



Impact of seed hydration level on seed preferences by carabid beetles (Coleoptera: Carabidae)

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Abstract. Carabid beetles are among the most important post-dispersal seed predators in agroecosystems, where their feeding habits contribute to the bioregulation of weed seedbanks. Although seed traits such as size, shape, and coat hardness can influence seed choice by carabids, the role of environmental factors, such as seed hydration level, on seed choice remains understudied. The current study investigated how the different levels of hydration of kochia (*Bassia scoparia*) and canola (*Brassica napus*) seeds affected seed choice by *Pterostichus melanarius* and *Poecilus corvus*, two omnivorous carabids. Seeds hydrated for 6 h were significantly more preferred by the carabid species tested over dry seeds, but hydration beyond that period provided no additional increase in preference. Olfactometric bioassays indicated that these feeding responses were more likely driven by the moisture-induced changes in seed physical properties rather than changes in seed-derived chemical cues. As such, early water uptake seemed to induce measurable structural modifications while extended hydration caused little or no further change. The strength of seed hydration effects varied among both carabid and seed species, suggesting that seed predation pressure in agroecosystems is likely to depend on the temporal patterns of seed hydration in the field in addition to the species composition of both carabid communities and soil seedbanks.

INTRODUCTION

Ground (carabid) beetles (Coleoptera: Carabidae) are among the most important post-dispersal seed predators in ecosystems of temperate regions (Honěk & Kocian, 2003; Olfert et al., 2005; Vankosky et al., 2017), with numerous species routinely incorporating seeds into their diets alongside prey (Saska, 2007; Saska et al., 2019; Guenay-Greunke et al., 2024). Thus, seed feeding habits are more widespread among carabids than previously thought (Frei et al., 2019; Guenay-Greunke et al., 2024), suggesting that seeds may be sought by carabids to meet certain biological and nutritional needs (Ali & Willenborg, 2021). Carabids, through their seed feeding activities, can, therefore, remove significant proportions of weed seeds from the soil seedbanks (60–90% under optimal conditions) and hence contribute to natural suppression of weed populations (Holland & Luff, 2000; Bohan et al., 2011; Kulkarni et al., 2015).

Seed choice by carabids does not appear to be random, as they often prefer certain seed species over others when seeds of different species are available in the environment (Ali & Willenborg, 2021). In this sense, seed choice by carabids is often mediated by seed-derived chemical cues (Kulkarni et al., 2017; Ali et al., 2022) and generally appears to be influenced by various seed traits, including seed

size and mass, coat hardness, and nutritional content (Gaba et al., 2019; Foffová et al., 2020b; Ali & Willenborg, 2023). Such traits are predicted to influence the handling time of seed species and, as such, determine which seed species is more nutritionally and/or physically suitable for consumption under different ecological and environmental contexts (Ali & Willenborg, 2021, 2023).

Seed hydration is the process by which seeds absorb water from the environment to initiate germination (Woodstock, 1998; Louf et al., 2018). This is a critical physiological event in the life cycle of plant species, as seed hydration sets in motion a cascade of events that ultimately leads to radical emergence, including the activation of metabolic enzymes, and the alteration of seed tissue properties, which often alter the physical properties of the seed as whole, increasing its vulnerability to predators and/or pathogens (Orozco-Segovia & Sánchez-Coronado, 2009). Although water uptake is a prerequisite for germination, it does not always terminate dormancy, as some seeds may remain metabolically active yet physiologically dormant until other environmental conditions are met (Bewley & Black, 2013). These metabolically active but physiologically dormant seeds are potentially prone to elevated predation risks in the field, where carabid beetles are often active and likely to encounter seeds in various physiological states, in-

cluding dry, hydrated, dormant, and even nonviable seeds (Petit et al., 2014; Baskin & Baskin, 2025).

