The three-way interaction between allelopathy, aging, and species explained a substantial portion of the variation, indicating that the combined effects of allelopathy level and seed aging on germination varied among species. Regarding germination rate, except for *A. palmeri,* aged seeds were prone to germinate more slowly when exposed to allelochemicals than non-aged seeds, and the magnitude of that response was species dependent, as was hypothesized.

While most germination studies utilize fresh, vigorous seeds, the results of the present research indicated that the responses of weed seeds are contingent upon their specific age/vigor. Therefore, relying exclusively on fresh seeds in germination studies provides a major underestimation of the benefits allelochemicals may provide for weed suppression under field conditions. This is not necessarily a problem, because researchers may want to consider a "worst-case scenario" in which allelopathy activity is tested against the most vigorous seed lots (i.e., minimum suppression). However, the reality is that weed seedbanks have considerable variation in age composition (Nunney 2002). This is the result of dormancy mechanisms that allow seeds to stay in the soil for several years without germinating. Meanwhile, compared with fresh seeds, aged seeds exhibit delayed germination; progressively declining viability, as indicated by an increasing proportion of ungerminated seeds; and a more pronounced dormancy over time (Graeber et al. 2012; Pirredda et al. 2023; Thompson et al. 2003). Consequently, the germinable seedbank can have differences in the number of seeds with high or low vigor, and this can determine how effectively allelochemicals will suppress weeds each year. In the case of the three weed species studied here, previous research demonstrated that the number of viable seeds in the soil decreases in more than 70% in less than 3 yr (Buhler et al. 2001; Masin et al. 2006; Sosnoskie et al. 2013). This decrease is strongly influenced by loss of seed viability in addition to seed germination and mortality.

A simple application of this information is that allelopathic suppression might not be evident or effective immediately following large weed escapes and seed rain, because a large portion of the seedbank will be viable and vigorous. In contrast, if seed production is consistently impeded, allelopathy will progressively become more effective by reducing weed emergence and establishment as seeds lose vigor over time. Additionally, our results documented the specificity in the response to allelochemicals depending on species and seed vigor. Thus, cereal rye may favor those weed species that are more tolerant to allelochemicals, potentially causing shifts in weed community composition. Nevertheless, the dynamics related to seed vigor loss would influence those shifts, likely also favoring species with more persistent seeds.

The fact that allelopathic activity is detected in vitro does not provide much information about its real impact in the field. Laboratory studies allow us to characterize the mechanisms that could operate in the field, but as other studies have shown, allelopathic activity is highly dependent on soil properties determining allelochemical availability and residuality (Rice et al. 2022; Teasdale et al. 2012). The selectivity and seed age effects observed in the present study are likely intrinsic to the seeds themselves and independent of soil-mediated influences. However, the concentration of allelochemicals in soil will certainly influence the likelihood of seed or seedling being injured to levels that prevent seedling emergence and establishment.

The most important limitation of this and most studies characterizing allelopathy in cover crops is that it has not been possible to determine how important allelopathy is for weed suppression in comparison to cover crop biomass. Distinguishing the relative importance of the physical barrier resulting from the large amounts of biomass produced by the cover crop from the allelopathic suppression has proven elusive. Nevertheless, a plausible application of the present results is that in cases in which allelopathic suppression is relatively low, such as for *A. palmeri* and *D. sanguinalis* (Figure 2), cover crop biomass production will likely be the driver of suppression. Conversely, for highly susceptible species, allelopathy might be sufficient to reduce the number of seedlings emerging and facing the challenge of growing through the cover crop before exhausting energy reserves. Many weed species persist in agricultural systems due to their extensive seed longevity in the seedbank, with dormancy and seed vigor allowing them to remain viable across multiple seasons (Bajwa et al. 2022). In such cases, cereal rye allelopathy could play a valuable role by weakening or disrupting the dormancy cycle and reducing vigor, making these persistent seeds more susceptible to suppression. In cases in which cover crop biomass is not high enough for adequate weed suppression, the interaction between seed age and allelopathy susceptibility may be valuable (Wiggins et al. 2017). For example, older weed seeds, which are less vigorous than younger ones, are more likely to reduce their germination rate when exposed to cereal rye allelochemicals (e.g., *S. faberi* and *L. sativa*; Figure 1). In this context, and considering such reduction a reflection on additional vigor loss, the seedlings that emerge through the soil surface will be weaker. Thus, the chances these seedlings have of successfully growing through the cover crop will be further reduced. The results of the present study provide an example of the value of considering the demographic structure of the seedbank to better assess weed suppression potential of cover crops. Cereal rye allelopathy may not control weeds with the high levels of efficacy provided by synthetic herbicides, but its contribution to reducing the emergence potential of older weed seeds can have important benefits from an integrated weed management perspective to reduce weed control variability over time.

