

# Mechanisms of Seed Discrimination and Selective Seed Foraging in Carabid Weed Seed Predators

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University of Saskatchewan  
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By  
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## ABSTRACT

Ground (carabid) beetles (Coleoptera: Carabidae) are among the most prevalent biological agents in temperate agroecosystems, with numerous species functioning as predators of both insect pests and weed seeds. Several carabid species are omnivorous, and the diversity and complexity of their feeding habits makes it difficult to predict the magnitude of population suppression they impose on pest and weed species. Thus, it remains unclear why omnivorous carabid beetles choose to feed on seed species when alternative food sources are available. It was first thought that seed feeding in carabids was driven by chance alone and hence, take place upon random encounter. Recent lines of evidence show that carabid predators can be quite choosy or selective about the seeds they consume when seeds of different species are offered in laboratory or field feeding experiments. Seed feeding habits in carabids thus seem driven by active seed discrimination and selection behaviors. Yet, little is known about the sensory, behavioral, nutritional, and biophysical mechanisms that underlie seed preferences in carabid seed predators.

In this thesis I explore the feeding ecology of omnivorous carabid species and describe aspects of the sensory, behavioral, nutritional, and biophysical ecology involved in their seed feeding habits. Multiple-choice feeding bioassays coupled with sensory manipulations of carabid predators showed that carabids rely on their olfactory system to detect seeds of different species and identify the suitable seed species among them. Seed choice was driven by the perception of long chain volatile chemicals derived from the epicuticular lipids located on the seed coat surface. Seed surface volatiles seemed to encode information about the nutritional quality of seed species, especially their fatty acid content. Experiments with synthetic diets showed that omnivorous carabids potentially seek seed consumption to overcome the scarcity of some lipids in their diets.

Carabids most likely choose seed species based on desirable lipid content if the physical properties of seed species in the environment pose no challenges to efficient seed handling. However, if physical seed traits such as mass or size are highly variable among seed species, seed handling costs are likely to vary widely among seed species and thus, constrain the active selection of nutritious seeds. Carabids would more preferably select seed species that are easier to handle in such a case, irrespective of their nutritional quality. The findings of this thesis bridge important knowledge gaps in the seed feeding ecology of carabid species as the sensory basis of seed perception and discrimination is elucidated, and some of the key factors that render seed of certain species more vulnerable to elevated carabid attacks are identified.

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## DEDICATION

*To my beloved parents, Ali and Fadiyah. Thank you for teaching me the power of devotion. I hope this achievement will complete the dream you had for me all those many years ago when you chose to give me the best education you could.*

*To my beloved wife, Safaa. Thank you for teaching me the power of true love. Without you, many stories would have been left untold, and new horizons would have been left unexplored.*

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# CHAPTER 1 Introduction

## 1.1. Introduction

Biological control (biocontrol) is an important service provided by insects in both natural and managed ecosystems (Bengtsson, 2015). In many cases, biocontrol agents (i.e., natural enemies), including insect predators and parasitoids, are endemic in agricultural fields and thus offer free natural control services (Zalucki *et al.*, 2015). In agroecosystems, natural control services help to maintain ecosystem balance and reduce reliance on pesticide inputs, which in turn helps to mitigate pesticide resistance problems (van Lenteren *et al.*, 2018). Reaping such bio-ecological benefits is not an easy task to achieve in modern agroecosystems. To determine and promote the beneficial services that natural enemies provide in the ecosystem, it is essential to understand the basic interactions between insect predators or parasitoids, and the organisms they target in the field (Symondson *et al.*, 2002; Bianchi *et al.*, 2006). One of the first steps is to understand how insect predators and parasitoids perceive their target organisms and assess their suitability, either for feeding or oviposition. Better understanding this will improve our ability to predict the efficacy of these natural biocontrol agents under realistic situations (Symondson *et al.*, 2002; van Nouhuys *et al.*, 2012). Such knowledge becomes even more crucial when the natural agents of interest are generalists or omnivorous, able to feed on or oviposit into a wide array of organisms (Hedlund *et al.*, 1996; Evans *et al.*, 1999). By understanding these core ecological aspects of feeding habits in natural enemies, agroecosystems can be managed to improve the diversity and abundance of insect predators and parasitoids to enhance their ecological functioning in agroecosystems.

Ground (carabid) beetles (Coleoptera: Carabidae) are one of the most important groups of predatory insects in temperate ecosystems, where they function as epigeic polyphagous predators (Lovei and Sunderland, 1996; Kromp, 1999). Carabid activity can be diurnal or nocturnal depending on their species and/or habitat properties; most are generally voracious feeders able to consume close to their body weight of food each day (Luff, 1978; Kromp, 1999; Tuf *et al.*, 2012). The majority of carabid predators are omnivorous and prey on various agricultural pests as well as seeds of annual weeds (Honek *et al.*, 2003; Lundgren, 2009). The diverse feeding habits of carabids place them amongst the most formidable, versatile predators in agroecosystems, where they have been suggested to have the potential to regulate populations of various pests and weeds (Lovei and Sunderland, 1996; Honek *et al.*, 2007, 2009).

Despite the high potential of omnivorous carabids as versatile biocontrol agents in agroecosystems, their complex feeding habits make it difficult to predict their biocontrol efficiency against specific pest or seed species. Seed feeding, in particular, remains relatively poorly understood, as it is unclear why seed feeding habits arose given the abundance of prey in arable fields (Denno and Fagan, 2003; Wilder *et al.*, 2013). Two hypotheses have been proposed to explain carabid seed feeding: 1) omnivorous carabids seek seed consumption only when prey species are scarce or inaccessible; or 2) carabids mix both food types because prey feeding alone is insufficient for survival and development (Lovei and Sunderland, 1996; Saska and Jarosik, 2001). Seed feeding habits actually seem to be more common among carabid taxa than previously thought as seeds are often featured in the diets of a large proportion of carabid species (Fawki *et al.*, 2003; Frei *et al.*, 2019; Carbonne *et al.*, 2020a). Therefore, it is vital to explore the ecology of seed feeding habits in omnivorous carabids, and to elucidate their impact on the ecological functioning of carabids in agroecosystems.

Random seed encounters were assumed to be the main driver of seed feeding and seed feeding behaviors in carabids (Lovei and Sunderland, 1996). However, field and laboratory studies have demonstrated that carabid predators can be choosy or selective about the seeds they consume, often showing an active selection of specific seed species when seeds of different species are accessible (Kulkarni *et al.*, 2016; Saska *et al.*, 2019a,b). Seed species preferences require carabids to discriminate among seeds of different species, assess their suitability, and then identify the seed species most suitable for consumption (Sih and Christensen, 2001). To perform these tasks, carabids need to collect reliable seed-derived information through their different sensory systems (Barron *et al.*, 2015). Our current understanding of the sensory and behavioral mechanisms that underlie discrimination of, and preference for, seeds in carabids remains rather rudimentary (Kulkarni *et al.*, 2015a). It is also difficult, based on our current knowledge, to ascertain the nutritional basis of seed feeding and whether seed consumption replaces or complements prey feeding in omnivorous carabids for fulfilling the needs of survival and development (Saska and Jarosik, 2001; Saska, 2005). These important knowledge gaps make it essential to study the biology and ecology of seed detection and discrimination in omnivorous carabids to better understand the importance of seed feeding habits to the biology and ecology of carabid predators. This is expected to further our understanding of the ecological functioning of omnivorous carabids in agroecosystems in terms of the mechanisms of seed perception and the factors influencing seed

selection decisions. This in turn is likely to help us ascertain the ecological conditions under which seed feeding may become a priority to the survival and reproduction of omnivorous carabid predators.

## **1.2 Research Objectives**

In this thesis, I adopted a mechanistic approach to elucidate aspects of the sensory, behavioral, nutritional, and biophysical ecology of seed feeding habits in carabid seed predators. For this purpose, laboratory experiments were carried out using different species of omnivorous carabid predators and three species of brassicaceous weeds as a model system. Omnivorous carabid predators represent the carabid species that are able to feed on both animal and plant materials, and are usually the major group of carabid predators in temperate agroecosystems (Carbonne *et al.*, 2020a). The main objectives of the study can be outlined as follows:

1. To elucidate the sensory mechanisms that enable carabid seed predators to detect and discriminate among seeds of different species.
2. To isolate and identify the seed-derived sensory cues that guide seed selection decisions in carabid seed predators.
3. To explore the nutritional basis of seed preference in carabid seed predators, and to identify the nutritional factors that may influence the selection of suitable seed species.
4. To explore whether mass-ratio scaling relationships and their biophysical attributes impact seed selection decisions in carabid seed predators.

## **1.3. Overall Outline of the Thesis**

In the first chapter I provide a general introduction to the seed feeding ecology in carabid species and why it matters to the study of biocontrol services provided by carabid predators in agroecosystems. The second chapter offers an extensive review of carabid seed predation literature and a synthesis of the current knowledge around seed predation interactions in a mechanistic context. Knowledge gaps that need further research are also highlighted in the literature review. The third chapter contains an experimental study to uncover the sensory mechanisms of seed



detection and discrimination in omnivorous carabid species. Sensory manipulation techniques coupled with multiple-choice seed feeding bioassays were adopted to determine whether chemoperception could guide seed detection and discrimination in carabids. In the fourth chapter, the seed-derived sensory cues that carabid predators exploit for seed perception and suitability assessment were isolated and identified. The identified seed cues were used in a series of behavioral experiments to test if they could trigger any significant changes in the feeding responses of carabids. In the fifth chapter, synthetic diets of different lipid-to-protein ratios were prepared to decipher which of these two macronutrients was more limiting to nutrient foraging in carabids. Diet quality was also manipulated by reducing protein quality in the diet, or adding seed secondary metabolites to the normal diet. The objective here was to test if the presence of low-quality protein or seed toxins in food sources of carabids would influence their nutrient intake regulation, which in turn might affect seed foraging decisions. In the sixth chapter, the mass-ratio scaling relationships between carabids and seed species were studied to clarify how seed mass and carabid body mass interact, and test whether seed selection decisions in carabids would be mass-based and thus, give rise to size-structured seed predation dynamics as predicted by predator-prey models. Finally, the major findings of this thesis are summarized in the seventh chapter, and the general implications they hold for seed predation ecology and weed biocontrol are discussed. Also, some recommendations for future research studies are given in the seventh chapter.

## CHAPTER 2 The Biology of Seed Discrimination and its Role in Shaping the Foraging Ecology of Carabid Weed Seed Predators<sup>†</sup>

<sup>†</sup>Some sections of this chapter have been published as a review paper: Ali, K.A., and C.J. Willneborg. 2021. The biology of seed discrimination and its role in shaping the foraging ecology of carabids: a review. *Ecology and Evolution*. 11: 13702-13722.

### 2.1. Introduction

Weeds are becoming more difficult to manage in modern agriculture due to the growing number of weed species with high levels of resistance to the major groups of chemical herbicides (Mithila and Godar, 2013; Délye *et al.*, 2015). Non-chemical weed control measures are being sought in light of the challenges posed by herbicide resistance. Historically, invertebrate herbivores have been among the most effective and environmentally safe biological agents adopted for implementing weed biocontrol programs (Gassmann, 1996; Wheeler *et al.*, 2003). In fact, most of the successful weed biocontrol attempts relied on insect species of foliage feeders or stem borers, which were reared and then released to bring weed infestations under control in different parts of the world (Schawrzlander *et al.*, 2018). Weed biocontrol researchers have more recently started to focus more on invertebrate species that feed on the seeds of weedy plants. Seed eaters destroy seeds species and prevent them from entering the soil seed bank and persisting in the field (Gallandt, 2006; Bohan *et al.*, 2011). In so doing, seed eaters contribute to the natural mortality of seed species in agroecosystems and thus, can function as genuine seed predators (Lima, 1998).

Seed predation interactions fall into two main categories: pre-dispersal and post-dispersal (Crawley, 2000; Lundgren, 2009). Pre-dispersal seed predation, generally speaking, is largely carried out by specialist species usually belonging to insect orders of Diptera, Lepidoptera, Hymenoptera, and Coleoptera (Kolb *et al.*, 2007). By and large, adults of these specialized insects oviposit on seed heads or seed pods of weedy plants, and the hatching larvae then attack and destroy the seeds before dispersal (Crawly, 2000; DeSousa and Swanton, 2003). Data documenting the magnitude of pre-dispersal seed predation pressure and how effective it could be in bio-regulating weed populations remain inconsistent and difficult to generalize (Kolb *et al.*, 2007). Some studies, for instance, have shown that pre-dispersal weed seed predation could significantly depress the seedling recruitment of some shrubs and perennials (Anderson, 1988; Moles *et al.*,

2003). Studies on annual weeds have revealed that larvae of the specialized micro-lepidopterans *Coleophora lineapuvella* (Chambers) and *Scrobipalpa atriplicella* (Fischer von Röslerstamm) could destroy large numbers of the seed heads of *Amaranthus retroflexus* L. and *Chenopodium album* L., respectively, in corn and soybean fields in Eastern Canada (Nurse *et al.*, 2003). However, the considerable seed-destructive effects of those pre-dispersal predators were sporadic, and were observed only in certain cases. In this regard, the overall pressure of pre-dispersal seed predation remained rather low over the season, showing high variability across locations and years (DeSousa and Swanton, 2003; Nurse *et al.*, 2003). Ultimately, these findings have led the authors to suggest that pre-dispersal weed seed predation alone is unlikely to bring about significant suppression against populations of annual weeds in arable fields. By contrast, the presence of the gall midge *Clinodiplosis cilicrus* (Kieffer) larvae in the flower heads of *Centaurea cyanus* L. was found to be associated with a four-fold reduction in seed numbers, and an approximately 40% drop in seed viability in a field study conducted in France (Koprdoва *et al.*, 2015). This has led authors to suggest that *C. cilicrus* larvae can potentially depress populations of *C. cyanus* in the field. However, the presence of gall midge larvae was not associated with visible seed damage but rather, reduced ovule fertilization. Therefore, seed reduction effects in such cases cannot be attributed to genuine seed predation, but rather to fertilization disruption by consuming resources necessary for successful fertilization or repelling pollinators (Kolb *et al.*, 2007; Koprdoва *et al.*, 2015).

Post-dispersal weed seed predation, on the other hand, is carried out by a wide range of seed predators that span both vertebrate and invertebrate taxa (Moles *et al.*, 2003; White *et al.*, 2007). By and large, arable fields are richly populated with invertebrates that exhibit post-dispersal weed seed feeding habits (Lundgren, 2009). Species of ground (carabid) beetles (Coleoptera: Carabidae), crickets (Orthoptera: Gryllidae), ants (Hymenoptera: Formicidae), and slugs (Mollusca: Gastopoda) are usually among the main post-dispersal weed seed predators in temperate arable fields (Lundgren and Harwood, 2012; van der Laat *et al.*, 2015). Slugs remain the least studied group, and laboratory studies have reported that slugs can consume seeds of certain weed species (Cardina *et al.*, 1996). But data from field studies suggest that the contribution of slugs to post-dispersal weed seed predation is most likely minor (Dudenhoffer *et al.*, 2016). Ants, by contrast, engage in a wide range of ecological interactions with seeds species, spanning from mutualism to antagonism (Gammans *et al.*, 2005). The mutualistic interactions relate to ant species that function as seed dispersers, whereas the antagonistic actions are usually undertaken

by ant species that forage for seeds to feed their colonies (Fischer *et al.*, 2008). In fact, the borderline between seed dispersal and seed predation is indistinct because numerous ant species function as both seed dispersers and seed predators (Ohara and Higashi, 1987). A prominent example in this regard is harvester ants, which have been found to remove large numbers of weed seeds from arable fields in some regions of Europe (Westerman *et al.*, 2012; Torra *et al.*, 2016). Harvester ants as such are likely to significantly contribute to the post-dispersal removal of weed seeds from arable fields, delivering valuable weed biocontrol services in agroecosystems. Still, this distinctive role undertaken by harvester ants remains limited to warm and dry regions, largely because carabid activity is rather low in these regions and carabid predators cannot compete with harvester ants for weed seeds (Sasakawa, 2010; Evans and Gleeson, 2016). This leaves carabid beetles and crickets as the two dominant post-dispersal seed predatory groups in temperate regions (Carmona *et al.*, 1999; Lundgren *et al.*, 2013). The role of crickets as post-dispersal weed seed predators of annual weeds is well documented, but they are difficult to trap and not widely studied (White *et al.*, 2007; Lundgren, 2009). By contrast, carabid beetles are widely distributed, easy to trap, and show a general high species richness in arable fields (Irmiler, 2003; Gaines and Gratton, 2010). Therefore, they are the most extensively studied group of invertebrate post-dispersal weed seed predators and thus, are the focus of this thesis.

## **2.2. Weed Seed Consumption by Carabid Beetles**

Carabid (ground) beetles, generally speaking, function as epigaeic polyphagous predators in agroecosystems (Lovei and Sunderland, 1996). Adults of carabid species can show diurnal or nocturnal activities depending on their species and/or habitat properties, and most are voracious feeders, often able to consume close to their body weight of food each day (Luff, 1978; Kromp, 1999; Tuf *et al.*, 2012). Predacious carabids prey upon a wide array of agricultural pests including aphids (Firlej *et al.*, 2013), lepidopteran caterpillars (Clark *et al.*, 1994; Suenaga and Hamamura, 1998), dipteran eggs and midges (Floate *et al.*, 1990), wireworms (Barsics *et al.*, 2013), slugs (Oberholzer *et al.*, 2003), and non-pest species such as springtails and earthworms (King *et al.*, 2010; Seric Jelaska *et al.*, 2014). In addition, numerous species of carabid predators are known to feed on seeds of weed species after seeds are shed (Lundgren, 2009; Kulkarni *et al.*, 2015a; Carbonne *et al.*, 2020a). Some species of *Harpalus* sp. and *Amara* sp. can even attack weed seeds

on the mother plant prior to seed shed (Sasakawa, 2010a). Data from field studies have shown that carabid beetles in some cases are responsible for removing large numbers of weed seeds from arable fields each year (see Table 1.1 for a summary of a selection of studies documenting estimates of post-dispersal removal rates of weed seeds by carabid beetles and other invertebrate groups). Overall, the seed predation literature promotes carabid beetles as effective natural agents capable of destroying large numbers of weed seeds in arable fields (Bohan *et al.*, 2011; Carbonne *et al.*, 2020a). Such elevated seed mortality pressures imposed by carabid seed predators are likely to bring about considerable disruption in abundance, distribution, and demography of weed communities in arable fields (Janzen, 1971; Jermeý, 1984; Davis *et al.*, 2011).