Carabids generally rely on both physical and chemical cues to determine seed suitability for consumption as discussed above (Foffová et al., 2020a; Ali et al., 2022; Ali & Willenborg, 2023). Thus, any moisture-induced changes in the physical and chemical properties of the seeds can potentially influence seed detection and selection. Hydrated seeds often exhibit changes in their biochemical activity in addition to changes in their biophysical properties (see above), which may directly or indirectly affect the ability of carabid seed predators to detect and consume them (Nelson, 2018; Pompelli et al., 2023; Upreti et al., 2024). For instance, water uptake may soften the seed coat, increase seed mass, or alter volatile emissions, which may affect seed detectability, alter handling time costs, and/or change seed nutrient accessibility, all of which can alter the palatability of hydrated seeds to carabid predators (Bradbeer, 1988; Willis et al., 2014; Long et al., 2015). In other words, hydrated seeds may incur lower handling costs or facilitate access to seed nutrients for carabids, whereas hard and dry seeds may be more mechanically difficult, demanding more energy and effort expenditure to handle and consume (Louf et al., 2018; Wu & Shen, 2021). Also, hydration may bring about changes in seed volatile emissions, which may influence both the detection and selection of seed species by seed-feeding carabids (Kulkarni et al., 2017). However, detailed studies that look into the impact of different levels of seed hydration on seed volatile emissions remain wanting. Previous research has shown that hydrated seeds are generally more palatable to carabids than dry seeds, likely due to changes in seed coat properties, volatile emission, and/or nutrient availability (Law & Gallagher, 2015; Foffová et al., 2020a, b; Deroulers et al., 2020). Nevertheless, it is worth mentioning that some carabid species are also known to consume dry seeds as well, indicating that hydration is not a universal prerequisite for seed selection and predation within carabid seed-feeders (Kulkarni et al., 2016, 2017). Under field conditions, rainfall or dew events can lead to water uptake by the seed and create a time window during which the metabolically active seeds that are near the soil surface are generally more vulnerable to carabid predators (Saska et al., 2008; Kulkarni et al., 2017). However, less is known about how different levels of seed hydration may affect seed choice by carabid beetles in terms of seed removal rate as a function of the amount of water in the seeds.

The impact of hydration on both seed detectability and palatability by carabids is likely to be shaped by multiple interacting factors. Seed species identity, hydration duration, as well as environmental factors such as temperature, soil texture, crop residue cover, diurnal temperature fluctuations, and microtopography, can all influence moisture retention and the rate of water uptake by the seed in the field (Meeks et al., 2023; Pompelli et al., 2023). This complexity renders the predation pressures that carabids impose on seed species in agroecosystems hard to predict. Some studies suggest that seeds that imbibe water more quickly

tend to become softer, more detectable, and thus more susceptible to carabid predation (Lundgren, 2009), whereas those that absorb water more slowly may be less detectable during foraging bouts by carabids (White et al., 2007; Paulsen et al., 2013). Such differences in how seed species interact with water gradients in the environment add more complexity to predicting the response of carabid beetles to seed hydration levels in the field. Therefore, generalizing the effects of seed hydration on seed detection and consumption by carabids remains untenable at this point.

In the carabid literature, most studies adopt a standard 24 h hydration treatment to study the impact of water uptake on seed detection and choice by carabids (e.g., Law & Gallagher, 2015; Kulkarni et al., 2016; Deroulers et al., 2020; Foffová et al., 2020b). It remains unclear if the 24-h period, despite its wide use, represents the optimal hydration level for bringing about the most significant (and positive) changes in seed detectability and palatability for carabids. Detailed studies that look into the impact of different seed hydration levels (i.e. different seed hydration durations) are still needed to explore how different levels of water content in the seed would affect the physical and chemical properties of different seed species and, in turn, influence seed detectability and acceptability by different species of carabids.

In this study, we examined the impact of different seed hydration levels (i.e. hydration durations) on seed choice responses by carabid beetles. To that end, seeds of *kochia* (*Bassia scoparia*) (L.) A.J. Scott, a common weed in Western Canada characterized by its persistence and herbicide resistance (Kumar et al., 2019), and canola (*Brassica napus*) L., a crop species that frequently persists in the soil after harvest and emerges as a volunteer weed, were subjected to four different hydration durations (i.e., hydration treatments) and then offered to *Pterostichus melanarius* Illiger and *Poecilus corvus* Leconte omnivorous carabids in both multiple-choice feeding and olfactometric bioassays. We hypothesized that increased levels of hydration would progressively increase seed detectability and palatability to carabid predators compared to dry seeds.

MATERIAL AND METHODS

Carabid species

Live adults of *P. melanarius* and *P. corvus* were collected during the summer of 2024 using dry pitfall traps placed in canola and cereal fields at the Kernen Crop Research Farm near Saskatoon, Saskatchewan (52.1508°N, 106.5437°W), and the Aberdeen Crop Research Farm in Aberdeen, Saskatchewan (57.8621°N, 102.8884°W). Both species are considered omnivores, with a body length that ranges between 12–18 mm for the first species, and 9–12 mm for the second species (Lindroth 1961–1969; Larochel & Larivière, 2003). The pitfall traps consisted of two plastic cups (1.5 L; 10 cm height × 8 cm diameter), one acting as a sleeve and was buried into the soil flush with the soil surface, while the other was inserted into the first cup and acted as the actual trap (Spence & Niemelä, 1994). The pitfall traps were enclosed in exclusion cages made of fine wire mesh ($\sigma = 1.1$ cm) to exclude rodents. The traps were emptied every three days, and the carabids captured were transferred to the laboratory for identification and