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Competing interests. The authors declare no conflicts of interest.

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Table 1. ANOVA final germination percent in response to three allelopathy treatments, two aging treatments, four species, and their interactions, with replication and experiment run as random effects

Source	DF	SS	MS	*F*-value	Pr(>*F*)
Allelopathy (Al)	2	11.86	5.93	11.84	<0.001
Aging (Ag)	1	0.05	0.05	0.05	0.83
Species (Sp)	3	16.08	5.36	16.05	<0.01
Al × Ag	2	11.14	5.57	11.12	<0.01
Al × Sp	6	271.48	45.25	271.00	<0.001
Ag × Sp	3	12.43	4.14	12.41	<0.01
Al × Ag × Sp	6	181.33	30.22	181.01	<0.001
Residuals	215	215.38	1.00		

Table 2. Time to reach 50% of the final cumulative germination (GR_{50}) for 21-d germination of *Amaranthus palmeri*, *Digitaria sanguinalis*, *Setaria faberi*, and *Lactuca sativa*, with (AA) and without (NA) accelerated aging of seeds, germinated in agar containing high and low levels of allelochemicals and an allelochemical-free agar (control)

Species	Seed aging	Allelopathy	GR_{50}	SEM[a]	CV[b] %
Amaranthus palmeri	NA	Low	2.48	0.03	3.6
		High	3.00	0.05	5.0
		Control	2.81	0.03	3.2
	AA	Low	7.53	0.07	2.8
		High	8.33	0.10	3.6
		Control	7.65	0.06	2.4
Digitaria sanguinalis	NA	Low	13.01	0.30	6.9
		High	11.40	1.92	50.5
		Control	4.15	0.03	2.2
	AA	Low	7.05	0.09	3.8
		High	7.76	0.15	5.8
		Control	7.32	0.15	6.1
Setaria faberi	NA	Low	6.68	0.11	4.9
		High	9.01	0.16	5.3
		Control	2.68	0.04	4.5
	AA	Low	6.81	0.35	15.4
		High	6.67	0.30	13.5
		Control	7.23	0.11	4.6
Lactuca sativa	NA	Low	3.48	0.05	4.3
		High	3.17	0.09	8.5
		Control	1.79	0.02	3.4
	AA	Low	7.19	0.29	12.1
		High	6.51	0.13	6.0
		Control	7.80	0.09	3.5

[a] SEM, standard error of the mean.

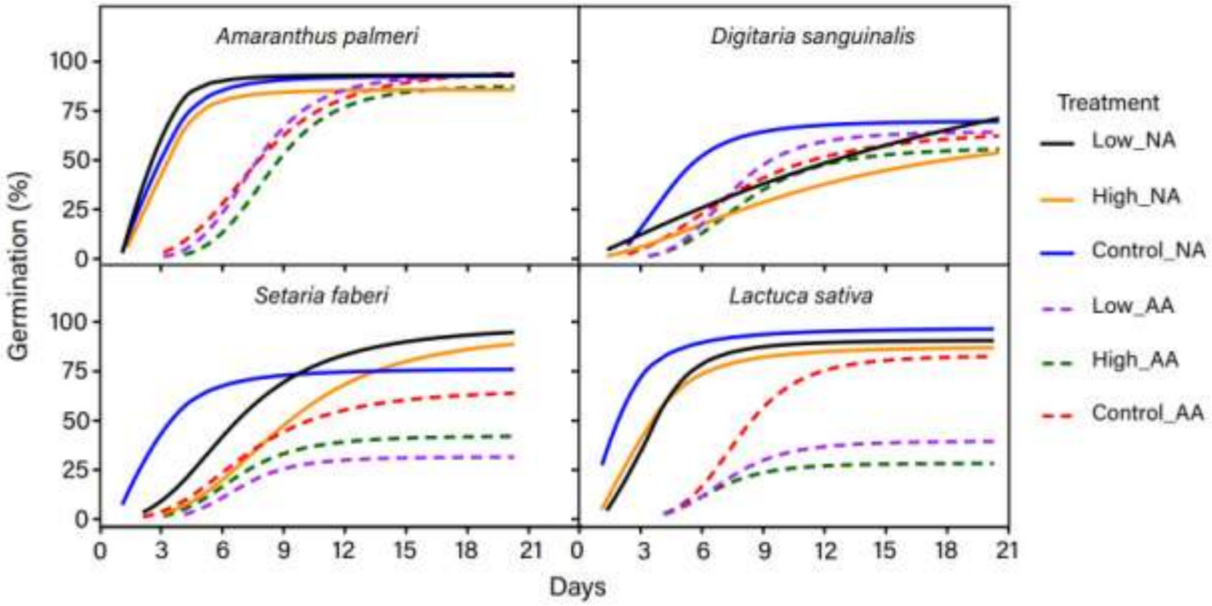

Figure 1. Cumulative germination (GR_{50}) over 21 d of *Amaranthus palmeri*, *Digitaria sanguinalis*, *Setaria faberi*, and *Lactuca sativa*, with (AA) and without (NA) accelerated aging of seeds, germinated in agar containing high and low levels of allelochemicals and an allelochemical-free agar (control). Lines were fit using a log-logistic model.