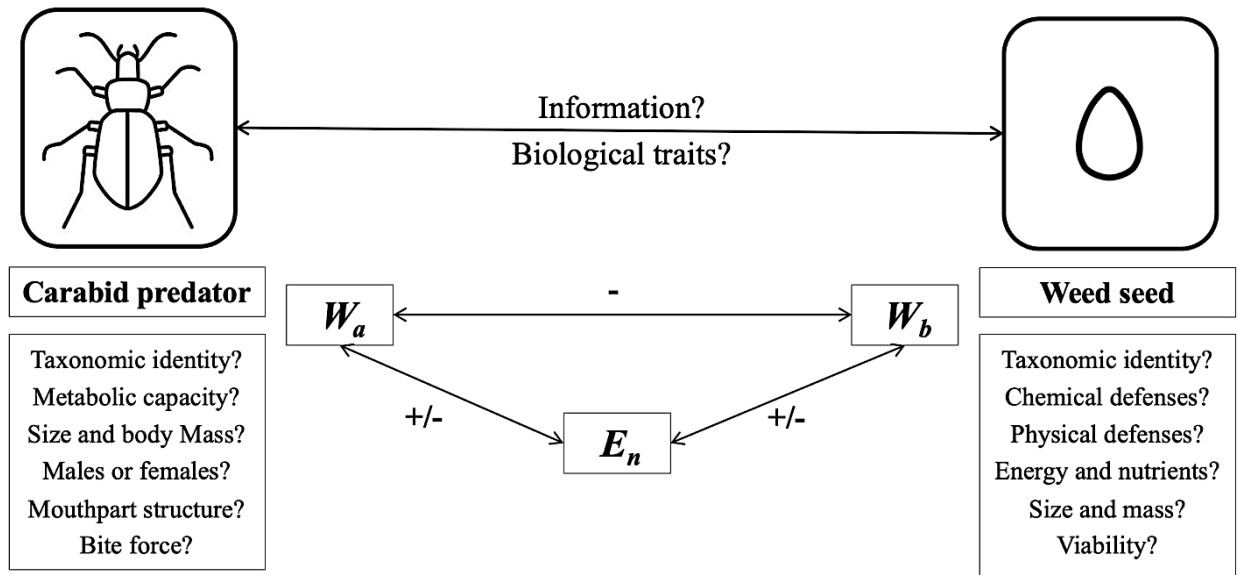
Larval carabids, on the other hand, are also predaceous but their feeding ecology remains poorly studied. Based on the evidence to date, carabid larvae seem to feature seed feeding habits similar to adults (Sasakawa, 2010b; Sasakawa *et al.*, 2010). For instance, larvae of *Amara* sp. and *Harpalus* sp. were reported to consume large numbers of weed seeds in laboratory feeding bioassays (Saska and Jarosik, 2001; Saska, 2015). Weed seed consumption by larval carabids could reach fairly high levels in some cases, similar in number to levels reported for adults (Klimes and Saska, 2010). Such findings are intriguing but should be approached with caution as they were observed in laboratory feeding experiments only. There have been no direct measurements of weed seed removal rates by larval carabids in the field to date.

It remains unclear why carabid beetles choose to consume weed seeds or how seed feeding preferences evolve given the abundance and diversity of prey carabids have access to in arable fields (Booij *et al.*, 1994; Lovei and Sunderland, 1996). These two big questions have surrounded seed predation ecology for a long time, spurring a lot of speculation without reaching definitive conclusions (see Figure 1.1). Traditionally, seed feeding habits in carabid beetles were considered opportunistic and occur in carabids that belong in two main trophic guilds; omnivores and granivores (Fawki and Toft, 2005; Talarico *et al.*, 2016). This dichotomous grouping suggests that seeds are not primary to the diets of omnivorous carabids, and are mainly consumed upon random encounter rather than carabids foraging for seeds specifically (Lovei and Sunderland, 1996; Cardina *et al.*, 1996). Studies on seed feeding habits have revealed that the distinction between omnivory and granivory in carabid species is ambiguous, however, as the two feeding habits often overlap (Fawki *et al.*, 2003; Fawki and Toft, 2005). For example, some carabid species originally proposed to feed strictly on animal prey were found to also include considerable

amounts of weed seeds in their diets (Carbonne *et al.*, 2020a). Similarly, multiple species of carabids within the genera *Amara* sp. and *Harpalus* sp. previously were assumed to subsist on diets composed mainly of seeds. However, many of these species turned out to include nontrivial amounts of animal prey in their diets as well (Loughride and Luff, 1983; Frank and Brambock, 2016). Strict granivory overall is quite rare among carabids, and carabid species that feed on seeds exclusively are found in only a few genera such as *Amara* sp., *Ophonus* sp., *Ditomus* sp., *Dixus* sp., and *Carterus* sp. (Saska, 2015; Talarico *et al.*, 2016). Together, these findings indicate that the most seed-eating carabid species belong in the mixed-feeding omnivorous group, whereas true granivory remains scarce. Molecular gut content analyses of carabid species spanning both mixed-feeders (omnivores) and specialized seed feeders (granivores) collected from multiple field sites in Europe found high levels (>70%) of seed DNA from weedy species in the guts of both groups (Frei *et al.*, 2019). The significant presence of weed seed DNA in guts of mixed-feeding carabids contradicts the opportunistic seed predation reasoning introduced in the early carabid literature. Instead, seed foraging behaviors in carabid seed predators are more likely driven by specific biological needs that are expected to influence predation dynamics in specific ways (Headrick and Goeden, 2001; Davis *et al.*, 2011; Carbonne *et al.*, 2020a).

The kind of biological needs that are likely to underlie seed feeding habits and the choice of suitable seeds in carabids remain unclear. Multiple attempts to uncover the key ecological factors that render seeds of specific weed species more likely to incur elevated carabid attacks in arable fields have not been fruitful thus far (Kulkarni *et al.*, 2015a). Large-scale weed seed predation studies show that predation pressures fluctuate considerably in space and time, and do not follow general trends or patterns (Hulme, 1998; Honek *et al.*, 2003; Hatton *et al.*, 2005; Jacob *et al.*, 2006; Kulkarni *et al.*, 2017a). Although some correlations between carabid activity-density and regulation of soil seedbanks could be established in a few large-scale studies (Bohan *et al.*, 2011; Carbonne *et al.*, 2020a), such relationships were absent in several small-scale studies (Saska *et al.*, 2008; Petit *et al.*, 2014). Such mixed results, in addition to the correlational nature of the evidence, preclude establishing a general and definitive functional link between activity-density of seed predatory carabids and the regulatory pressures they impose on soil seedbanks. Mechanistic studies would be more informative in this regard, with a better ability to elucidate how and why weed seeds are attacked by carabid predators. Studies carried out within the mechanistic paradigm would help to tease out the key factors that underlie correlational

relationships in the seed predation literature. They would also help to bridge the knowledge gaps that remain in weed seed predation ecology (van Regenmortel, 2004; Dean and Thornton, 2007; Baskett, 2012). Therefore, in addition to studying seed predation ecology on large scales in the field, seed predation should also be studied on small scales and at mechanistic levels. This way, numerous confounding factors could be filtered out, and pseudo-replication issues could be avoided to uncover the functional aspects of weed seed predation interactions. This would further allow one to identify the ecological factors that determine interaction strength and power predation dynamics (Hurlbert, 1984; Davis *et al.*, 2015). Uncovering the mechanistic aspects of seed predation ecology is likely to improve the design and conduct of weed seed predation studies on larger scales (Denny and Benedett-Cecchi, 2012).



**Figure 2.1.** A hypothetical ecological matrix illustrating the possible ecological factors that are likely to influence weed seed predation dynamics in agroecosystems - question marks indicate a possible but not widely understood role for the proposed factor. ( $W_a$ ): fitness of the carabid predator; ( $W_b$ ): fitness of the weed species; ( $E_n$ ): various biotic and abiotic environmental factors that can possibly influence predation dynamics (adopted from Ali and Willenborg, 2021).

**Table 2.1.** A selection of studies summarizing field data of seed removal rates of some weed species from arable fields by post-dispersal weed seed predators (adopted from Ali and Willenborg, 2021).

Weed species	Average seed removal rates	Study duration	Seed predatory group	Crop	References
<i>Ambrosia artemisifolia</i> L., <i>Amaranthus retroflexus</i> L., <i>Casia obtusifolia</i> L., <i>Datura stramonium</i> L.	4.2-4.8% day <sup>-1</sup>	5 weeks	Carabid beetles, crickets, and ants	Corn and soybean	Brust and House, 1990
<i>Alopecurus myosuroides</i> Huds., <i>Bromus sterilis</i> L., <i>Avena fatua</i> L.	1.43-7.2% day <sup>-1</sup>	1 month	Invertebrates	Grassy margins of cereal fields	Povey <i>et al.</i> , 1993
<i>Chenopodium album</i> L., <i>Echinochloa crusgalli</i> (L.) Beauv.	22-28% day <sup>-1</sup>	2 years	Invertebrates	Corn, soybean, and wheat	Cromar <i>et al.</i> , 1999
<i>Digitaria sanguinalis</i> (L.) Scop.	11% day <sup>-1</sup>	2 weeks	Invertebrates	Corn	Menalled <i>et al.</i> , 2000
<i>Setaria faberi</i> Herm.	58% 2-days <sup>-1</sup>	3 months	Carabid beetles and crickets	Wheat with red clover	Davis and Liebman, 2003
<i>Ambrosia trifida</i> L.	57-70% year <sup>-1</sup>	12 months	Carabid beetles	Corn (no-till)	Harrison <i>et al.</i> , 2003
<i>Stellaria media</i> L., <i>C. album</i> , <i>A. fatua</i>	38-74% year <sup>-1</sup>	2 years	Carabid beetles and mice	Organic cereal fields	Westerman <i>et al.</i> , 2003
<i>Abutilon theophrasti</i> Medik.	17% 2-days <sup>-1</sup>	2 years	Carabid beetles, crickets, and prairie deer mice	Corn and soybean	Westerman <i>et al.</i> , 2005
<i>A. theophrasti</i>	32% 2-days <sup>-1</sup>	2 years	Carabid beetles, crickets, and prairie deer mice	Corn, soybean, triticale, and alfalfa-alfalfa	Westerman <i>et al.</i> , 2005
<i>Polygonum aviculare</i> L., <i>Sinapis arvensis</i> L., <i>S. media</i> , <i>C. album</i>	35% week <sup>-1</sup>	2 weeks	Carabid beetles	Spring barley	Mauchline <i>et al.</i> , 2005
<i>Setaria faberi</i> Herm., <i>A. theophrasti</i>	16-30% day <sup>-1</sup>	4 months	Invertebrates	Cereals and legumes	Heggenstaller <i>et al.</i> , 2006
<i>Lolium rigidum</i> Gaudin	57% 2-weeks <sup>-1</sup>	3 months	Ants and invertebrates	Postharvest cropping field	Jacob <i>et al.</i> , 2006
<i>A. fatua</i>	42% 2-weeks <sup>-1</sup>	3 months	Ants and invertebrates	Postharvest cropping field	Jacob <i>et al.</i> , 2006



<i>Raphanus raphanistrum</i> L.	45% 2-weeks <sup>-1</sup>	3 months	Ants and invertebrates	Postharvest cropping field	Jacob <i>et al.</i> , 2006
<i>Panicum dichotomiflorum</i> Mchx., <i>C. album</i>	10-90% day <sup>-1</sup>	3 months	Carabid beetles	Corn (organic field)	Menalled <i>et al.</i> , 2007
<i>Taraxacum officinale</i> Weber	34-40% year <sup>-1</sup>	2 years	Carabid beetles and isopods	Grassland	Honek <i>et al.</i> , 2009
<i>S. faberi</i> , <i>A. trifida</i> , <i>A. theophrasti</i>	11% day <sup>-1</sup>	4 weeks	Carabid beetles	Potato	Gaines and Grandon, 2010
<i>Amaranthus retroflexus</i> L.	5% day <sup>-1</sup>	4 months	Carabid beetles	Potato	Gaines and Grandon, 2010
<i>Lolium multiflorum</i> Lam.	11.5-29.8% 18-days <sup>-1</sup>	18 days	Carabid beetles	Winter wheat	Baraibar <i>et al.</i> , 2011a
<i>Avena ludoviciana</i> Durieu	33-63% 6-weeks <sup>-1</sup>	6 weeks	Invertebrates	Barley	Noroozi <i>et al.</i> , 2012
<i>Hordeum spontaneum</i> (K. Koch) Thell.	27-33% 6-weeks <sup>-1</sup>	6 weeks	Invertebrates	Barley	Noroozi <i>et al.</i> , 2012
<i>Galium spurium</i> L.	60-70% 2-days <sup>-1</sup>	2 years	Harvester ants <i>Messor barbarus</i> L.	Dryland cereals	Westerman <i>et al.</i> , 2012
<i>Lolium rigidum</i> Gaud	90-100% 2-days <sup>-1</sup>	2 years	Harvester ants <i>M. barbarus</i>	Dryland cereals	Westerman <i>et al.</i> , 2012
<i>Papaver rhoeas</i> L.	90% 2-days <sup>-1</sup>	2 years	Harvester ants <i>M. barbarus</i>	Dryland cereals	Westerman <i>et al.</i> , 2012
<i>Bromus diandrus</i> Roth	20% 2-days <sup>-1</sup>	2 years	Harvester ants <i>M. barbarus</i>	Dryland cereals	Westerman <i>et al.</i> , 2012
<i>Viola arvensis</i> Mur., <i>Capsella bursa-pastoris</i> (L.) Medik., <i>A. myosuroides</i>	30% week <sup>-1</sup>	5 weeks	Carabid beetles	Winter cereals	Trichard <i>et al.</i> , 2013
<i>C. album</i>	53-65% 2-days <sup>-1</sup>	3 months	Carabid beetles and crickets	Corn and soybean	van der Laat <i>et al.</i> , 2015
<i>Amaranthus tuberculatus</i> (Moq.)	80-85% 2-days <sup>-1</sup>	3 months	Carabid beetles and crickets	Corn and soybean	van der Laat <i>et al.</i> , 2015
<i>Brassica napus</i> L.	42.3-69.7% week <sup>-1</sup>	3 weeks	Carabid beetles	Canola	Kulkarni <i>et al.</i> , 2016
<i>S. arvensis</i>	41-58.9% week <sup>-1</sup>	3 weeks	Carabid beetles	Canola	Kulkarni <i>et al.</i> , 2016
<i>Thlaspi arvense</i> L.	16.2-28.3% week <sup>-1</sup>	3 weeks	Carabid beetles	Canola	Kulkarni <i>et al.</i> , 2016
<i>Avena sativa</i> L. (used as a substitute for weed seeds)	78-100% day <sup>-1</sup>	3 weeks	Harvester ants <i>M. barbarus</i>	Dryland cereals	Torra <i>et al.</i> , 2016

## 2.3. The Ecological Processes of Post-Dispersal Weed Seed Predation by Carabid Beetles

### 2.3.1. *Seed and Carabid Predator Co-occurrence in Space and Time*

Seed predation requires synchronicity between seed availability and predators' activities both in space and time. Seeds of annual weeds are usually shed into arable fields at certain times, and can persist in the seedbank for extended periods (Baskin and Baskin, 1998). Hence, the window of seed availability for post-dispersal carabid weed seed predators could be considerably wide in time (Meiners, 2015). This would seem to suggest that finding weed seeds in time should not be an exacting task for carabid weed seed predators.

By contrast, the spatial overlap between weed seed shed (i.e., the seed shadow) and carabids is more complex. Some of the early carabid literature had proposed that carabid weed seed predators locate weed seeds upon random encounter (Lovei and Sunderland, 1996; Cardina *et al.*, 1996). That is, if carabid beetles came across a weed seed, they would simply consume it. Beyond that, they would not spend considerable time and energy foraging for weed seeds that are less nutritious and more difficult to locate compared to prey (Kolb *et al.*, 2007). I suggest that such assumptions in the literature have originated from the old 'no-choice' laboratory feeding bioassays, where carabids would accept the majority of food types offered to them (e.g., Shough, 1940). Such observations led carabid ecologists to assume that carabid species were scavengers or opportunistic feeders for the most part (Forsythe, 1982; Wheater, 1989). This idea still transcends the modern carabid literature, nonetheless. Recent evidence now points to random encounters as the exception rather than the rule, at least for carabid weed seed predators (Ward *et al.*, 2014; Saska *et al.*, 2019b). The emerging evidence demonstrates that carabid beetles are likely to employ active foraging behaviors in search of weed seeds (Honek *et al.*, 2007; Lundgren and Harwood, 2012). Carabid species in other words not only locate weed seeds in space, they also show an ability to discriminate among seeds of different species and choose the most desired (Kulkarni *et al.*, 2016). Therefore, finding seed species in space entails multiple exacting tasks for the foraging carabid beetle, especially regarding seed detection and location.

### 2.3.2. Seed Detection and Location

Interactions between insects and plants that lead to weed seed predation should be viewed as a special case of plant-insect interactions (Jermy, 1984). The main difference here, though, is that the insects attack the reproductive units of the plant rather than its somatic tissues (McArt, 2013). It is well established that herbivorous insects rely on various types of visual and/or chemical cues to locate their host plants and assess their quality as sources of food (Bruce *et al.*, 2005; Bruce, 2015). Chemical cues, such as volatile and non-volatile compounds, have been proven to act as major drivers of host selection decisions in plant-feeding insects (Eigenbrode and Esplie, 1995; Baldwin, 2010; Bruce and Pickett, 2011). By the same token, carabid weed seed predators are expected to rely on similar cue-based systems for seed location and suitability assessment (Kielty *et al.*, 1996; Merivee, 2007, 2008).

Post-dispersal weed seed predators have to search for seeds that are imbedded in complex, often cryptic environments (Forister *et al.*, 2012; Aartsma *et al.*, 2019). In other words, seed searching for carabid seed predators is a multi-layered behavioral process fraught with challenges. Seed abundance is highly variable in both space and time (Henderson, 1990; Dessaint, 1991). Moreover, seeds are small in size and randomly scattered on the soil surface, or even buried underneath it (White *et al.*, 2007; Menalled *et al.*, 2007). This essentially renders any reliable cues for seed location and suitability assessment sparse and difficult to detect (Baldwin, 2010; Poisot *et al.*, 2011). Unlike random encounters, active foraging behaviors require the foraging animal to have highly developed motor abilities coupled with sensitive sensory systems for picking up and deciphering cues of low detectability (Meiners, 2015; Aartsma *et al.*, 2019). This assumption has been borne out as carabids have been shown to possess highly developed motor abilities along with a formidable arsenal of sensory receptors to guide their behaviors and food choice responses (Forsythe, 1983a; Merivee *et al.*, 2000-2008). Behavioral data overall indicate that food searching and food choice in carabid beetles, be that seed or prey, is an active and directed process guided by accurate sensory information collected from the environment (White *et al.*, 2007; Harrison and Gallandt, 2012; Kulkarni *et al.*, 2015b). I would suggest that carabid predators should be viewed as active and selective prey and seed foragers, clearly able to decipher between food types irrespective of random encounters.

### 2.3.3. *Discrimination Among Different Seed Species and Identifying the Suitable Seed Species*

The logic of optimal foraging theory assumes that food recognition and acceptance in selective foragers is guided by ‘search images’ hardwired in the brain or learned from the environment (Krier and Breer, 1999; Garay *et al.*, 2018). Technically speaking, search images could be visual, chemical, or complex (visual and chemical) (Vet and Dicke, 1992). Carabid beetles as selective foragers are expected to employ search images to guide their food discrimination and selection decisions so that the most suitable food types are chosen for consumption. Active food selection processes need accurate sensory information, and carabid species are thus expected to use their different sensory modalities (i.e., vision, olfaction, and gustation) to sample their environments in search of the sensory information necessary for food detection and selection (Ramaswamy, 1988; Ploomi *et al.*, 2003). Technically, gustation could be considered a special case of olfaction because olfactory and gustatory receptors show similar physiological structure and function, and collect chemical information of similar nature (Krier and Breer, 1999; Isono and Morita, 2010). Henceforth, both smell and taste in carabid beetles will be treated as chemoperception in sections that follow.