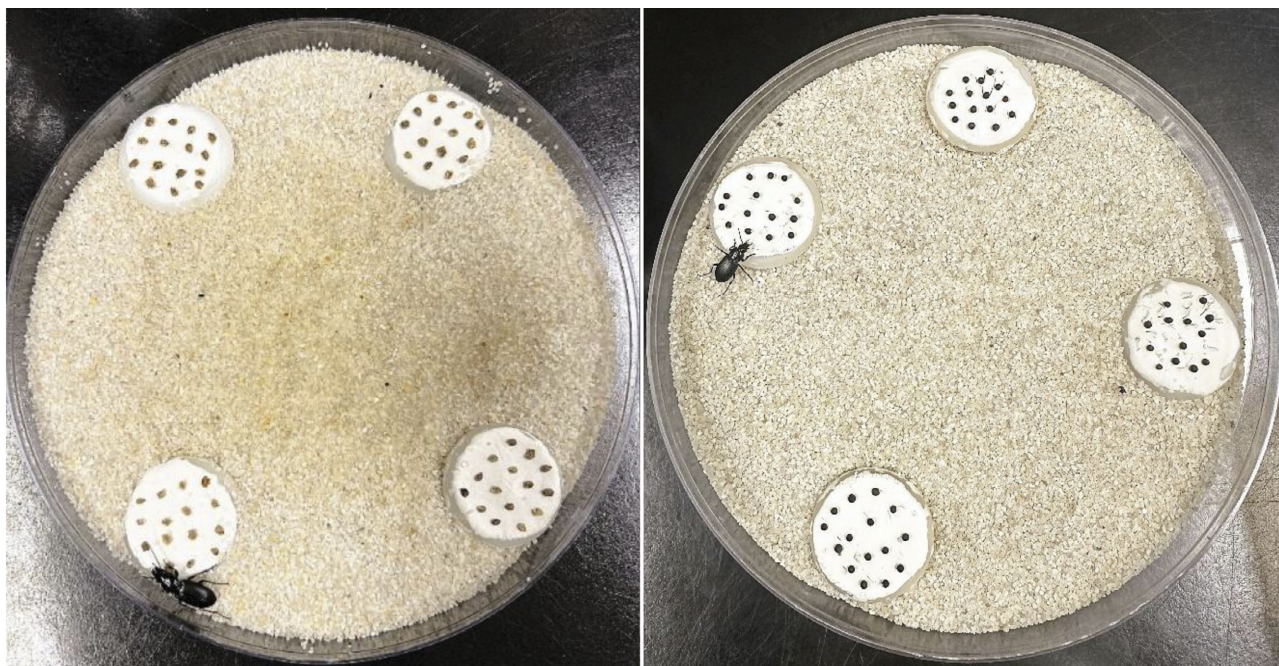


Fig. 1. Feeding arenas containing seeds of kochia (left) and canola (right). These setups were used to evaluate the feeding preferences of *P. melanarius* and *P. corvus* to different seed hydration treatments.

experimentation. Species were identified based on the taxonomic keys in Lindroth (1961–1969).

Seed material

Seeds of kochia (*Bassia scoparia*) and canola (*Brassica napus*) were used in feeding and olfactometric experiments. The seeds of the two species differed markedly in their size, with a mean dry mass between 0.6 and 1.0 mg for kochia (Schwinghamer & Van Acker, 2008) and 3–4 mg for canola (Diepenbrock, 2000). Seeds of both species were hand-harvested from different field locations at the Kernen Research Farm in 2023 and stored at $5 \pm 1^\circ\text{C}$ until use. Kochia was selected due to its importance as a problematic weed in Western Canada, especially the increasing spread of herbicide resistance in this weed species (Beckie et al., 2015; Kumar et al., 2019). Raw kochia seeds, including the full calyx structure, were used in the feeding and olfactometric experiments. Canola was chosen for its significance as a volunteer weed in Western Canada, in addition to this seed species being highly preferable to carabid beetles, as reported by previous studies (e.g. Koprlová et al., 2012; Kulkarni et al., 2016).

Multiple-choice feeding experiments

Prior to experimentation, beetles were starved for 72 h to clear their digestive tracts and standardize their hunger level (Cheeseman & Gillott, 1987; Honěk & Martinková, 2003). During the starvation period, individuals were housed separately in small Petri dishes (Ø 10 cm, 1.5 cm depth), each lined with a moist filter paper and maintained in a growth chamber (Thermo Scientific Precision Model 818, USA) at $20 \pm 1^\circ\text{C}$, 16L : 8D photoperiod, and 55% relative humidity (RH).