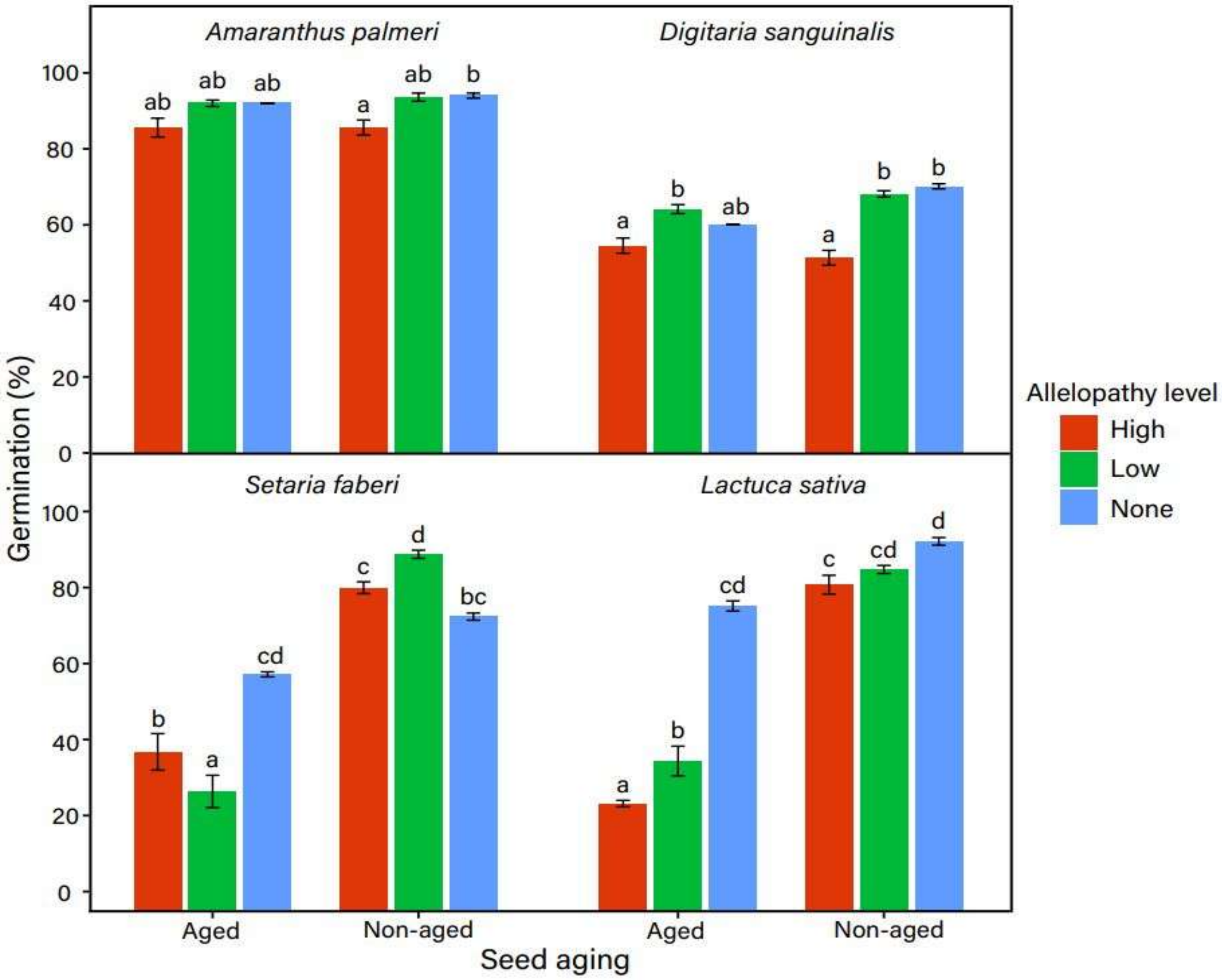

Figure 2. Estimated marginal means (± standard error of the mean) of seed germination (%) across seed aging treatments and allelopathy levels, analyzed separately for each species: *Amaranthus palmeri*, *Digitaria sanguinalis*, *Setaria faberi*, and *Lactuca sativa*. Bars with the same letter were not significantly different within species based on Tukey's honestly significant difference α = 0.05.