It is still uncertain which sensory modality (vision or chemoperception) carabid species rely upon most to guide their food location and selection behaviors. Despite this uncertainty however, the evidence strongly hints that chemoperception is likely the top candidate for roles related to food searching and food choice in carabid predators (Wheater, 1989; Oster *et al.*, 2004; Law and Gallagher, 2015). It can be proposed, therefore, that food selection decisions in carabid beetles, be that seed or prey, are generally guided by chemical cues encoding information that is interpreted based on innate or learned ‘templates’ or ‘images’ in the brain (Vet and Dicke, 1992; Oster *et al.*, 2004). As such, the chemical cues emitted from different species of weed seeds in arable fields are expected to mediate seed recognition and seed selection in carabid seed predators (Lundgren *et al.*, 2013; Law and Gallagher, 2015; Kulkarni *et al.*, 2017b). However, given the limited number of sensory studies and the highly diverse feeding habits of carabid species in arable fields, it cannot be ruled out that vision may also play some role in guiding seed selection decisions, leading to the formation of complex search images in some cases (Wheater, 1989; Ducas and Kamil, 2001).

The uncertainty around the sensory basis of seed detection and discrimination stems from reports showing diurnal carabid species, which usually actively hunt live mobile prey, often carry larger compound eyes and shorter antennae that house significantly fewer olfactory receptors compared to nocturnal species (Bauer and Kredler, 1993; Merivee *et al.*, 2001, 2002). Such sensory differences seem to suggest that visual cues are likely to play more vital roles in guiding food searching behaviors in diurnal carabids compared to nocturnal ones (Wheater, 1989). But the same studies also showed that visually-driven hunting behaviors in carabid predators broke down when prey items were immobilized. Therefore, it could be suggested that carabid visual receptors are more finely-tuned towards detecting movement and are not expected to be of much help when carabid predators are searching for immobile and often cryptic food items like seed species (Srinivasan *et al.*, 1999; Oster *et al.*, 2004). Still, a possible role for vision in seed foraging among carabid seed predators cannot be decisively ruled out based on our current knowledge, and more studies are warranted.

Evidence in support of chemically-mediated food detection, by contrast, is more abundant and comes from sensory and behavioral data pertaining to carnivorous and omnivorous carabids. Some carabid carnivores, for instance, showed positive orientation towards volatile chemicals originating from prey habitat, as well as volatile chemicals specific to prey itself or to its pheromones (Keilty *et al.*, 1996; Mundy *et al.*, 2000; Tréfás *et al.*, 2011). Moreover, electroantennographic detection (EAD) studies have shown that antennal preparation of *Pterostichus melanarius* Illiger adults could respond to odor chemicals originating from live or dead slugs, or even from the slug trails (McKemey *et al.*, 2004). Similarly, antennal preparations of *P. melanarius* larvae produced detectable electrical signals when exposed to chemical odors of different prey species (Thomas *et al.*, 2008). Strong electrical signals were likewise recorded from labial palps of the ant-specialist carabid *Siagona europaea* Dejean when exposed to formic and acetic acid secretions from its ant prey (Talarico *et al.*, 2010).

Laboratory studies have also reported that the seed eating carabid *Harpalus pensylvanicus* (Degeer) exhibited positive olfactory orientation towards weed seed odors in olfactometric bioassays (Law and Gallagher, 2015). Similar positive orientation responses towards seed odors of different brassicaceous weed species were also reported for *Harpalus affinis* Schrank, *Amara littoralis* Dejean, and *P. melanarius* when seed masses were placed into the odor chambers of a four-arm olfactometer (Kulkarni *et al.*, 2017b). Carabids used in the experiment spent significantly

more time in odor arms harboring the highly preferable weed species (i.e., *Brassica napus* L. seeds in this case), indicating that they were able to discriminate among the seed species offered in the olfactometer based on seed odor alone. Moreover, the species of carabid weed seed predators were able to excavate weed seeds buried down to 1 cm below the soil surface, without considerable loss in seed-finding efficiency (White *et al.*, 2007; Kulkarni *et al.*, 2015b). The efficiency for excavating seeds that were buried at the same depth differed between the carabid species under study, yet seeds were detected and dug out in most cases. It is plausible to assume that differences in seed excavation efficiency were due to species-specific differences in soil-burrowing responses among the tested carabids, not the absence of visual cues due to seed burial (Evans and Forsythe, 1985; Wallin and Ekblom, 1988). While there may be some role for vision in seed foraging, I suggest that any such role is likely minor given the findings of seed burial experiments. Overall, evidence to date clearly suggests that chemoperception is likely the primary mechanism guiding seed finding and seed selection responses in carabid weed seed predators.

It is crucial to reiterate that carabid predators forage in complex environments. Weed seeds are imbedded in an intricate matrix of environmental variables that could influence the foraging decisions of carabid predators in unpredictable ways (Sarabi, 2019, De Heij and Willenborg, 2020). Under such conditions, there will be certain cases where predicting the suitable seed species based on chemical cues alone fails to explain the observed seed selection responses of carabid weed seed predators (Foffova *et al.*, 2020a). Factors relating to habitat properties, chemical and physical seed traits, composition of the local carabid community, fear, and effects of learning and experience could all influence the processes of seed selection decision-making, or perhaps override it altogether (Ishii and Shimada, 2010; De Heij and Willenborg, 2020). Overlooking such influential ecological and environmental factors is likely one main reason why field data involving seed predation remain ambiguous (e.g., Honek *et al.*, 2003; Petit *et al.*, 2014). Therefore, it is likely that weed seed selection by carabid weed seed predators entails complex and multi-layered processes that are unlikely to be explained by chemoperception-based decisions *per se*. Hence, in sections that follow I will attempt to identify other possible ecological forces likely to shape seed selection decisions in carabid weed seed predators. The discussion will be reserved for analyzing functional traits for both weed seeds and carabid weed seed predators, and how their interactions might determine the predation strength under realistic situations.

## **2.4. Biological Traits Powering the Ecological Processes of Weed Seed Predation by Carabid Beetles**

### **2.4.1. Biological Traits of Weed Seeds Underlying Vulnerability to Predation Risks**

#### *2.4.1.1. Seed Nutritional Content*

Seeds of plant species usually contain large amounts of essential nutrients that are vital for embryo survival (Agrawal, 2007; Wang and Yang, 2020). Nutrients in seeds are typically comprised of carbohydrates (starch), protein (amino acids), and fatty acids (lipids), and these show considerable variations across plant families and genera (Bretagnolle *et al.*, 2015). It remains unclear which nutrient or combination of nutrients, if any, bring about a higher vulnerability to seed predation. Early studies with rodents reported that seeds containing high levels of protein suffered elevated predation rates (Henderson, 1990; Herms and Mattson, 1992; Halkier and Gershenzon, 2006; Gong *et al.*, 2015). However, similar data for arthropod seed predators are scarce. Studies on ant-dispersed seeds, for example, showed that seeds with lipid-rich elaiosomes were usually picked up at higher rates by ants (Brew *et al.*, 1986). Indirectly, this could be an indication that seed-feeding arthropods suffer some lipid limitations in their environments, as ants usually eat only the elaiosome and leave the seed intact (Brew *et al.*, 1986; Rodgerson, 1998). In line with this, some carabid predators were found to interfere with ants and compete with them for seed elaiosomes (Ohara and Higashi, 1987). Carabid predators here consumed only the lipid-rich elaiosomes and left the seeds intact in much the same way as ants. This could be an indirect indication that carabid predators also suffer lipid limitations in their diets and thus, seek out seed consumption to acquire essential fatty acids from seed species rich in lipids.

Recent field studies have indeed reported that weed seeds with high lipid content are usually more preferable to carabid weed seed predators than are seeds with low lipid contents (Petit *et al.*, 2014). Laboratory feeding experiments also have shown that, within certain limits of seed size, weed seeds with high lipids suffer higher rates of attack by carabid weed seed predators (Gaba *et al.*, 2019). Together, field and laboratory findings fall nicely in line with nutritional ecology data demonstrating that carabid species in arable fields usually forage under lipid-limited conditions (Raubenheimer *et al.*, 2007; Jensen *et al.*, 2012; Toft *et al.*, 2019). This agrees with

other studies reporting that arthropod predators, including spiders and other insects, also suffer considerable lipid limitations in their natural habitats as well (Wilder and Eubanks, 2010; Wilder *et al.*, 2013). Plus, carabids seem to maintain a strong preference for weed seeds in the field even when seeds are offered along with prey (Frank *et al.*, 2011; Lundgren and Harwood, 2012; Blubaugh *et al.*, 2016). Taken together, the totality of evidence thus far suggests that seeds of some weed species must contain specific lipids essential for carabid physiology, which might be absent from protein-rich insect prey (Booij *et al.*, 1994; Wilder *et al.*, 2013). Thus, weed seeds may be salient elements of the general feeding habits of carabid seed predators. While it is compelling to assume that lipid limitations are one primary reason carabid seed predators consume seeds of specific species, much more detailed studies are needed to ascertain if other important nutrients like protein or carbohydrates might also impact carabid feeding ecology and therefore, seed selection decisions (Denno and Fagan, 2003).

#### 2.4.1.2. *Seed Chemical Defenses*

Plants cannot compromise on the nutritional needs for embryo survival, so they deploy different layers of defense that make seed nutrients difficult to access and costly to extract by seed predators (Rees and Long, 1993; Dalling *et al.*, 2011). Plants, including weeds, mobilize a wide array of secondary metabolites to maturing seeds, many of which serve multiple defensive functions (Rattan, 2010; Trowbridge, 2014). While seed nutrients (i.e., primary metabolites) are likely to act as major feeding stimulants for carabid weed seed predators, the presence of defensive chemicals (i.e., secondary metabolites) could act against the phago-stimulatory effects of nutrients (Chapman, 1999, 2003). Presumably, the acceptability of weed seeds would be determined by the overall balance between primary and secondary metabolites in seed tissues (Bernays *et al.*, 2004; Agrawal and Fishbein, 2006). That is, seed species with low levels of chemical defenses should be more preferable to carabid seed eaters, perhaps irrespective of nutritional content. However, the assumption that insects choose to feed on plant tissues based on chemistry alone does not hold true in all situations. The levels of secondary metabolites in plant tissues do not always determine the acceptability of those tissue to insect herbivores as shown by a meta-analysis study (Carmona *et al.*, 2011). This should not be surprising because the levels of primary and secondary metabolites in plant tissues are determined by complex interactions and trade-offs between multiple plant traits



(Blate *et al.*, 1998; Davis *et al.*, 2016). In line with this, studies that tested the impact of secondary metabolite levels on seed choice by rodent seed predators also produced conflicting results (Wang *et al.*, 2012). Rodent seed predators, for example, avoided seeds with high levels of phenols in some cases (Henderson, 1990; Gong *et al.*, 2015). In another study, seed preferability to scatter-hoarding rodents was influenced not only by the concentration of tannins (water-soluble phenolic compounds), but also the amount of protein and fatty acids in the seed (Wang and Chen, 2012). In one more extensive study, effects arising from seed toxins on seed selection responses in rodent seed predators were totally absent (Rodgers, 1998). More interestingly, studies investigating how the chemistry of weed seeds affect their persistence in seed banks showed that physical traits like seed mass, size, and coat hardness were much more important to long-term seed persistence than chemical traits (Davis *et al.*, 2008). Similarly, for seeds of multiple weed species, the physical characteristics of the seed were more crucial for avoiding predation by carabid predators than was seed chemistry (Foffova *et al.*, 2020a). Given the evidence to date, it appears that seed chemistry alone is unlikely to be the sole driver of seed selection decisions in carabid seed predators. Instead, seed biophysical properties may profoundly interact with, and perhaps even override, the effects of seed chemistry.

#### 2.4.1.3. *Seed Size and Mass*

Size is one physical trait of special interest to seed ecology (Baskin and Baskin, 1998; Dalling *et al.*, 2020). Seed size directly relates to many seed quality parameters (Petit *et al.*, 2014; Wang and Yang, 2020). Larger seeds, for instance, usually contain more energy and nutrients (Gong *et al.*, 2015), but could also contain more chemical defenses (Agrawal and Fishbein, 2006; Wang and Chen, 2012). Also, larger seeds tend to have thicker and harder seed coats (Moles *et al.*, 2003). Overall, the relationships between seed size, seed chemistry, and seed physical properties are quite complex and not well understood (Wang and Chen, 2012; Dalling *et al.*, 2020). Nonetheless, the size of weed seeds was observed to be among the factors determining which seeds of weed species would be more preferable to carabid weed seed predators in laboratory trials (Lundgren and Rosentrater, 2007; Saska *et al.*, 2019b). In a laboratory study, Honek *et al.* (2007) produced measurements of dry mass for 25 different weed species and the body mass of 30 carabid weed seed predators in laboratory experiments. The authors managed to establish predator-seed

mass allometric scaling relationships that strongly influenced seed selection responses of the carabid predators under study. Mass-ratio scaling relationships have been shown to be vital for determining the strength of predator-prey interactions in vertebrate systems (Freeman and Leman, 2008), but the mechanistic aspects of size-based prey choices remain relatively poorly understood. Likewise, mechanisms of the size-based seed selection responses in carabid weed seed predators remain to be discovered. More studies are needed in this regard to elucidate the factors underlying mass-ratio scaling relationships and their impact on strength of the interactions between weed seeds and carabid seed predators.

#### 2.4.1.4. *Seed Coat Hardness*

Seed coat hardness is determined by the amount of sclerenchyma deposited in the palisade cells of seeds (Radchuk and Borisjuk, 2014). Weed seed coat hardness, in general, decreases with increasing seed mass (Janzen, 1969; Lundgren and Rosentrater, 2007). Yet, the opposite relationship patterns were observed for some species (van der Meij and Bout, 2000). Functionally, the relationship between seed mass and seed coat hardness differs from one weed species to another, and is more likely to follow phylogenetic patterns rather than simple general linear patterns (Lovas-Kiss *et al.*, 2020). Despite these species-specific differences, what seems to be of most consequence to the carabid weed seed predator is seed coat hardness (Jorgensen and Toft, 1997; Petit *et al.*, 2014). The authors of two field studies observed that weed seeds with soft coats were much more susceptible to carabid weed seed predators than seeds with hard coats (Jorgenson and Toft, 1997; Noroozi *et al.*, 2012). This piece of evidence remains anecdotal because neither study tested the effects seed coat hardness on removal rates of weed seeds. More direct testing of the relationship between seed coat hardness and predation avoidance has found that seed coat hardness was crucial in determining the susceptibility of weed seeds to predation by carabids (Lundgren and Rosentrater, 2007; Foffova *et al.*, 2020a,b). This suggests that weed seed coat hardness could decisively determine vulnerability to seed predation if coat hardness differs considerably among seed species in any given environment (Foffova *et al.*, 2020a,b). Still, it remains unclear if coat hardness acts alone or together with other seed traits as syndromes influencing predation avoidance (Agrawal and Fishbein, 2006; Dalling *et al.*, 2011). A more plausible scenario is that the relative importance of any given seed trait with regard to predation

avoidance is likely to be determined by how other seed traits also match or mismatch with the functional traits of the predator (Dalling *et al.*, 2020; Foffova *et al.*, 2020a,b). In other words, trait values for the different seed defensive traits are only one part of the interaction. Predation pressure would also be determined by how many seed defensive traits actually match or mismatch with the functional traits of carabid predators with regard to the ability of the carabid to neutralize seed defenses (Quieter *et al.*, 2007; Ball *et al.*, 2015). This appears crucial because morphological traits of the seed such as seed mass, size, and coat hardness undergo considerable changes over time in the soil seed bank, and these changes are likely to affect seed vulnerability to carabid predation (Martinkova *et al.*, 2006; Saska *et al.*, 2019a,b; Saska *et al.*, 2020). For a summary of the different seed traits influential on weed seed vulnerability to carabid predators, see Table 2.2.

**Table 2.2.** Summary of potential seed traits that mediate seed vulnerability or avoidance to carabid seed predators (adopted from Ali and Willenborg, 2021).

Seed trait	Vulnerability to seed predation	References
Seed nutrients (lipids)	+	Petit <i>et al.</i> , 2014; Gaba <i>et al.</i> , 2019
Seed chemical defenses	0	Foffova <i>et al.</i> , 2020a
Seed size and mass <sup>‡</sup>	±	Lundgren and Rosentrater, 2007; Honek <i>et al.</i> , 2007; Saska <i>et al.</i> , 2019b; Foffova <i>et al.</i> , 2020a; Saska <i>et al.</i> , 2020
Seed coat hardness	-	Jorgenson and Toft, 1997; Lundgren and Rosentrater, 2007; Noroozi <i>et al.</i> , 2012; Foffova <i>et al.</i> , 2020a,b; Saska <i>et al.</i> , 2020
Seed viability	0	Saska <i>et al.</i> , 2019a

(+): indicates a positive effect; (-): indicates a negative effect; (0): indicates no documented effect; <sup>‡</sup> (±): indicates that the net effect depends on the predator-to-seed mass-ratio scaling relationship.

## 2.4.2. Biological Traits of Carabid Predators of Importance to Weed Seed Feeding

### 2.4.2.1. The Physiological Traits of Carabid Predators

Through feeding interactions, carabid predators obtain nutrients to address the nutritional limitations they face in their environments and thus, fulfill the nutritional requirements for survival and reproduction (Frank *et al.*, 2011; Potter *et al.*, 2018). Generally speaking, protein and lipids

are the two major macronutrients that drive the nutrient foraging efforts of arthropod predators, including carabids (Jensen *et al.*, 2012; Schmidt *et al.*, 2012; Wilder *et al.*, 2019). Seeds of weed species offer both types of macronutrients, but to different extents (Bretagnolle *et al.*, 2015). Given that most carabid species generally suffer lipid limitations in their agricultural environments, foraging for lipids could be, in principle, the main trophic link binding together weed seeds and carabid seed predators (Raubenheimer *et al.*, 2007; Toft *et al.*, 2019; Gaba *et al.*, 2019). Although the main goal of trophic interactions is the acquisition of nutrients from the environment, there are other aspects of nutrient foraging behaviors than food extraction *per se* (McArthur and Pianka, 1966; Ydenberg *et al.*, 1994). Setting out on foraging bouts is associated with different risks and entails high costs related to food handling and processing (Schoener, 1971; Pyke *et al.*, 1977). Carabid seed predators in this sense need not only to find weed seeds, but the chosen seed species or seed patch should also offer high nutritional rewards at a low handling cost (Sih, 1984; Fawki *et al.*, 2003). Clearly, the balance between reward and cost of different seed species (or types) is likely a major factor influencing seed selection decisions (Bretagnolle *et al.*, 2015; Brousseau *et al.*, 2018). Moreover, how rewarding a certain seed species is to any given carabid species is determined by the biophysical and biochemical functional traits specific to predatory species itself (Evans and Forsythe, 1985; Forsythe, 1987; Brousseau *et al.*, 2018). In other words, the carabid predator needs to be able to neutralize seed defenses to gain more sufficient nutrition at low costs. Should this not be the case, the carabid predator will suffer great fitness costs (Emlen, 1966; Pyke *et al.*, 1977). Therefore, the functional traits specific to carabid species are expected to strongly affect the species (or types) of seeds they come to accept for consumption. Among the various functional traits of carabid species, physiological and biochemical traits remain the least understood. Hence, it could be much more informative to examine the feeding ecology of carabid predators from a biophysical standpoint.