The hydration level treatments were carried out by placing seeds from each species in large Petri dishes (Ø 15 cm, 2 cm depth) lined with a filter paper (Fisherbrand, P5, Ø = 15 cm) moistened with 10 mL of distilled water. The seeds were arranged in a single uniform layer, ensuring that all seeds were in direct contact with the fully moist filter paper (excess water was removed) for uniform water absorption. Each dish contained approximately 100 seeds, and this protocol was repeated as many

times as necessary to produce the sufficient hydrated seeds required for each experiment. The exact number of seeds per dish was not estimated, as this was not expected to affect the hydration treatments, since all seeds were hydrated (to different durations) in a single, uniform layer that provided sufficient water for all seeds. Seeds were allowed to absorb water for either 6 h, 24 h, or 96 h in a growth chamber at $5 \pm 1^\circ\text{C}$. This low temperature was adopted to prevent/delay germination during the hydration treatments, and hence, the confounding effects of radicle emergence and nutrient depletion could be minimized. Air-dried seeds (i.e., no hydration 0 h) served as the control in the experiments.

The feeding arenas consisted of large Petri dishes (Ø 15 cm, 2 cm depth) containing 15 seeds of each hydration treatment (0 h, 6 h, 24 h, and 96 h) of a single seed species. Seeds of kochia and canola were presented separately to each carabid beetle species (Fig. 1). The seeds were placed in round plastic trays (Ø 28 mm, 6 mm depth) filled with white plasticine and positioned near the perimeter of the Petri dishes (Honěk et al., 2007; Ali & Willenborg, 2023). Sterilized sand (CaribSea Inc., Canada) was added as a substrate to simulate natural conditions (Ali et al., 2022; De Heij et al., 2023), and a single adult carabid beetle, previously starved for 72 h, was released into each dish.

Beetles were allowed to feed on the seeds for five consecutive days in a growth chamber set at $20 \pm 1^\circ\text{C}$, 16L : 8D photoperiod, and 55% relative humidity (RH). These environmental conditions were selected to maintain both beetle activity and seed moisture, preventing substantial drying or further water uptake by the seed during the experimental period. Therefore, such confounding effects were minimal during the experiment, negating the need to remeasure the seed water content over the 5-day feeding period, as we did not expect it to change significantly under the experimental conditions described above. At the end of the experiment, the beetles were removed, and the remaining seeds were counted to determine seed consumption rates. Seed consumption was expressed as mass (mg) to standardize the number of seeds consumed and account for size differences between canola and kochia. All feeding experiments were replicated 15 times for each carabid and seed species.

Olfactometric experiments

The olfactometric experiments were conducted to determine whether *P. melanarius* and *P. corvus* exhibited behavioral responses to any potential volatile compounds emitted by canola and kochia seeds, both in their dry state and after water uptake. A four-arm olfactometer (Sigma Scientific CADS-4CPP, USA) paired with an EthoVision® tracking system (Noldus, Leesburg, VA, USA) was used to examine the responses of beetles to different odor stimuli. The four-arm olfactometer used in this study was equipped with a Clean Air Delivery System (four valves) and a Century vacuum system (one valve) to maintain controlled air-flow conditions. The pressurized supply air did not exceed 100 PSI, with the system pressure regulated at 15 PSI. The vacuum pressure was maintained at 10 PSI, ensuring natural air movement within the setup. The Clean Air Delivery System was configured to provide 0.5 L/min of airflow per valve, totalling 2 L/min across the four arms. The venturi vacuum system was set at 2 L/min, combining the output of the four positive flow meters. This setup ensured a stable airflow and a homogenous delivery of the odor stimuli through the olfactometric arena (Roberts et al., 2023).

The experiments were conducted under complete darkness, with the olfactometric arena being illuminated only with red light to facilitate the video tracking of beetles. Each beetle in the olfactometer was simultaneously exposed to four different odor treatments. Each odor chamber contained 200 mg of seeds that had been hydrated for 0, 6, 24, or 96 h (i.e., four hydration treatments). After every six experimental runs, the chambers were randomly reassigned for an additional six runs, resulting in an average of 12 trials per day and approximately six trials per carabid species. The central arena was sanitized with 70% ethanol between reassignments to eliminate potential residual scent cues left by previously tested carabid species. At the end of the experiments, the arena was thoroughly rinsed with soap, water, and 70% ethanol before use to remove any residual odors. The glassware was also thoroughly washed with soap and water, followed by three to four rinses with acetone and hexane before sterilizing in a dry oven at 250°C for 6 h.