#### 2.4.2.2. *The Biophysical Traits of Carabid Predators*

The relationship between morphological traits and feeding habits is well established for insect herbivores, as well as for carabid predators (Bernays, 1991; Knapp and Knappova, 2013, Kanpp and Uhnava, 2014). Curiously though, these relationships have remained largely overlooked in the study of carabid feeding ecology. For instance, there is generally strong

functional links between mouthpart structure and the type of foods adult carabids can consume (Forsythe, 1982, 1983b; Deroulers and Bretagnolle, 2018). Recently, similar functional links between mouthpart morphology and the degree of feeding specialization have been uncovered for carabid larvae as well (Sasakawa, 2016). By and large, the morphology and structure of mouthparts in carabid species (both adults and larvae) seem to be among the fundamental functional traits that determine key aspects of the feeding specialization niche in terms of carnivory, omnivory, or granivory (Evans and Forsythe, 1985; Forsythe, 1987; Paarmann *et al.*, 2006). Within each of these feeding niches however, functional traits other than mouthpart structure seem to determine aspects of food choice and preference.

Predator-prey mass allometry has been found to be highly predictive of prey selection decision in carnivorous carabids preying on insects including aphids, collembola, and caterpillars (Bell *et al.*, 2008; Schmitz 2009; Rusch *et al.*, 2015). Similarly, predator body mass strongly affected seed selection decisions in carabid weed seed predators in laboratory studies (Honek *et al.*, 2007; Martinkova *et al.*, 2019; Saska *et al.*, 2019b). Despite their strong impact, the mechanisms by which predator body mass affects the choice of suitable prey or seed species remain poorly understood. Body mass is a complex trait with links to multiple other traits that directly relate to foraging behaviors, food handling capabilities, and functional responses of carabid predators (Aljetlawi *et al.*, 2004; Brose, 2010; Reum *et al.*, 2019). In this respect, a positive scaling relationship has been reported between carabid body mass (but not body length) and size of the muscle mass that powers the mandibles, ultimately exerting control over the strength of bite force (Evans and Forsythe, 1985; Wheeler and Evans, 1989).

The relationship between body mass, jaw musculature, and bite force have been found to shape vital aspects of the feeding ecology in mammalian predators (Wore *et al.*, 2004; Freeman and Leman, 2008), as well as granivorous birds (van der Meij and Bout, 2004). However, the importance of body mass and biting force for the feeding ecology of insect species remains poorly understood. Emerging evidence in this regard suggests that body mass and bite force underlie fundamental aspects of the feeding ecology of predatory insects as well (Weihmann *et al.*, 2015; Blanke *et al.*, 2018). Recent studies, for instance, have shown that the match between bite force and cuticular hardness of prey species was the most powerful factor in predicting prey preferences when carabid species were offered different species of prey (Konuma and Chiba, 2007; Brousseau *et al.*, 2018). There are no similar studies looking into the role of bite force in seed selection

decisions in carabid weed seed predators. Some authors speculate that bite force should play a key role in weed seed selection as well (Brousseau *et al.*, 2018). Here, it is tempting to infer that the strong effects of carabid body mass in shaping the feeding response of carabid predators are most likely derived from its intimate relationship with bite force. This reasoning may explain why the relationship between carabid body mass and metabolic rates often provide only a partial explanation of the interaction strength between carabid predators and prey species (Brown *et al.*, 2004; Brose *et al.*, 2006, 2008; Rumen, 2018).

The bio-morphological reasoning laid out above seems to apply to larval carabids as well. Larvae of carabid weed seed predators, for example, have been shown to deliver stronger bite forces compared to strictly carnivorous larvae (Brandmayr *et al.*, 1998; Paarmann *et al.*, 2006). This is an essential adaptation for handling the hard coats of weed seeds. Moreover, the feeding niche of carabid larvae expands as they grow and molt from one instar to another (Saska, 2005; Klimes and Saska, 2010). Such changes in feeding habits are likely due to increases in body mass and strength of mandibular muscles of the growing larvae as they advance from one instar to the other (Refeseth, 1984; Sasakawa, 2016). Overall, bio-morphological traits are better in predicting the feeding ecology of carabid beetles compared to physiological traits (Bell *et al.*, 2007).

Gape size is not expected to constrain food choice in carabid predators in general, including seed predators, because larval and adult carabids are fragmentary feeders and often show no swallow feeding habits (Evans and Forsythe, 1985; Brousseau *et al.*, 2018). Based on the evidence discussed above, it is plausible to suggest that bio-morphology in terms of mouthpart structure and bite force are two traits that shape key aspects of feeding specialization in carabid species, including carabid seed predators. Again, this accentuates further the previous discussion as to why seed selection is unlikely to be based on seed chemistry alone. For a summary of carabid traits likely to affect ability of carabid predators to destroy weed seeds, see Table 2.3.

**Table 2.3.** Summary of potential carabid traits that influence the ability of carabid predators to destroy weed seeds (adopted from Ali and Willenborg, 2021).

Carabid trait	Ability for weed seed destruction	References
Mouthpart morphology	+	Forsythe, 1982, 1983b,1987; Evans and Forsythe, 1985; Sasakawa, 2016; Deroulers and Bretagnolle, 2018
Body mass	+	Honek <i>et al.</i> , 2007; Martinkova <i>et al.</i> , 2019; Saska <i>et al.</i> , 2019b
Bite force	+	Evans and Forsythe, 1985; Wheater and Evans, 1989; Brousseau <i>et al.</i> , 2018
Body length	0	Kulkarni <i>et al.</i> , 2016; Saska <i>et al.</i> , 2019b
Gape size	0	Evans and Forsythe, 1985; Brousseau <i>et al.</i> , 2018

(+): indicates a positive effect; (-): indicates a negative effect; (0): indicates no documented effect.

#### 2.4.3. *The Match-Mismatch Between Traits of Carabid Predator and Weed Seed and Their Impact on Seed Choice*

The discussions above have established that seed detection and suitability assessment are most likely mediated by olfactory mechanisms in some way. Following weed seed localization by a carabid seed predator, seed feeding will commence only if traits of both the predator and the seed overlap to large extents (Kalinkat *et al.*, 2015; Saska *et al.*, 2019a). Seed suitability rank in such trait-based foraging scenarios will be determined by the ability of the predator to break the physical and/or chemical defenses of the seed (Schmitz, 2009; Larios *et al.*, 2017; Foffova *et al.*, 2020a). Taking this into account, a brief hypothesis of the steps in post-dispersal weed seed predation is as put forward in the lines that follow. The ability of the carabid predator to break through the seed coat is an essential first step before feeding on seed tissues can commence (Forsythe, 1982,1983b; Brousseau *et al.*, 2018). It can thus be expected that if predators are able to efficiently break through the seed coat of different seed species without considerable costs, seed chemistry would rule and predators would select seed species of superior nutritional quality (Blate, 1998; Moles *et al.*, 2003). By contrast, if the seed coat hardness varies widely among the seed species available in the environment, predators would select the seed species that is easy to handle, regardless of its nutritional quality (Ananthakishan, 1990; Potter *et al.*, 2018).

## 2.5. Foraging Strategies and Effects of Carabid Weed Seed Predation on Weed Communities

It has been established in the previous section that seed selection in carabid seed predators is most likely determined by the magnitude of trait overlap between carabid and seed species. It is now more informative to build upon that trait-based seed selection discussion and explore its aspects at the community level. Weed communities in arable fields are composed of several coexisting weed species (Booth and Swanton, 2002; Petit *et al.*, 2011). Consequently, weedy plants in any given weed community produce seeds of different species (i.e., types) and shed them in various numbers each year (Dessaint *et al.*, 1991; Bagavathiannan and Norsworthy, 2012). There are thus two main sources of variability in weed seeds of arable fields; seed type as defined by species-specific seed traits (i.e., trait-based seed suitability rank) and seed density, as defined by seed numbers per unit area (Albercht and Auerswald, 2009). The optimal foraging theory predicts carabid predators, when choosing among weed seeds, should base their choice not only on seed species (types) but also seed numbers (Sih and Christensen, 2001).

The optimal seed foraging assumption predicts that carabid seed predators should keep track of any changes in abundance of seed species they prefer, and adjust their foraging decisions accordingly (Pyke *et al.*, 1977; Hubbard and Cook, 1978). Foraging decisions in carabid seed predators should therefore be dynamic, showing changes through time and/or space. This assumption has been borne out by data from laboratory and field studies as carabid seed predators were shown to respond to changes in the weed seed abundance in their environments (Frank *et al.*, 2011; Dudenhoffer *et al.*, 2016). In many cases, the rates of weed seed removal by carabid predators progressively increased as a function of seeds numbers per unit area (Honek *et al.*, 2003; Westerman *et al.*, 2008). Technically, these patterns suggest that carabid predators destroy more weed seeds as seed numbers increase, and are therefore expected to have a stabilizing influence on weed populations as suggested by models of predator-prey dynamics (Sih, 1980, 1984; Abrams, 2000). The mechanisms underlying the density-dependent responses exhibited by carabid seed predators in arable fields remain unknown, nonetheless. The phenomenon could arise from individual carabid predators consuming more weed seeds as they become more abundant per unit area, perhaps giving rise to foraging strategies ruled by functional responses (Holling, 1959). Alternatively, the higher rates of weed seed predation at increasing seed densities could come about by larger numbers of individual carabid seed predators being attracted to dense seed patches,



giving rise to foraging strategies ruled by numerical responses (Hulme, 1997; Marino *et al.*, 2005). Some evidence has shown that dense seed patches attract higher numbers of carabid seed predators (Honek and Martinkova, 2001), but the correlation between carabid numbers aggregating to the dense seed patch and consumption rates of the preferable seed species in the patch was quite poor in some cases (Honek *et al.*, 2005). Numerical responses are thus unlikely to always explain seed removal rates in response to changes in seed density. Instead, both functional and numerical responses are most likely to work together and create complex seed predation dynamics in the field (Lester and Harmsen, 2002; Kuang and Chesson, 2010). Hence, a deeper treatment of seed foraging strategies requires elucidating how functional and numerical responses are affected by changes in density of the preferable seed species relative to densities of other seed species available in the environment (Symondson *et al.*, 2002; Lester and Harmsen, 2002).

It has been suggested that frequency-dependent functions rule seed foraging strategies in arable fields (Greenwood, 1985; Horst and Venable, 2018). The key factor that powers seed predation dynamics in such a case would be seed encounter rate (Kuang and Chesson, 2010). The seed species encountered most frequently in the environment would suffer most of the carabid attacks, whereas seed species that are less abundant would be largely ignored by carabid predators (Merilaita, 2006). Frequency-dependent models may help explain some patterns of weed seed removal by carabids in arable fields, but such explanations are based on seed numbers alone. Frequency-dependence assumptions ignore that weed seeds shed in arable fields differ not only in number, but also in suitability in terms of species-specific traits (Dessaint *et al.*, 1991; Bagavathiannan and Norsworthy, 2012). Basically, frequency-dependence reasoning turns weed seed predation interactions into a numbers game, leaving no leeway for predators to choose seed species that suit them best as optimal foraging models predict (Pyke *et al.*, 1977; Merilaita, 2006).

Ignoring this important shortcoming in frequency-dependent models has led to wide contradictions in weed seed foraging data. For example, data from some field studies have shown that weed seed predation rates followed inverse density-dependent patterns, and seed consumption rates declined as more seeds were offered per unit area (Cardina *et al.*, 1996; Marino *et al.*, 2005; Westerman *et al.*, 2008). In such cases, seed predation would exert bio-regulatory effects on weed populations only when weed seeds are scattered at low densities in the field. Higher densities of weed seeds would overwhelm the environment and saturate the functional and/or numerical responses of carabid seed predators, breaking down any bio-regulatory effects against weed

populations (Petit *et al.*, 2014; Pannwitt *et al.*, 2017). Intriguingly, the density-dependent removal of weed seeds was totally absent in some feeding trials conducted under field conditions (Baraibar, 2011b; Noroozi *et al.*, 2012; Pufal and Klein, 2013). The absence of density-dependent effects in such situations suggests that factors other than seed numbers alone also play into seed selection decisions. Hence, weed seed predation interactions in arable fields are unlikely to be a game of numbers.

Density-dependent and selective (trait-based) seed foraging should not be mutually exclusive in weed seed predation interactions, as frequency-dependent models presume (Mongel and Clark, 1986; Baraibar *et al.*, 2011a). Instead, the two strategies could work together, and the abundance of preferable seed species relative to other less preferable seed species would determine seed selection decisions (Pyke *et al.*, 1977). One key difference from frequency-dependence here is that upon an initial successful encounter with the preferable food type in the environment, the selective forager would alter its foraging behavior towards increasing the chances of coming across the preferable food type (Sih, 1980, 1984; Garay *et al.*, 2018). Thus, food searching behavioral patterns exhibited by selective foragers should be directed rather than random, and not driven by encounter rates alone (Pyke *et al.*, 1977; Hassell and Southwood, 1978). This fits nicely with the core assumptions of optimal foraging models, as highly preferable seed species should always be selected when they are abundant in the environment (Westoby, 1978; Sih and Christensen, 2001). Less preferable seed species should be consumed only when the highly preferable seed species is no longer available or is available only in small numbers (Hassell and Southwood, 1978).

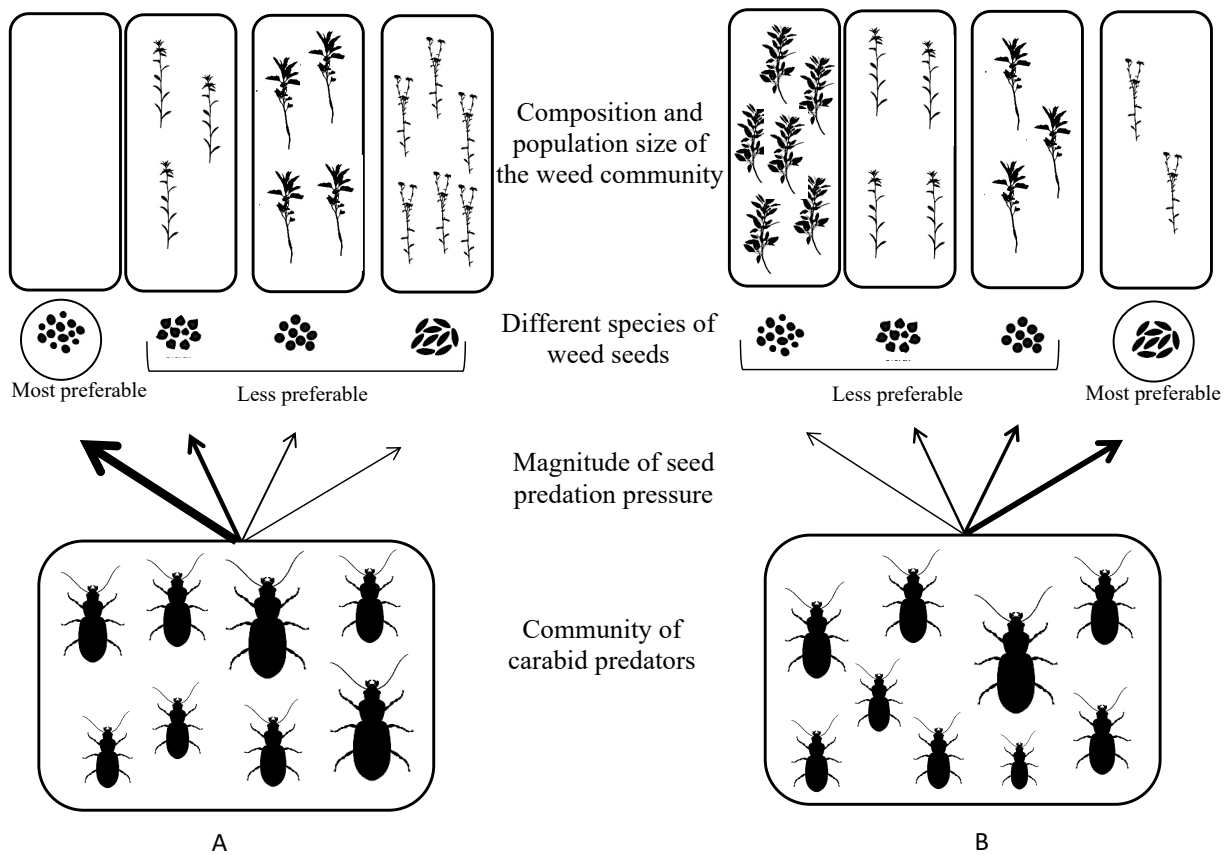
Given the above, it could be suggested that within certain ranges of seed densities, attack rates against the preferable seed species are likely to follow positive density-dependence patterns (Cardina *et al.*, 1996; Westerman *et al.*, 2008). When the density of the highly preferable seed species starts to run low, the seed foraging strategies of carabid seed predators can be summarized under the following two scenarios. The first scenario would involve seed predators continuing to attack the highly preferable weed seed type until it is depleted or no longer detectable (Holling, 1959; Zhang *et al.*, 1992). Then, switching to less preferable seed species would take place. As a result, the highly preferable seed species would suffer significantly higher predation pressures when scattered in the environment at low densities compared to moderate or high densities. In such cases, inverse-density-dependent (type II) functions would power the seed predation dynamics (Holling, 1959). That is, the highly preferable species would suffer strong negative effects when

its abundance is low, and its population could be extinguished in some local environments (Murdoch and Oaten, 1975; Figure 2.2 A). There are field data to support the assertion of this scenario, suggesting that events of inverse-density-dependent weed seed predation could unfold under some realistic situations (e.g., Cardina *et al.*, 1996; Westerman *et al.*, 2008; Pannwitt *et al.*, 2017). Similar inverse-density-dependent prey foraging patterns were also reported for carabids predating on colonies of soybean aphids (Firlej *et al.*, 2013). It could be inferred, therefore, that destabilizing inverse density-dependent effects are potentially the hallmark of carabid predation against seed and prey species in agroecosystem, but more studies are needed before any firm conclusions can be drawn here.

In the other possible scenario, carabid weed seed predators would switch their foraging mode towards the less preferable seed species when abundance of the preferable seed species drops below a certain threshold level (Chernov, 1976; Pyke *et al.*, 1977). Below that abundance threshold, attacks against the preferable seed species start to wane as predators start to seek more rewarding seed patches (Sih, 1980, 1984; Pyke, 1984). Predation dynamics in such a case would be powered by density-dependent (type III) functions (Holling, 1959). This would give the preferable seed species an ‘escape density’ below which predation risks are diminished (Sentis and Boukal, 2018). Instead of causing local population extinctions, carabid seed predators could in the density-dependent scenario would promote the coexistence of multiple weed species in the weed community (Juliano, 2001; Kuang and Chesson, 2010). This is a less attractive scenario for weed bio-control programs because the predation pressure is more distributed among the different weed species in the community (Kuang and Chesson, 2010). But given that the abundance of the highly preferable weed seed species is kept below certain thresholds, some suppression against its populations should be expected (Holling, 1959; Lester and Harmsen, 2002; Figure 2.2 B). Data in support of this scenario remain wanting, however, and studies testing this prediction are needed.

The scenarios laid out above need not to be mutually exclusive since it is possible for both scenarios to take place in the field, and give rise to complex seed predation dynamics. Composition of the carabid community would be the main factor to determine which seed foraging strategy would be predominant, and hence determine the magnitude of seed predation pressure against seed species in the field. Carabid communities in arable fields usually comprise numerous species and show considerable changes in their species composition through space and time (Jacob *et al.*, 2006; Pufal and Klein, 2013). Hence, the exact composition of the carabid community at any point in

time or space would determine which weed species are likely to incur the strongest predation pressures (Petit *et al.*, 2014; Charalabidis *et al.*, 2019). However, carabid species within those communities also are expected to interact, interfere, or even compete with one another in ways that remain poorly understood (Niemela, 1993; Niemela *et al.*, 1997; Currie *et al.*, 1996). This paints a very complex picture for seed predation interactions at the community level, leaving the discussions laid out above rather simplistic. Notwithstanding, analysis of functional traits offers a powerful tool that may improve our ability to drill down through these complex layers and untangle the possible ecological forces driving weed seed predation dynamics at the community level (Rall *et al.*, 2102; Reum *et al.*, 2019).



**Figure 2.2.** Possible scenarios of the foraging strategies of carabid seed predators and their effects on weed communities in arable fields. (A): the preferable type of weed seeds suffers higher attacks at lower densities, and switching to less preferable seed types takes place only when the preferable seed type is depleted to large extents. Predation pressure is strongly directed against the preferable seed species leading to local extinctions in some cases. (B): the preferable type of weed seeds suffers lower attacks at lower densities, and switching to less preferable seed types takes place when abundance of the preferable drops below a certain threshold. Predation pressure is more distributed among the different seed species in the environment, and no local extinctions are expected take place (adopted from Ali and Willenborg, 2021).

Functional trait analysis of carabid communities has revealed that average values of predator-prey mass-ratio scaling parameters at the community level are key predictors of the suppression pressure carabid predators impose on different prey species in the field (Roubah *et al.*, 2014; Rusch *et al.*, 2015). Mechanisms that link mass-ratio scaling effects and the magnitude of predation pressure carabids come to exert on prey species at the community level remain unclear. Predator-prey models predict that mass-ratio scaling between arthropod predators (including carabids) and their prey determine the type of functional response (type II or III) that predators employ in their prey foraging strategies (Rudolf, 2008; Kalinkat *et al.*, 2013). That is, if the structure of body mass in the carabid community overlaps with the mass distribution in the prey community, type II functional responses (inverse-density-dependent) would rule the prey foraging strategies in the field (Kalinkat *et al.*, 2013). Beyond that, functional responses would shift towards type III functions (density-dependent) and rule prey foraging strategies (Kalinkat *et al.*, 2013). It is unclear whether the interplay between mass-ratio scaling effects and functional responses can also be applied to the study of carabid seed predation systems, as trait analytical studies in this regard remain rather scarce. This accentuates even further the need for mechanistic studies to sieve out the core traits that determine the strength of interactions between carabid predators and seeds of weed species. Such knowledge would clarify where the line between ecological and environmental effects should be drawn with regard to predicting the magnitude of seed predation pressures in arable fields. In this way, large-scale studies could be better designed to elucidate the impact of agricultural practices on predation dynamics (Headrik and Goeden, 2001). Conservation biocontrol measures could then be tailored to enhance the functioning of carabid communities based on the local agricultural practices.

## CHAPTER 3 Olfactory Chemoperception Mediates Detection and Discrimination of Seed Species in Carabid Weed Seed Predators

### 3.1. Introduction

Carabid (ground) beetles are among the most studied and characterized arthropods in the temperate arable land (Kromp, 1999). This is due in part to their importance and prevalence in agroecosystems of temperate regions, where they function as important predators of numerous insect pests and seeds of annual weeds (Crawley, 2000; Lundgren, 2009). The ecology of seed feeding habits is widely studied in the carabid literature (Kulkarni *et al.*, 2015a), and data suggest that seed feeding habits in carabid beetles generally tend to take place in two main trophic guilds; omnivores and granivores (Fawki and Toft, 2005; Talarico *et al.*, 2016). Based on this, granivorous carabids are expected to concentrate their food searching efforts towards foraging for seeds specifically, while omnivorous carabids are supposed to more likely locate and consume seeds through random encounters (Lovei and Sunderland, 1996; Cardina *et al.*, 1996). Seed feeding habits in omnivorous carabids as such seem to be opportunistic, driven by chance mostly, and tend to occur only under certain occasions of prey scarcity.

This classic and dichotomous view of seed feeding habits in carabids still transcends modern carabid literature, but an alternative, more comprehensive view is needed in light of the current evidence. Recent studies have shown that the feeding ecology of carabid species is much more diverse and complex than what the trophic guilds dichotomy seems to predict (Talarico *et al.*, 2016; Carbonne *et al.*, 2020a). In fact, the borderline between omnivorous and granivorous feeding habits is ambiguous, as the two feeding habits often overlap in carabid predators (Talarico *et al.*, 2016; Frei *et al.*, 2019). Also, the occurrence of seed feeding habits among the carabid taxa tends to exceed previous expectations (Carbonne *et al.*, 2020a). Together, these lines of evidence seem to suggest that feeding habits in carabid species possibly arose to satisfy specific, yet unexplored, biological needs that are not exclusive to granivorous carabids as the logic of the trophic guilds concept seems to suggest.

Seed feeding in carabid predators seems to be driven by non-random behaviors that are directed towards satisfying specific biological needs. This renders random encounters insufficient as the main driver of seed feeding and seed preference in carabids. Indeed, field and laboratory

data have demonstrated that carabid predators select specific species for consumption when seeds of different species are available to the predators (Kulkarni *et al.*, 2016; Saska *et al.*, 2019a,b). The mechanistic aspects of seed selection decisions in carabids are not very well understood. Numerous biotic and abiotic factors have been shown to drive or influence seed selection decisions in carabids. Seed preferences in carabids seem to vary depending on habitat properties (Blubaugh *et al.*, 2016; Carbonne *et al.*, 2022), the chemical and biophysical traits of seed species (Gaba *et al.*, 2019; Foffova *et al.*, 2020a,b), the composition and structure of local carabid communities (Sarabi, 2019, De Heij and Willenborg, 2020), in addition to inter- and intra-guild predation risks (Charalabidis *et al.*, 2017, 2019).

Despite the well-evidenced seed choosiness in carabid seed predators, the sensory mechanisms that enable carabids to distinguish among seeds of different species, and then identify seed species that are more suitable for consumption, remain poorly understood. Selection of specific seed species requires carabids to distinguish among seeds of different species, assess their suitability aspects, and then decide which species should be chosen for consumption based on the biotic and abiotic conditions of local environments (Sih and Christensen, 2001). To do that, carabids need to collect reliable seed-derived information through the different sensory packages they are equipped with (Barron *et al.* 2015). The current knowledge around the sensory basis of seed detection and discrimination in carabids is limited to a few reports that seed odors can influence the orientation responses of carabid seed predators in the olfactometer (Law and Gallagher, 2015; Kulkarni *et al.*, 2017b). It thus remains unclear whether olfaction alone can decisively guide seed selection decisions in carabid seed predators, thereby enabling carabids to make accurate seed choices. The accurate choice of suitable seed species perhaps cannot take place without sensory inputs from the visual and/or gustatory systems. It is therefore essential to elucidate the sensory mechanisms that enable carabids to detect and discriminate among seeds of different species. This is expected to further our understanding of the sensory ecology behind seed selection decisions in carabid seed predators.

The dearth of detailed sensory studies coupled with the complexity of feeding habits in carabid predators make it difficult to decipher the sensory ecology of seed detection and discrimination (Kromp, 1999; Talarico *et al.*, 2016). Diurnal carabids, for instance, usually hunt live mobile prey and have been shown to carry large compound eyes and short antennae that house significantly fewer chemoreceptors compared to nocturnal species (Bauer and Kredler, 1993;

Merivee *et al.*, 2001, 2002). These sensory differences have led carabid ecologists to assume that the sensory cues exploited by carabids to guide food searching behaviors may differ depending on the sensory biology and/or their activity rhythms (diurnal versus nocturnal) of carabid species, not the nature of food type (mobile versus sessile) that carabids are in search of. Comparing the sensory biology among carabid species of different ecologies can give important insights into the sensory mechanisms that guide food searching behaviors, but this approach is speculative and can even be misleading in some cases. For instance, prey hunting behaviors in diurnal carabids are often driven by visual cues, yet these behaviors tend to break down if prey items are immobilized (Wheater, 1989). Carabid visual receptors thus seem more attuned for detecting prey movement and should be more helpful for hunting down highly mobile prey (Srinivasan *et al.*, 1999; Gadenne *et al.*, 2015). Carabids are, therefore, not expected to rely on visual receptors to detect sessile prey or weed seeds, especially that seeds are usually cryptic and difficult to distinguish against the soil.

Alternatively, carabids are expected to rely on their chemoreceptors to detect and distinguish among different species of seeds and/or immobile prey. Indeed, mechanistic studies have shown that chemoperception is the primary sensory mechanism that guide prey detection and selection decisions in both specialized (carnivorous) and unspecialized (omnivorous) carabid predators (Kielty *et al.*, 1996; Mundy *et al.*, 2000; Tréfás *et al.*, 2001). Chemoreceptors located on antennae and palps of larval and adult carabids detect prey-derived volatile chemicals (i.e., prey odors), and guide the identification of prey species that are suitable or desirable for consumption (McKemey *et al.*, 2004; Thomas *et al.*, 2008; Talarico *et al.*, 2010). Similar mechanistic knowledge about seed detection and discrimination is still wanting in the carabid literature. Thus, it remains to be determined if the perception of seed odors is also the primary sensory mechanism behind seed preferences in carabid seed predators.

I carried out mechanistic experimental studies that combined multiple-choice feeding bioassays with sensory manipulations to determine the sensory basis of seed detection and discrimination in carabid seed predators. I hypothesized that carabids would rely on their olfactory receptors to gather the sensory information necessary for guiding their seed selection decisions. I tested three species of omnivorous carabid predators to check if the sensory mechanisms of seed perception might differ among carabid species. I was also interested in clarifying whether seed perception mechanisms might be sexually dimorphic and thus, differ among males and females of carabid species.



## 3.2. Materials and Methods

### 3.2.1. Seed Material

Seeds of three different brassicaceous weed species (Brassicaceae: *Brassica napus* L., *Sinapis arvensis* L., *Thlaspi arvense* L.) were used in this study. The seed species selected for this study are all considered high in lipids, and all are weeds of considerable importance in arable fields of the Northern Great Plains region of North America (Kulkarni *et al.*, 2016). Also, the seed species under study often differ in preferability or suitability rank to carabids (Kulkarni *et al.*, 2016, 2017b) and thus offer a good model for studying seed selection decisions in carabid seed predators. Based on that, seeds of canola (*B. napus*) were used as a highly preferable seed type, whereas seeds of wild mustard (*S. arvensis*), and field pennycress (*T. arvense*) represented moderately and weakly acceptable seed types, respectively. Seed masses of the three weed species were hand collected from different field sites at the Kernen Crop Research Farm near Saskatoon, SK, Canada (52°09'10.3" N 106°32'41.5" W) in summers of 2017-18 and stored at 5 °C until use.

### 3.2.2. Carabids

Adults of the omnivorous carabid species *Poecilus corvus* (Leconte), *Pterostichus melanarius* (Illiger), and *Amara littoralis* Dejean, which are known to consume weed seeds, were used as the seed predatory species. Live adults of those carabids were collected from different field sites at the Kernen Crop Research Farm outside of Saskatoon, SK, Canada (52°09'10.3" N 106°32'41.5" W) in summers of 2018-19 via dry pitfall trapping. Field sites chosen for carabid trapping were seeded with canola, pulse, or cereal crops. Pitfall traps consisted of two plastic 0.5 L cups (10 cm height × 8 cm diameter); one acted as a sleeve and was buried into the soil and kept flush with the soil surface, while the other cup (the actual trap) was inserted into it (Spence and Niemela, 1994). In all field sites, pitfall traps were enclosed into cages of fine wire mesh ( $\sigma = 1.1$  cm) to prevent vertebrates from entering the traps and ravaging the catches. Traps were emptied every three days and the collected insects were placed into plastic boxes (40 cm × 25 cm, 25 cm depth) lined with plant material and moist filter paper. All boxes were then brought to the laboratory for identification and further experimentation. Carabid beetles used in the experiments

were identified down to the species and were also sexed using keys in Lindroth (1961-1969). Unless mentioned otherwise, voucher specimens of all carabid species used in this thesis were deposited in the Laboratory of Entomology at Plant Sciences Department, University of Saskatchewan.

### 3.2.3. *Cafeteria Multiple-Choice Seed Feeding Bioassays*

Seeds of the three brassicaceous weed species were offered to carabid species in multiple-choice feeding bioassays. Feeding bioassays were laid out in an arena consisting of a large Petri dish ( $\text{\O} = 25$  cm, 5 cm depth) lined with a 2-cm layer of sterilized, moist sand. Sand was used as a neutral and easy-to-sterilize substrate because soil samples could have seed carry-over and/or organic components that might offer an alternative food source for carabids (Saska *et al.*, 2014). The feeding arenas were designed to offer seeds of the different weed species as ‘seed patches’ of equal size. For this purpose, seeds were placed into plastic tray rings ( $\text{\O} = 28$  mm, 6 mm depth) filled with white plasticine and placed into the Petri dishes. Plasticine has been shown to not interfere with seed preference in carabid seed predators (Honek *et al.*, 2007). In each ring, 25 seeds of one weed species were pressed halfway into the plasticine layer and seed trays were then placed near the perimeter of the Petri dishes. A total of three trays (one of each weed species) were placed into each Petri dish so that the seed patch was at the same level with sand layer. Imbibed seeds were used in all feeding experiments. Seeds were imbibed by placing seed masses on wet filter paper in Petri dishes ( $\text{\O} = 6$  cm, 2 cm depth), and leaving seeds to absorb moisture for 24 h in a growth chamber at  $21 \pm 1$  °C (Kulkarni *et al.*, 2016). Seed imbibition was used to standardize the water content of the seed species under study, as seed water content can affect volatile emission and/or the acceptability of seed species to carabid beetles (Jorgenson, 2001; Kulkarni *et al.*, 2017b; Foffova *et al.*, 2020b).

### 3.2.4. *Sensory Manipulation Treatments of Experimental Carabids*

Beetles were not fed after collection and were starved for 72 h prior to feeding experiments, to empty their guts and standardize their hunger level (Law and Gallagher, 2015). Starvation was carried out by placing a single beetle (to prevent cannibalism) into a clean and

sterile Petri dish ( $\varnothing = 6$  cm, 2 cm depth) lined with a moist filter paper. Petri dishes were then placed into a growth chamber at  $21 \pm 1$  °C and 16:8 L:D photoperiod (White *et al.*, 2007). This procedure was also useful for negating the effects of learning and experience, as any olfactory memory that might form through learning and experience while beetles are foraging in the field usually decays within 72 hours (Glinwood *et al.*, 2011).

After 72 hours of starvation, the Petri dishes containing the predators were placed in a refrigerator at 5 °C for 20 min to reduce their activity (Booij *et al.*, 1994). Following that, each immobilized beetle was randomly assigned to receive a sensory manipulation treatment (see Table 3.1). Sensory manipulation was conducted by placing the immobilized beetle in a dissection plate, and then ablating one or more of the sensory appendages under a stereoscope (DeBoer and Hansen, 1987; Sablon *et al.*, 2013). Ablation of sensory appendages was chosen for this study because blocking sensory appendages with glycerol or nail varnish failed to work in the pilot experiments. Beetles were able to remove the blocking substances with the antenna cleaning apparatus and/or the strong spines on their front legs (Ali, 2018 personal observations). Ablation of insect sensory appendages is widely used for the study of sensory perception in insects, including species of Coleoptera (Sablon *et al.*, 2013), Orthoptera (Zhang *et al.*, 2017), Dictyoptera (Wada-Katsumata *et al.*, 2011), Lepidoptera (Guo and Lib, 2009), Diptera (Vosshall *et al.*, 2000), and Hymenoptera (Draft *et al.*, 2018). In treatment groups where vision needed to be disabled, carabid beetles were blinded by covering their compound eyes with permanent black ink (Pekar and Hurskova, 2006). Sensory treatments as above (i.e., ablation and blinding) enabled the creation of different groups of carabid beetles each lacking the ability to perceive sensory information of a specific nature; visual, olfactory, or gustatory.

Seed feeding responses of the sensory-manipulated carabids were then compared to three control groups: positive, unilateral, and negative. The positive control represented 'intact' beetles with a full complement of functional sensory organs. Beetles in the unilateral control had their sensory organs ablated and the compound eye covered only on one side of the head. The side on which the sensory treatment was carried out (left or right) was randomized to avoid bias. Finally, the negative control group was created by ablating all of the sensory appendages on the carabid head and blackening both compound eyes. As of this writing, no published reports have documented the presence of tarsal gustatory receptors in carabid beetles. Carabids in the negative control were, therefore, unable to perceive any sensory information about their environments.

**Table 3.1.** Treatment list for sensory mechanism of seed perception in three carabid species with associated treatment descriptions.

Treatment number and code	Treatment description	
1 Positive control (+/+)	Carabid beetles with <i>fully functional sensory organs</i> (intact carabid beetles)	Seeds of <i>Brassica napus</i> L. ••• Seeds of <i>Sinapis arvensis</i> L. •• Seeds of <i>Thlaspi arvense</i> L. •
2 Unilateral control (+/-)	Carabid beetles with <i>fully functional sensory organs on only one side of the body</i>	Seeds of <i>Brassica napus</i> L. ••• Seeds of <i>Sinapis arvensis</i> L. •• Seeds of <i>Thlaspi arvense</i> L. •
3 Negative control (-/-)	Carabid beetles with <i>all sensory organs blocked and removed</i> (no sensory perception)	Seeds of <i>Brassica napus</i> L. ••• Seeds of <i>Sinapis arvensis</i> L. •• Seeds of <i>Thlaspi arvense</i> L. •
4 (Antennae + Palps)	Carabid beetles with <i>functional antennae and palps</i>	Seeds of <i>Brassica napus</i> L. ••• Seeds of <i>Sinapis arvensis</i> L. •• Seeds of <i>Thlaspi arvense</i> L. •
5 (Antennae)	Carabid beetles with <i>functional antennae</i>	Seeds of <i>Brassica napus</i> L. ••• Seeds of <i>Sinapis arvensis</i> L. •• Seeds of <i>Thlaspi arvense</i> L. •
6 (Palps)	Carabid beetles with <i>functional maxillary and labial palps</i>	Seeds of <i>Brassica napus</i> L. ••• Seeds of <i>Sinapis arvensis</i> L. •• Seeds of <i>Thlaspi arvense</i> L. •
7 (Maxillary Palps)	Carabid beetles with <i>functional maxillary palps</i>	Seeds of <i>Brassica napus</i> L. ••• Seeds of <i>Sinapis arvensis</i> L. •• Seeds of <i>Thlaspi arvense</i> L. •
8 (Labial Palps)	Carabid beetles with <i>functional labial palps</i>	Seeds of <i>Brassica napus</i> L. ••• Seeds of <i>Sinapis arvensis</i> L. •• Seeds of <i>Thlaspi arvense</i> L. •
9 (Eyes)	Carabid beetles with <i>functional compound eyes and ocelli</i>	Seeds of <i>Brassica napus</i> L. ••• Seeds of <i>Sinapis arvensis</i> L. •• Seeds of <i>Thlaspi arvense</i> L. •

••• indicates seeds of highly preferable seed species; •• indicates seeds of moderately preferable seed species;  
• indicates seeds of weakly preferable seed species.