Each beetle was carefully placed at the center of the olfactometer arena to minimize abrupt movements that could interfere with its natural behavioral responses. Individuals were observed for up to 5 min to allow for acclimation and the onset of exploratory behavior. Trials in which the beetle remained inactive for more than 3 to 5 min were excluded from analysis. Behavioral recording started once the beetle initiated active movement, including walking and antennal movement, indicating the initiation of foraging behavior (Wallin & Ekblom, 1988). The latent period before the start of foraging behavior varied across the beetles used. Thus, no fixed acclimation period was imposed. Instead, behavioral recording was started when the beetle that was released in the olfactometric arena initiated foraging behavior as described above.

Each trial lasted 20 min, during which the software continuously tracked the position and movement of the beetle within and between the defined odor zones relative to the central neutral field. Odor preference was quantified using two metrics: frequency of visits (i.e. number of entries into an odor zone) and the total time spent in each odor zone (in s). From these, the mean duration per visit was calculated by dividing the cumulative duration the beetle spent in an odor zone by the number of entries for each odor zone. A preference response was defined as the greatest amount of time spent in one odor zone compared to the others, indicating a relative preference for that odor source. This allowed for inference of the odor-driven attraction based on the relative time the beetles spent in each odor zone (i.e. hydration treatment).

Other behavioral variables, which provided insight into searching and locomotory responses, were also recorded (data not shown). Velocity was calculated as the mean movement speed (cm/s) for each beetle. The number of alternations, defined as the frequency of transitions between zones, was also extracted directly from the software and used to infer the levels of indecision or exploratory behavior. These metrics allowed an assessment of how the different seed hydration treatments influenced detection, attraction, and exploratory responses by the two carabid species under study.

Estimating seed mass change

The seed mass values were measured for each of the four hydration treatments to assess how seed water uptake influences the physical properties of the seeds. The initial dry mass of seeds was measured using an electronic analytical balance (AD ER-120A), and seeds were reweighed after each hydration period to measure any mass change that could have been induced by moisture absorption. A sample of 50 seeds was weighed for each mass measurement, and 10 replicates were weighed for each hydration treatment per each seed species.

Data analysis

Data analyses were conducted using R version 4.4.3 (R Core Team, 2025). For the feeding arena experiment, the response variable was seed mass consumed (mg). Data were analyzed using linear mixed-effects models (LME), applying the *lme* function in the R package *nlme* (Pinheiro et al., 2025). The maximal model included hydration duration (0, 6, 24, 96 h), seed species (*B. napus*, *B. scoparia*), and carabid species (*P. melanarius*, *P. corvus*) as fixed effects, with replicate included as a random blocking factor to account for spatial nesting within Petri dishes.

Model assumptions were assessed graphically using residuals vs. fitted values and normal QQ plots. Heteroscedasticity was detected in the raw response variable; therefore, seed mass consumed was log-transformed [$\log(x + 1)$] prior to analysis. Post-hoc pairwise comparisons were conducted using estimated marginal means (EMMs) calculated with the *emmeans* package (Lenth, 2020), with Tukey-adjusted comparisons used to evaluate differences among hydration treatments within seed species.

For graphical presentation (Fig. 3), arithmetic means and associated variability were calculated from the original, untransformed observations and plotted on the original measurement scale (mg), whereas statistical analyses and pairwise comparisons were conducted on the $\log(x + 1)$ -transformed response variable.

For the olfactometer experiment, the response variable was preference for odor source (%). As with the feeding experiment, model assumptions were assessed using residual vs. fitted values and normal QQ plots. Diagnostic plots indicated that residuals were approximately normally distributed; therefore, no transformation of the response variable was required.

Post-hoc pairwise contrasts for both experiments were conducted using estimated marginal means (EMMs) calculated with the *emmeans* package in R. These comparisons were used to identify significant differences among treatment levels. Data visualization was performed using *ggplot2* in combination with the *tidyplots* and *multcomp* packages (Hothorn et al., 2008; Wickham, 2016; Yarberry, 2021).

To further investigate the effects of hydration treatments and seed species on seed mass change (%), data were analyzed using a two-way analysis of variance (ANOVA). The response variable was seed mass change (%) measured for canola (*Brassica napus*) and kochia (*Bassia scoparia*) after 0, 6, 24, and 96 h of hydration. Model assumptions were evaluated using diagnostic plots (residuals vs. fitted values and normal QQ plots). Because hetero-