### 3.2.5. Seed Detection and Discrimination by Sensory-Manipulated Carabids

Carabid predators after receiving sensory treatment (see above) were returned to the Petri dishes used for starvation and given 10 min to ‘acclimatize’ before being released into the feeding arenas. A single predator was then released into a Petri dish containing three patches of different weed seeds and left there to feed for five consecutive days, without replacing the consumed seeds.

Feeding arenas were incubated in a growth chamber at  $21\pm 1^{\circ}\text{C}$ , and 16:8 L:D photoperiod (White *et al.*, 2007). Throughout the experiments, each beetle was used only once and all treatments and controls were replicated 25 times for both *P. melanarius* and *P. corvus*, and 15 times for *A. littoralis*. The sex ratio in the experiment was close to 50♂:50♀ across all treatments and controls, to test whether the sensory mechanisms of seed detection and discrimination might differ between males and females.

At the end of the experiment, beetles were removed from the arena and the number of seeds consumed from each seed patch was recorded (Petit *et al.*, 2014). The seed was considered consumed if >50% of the seed was eaten as half-eaten seeds are unlikely to germinate (Honek *et al.*, 2005). The total number of seed consumption was calculated by adding up the number of seeds consumed from each of the three seed patches in the Petri dishes. Total seed consumption was used as a measurement of seed detection success under the different sensory manipulation treatments. The number of seeds consumed from each seed species offered in the feeding arenas was used as a measurement of seed selection responses under the different sensory manipulation treatments.

### 3.3. Data Analysis

The R Package Version 4.0.3 (R Development Team 2020) was used for all data analysis. The total number of seeds consumed by each carabid beetle over five days was used as the response variable to compare seed detection success under the different sensory treatments via three-way analysis of variance. The initial analysis was carried out on the full data set (all three carabid species together) by fitting a maximal model to the data including sensory manipulation treatment, insect species, insect sex, and their possible interactions as the main factors in the analysis. Significant differences between carabid species were detected, so data were analyzed and presented for each species separately. Model diagnostic plots showed no violations of the normality assumption of ANOVA throughout data analysis steps in this section. Tukey HSD test was used to perform post-hoc comparisons between the different treatments for each carabid species.

The number of seeds consumed by each carabid beetle within each seed species was used as the response variable to compare seed selection responses under sensory treatments. For this

comparison, I used mixed effects models using the function “lmer” (lme4 package, Bates *et al.*, 2015). The initial analysis was carried out on the data set as a whole (all three carabid species together) by fitting a maximal model including weed species, sensory manipulation treatment, insect species, insect sex, and their possible interactions as main effects. The experimental design featured a spatial structure (i.e., three weed species nested in each Petri dish). Therefore, replicate was used as a random blocking factor in the model to account for the error structure in the experimental design. Data were analyzed and presented for each species separately as significant differences between carabid species were detected. For each predatory species, the analysis was initiated by fitting a maximal model to the data including weed species, sensory manipulation treatment, insect sex, and their possible interactions as main effects. Replicate was used as a random blocking factor as discussed above. The packages “LmerTest” and “emmeans” were used to perform post-hoc comparisons on the final models (Lenth, 2019; Schielzeth *et al.*, 2020). Distribution of model residuals was examined to check for any violations of model assumptions throughout the steps taken to analyze the data in this section (Nobre and Singer, 2007).

### 3.4. Results

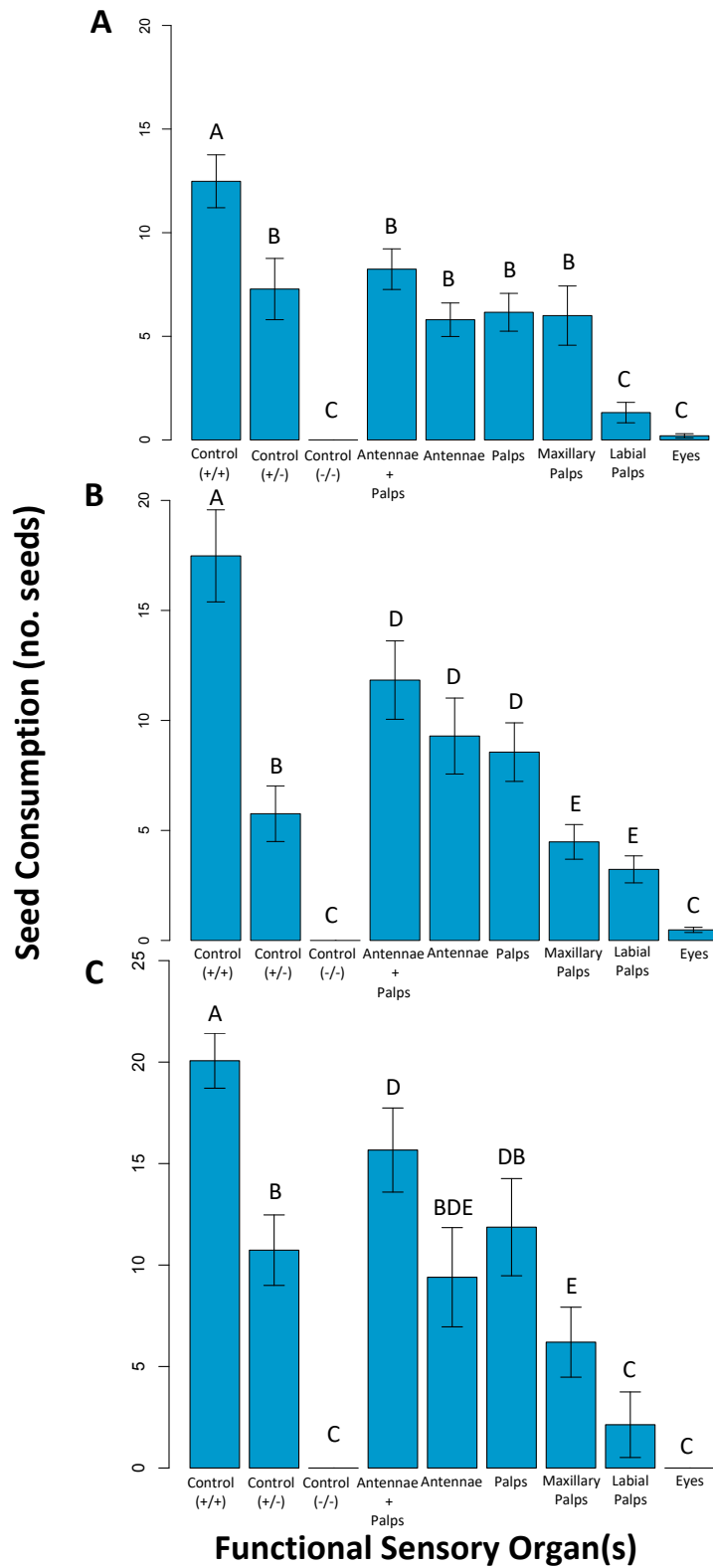
Total seed consumption was used as a measurement of carabid seed detection success under sensory manipulation treatments. Analysis of variance of the full data set (three carabid species) showed that disabling different sensory organs significantly affected the ability of carabid species to detect seeds in the feeding arenas ( $F_{8,530} = 21, P < 0.01$ ). The interaction between sensory treatments and insect species was statistically significant ( $F_{7,530} = 2.26, P < 0.03$ ). By contrast, there were no significant differences in the responses of carabids based on sex ( $F_{1,530} = 0.32, P = 0.56$ ). Given the species-specific differences detected here, the statistical analysis was carried out for each carabid species separately.

*Poecilus corvus* showed significant differences in seed detection success under different sensory treatments compared to the positive control ( $F_{8,207} = 17.9, P < 0.001, n = 25$ , Table 3.2). There were no significant differences between males and females of this species in their response to sensory treatments ( $F_{1,207} = 1.61, P = 0.2, n = 25$ ). Disabling of compound eyes and sensory appendages on one side of the carabid head (unilateral control) reduced the ability for seed detection by almost 40% (Figure 3.1 A), while carabids in the negative control group failed to detect any

seeds, and seed consumption was zero. Interestingly however, carabids with blackened compound eyes (all else functional) showed a significant reduction (almost 30%) in seed detection success compared to intact insects. Carabids with only functional eyes failed to detect the seeds, and their seed consumption was not significantly different from the negative control, which was zero. When the predators were left with antennae and/or maxillary palps (olfactory organs) they managed to find seeds with considerable success, as seed finding rates ranged between 50-70% compared to intact insects. Carabids left with labial palps only (gustatory organs) were significantly less successful at finding seeds, as seed consumption in this case was not statistically different from zero.

*Pterostichus melanarius* also showed significant differences in seed detection success under different sensory treatments compared to the positive control ( $F_{8,206} = 19.5$ ,  $P < 0.001$ ,  $n = 25$ , Table 3.2). Males and females of this species showed no significant differences in their response to sensory treatments ( $F_{1,206} = 0.17$ ,  $P = 0.67$ ,  $n = 25$ ). Antennae, palps, or both enabled this carabid to detect the seed with relative consistency, as seed detection success ranged between 50-70% compared to the positive control (Figure 3.1 B). Maxillary or labial palps alone significantly reduced the ability of this carabid species to successfully find the seeds, with detection success only around 25% compared to the positive control. Compound eyes were not useful for successful seed detection on this species; seed finding rates were not significantly different from zero when both antennae and palps were ablated.

Sensory-manipulated *A. littoralis* showed significant differences in their seed detection success of under sensory treatments compared to the positive control ( $F_{8,117} = 16.41$ ,  $P < 0.001$ ,  $n = 15$ , Table 3.2). Sex did not significantly affect seed detection success in this carabid species ( $F_{1,117} = 0.05$ ,  $P = 0.81$ ). In all treatments where carabids had functional antennae, four palps, or antennae and palps seed detection success was significantly different from zero and ranged between 55-75% compared to the positive control (Figure 3.1 C). Carabids with maxillary palps alone were able to detect the seeds to reasonable extents as seed finding success was around 30% compared to the positive control. By contrast, beetles with labial palps alone could not successfully detect the seeds and seed detection success was not significantly different from zero. Successful seed detection was also lost when antennae and palps were ablated and beetles were left with eyes only.



**Figure 3.1.** Total number of seeds consumed (mean total seed consumption  $\pm$  mean standard error) of *Poecilus corvius* (A), *Pterostichus melanarius* (B), and *Amara littoralis* (C) carabids under different sensory manipulation treatments. (+/+): positive control (intact insects); (+/-): unilateral control; (-/-): negative control.



**Table 3.2.** Analysis of variance results (P-values) for measured weed seed feeding responses of the tested carabid species as affected by sensory manipulation treatments and insect sex and their interactions.

Effect	Carabid species												
	<i>Pterostichus melanarius</i> (n = 25)				<i>Poecilus corvius</i> (n = 25)				<i>Amara littoralis</i> (n = 15)				
	ndf	ddf	F-value	P-value	ndf	ddf	F-value	P-value	ndf	ddf	F-value	P-value	
<b>Sensory treatment</b>	8	206	19.5	<0.0001	8	207	17.9	<0.0001	8	117	16.41	<0.0001	***
<i>Insect sex</i>	1	206	0.17	0.67	1	207	1.16	0.2	1	117	0.05	0.81	
<i>Sensory treatment</i> × <i>Insect sex</i>	8	206	0.93	0.49	8	207	1.02	0.41	8	117	0.83	0.57	

ndf: numerator degree of freedom, ddf: denominator degree of freedom

\* Indicates significant difference p<0.05

\*\* Indicates strong significant difference p<0.01

\*\*\* Indicates very strong significant difference p<0.001

Seed selection responses revealed significant effects of the sensory manipulation treatments on the ability of carabid predators to make accurate seed choices across the three carabid species under study ( $F_{16,1532} = 2.27$ ,  $P < 0.01$ , Table 3.3). The interaction between weed species and insect species was statistically significant ( $F_{4,1449} = 74.7$ ,  $P < 0.01$ ), indicating that seed choice responses differed significantly among the tested carabids. The interaction term between sensory treatments and insect species was also significant ( $F_{16,1527} = 2.22$ ,  $P = 0.003$ ). There was also a significant three-way interaction between weed species, sensory treatments, and insect species ( $F_{32,1543} = 8.77$ ,  $P < 0.01$ ). Responses did not differ between males and female in any of the carabid species tested ( $F_{1,1548} = 0.01$ ,  $P = 0.9$ ). Given the species-specific differences detected here, the statistical analysis was carried out for each carabid species separately.

**Table 3.3.** Mixed effects analysis (P-values) for measured weed seed feeding responses of the full data set of the three tested carabid species as affected by sensory manipulation treatments, weed species, insect species, and insect sex and their interactions.

Statistical term	ndf	ddf	F-value	P-value
<i>Weed species</i>	2	59.63	10.99	<b><math>P &lt; 0.0001</math></b>
<i>Sensory treatment</i>	8	1528	55.73	<b><math>P &lt; 0.0001</math></b>
<i>Insect species</i>	2	1447	8.6	<b><math>P &lt; 0.0001</math></b>
<i>Insect sex</i>	1	1548	0.01	$P = 0.9$
<i>Weed Species</i> × <i>Sensory treatment</i>	16	1532	2.27	<b><math>P = 0.002</math></b>
<i>Weed Species</i> × <i>Insect species</i>	2	1449	74.7	<b><math>P &lt; 0.0001</math></b>
<i>Sensory treatment</i> × <i>Insect species</i>	16	1527	2.22	<b><math>P = 0.003</math></b>
<i>Sensory treatment</i> × <i>Insect sex</i>	8	1562	0.94	$P = 0.47$
<i>Insect species</i> × <i>Insect sex</i>	2	1544	0.16	$P = 0.85$
<i>Weed species</i> × <i>Sensory treatment</i> × <i>Insect species</i>	32	1543	8.77	<b><math>P &lt; 0.0001</math></b>
<i>Weed species</i> × <i>Sensory treatment</i> × <i>Insect sex</i>	16	1531	0.8	$P = 0.68$
<i>Weed species</i> × <i>Insect species</i> × <i>Insect sex</i>	4	1552	0.86	$P = 0.48$
<i>Sensory treatment</i> × <i>Insect species</i> × <i>Insect sex</i>	16	1552	1.41	$P = 0.12$
<i>Weed Species</i> × <i>Sensory Manipulation</i> × <i>Insect Species</i> × <i>Insect sex</i>	32	1554	1.09	$P = 0.32$

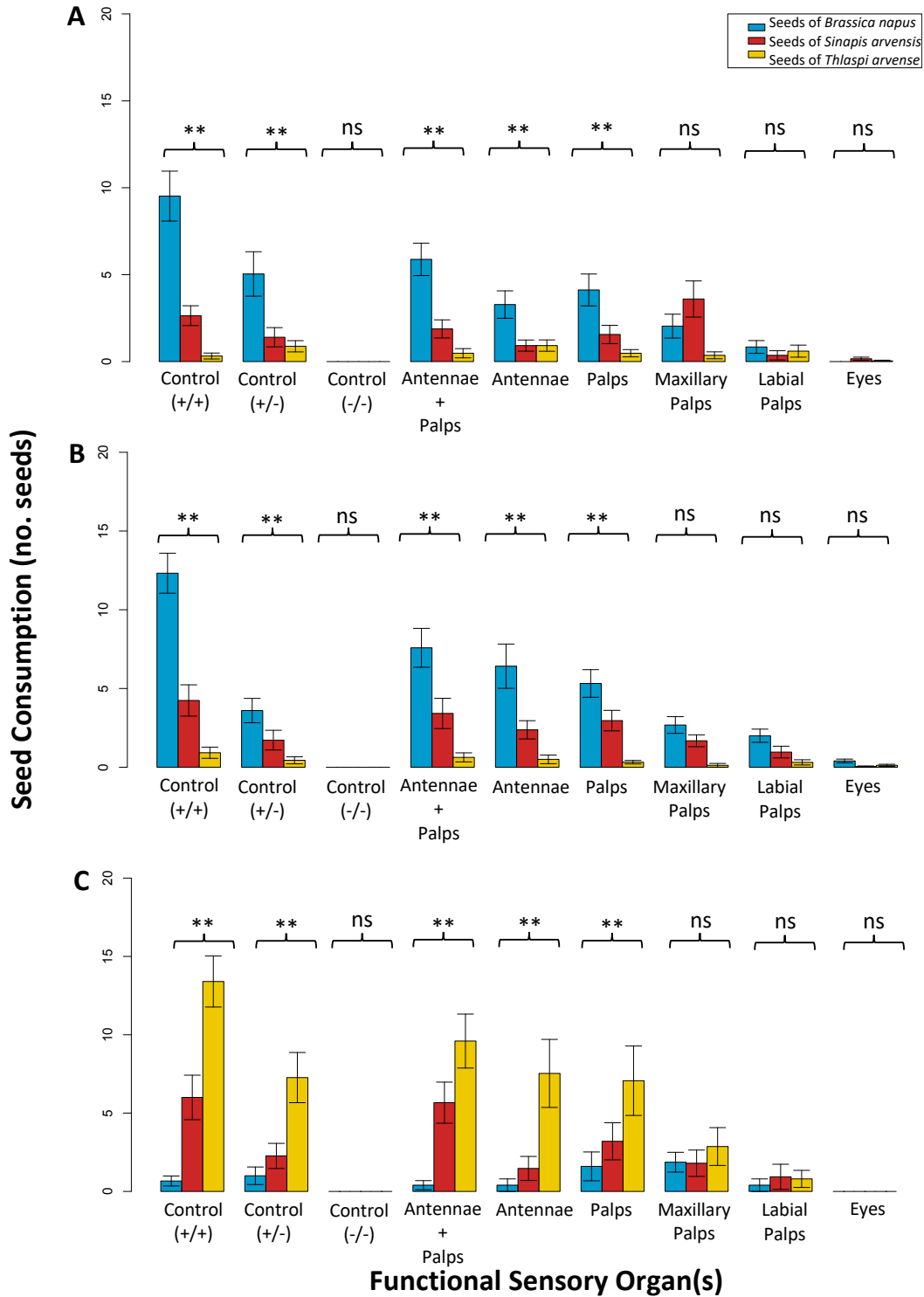
ndf: numerator degrees of freedom; ddf: denominator degrees of freedom

*Poecilus corvus* showed a strong preference for *B. napus* seeds, and this preference was maintained across all treatments where beetles had two antennae and/or four palps ( $F_{8,621} = 15.16$ ,  $P < 0.001$ ,  $n = 25$ , Table 3.4, Figure 3.2 A). Males and females of this species showed no significant differences in their responses ( $F_{1,621} = 1.06$ ,  $P = 0.3$ ,  $n = 25$ ). The most accurate seed choices (strongest preference for *B. napus* seeds) were observed almost always when antennae were

present (with or without palps). When antennae were ablated, beetles needed all four palps to make an accurate seed choice. Carabid beetles lost the ability to choose seeds accurately when left with only one pair of either palps since the preference for *B. napus* seeds lost statistical significance. Accurate seed choice was also lost when antennae and palps were ablated, leaving the beetles with functional eyes only.