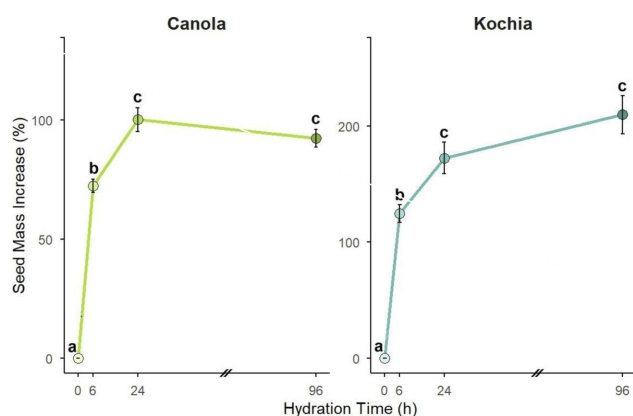


Fig. 2. Mean seed mass increase (%) of canola (*Brassica napus*) and kochia (*Bassia scoparia*) seeds following 0, 6, 24, and 96 h of hydration. Error bars represent $\pm$ standard error of the mean (SEM) calculated from the original observations. Statistical analyses were conducted on $\log(x + 1)$ -transformed seed mass increase data. Different letters indicate significant differences among hydration treatments within each seed species based on Tukey's HSD post hoc comparisons ($\alpha = 0.05$).

scedasticity was detected in the raw data, seed mass change (%) was log-transformed [$\log(x + 1)$] prior analysis.

Hydration duration was treated as a categorical factor with four levels ("0", "6", "24", and "96"), and seed species was included as a fixed factor. Tukey's HSD post-hoc tests were used to identify significant differences among treatment means and was implemented using *multcomp* package (Hothorn et al., 2008). Data summarization and visualization were performed using the R packages *dplyr*, *ggplot2*, and *stringr* (Wickham & Wickham, 2019). For graphical presentation (Fig. 2), arithmetic means and standard errors were calculated from the original, untransformed observations and plotted on the original percentage scale, whereas statistical analyses and post-hoc comparisons were conducted using $\log(x + 1)$ -transformed data.

RESULTS

Seed mass change

A two-way ANOVA conducted on $\log(x + 1)$ -transformed seed mass change (%) detected a significant hydration duration $\times$ seed species interaction ($F_{3,72} = 21.78$, $P < 0.0001$; Fig. 2), indicating that the temporal pattern of water uptake dynamics differed significantly between seed species. Canola showed a rapid increase in mass during early hydration, with mass increasing substantially after 6 h of water uptake, peaking around 24 h, and declining slightly by 96 h. This pattern corresponded to an approximately 100% increase relative to dry seeds, meaning that canola seeds roughly doubled their mass at peak water uptake. In contrast, kochia seeds nearly doubled their mass after 96 h of water uptake.

Multiple-choice feeding experiments

Seed consumption was analyzed using a linear mixed-effects model (LME) fitted to $\log(x + 1)$ -transformed seed consumption (mg) to account for heteroscedasticity. The three-way interaction among hydration duration, seed species, and carabid species was not significant ($F_{3,210} = 0.477$, $P = 0.699$). Among the two-way interactions, only the hydration $\times$ seed interaction was significant ($F_{3,210} =$

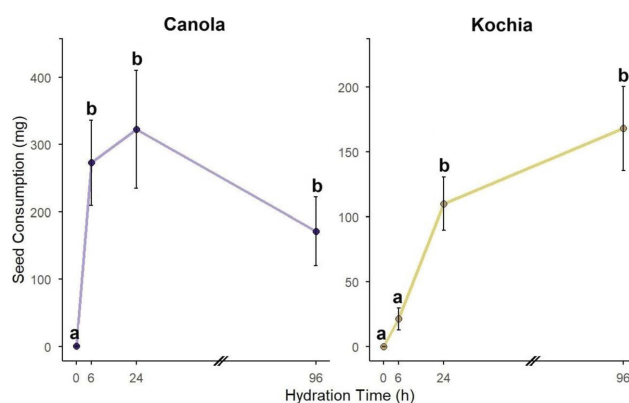


Fig. 3. Mean seed consumption (mg) of canola (*B. napus*) and kochia (*B. scoparia*) seeds following 0, 6, 24, and 96 h of hydration. Error bars represent $\pm$ standard error of the mean (SEM) calculated from the original observations. Statistical analyses were conducted using a linear mixed-effects model fitted to $\log(x + 1)$ -transformed seed consumption data, with replicate included as a random effect. Different letters indicate significant differences among hydration treatments within each seed species based on Tukey-adjusted pairwise comparisons of estimated marginal means derived from the fitted linear mixed-effects model ($\alpha = 0.05$).

4.885, $P = 0.002$, Fig. 3), indicating that hydration influenced consumption differently for canola and kochia seeds. Specifically, consumption of canola increased markedly between 6 and 24 h of hydration, whereas consumption of kochia seeds increased primarily after 24 h. In contrast, the hydration $\times$ carabid interaction ($F_{3,210} = 0.980$, $P = 0.403$) and the seed $\times$ carabid interaction ($F_{1,210} = 0.788$, $P = 0.376$) were not significant. Hydration durations had a significant overall effect on consumption ($F_{3,210} = 22.575$, $P < 0.0001$), and carabid species differed in total seed consumption ($F_{1,210} = 7.488$, $P = 0.006$).

Olfactometric experiments

Olfactometric responses were analyzed using a linear mixed-effects model (LME) and were significantly influenced by interactions. The three-way interaction among hydration durations, seed species, and carabid species was not significant ($F_{3,228} = 2.155$, $P = 0.094$). Among the two-way interactions, only the seed species $\times$ hydration treatment interaction was highly significant ($F_{3,228} = 5.540$, $P = 0.001$), with carabids showing an increasing preference for the odor of hydrated canola seeds, whereas preference for kochia odors declined with longer hydration. The hydration treatment $\times$ carabid species interaction was also significant ($F_{3,228} = 2.873$, $P = 0.037$, Fig. 4), as *P. corvus* showed little change in odor preferences across the different treatments, while *P. melanarius* exhibited a notable decrease in attraction towards hydrated seeds in general, as all hydration treatments beyond 6 h showed reduced preference.

DISCUSSION

The hydration of canola (*B. napus*) and kochia (*B. scoparia*) seeds brought about a substantial increase in seed mass across the different hydration treatments. Although most of the mass increase occurred within the first 24 h of seed hydration, carabid feeding responses seemed to

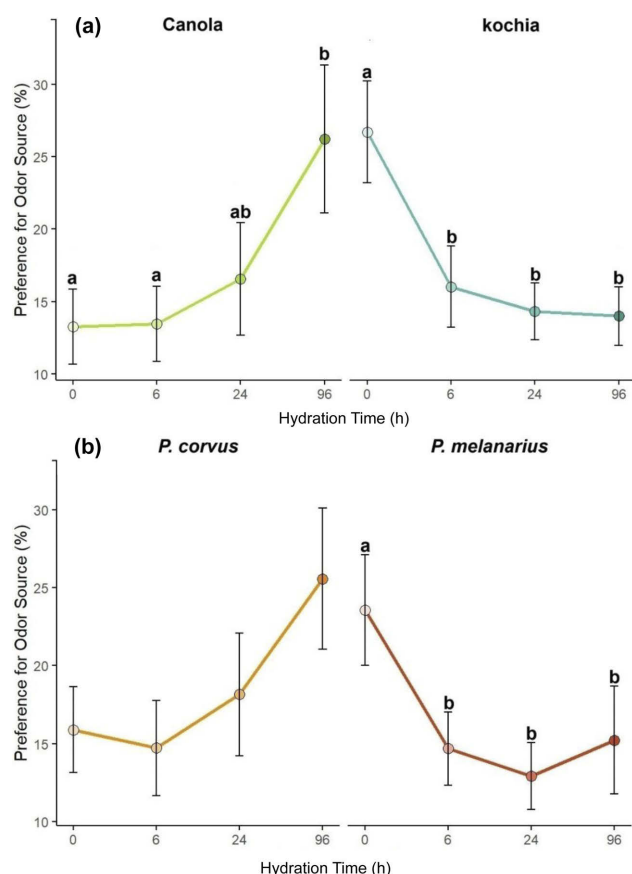


Fig. 4. Mean olfactory preference (%) of two carabid species toward the odors of canola and kochia seeds subjected to four different hydration durations (0, 6, 24, and 96 h). Points represent mean values $\pm$ standard error of the mean (SEM). (a) Interaction between seed species (canola and kochia) and hydration treatment. (b) Interaction between carabid species (*Pterostichus melanarius* and *Poecilus corvus*) and hydration treatment. Different letters above points indicate statistically significant differences among hydration treatments within each panel based on Tukey's HSD test ($\alpha = 0.05$).

change much earlier in the process, as only 6 h of hydration was sufficient to induce a significant increase in seed preference, which was relatively stabilized during the longer hydration durations. This 6 h time period is supposed to correspond with the hydration period during which seeds undergo substantial changes in their physical structure and metabolic activity prior to radicle emergence (Bewley & Black, 1994; Finch-Savage & Bassel, 2016; Upreti et al., 2024; Charalabidis et al., 2025). In other words, within the first few hours of seed hydration, the seed coat and the underlying tissues undergo structural changes, enzymatic activation, and alternation in turgor pressure, which all could reduce seed coat integrity, rendering the seed less costly to handle for carabids and thus, facilitating the extraction of seed nutrients by seed-foraging carabid predators (Woodstock, 1988; Louf et al., 2018; Pompelli et al., 2023). Therefore, even modest hydration seemed to induce sufficient structural changes that influenced how seeds were assessed and handled by carabids.