*Pterostichus melanarius* also showed significant differences in the ability for making accurate seed choices under the different sensory manipulation treatments compared to control ( $F_{8,601} = 26.99$ ,  $P < 0.001$ ,  $n = 25$ , Table 3.4). Responses of males and females did not significantly differ in this species ( $F_{1,615} = 0.13$ ,  $P = 0.71$ ,  $n = 25$ ). This carabid species showed a strong preference for seeds of *B. napus* (Figure 3.2 B). Antennae alone or with four palps enabled the predator to make the most accurate seed choices. Maxillary or labial palps alone did not enable beetles to accurately chose seeds of *B. napus*, as seed preference in this case lost statistical significance. Seed preference was also lost when beetles were left with only eyes.

Sensory-manipulated *A. littoralis* also showed significant differences in their ability for making accurate seed choices compared to control ( $F_{8,310} = 15.88$ ,  $P < 0.001$ ,  $n = 15$ , Table 3.4). The responses to sensory treatments were not affected by sex ( $F_{1,336} = 0.02$ ,  $P = 0.88$ ,  $n = 15$ ). Unlike the other two species, this carabid species showed a strong preference for *T. arvense* seeds (Figure 3.2 C). Antennae alone or with four palps enabled this carabid species to choose seeds of *T. arvense* accurately. Accurate seed choice was lost when beetles were left with only one type of palps (maxillary or labial) or only functional compound eyes.



**Figure 3.2.** Number of seeds consumed (mean number of seeds  $\pm$  mean standard error) by *Poecilus corvus* (A), *Pterostichus melanarius* (B), and *Amara littoralis* (C) carabids from patches of three different weed species under different sensory manipulation treatments. Asterisks above the bar (\*\*) indicate significant differences in the seed choice response within the treatment group; (ns): indicates no significant differences in seed choice within the treatment group. (+/+): positive control (intact insects); (+/-): unilateral control; (-/-): negative control.

**Table 3.4.** Mixed effects analysis (P-values) for measured weed seed feeding responses of the tested carabid species as affected by sensory manipulation treatments, weed species, and insect sex and their interactions.

Effect	<i>Poecilus corvus</i> (n = 25)			<i>Pterostichus melanarius</i> (n = 25)			<i>Amara littoralis</i> (n = 15)				
	ndf	ddf	P-value	ndf	ddf	F-value	P-value	ndf	ddf	F-value	P-value
<b>Sensory treatment</b>	2	621	<0.0001	2	593	88.51	<0.0001	2	43	24.58	<0.0001
<b>Weed species</b>	8	621	<0.0001	8	601	26.99	<0.0001	8	310	15.88	<0.0001
<b>Insect sex</b>	1	621	0.3	1	615	0.13	0.71	1	336	0.02	0.88
<b>Weed species × Sensory treatment</b>	16	621	<0.0001	16	593	9.26	<0.0001	16	310	5.44	<0.0001
<i>Weed species × Insect sex</i>	2	621	0.58	2	593	1.01	0.36	2	336	0.43	0.64
<i>Sensory treatment × Insect sex</i>	8	621	0.87	8	611	1.13	0.33	8	336	1.08	0.3
<i>Sensory treatment × Weed species × Insect sex</i>	16	621	0.68	16	593	0.43	0.97	16	336	1.34	0.16

ndf: numerator degrees of freedom, ddf: denominator degrees of freedom

\* Indicates significant difference p<0.05

\*\* Indicates strong significant difference p<0.01

\*\*\* Indicates very strong significant difference p<0.001

### 3.5. Discussion

This study has demonstrated that carabid seed predators rely mainly on chemoperception to detect and discriminate among seeds of different seed species. Chemoreceptors on antennae and palps of carabid seed predators were found to be responsible for detecting the sensory cues necessary for seed detection and discrimination. Seed-derived chemical cues emerged as the main mediators of interactions between carabid predators and seeds of weedy plants. By contrast, visual cues did not seem to elicit the sensory response necessary for seed detection and discrimination. These findings align with other pieces of evidence reporting chemoreceptors as the mediators of prey detection and selection in carabid predators (Kielty *et al.*, 1996; Mundy *et al.*, 2000; McKemey *et al.*, 2004; Thomas *et al.*, 2008). Together, these lines of evidence align with my hypothesis that chemoperception is likely the primary sensory mechanism that carabids employ to detect food types of low mobility such as sessile insect prey and seed species. It is important to mention here that sensory manipulations, although intrusive, did not seem to cause significant detriment to the ability of carabids to detect and discriminate among seeds of different species. Sensory-manipulated carabids carrying sufficient functional chemoreceptors (i.e., antennae, palps, or both) were still able to make accurate seed choices similar to those of intact insects (positive control). The sensory receptors located on the sensory appendages of insects collect sensory information from the surrounding environment, but play no major roles in processing the sensory input or releasing of behavioral responses (Sato and Touhara, 2009; Leal, 2013). Higher cognitive centers like optic lobes, antennal lobes, lateral horns, and mushroom bodies are responsible for processing the sensory input and releasing appropriate behavioral responses (Prokopy and Owens, 1983; Schmidt and Benton, 2020), and those remained intact in the carabids under study.

The carabid species we studied seem likely to carry most of the chemoreceptors responsible for seed detection on their antennae (Merivee *et al.*, 2008). Antennae, either alone or with palps, always enabled carabids to identify the suitable seed species with high accuracy. Maxillary and labial palps appear to carry significantly fewer of those chemoreceptors, as predators left with one pair of either palps failed to make accurate seed choices. Carabid antennae usually carry an abundance of olfactory receptors that enable the predator to collect different types of chemical information about their environment (Merivee *et al.*, 2000-2002; Giglio *et al.*, 2013). Olfactory receptors can also be found on maxillary and labial palps, but their abundance on these

appendages is usually rather low (van Naters and Carlson, 2007). On the contrary, gustatory receptors are usually more abundant on maxillary and labial palps (Amrein and Throne, 2005; Su *et al.*, 2009). Olfactory and gustatory receptors show considerable similarities in their structure and physiological function, and collect chemical information of similar nature (Krieger and Breer, 1999; Isono and Morita, 2010). Nonetheless, some authors suggest that sensory information perceived through olfactory receptors is usually more accurate and specific than information perceived via gustatory receptors (e.g., Chapman, 1998). Taken together, these lines of reasoning might explain why accurate seed choices could take place in all treatment groups where antennae were not ablated. This might also explain why all four palps (two maxillary and two labial palps) were needed for accurate seed choices to take place. Beetles carrying either type of palps alone did not seem to perceive the chemical sensory information necessary for making accurate seed choices. Given the above, it could be suggested that olfactory receptors are, in all likelihood, the type of chemoreceptors responsible for the perception of seed species in carabid seed predators.

It has been established in the discussion above that carabid seed predators rely on the olfactory receptors located on their antennae and palps to detect seed-derived chemical cues, and guide their seed selection decisions. The exact nature of chemical cues that originate from seeds of weed species and are exploited by carabids for seed perception remain uncertain at this point. Previous studies have reported that some species of carabid seed predators exhibit positive orientation responses to odors of certain weed seeds in the olfactometer (Law and Gallagher, 2015; Kulkarni *et al.*, 2017b). Therefore, it could be suggested that carabid perception of weed seeds is most likely guided by seed volatile chemicals of some sort. The identity of seed volatile chemicals that carabids perceive and exploit to guide their seed choice decisions remain unknown at this point, and further research to unmask their identity is warranted. It is also unclear which type (morphology and structure) of carabid olfactory chemosensilla is responsible for detection of seed volatiles. Basiconic receptors are usually the main receptors responsible for picking up food-related odor cues in insect species, and these receptors often serve both olfactory and gustatory functions (Ha and Smith, 2008; Van and Carlson, 2007). The sensory arsenal of carabid species encompasses basiconic sensilla as well (Ploomi *et al.*, 2003) and it thus can be proposed that basiconic receptors in carabids are the potential detectors of seed-derived volatile cues. Further research is needed to verify this proposal and explore the sensory physiology of seed perception in carabid seed predators in more depth and breadth.

Seed visual cues did not appear to play any role crucial for seed detection in the carabid species under study. Still, blackening the compound eyes of carabids and leaving antennae and palps intact brought about a significant drop (ca. 50%) in seed detection success compared to the positive control. These observations do not contradict our conclusion that visual cues are unlikely to be vital for seed detection. Alternatively, these observations suggest that seed selection decisions in carabid seed predators is the culmination of different types of sensory inputs being integrated at the higher centers of the brain (Wessnitzer and Webb, 2006; Greene *et al.*, 2013; Riffel, 2020). The absence of visual input, or any other sensory input, in such multi-modal sensory integration brings about an imperfect perception of the environment in the brain of the predator (Henze and Pfeiffer, 2018). The dearth of visual input inflicted on carabid predators by blocking their visual receptors is expected to bring about a gap in their sensory integration system (Yv *et al.*, 2015; Riffel, 2020). This would cause the affected predator to take more time than normal to sample the environment, identify the suitable food types, and handle the chosen food items afterwards (Hassel and Southwood, 1978; Goyret *et al.*, 2007). The extensive time spent on environmental sampling and food handling would ultimately result in predators consuming less food per unit time (Pyke *et al.*, 1977; Giller, 1980). Data in the current study seem to align with the multi-modal sensory integration reasoning as blinded carabid predators, even though their chemoperception was left intact, consumed significantly fewer seeds during the time allotted for seed feeding compared to positive controls.

Seed perception in carabid species seems to be mediated by sensory mechanisms that are similar across species. The response patterns to sensory manipulations showed no fundamental differences when compared among the carabid species under study (see results section). The same sensory mechanisms guided accurate seed choices across species, even when the most preferred seed species differed among carabid species. Some minor differences in carabid responses could be detected among carabid species, nonetheless. Perhaps these subtle differences stem from some differences in the sensillar package each species is equipped with, or differences in the processing and interpretation of sensory information at higher cognitive centers (Andersson *et al.*, 2015; Schmidt and Benton, 2020). The diel rhythms of carabids can, in some instances, be correlated with differences in the sensory and behavioral ecology specific to each species (Luff, 1978; Bauer and Kredler, 1993; Kamenova *et al.*, 2015). This is a less plausible causal factor under the conditions of this study, as all of the carabid species tested are generally nocturnal in their activities



(Lindroth 1969; Allema *et al.*, 2012). Further research is thus needed to explore whether the ecology of carabid species in terms of their diel rhythms (diurnal versus nocturnal) may entail any considerable differences in the sensory mechanisms of seed perception. Male and female carabids, on the other hand, exhibited no significant differences in their responses to sensory manipulations throughout the study. Gender of carabid predators usually has no considerable impact on sensillar packages (Ploomi *et al.*, 2003). It could, therefore, be suggested that seed perception mechanisms in carabid seed predators are unlikely to be sexually dimorphic.

Carabid seed predators exploit olfactory seed cues for seed detection and discrimination. Carabids may, therefore, employ ‘olfactory templates’ or ‘search images’ to identify seed species that are suitable for consumption (Vet and Dicke, 1992). Olfactory search images as mediators of food foraging behaviors have been reported for several species of predatory insects and spiders (Pellegrino and Nakagawa, 2009; Ishii and Shimada, 2010; Cross and Jackson, 2010). It is yet unclear whether the olfactory search image concept applies to carabid predators as well. Applying the search image concept in its strict sense to omnivorous feeders such as the majority of seed-feeding carabids is problematic, as it constrains their cognitive ability for food discrimination (Dukas and Kamil, 2001; Ishii and Shimada, 2010). That is, the recognition of suitable seed or prey would be limited to only a narrow range of species that match the olfactory templates or images hardwired in the brain of the carabid predator (Messing and Rabasse, 1995). Search images of such sort would be nonadaptive for omnivorous carabid predators foraging in complex environments where food types are diverse and of heterogeneous abundance (Forister *et al.*, 2012; Aartsma *et al.*, 2019). Notwithstanding, the search image concept can still be applied to carabid predators if elements of flexibility are added to it. Learning and experience can add some flexibility to the search image concept, allowing for food selection decisions to be more adaptive (Huigens and Fatouros, 2013). Indeed, learning and experience have been shown to play important roles in the formation and adjustment of olfactory search images that guide food selection decisions in polyphagous insect predators and parasitoids (Papaj and Prokopy, 1989; De Boer, and Dicke, 2006). Still, it is unknown if olfactory learning may also mediate some important aspects of seed selection decisions in carabids. Our data allow for no conclusions to be drawn in this respect, and further research is needed to explore the roles of olfactory learning (associative and non-associative) in seed choices of carabid seed predators.

Finally, carabids in the current study were given five days to feed on seed species in the feeding arenas. This is a relatively long period of time, and thus one might assume that hunger might have considerably affected the seed feeding responses of carabids. Although hunger can affect nutrient intake decisions in carabids (Toft *et al.*, 2021), the effects of hunger are usually limited to slight increases in food consumption without significant changes in food preferences (Lang and Gsodl, 2001). Thus, hunger was unlikely an influential factor in this study in terms of seed selection responses, as the level of hunger was standardized for all carabids prior to experimentation. Moreover, all carabid species showed a clear and statistically significant tendency to favorably consume seeds of a specific seed species among the seed species offered in the feeding arenas. Therefore, seed feeding responses of the carabids tested were not random or passively driven by hunger alone (Deroulers and Bretangolle, 2018). Instead, seed selection decisions were most likely based on the active assessment of seed suitability, which was guided by olfactory seed cues detected by the chemoreceptors of carabids. Disabling the chemoreceptors of the carabid species under study rendered them unable to detect and select among seed species.

It should be noted here, however, that seed selection decisions were studied under the artificial conditions of laboratory experiments. The artificial experimental conditions may have affected seed selection decisions in the carabids tested (Luff, 1974), but this does not mean that carabids will not exploit olfactory seed cues to guide their seed selection decisions under realistic situations. Rather, carabids will still rely on olfactory seed cues to identify suitable seeds in the field, but identity of the suitable seed species may differ under realistic conditions depending on habitat properties and the composition of seed bank (Blubaugh and Kaplan, 2014; Blubaugh *et al.*, 2016; Foffova *et al.*, 2020b). The biotic and abiotic properties of carabid habitats affect the abundance of plant and animal foods, and the microclimatic and microsite conditions (Petit *et al.*, 2017; Carbonne *et al.*, 2022). These factors can profoundly affect the composition and structure of the carabid community, and the biocontrol services carabids provide in agroecosystems. In addition to habitat properties, factors relating to physical seed traits, learning and experience, fear, and identity of the dominant carabid species in the local carabid community can also influence seed selection decisions (Ishii and Shimada, 2010; De Heij and Willenborg, 2020; Foffova *et al.*, 2020b). Therefore, seed selection decisions in carabids are complex and sensitive to multiple biotic and abiotic factors, but are generally guided by seed-derived chemical cues that are detected by the antennae and palps of carabid predators.

## Chapter 4 Volatile Derivatives of Seed Coat Surface Lipids Guide the Choice of Seed Species in Carabid Weed Seed Predators

### 4.1. Introduction

The biology and ecology of interactions between carabid species and seeds of weedy plants remain relatively poorly understood. It is thus difficult to ascertain the processes that bring carabids and seed species together, and trigger their interactions. It could, therefore, be more informative to treat seed predation interactions as a special case of plant-insect interactions (Jermeý, 1984; Herrera *et al.*, 2002). Carabid predators in this case interact directly with seeds that are scattered on the soil surface and no longer attached to weed plants. Despite this, the ecological processes that rule seed predation interactions are probably not fundamentally different from those that govern plant-insect interactions in general.

Plant-insect interactions are generally initiated by insects seeking chemical and visual plant-derived cues to guide their searching behaviors toward increasing the chances of locating potential host plants (Prokopy and Owens, 1983; Anton *et al.*, 2007; Rusman *et al.*, 2018). Insects also exploit the chemical and visual characteristics of the different plant species they locate to obtain information about the quality of those plant species, and use this information to identify the species of suitable quality and choose it as a host (Bruce *et al.*, 2005; Beyaert *et al.*, 2010; Reeves, 2011). Without this ability, insects would fail to find their host plants or might select hosts that are unsuitable for feeding and/or oviposition, and suffer significant costs to their survival and reproduction (Mayhew, 1988; Doak *et al.*, 2006). Plant-derived chemical and visual cues (sensory information) are thus the key link in the chain of events that bring insects and plants together, and mediate the initiation of their interactions (Heisswolf *et al.*, 2007; Haverkamp *et al.*, 2018).

Carabid predators, when seeking to interact with seed species, are similarly expected to employ cue-guided behaviors to locate seed species and assess their suitability for consumption. The sensory cues needed for guiding the behavioral tasks of seed searching and suitability assessment need to be obtained mainly from the seeds and not their mother plants (Crawley, 2000; Kulkarni *et al.*, 2017b). After dispersal seeds are usually scattered randomly on the soil surface or even imbedded into the soil, and seed abundance often varies greatly in space and time (Dessaint, 1991; Aartsma *et al.*, 2019). These factors render the seed-derived cues necessary for seed finding

and suitability assessment sparse, and not easily detected (Baldwin, 2010; Borges, 2105). Successful seed finding in such cases would require carabids to engage in active searching for seed-related cues in the environments they come to inhabit. Carabids seem to have the motor abilities along with sensory packages necessary for active seed searching behaviors as many pieces of evidence suggest (e.g., Forsythe, 1983a; Bauer and Kredler, 1993; Merivee *et al.*, 2000-2012).

The sensory basis of seed perception in carabid seed predators remained unclear for a long time in the carabid literature. In the previous chapter, I have managed to show that carabids exploit seed-derived olfactory cues for seed detection and discrimination purposes. The structure and identity of these seed chemical cues remain unknown, however. Previous attempts to sample the volatile chemistry that pervade the headspace of weed seeds could only detect CO<sub>2</sub> and ethylene (Law, 2012; Law and Gallgher, 2015). Still, carabid species showed no responses to either carbon dioxide or ethylene in olfactometric bioassays. It was unclear why no organic volatile chemicals could be detected through sampling weed seed headspaces in those studies. Perhaps technical shortcoming and/or lack of powerful chemical analytical tools precluded the detection of organic seed volatile chemicals in those cases (Tholl, 2006). Profiling the seed headspace via more advanced techniques managed to detect volatile alcohols, ketones, and aldehydes of low molecular weight (e.g., Valette *et al.*, 2006; Paulsen *et al.*, 2013; Foffova *et al.*, 2020a). Still, such volatiles showed no significant bearing on the seed selection decisions of carabids (Foffova *et al.*, 2020a).

Seeds of plant species emit a wide range of volatile alcohols, ketones, and aldehydes that can be detected in the seed headspace, usually reflecting the metabolic state and water content of the seed (Buckely and Buckley, 2009; Mira *et al.*, 2010; Jorgensen, 2011; Colville *et al.*, 2012). As such, the ubiquitous volatile chemicals that usually pervade the seed headspace do not seem to encode information specific to the seed such as species (taxonomic) identity or nutritional quality, as plant volatiles often do (Heil, 2014; Bruce *et al.*, 2015). Thus, ubiquitous seed volatiles are unlikely to provide the accurate and reliable information to make seed discrimination possible in carabid seed predators. Moreover, there is no evidence at this point to suggest that carabids can detect or respond to the ubiquitous alcohols, ketones, or aldehydes that often pervade the seed headspace (e.g., Law, 2012; Foffova *et al.*, 2020a). Based on this, it can be assumed that seed discrimination in carabids is more likely guided by seed volatiles that encode specific information about seed species identity and/or its nutritional quality. These seed volatiles, which are likely to

encode seed-specific information and make seed discrimination in carabids possible, remain to be discovered.