The hydration dynamics differed strongly between species. However, canola seeds absorbed water rapidly, doubling their mass by 24 h before slightly declining at 96 h.

By contrast, kochia seeds hydrated more slowly but continued absorbing water throughout the 96 h period, ultimately reaching a $\sim 200\%$ mass increase. These species-specific hydration patterns likely reflect differences in seed coat composition, permeability, and internal tissue structure (USDA Forest Service). Such differences determine how quickly seeds transition from a dry, low-profitability state to a hydrated, metabolically active state that seems to be more attractive to carabid predators. This aligns with Hulme & Benkman (2002) and with Foffová et al. (2020b), who found that hydrated seeds were more frequently consumed than dry ones. Other studies have also shown that hydrated seeds are more vulnerable to carabid predation than dry seeds, but the seeds in these studies were hydrated for only 24 h (e.g. Law & Gallagher, 2015; Kulkarni et al., 2016; Foffová et al., 2020a). However, not all studies report consistent effects: Honěk et al. (2007).

The significant increase in seed consumption that occurred after only 6 h of hydration in the current study suggests that the early stages of water uptake by the seed are sufficient to alter the mechanical and potentially the chemical properties of the seed in ways that make the seed more preferable to carabid predators. This could potentially be a sort of “threshold response”; the key palatability-enhancing changes occur within the first 6 h of seed hydration, with any additional hydration providing only a little added benefit. Therefore, short hydration events such as dew or light rainfall may be sufficient to bring about substantial increases in seed predation by carabids; such events can overlap with a high carabid activity in the field (Baraibar et al., 2009; Jonason et al., 2013; Sano et al., 2016). Still, this conclusion is based on testing only two seeds and carabid species. Thus, further research is needed to confirm if this would be the case for other seed and carabid species as well.

Hydration did not seem to enhance olfactory detection in olfactometric experiments. Although water uptake can trigger changes in seed volatile emission (Upreti et al., 2024; Özden et al., 2025), the lack of a significant preference toward hydrated seeds by carabids suggests that hydration did not induce a strong change in volatile emissions in either seed species. The hydration-induced changes in seed volatiles are often species-specific and might depend on lipid composition and the metabolic and biochemical pathways (Bewley et al., 2012; Pompelli et al., 2023). Consequently, seeds with low lipid reserves or lower metabolic activity may show minimal changes in their odor profile even after water uptake (Finch-Savage & Leubner-Metzger, 2006). Work from Ali et al. (2022) has shown that surface hydrocarbons were the main olfactory cues detected by carabids and mediate seed detection and selection. However, those chemicals are too heavy and require the carabid to touch the seed with the antenna to detect them. Therefore, we do not expect seed surface chemicals to play a major role in olfactometric experiments, as the carabid needs to be sufficiently close to the seed to detect such chemicals, and perhaps, this could be one reason behind the lack of effect in olfactometric experiments. Still, the exact mechanism

behind the lack of effect in the olfactometric experiments remains unknown at this point. Thus, it could be proposed that seed hydration in the current study induced physical changes in the seed structure that enhanced seed palatability, without significantly affecting the olfactory seed-derived compounds that mediate detectability.

The olfactometric results also revealed species-specific differences between the two carabid species tested in terms of their behavioral responses to seed hydration in general. *P. melanarius* exhibited a notable decrease in attraction to hydrated seeds, while *P. corvus* showed little change in this regard. However, these odor-mediated responses did not translate into differences in feeding behavior for both species, indicating the differences in seed preferences exhibited by the two carabid species in the feeding experiments were not driven by olfactory cues, but more likely driven by certain changes in the physical characteristics of the seeds as induced by hydration and potentially affecting seed handling costs. Seed handling costs were not quantified directly in the current study, and the impact of seed handling costs on seed choice by carabids remains understudied in the literature.

Based on the current study, it could be concluded that short hydration events, such as dew, light rainfall, or irrigation, could be sufficient to trigger a substantial increase in seed predation pressure against certain vulnerable seeds (Hulme & Benkman, 2002; Saska et al., 2010; Pannwitt, 2019; Lundgren & Anderson, 2023). Short natural hydration events may trigger episodes of intense seed predation events against the hydrated seeds of vulnerable seed species, which are likely to unfold quickly instead of occurring gradually over time as would be the case for dry seeds. But given the magnitude of hydration effects varied across the carabid and seed species tested, it could be predicted that the ecological consequences of seed hydration events in terms of seed vulnerability to carabid predation will most likely depend on the composition of local carabid communities and the composition of the soil seedbank regarding species-specific hydration dynamics of weed seeds. Identifying weed species that become highly vulnerable to carabid predation after brief hydration events could help improve carabid conservation and weed management strategies towards accelerating seedbank depletion (Fischer et al., 2021).

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