Carabid seed predators exploit seed-derived olfactory information for seed detection and discrimination, as follows from the discussion above and from findings of the previous chapter. Carabid seed predators, as olfactory-oriented foragers, are expected to employ olfactory ‘templates’ or ‘search images’ to guide their seed foraging efforts (Vet and Dicke, 1992; Krier and Breer, 1999). Innate or inflexible olfactory templates or search images are unlikely to be adaptive for omnivorous carabids foraging for food in complex and heterogenous environments (Ducas and Kamil, 2001; Forister *et al.*, 2012; Webster *et al.*, 2013). Alternatively, flexible olfactory templates or search images that can be modified through learning and experience are likely more adaptive for carabid omnivores (Papaj and Prokopy, 1989; Ishii and Shimada, 2010). Indeed, mechanisms of non-associative and associative olfactory learning have been shown to strongly influence food preferences in insect predators and parasitoids (Turlings *et al.*, 1993; De Boer and Dicke, 2006). Sensitization of predators or parasitoids to odors of certain prey or host species via non-associative learning could, in some cases, suffice to create an olfactory bias that induced profound changes in food preferences (Seenivasagan *et al.*, 2010; Schusberger and Pender, 2017). In other cases, however, the olfactory bias necessary for altering prey or host preferences could only be induced through associative learning (Meiners *et al.*, 2003; Glinwood *et al.*, 2011). Still, almost nothing is known about the impact of learning and experience on seed selection decisions in carabid seed predators, and detailed research studies in this regard are needed.

I carried out an experimental study to isolate and identify seed cues that mediate carabid seed predation interactions. I hypothesized that carabids exploit seed volatiles that encode specific information about seed identity and/or its nutritional suitability. Seed headspace sampling protocols and direct extractions of seed surface chemicals were developed to isolate and identify seed cues. Behavioral studies were then conducted to test if the identified seed cues could influence the feeding responses of carabids. Finally, an olfactory priming protocol was developed to test if seed preferences in carabids can be altered by sensitization as a mechanism of non-associative learning. I hypothesized that if seed selection decisions in carabid seed predators were guided by seed odor alone, simple non-associative olfactory priming would suffice to alter seed preferences by selective attention. Beyond that, more complex olfactory learning mechanisms would be needed for learned seed preferences to arise and drive seed selection decisions in carabid seed predators.

## 4.2. Materials and Methods

### 4.2.1. Seed Material

Seeds of the same three brassicaceous weed species mentioned in the previous chapter were used in this study. In brief, seeds of canola (*B. napus*) were used as a highly preferable seed type, whereas seeds of wild mustard (*S. arvensis*), and field pennycress (*T. arvense*) represented moderately and weakly acceptable seed types, respectively. Seed masses of the three weed species were obtained from stored samples (5 °C) collected in summers of 2016-17. Seeds were collected from different field sites at the Kernen Crop Research Farm near Saskatoon, SK, Canada (52°09'10.3" N 106°32'41.5" W).

### 4.2.2. Carabids

Adults of the omnivorous carabid species *Poecilus corvus* (Leconte), *Harpalus amputatus* Say, *Pterostichus melanarius* (Illiger), and *Amara littoralis* Dejean, which are known to consume weed seeds, were used as the seed predatory species in this study. Live adults of carabids were collected from different field sites at the Kernen Crop Research Farm near of Saskatoon, SK, Canada (52°09'10.3" N 106°32'41.5" W) summers of 2019-20 via dry pitfall trapping as described in the previous chapter.

### 4.2.3. Dynamic Headspace Sampling of Seed Volatiles

Dynamic headspace sampling was used to collect samples of weed seed volatile organic compounds (VOCs) from the three brassicaceous weed species mentioned above. The dynamic headspace sampling was carried out using a Sigma Air Delivery System (Sigma Scientific, USA). The sampling procedure was initiated by placing a mass of 500 mg of imbibed seeds on a 2 × 4 cm clean filter paper into a clean and sterilized glass odor collection chamber (Sigma Scientific, USA). At one end, the chamber was connected to source of clean and filtered air. On the other end, a volatile collection trap was attached to the chamber. The volatile traps were built by using 3.5" clean and sterilized Pasteur glass pipettes 5 mm internal diameter (Sigma Scientific, USA). In each

glass pipette, Porapak Q (150 mg, 80 – 100  $\mu\text{m}$ ) was positioned between 3 glass wool plugs (100 + 50 mg of Porapak Q, respectively). Volatile sampling was carried out by pushing clean and filtered air into the glass chamber containing the seed mass, and then through the VOC trap. Volatile sampling via the air entrainment system for each seed mass (replicate) was maintained for 48 hr. Five independent collections were carried out per each weed species, each representing a replicate. Also, a blank sample (filter paper only) was used as a negative control for each round of volatile sampling (Zhu and Park, 2005). Between sampling sessions, all glassware was washed with three rinses of *n*-hexanes, three rinses of ethanol, and three rinses acetone, then baked overnight in a dry oven at 130 °C (Vutz *et al.*, 2015). At the end of the air entrainment sessions, the collected volatiles were eluted (desorbed) from the Porapak Q traps with 3 ml of *n*-hexanes (HPLC-grade) containing 250 ng of pentadecane as an internal standard. Following elution, extracts were concentrated down to 200  $\mu\text{l}$  under a gentle stream of liquid nitrogen and then stored at -80 °C until gas chromatography – mass spectrometry (GC-MS) analysis (Bartlet *et al.*, 2004).

#### 4.2.4. *Static Headspace Sampling of Seed Volatiles Using Solid Phase Microextraction Fibers*

Seed volatile chemicals were sampled in this experiment by placing a 500 mg mass of imbibed weed seeds in a clean and sterile 5 ml crimp-top glass tube. A solid phase microextraction (SPME) fiber coated with polydimethylsiloxane (Supelco, Sigma Aldrich, Canada) was then inserted through the tube into the vial and positioned above the seed mass without contacting it (Tholl *et al.*, 2006). The preparation was then incubated at 21 $\pm$ 1°C in a growth chamber for 24 hours. Five independent preparations were made for each weed species, with each representing a replicate. The same steps were repeated, but without placing seed masses in the glass tube, and those blank preparations served as negative controls. After incubation, chemicals trapped on the fiber were extracted by thermo-desorption at 250 °C for 5 minutes, then injected into the gas chromatography (GC) column for thermal fractionation. The GC conditions and thermal fractionation were as described below.

#### 4.2.5. Direct Extraction of Seed Surface Chemicals via Organic Solvents

Seed surface chemicals were extracted directly by placing 500 mg masses of imbibed seeds in clean and sterile 5 ml glass tubes. Following this, 3 ml of a 9:1 mixture of *n*-hexanes: dichloromethane (non-polar and polar solvents, respectively) was added to each seed mass and shaken thoroughly for 15 minutes (Ardenghi *et al.*, 2017). Preparations were then sealed with parafilm and incubated in a growth chamber at  $21 \pm 1^\circ\text{C}$  for 72 hours. After incubation, the solvent mixture was pipetted out and placed into a new clean and sterile glass tube. Five independent extractions were carried out for each seed species. Extracts were then completely dried under a gentle stream of nitrogen, then re-eluted into 200  $\mu\text{l}$  of *n*-hexanes and then stored at  $-80^\circ\text{C}$  until GC-MS analysis (Ardenghi *et al.*, 2017). The same protocol was repeated without placing any seed mass in the tubes, and those blank extracts served as negative controls.

#### 4.2.6. Analysis of Seed Volatiles Using Gas Chromatography - Mass Spectrometry

The chemical extracts were analyzed by the GC-MS to identify any volatile chemical compounds isolated. The GC-MS analysis was initiated by injecting aliquots of the volatile extracts (2  $\mu\text{l}$ ) into a HP-1 capillary GC column (50 m  $\times$  0.32 mm i.d., 0.55  $\mu\text{m}$  film thickness) equipped with a cool on-column injector and coupled to a mass spectrometer (JEOL AccuTOF 4G, USA). The GC (Agilent 7890A, USA) was programmed as follows: oven temperature was maintained at  $50^\circ\text{C}$  for 2 min and then programmed at  $5^\circ\text{C}/\text{min}$  to  $250^\circ\text{C}$  using helium as carrier gas. The initial identification of any detected compounds was done by comparing retention indices (Kovats Index) of the detected peaks to published spectrum libraries. The TSS Utility software with a link to the NSIT Library was used for analyzing the chromatograms and identifying the detected peaks. Authentic samples of the compounds identified by GC-MS analysis were purchased from Sigma Aldrich (Sigma Aldrich, Canada) for structural confirmation. Purity of each synthetic compound was checked by injecting two independent aliquots of each standard compound into GC-MS. Aliquots were prepared as solutions containing 200 ng of each standard compound in 2  $\mu\text{l}$  of hexane. Compound purity ranged between 98-99.5%.



#### 4.2.7. Checking for Non-Volatile Chemical Seed Cues

Gas chromatography can only detect volatile compounds, and given that the extraction was done from the seed surface, other non-volatile chemicals could have been missed in GC-MS analysis. Therefore, we wanted to make sure that the seeds did not carry any non-volatile chemical cues that could have been overlooked in the GC-MS. The steps described for the direct chemical extraction with a mixture of *n*-hexanes and di-chloromethane (9:1) were repeated. Seed that had undertaken extraction with organic solvents were then offered in two-choice feeding arenas against intact seeds representing control (no solvent treatment). Seeds were offered into plastic trays filled with plasticine and placed in Petri dishes lined with a layer of sand. One tray of treated seeds and another tray of intact seeds each harboring 25 seeds of the same weed species were placed in each Petri dish. *Poecilus corvus*, *A littoralis*, and *H. amputatus* carabids were released in the feeding arenas after 72 hours of starvation. Petri dishes were kept in a growth chamber at  $21\pm 1^{\circ}\text{C}$  and 16:8 L:D photoperiod. Feeding trials were replicated 10 times for each carabid species, and insects were given five consecutive days to feed on seed species. Carabids were removed at the end of experimental time and seed consumption rates were recorded. The feeding interaction matrix in this experiment was established based on pilot seed preference data as follows: 1) *Poecilus corvus*  $\times$  *Brassica napus*; 2) *Amara littoralis*  $\times$  *Thlaspi arvense*; 3) *Harpalus amputatus*  $\times$  *Sinapis arvensis*.

#### 4.2.8. Coating of Protein Pellets with Extracts of Seed Surface Chemicals

The aim of this experiment was to test if seed volatile chemicals identified in previous sections would influence feeding responses of carabid weed seed predators. For this purpose, protein pellets were used as the food source offered in multiple-choice feeding bioassays. Protein pellets used here were composed of pure animal protein (100% shrimp protein and no fat). The use of protein pellets was not intended to mimic the seeds as seeds are not made up of protein only. Protein pellets offered a simple and homogenous (physically and chemically) food source to measure carabid feeding responses to seed surface extracts under controlled conditions. It also offered a good model for testing whether animal protein would become less acceptable when coated with seed surface chemicals. Treatments represented coating the pellets with seed surface

extracts. Seed extracts were collected by direct extraction in a mixture of *n*-hexanes and dichloromethanes (9:1) as described in previous sections. Chemical coating was carried out by soaking the pellets in 2 ml of concentrated of a specific seed extract for 30 min. After soaking, pellets were placed on clean filter paper and left for 10 min for the hexanes to evaporate. In the first experiment, pellets coated with extracts of seeds of *B. napus* were offered against pellets treated with *n*-hexanes only (soaked in 2 ml of pure *n*-hexanes for 30 min then air dried for 10 min) in two-choice feeding arenas in Petri dishes lined with a 2-cm layer of sand. Pellets were placed into plastic tray rings filled with white plasticine and placed into the Petri dishes with 5 pellets in each tray. Prior to placing the pellets into the plasticine tray, the five pellets assigned for each tray were weighed to the nearest 0.1 mg.

Following feeding setup preparations, individuals of *P. melanarius*, *P. corvus*, or *H. amputatus* were released after 72 hours of starvation. Petri dishes were then kept in a growth chamber at  $21\pm 1^{\circ}\text{C}$  and 16:8 L:D photoperiod. Feeding bioassays were replicated 10 times for each species, and insects were allowed to feed for five consecutive days. Carabids were removed at the end of the experiment, and food consumption was recorded by weighing food remnants from each patch to the nearest 0.1 mg. The exact same steps were repeated in the second experiment by offering pellets coated with surface extracts of *B. napus* seeds against pellets coated with surface extracts of *T. arvensis* seeds. In the third experiment, the same steps were repeated but pellets were offered in three-choice feeding arenas. Three patches of protein pellets were offered in the feeding experiments, one coated with surface extracts of *B. napus* seeds, the other coated with surface extracts of *S. arvensis* seeds, and the third coated with surface extracts of *T. arvensis* seeds. Pellets in this experiment were offered to *P. corvus*, or *H. amputatus*, and *A. littoralis* carabids.

#### 4.2.9. Coating the Seed with Surface Chemicals of Another Seed Species

The aim of this experiment was to test if seed surface chemistry, and therefore their preferability to carabid predators, could be changed by chemical coating procedures. Treatments here represented coating seeds of *B. napus* (highly preferable to carabids) with surface extracts of *T. arvensis* seeds (weakly preferable to carabids). Seed chemicals were collected by direct extraction in a mixture of *n*-hexanes and di-chloromethanes (9:1) as described in previous sections. The chemical coating was then carried out by soaking the seeds in 2 ml of concentrated seed extract

suspended in Triton X-100 (2% v/v) with for 30 min (Takahashi and Gassa, 1995). Control *B. napus* seeds underwent the same coating procedure but without adding seed extracts of *T. arvense* to the mix. Seeds after soaking were placed on clean filter paper and left for 10 min for the *n*-hexanes to evaporate. Coated and uncoated *B. napus* seeds were then placed in plasticine trays and offered in two-choice feeding arenas in Petri dishes lined with a 2-cm layer of sand. Each tray harbored 25 seeds and those were exposed to *P. corvus*, *H. amputatus*, or *A. littoralis* carabids after 72 hours of starvation. Petri dishes were then kept in a growth chamber at 21±1°C and 16:8 L:D photoperiod. Feeding trails were replicated 10 times for each species, and insects were allowed to feed for 5 consecutive days. Carabids were removed at the end of the experiment and seed consumption rates were recorded. The exact same steps were repeated to coat seeds of *T. arvense* with surface extracts of *B. napus* seeds and offer them to carabids in two-choice feeding arenas.

#### 4.2.10. Olfactory Priming Experimentation

I have found in the previous chapter that carabids seed predators rely mainly on their olfactory system to detect seeds of different species. The aim of this experiment was to test if seed preference in carabid seed predators could be changed through olfactory priming (i.e., sensitization) as a non-associative olfactory learning mechanism. An olfactory priming procedure was carried out by placing a 500 mg mass of seeds pertaining to a specific weed species into a microbiological sieve, and then sealing it at the bottom with filter paper and tape. The seed mass inside the sieve was then placed in a clean and sterile 250 ml glass beaker. A carabid predator that has been starved for 72 hours was then introduced into the beaker, and the top of the beaker covered with a perforated plastic lid. After that, the preparation was placed into a growth chamber at 21±1°C and 16:8 L:D photoperiod, and left there for 24 hours (Glinwood *et al.*, 2011). These steps were adopted as an ‘olfactory priming’ protocol as carabid species were exposed to odors of specific seed species for extended periods of time to make them experienced with the odor of the seed species used for priming (Tumlinson *et al.*, 1993, see Table 4.1). Carabids undergoing olfactory priming treatments could smell seed odor but could not probe or handle it. The olfactory priming steps described above were applied to predators of *P. corvus* (10 replicates), *A. littoralis* (10 replicates), and *H. amputatus* (5 replicates). Seed masses of *B. napus*, *S. arvensis*, and *T. arvense* were the three different odor sources in the olfactory priming treatments. The same

priming steps were repeated but without placing seeds into the sieves. These empty sieves represented blank treatments and served as procedural controls in the experiment. After carabid predators had been primed, they were released in multiple-choice feeding arenas offering seeds of *B. napus*, *S. arvensis*, and *T. arvense* as described in previous sections.

**Table 4.1.** Treatment list for the olfactory priming experiments with associated treatment descriptions.

Treatment number and code	Treatment description	
1	<i>Poecilus corvopus</i> beetles primed with canola odor	Seeds of <i>Brassica napus</i> L. Seeds of <i>Sinapis arvensis</i> L. Seeds of <i>Thlaspi arvense</i> L.
2	<i>Poecilus corvopus</i> beetles primed with wild mustard odor	Seeds of <i>Brassica napus</i> L. Seeds of <i>Sinapis arvensis</i> L. Seeds of <i>Thlaspi arvense</i> L.
3	<i>Poecilus corvopus</i> beetles primed with field pennycress odor	Seeds of <i>Brassica napus</i> L. Seeds of <i>Sinapis arvensis</i> L. Seeds of <i>Thlaspi arvense</i> L.
4 (Control)	<i>Poecilus corvopus</i> beetles with no odor priming	Seeds of <i>Brassica napus</i> L. Seeds of <i>Sinapis arvensis</i> L. Seeds of <i>Thlaspi arvense</i> L.
5	<i>Amara littoralis</i> beetles primed with canola odor	Seeds of <i>Brassica napus</i> L. Seeds of <i>Sinapis arvensis</i> L. Seeds of <i>Thlaspi arvense</i> L.
6	<i>Amara littoralis</i> beetles primed with wild mustard odor	Seeds of <i>Brassica napus</i> L. Seeds of <i>Sinapis arvensis</i> L. Seeds of <i>Thlaspi arvense</i> L.
7	<i>Amara littoralis</i> beetles primed with field pennycress odor	Seeds of <i>Brassica napus</i> L. Seeds of <i>Sinapis arvensis</i> L. Seeds of <i>Thlaspi arvense</i> L.
8 (Control)	<i>Amara littoralis</i> beetles with no odor priming	Seeds of <i>Brassica napus</i> L. Seeds of <i>Sinapis arvensis</i> L. Seeds of <i>Thlaspi arvense</i> L.
9	<i>Harpalus ampuatus</i> beetles primed with canola odor	Seeds of <i>Brassica napus</i> L. Seeds of <i>Sinapis arvensis</i> L. Seeds of <i>Thlaspi arvense</i> L.
10	<i>Harpalus ampuatus</i> beetles primed with wild mustard odor	Seeds of <i>Brassica napus</i> L. Seeds of <i>Sinapis arvensis</i> L. Seeds of <i>Thlaspi arvense</i> L.
11	<i>Harpalus ampuatus</i> beetles primed with field pennycress odor	Seeds of <i>Brassica napus</i> L. Seeds of <i>Sinapis arvensis</i> L. Seeds of <i>Thlaspi arvense</i> L.
12 (Control)	<i>Harpalus ampuatus</i> beetles with no odor priming	Seeds of <i>Brassica napus</i> L. Seeds of <i>Sinapis arvensis</i> L. Seeds of <i>Thlaspi arvense</i> L.

Petri dishes were incubated in a growth chamber at  $21\pm 1^{\circ}\text{C}$  and 16:8 L:D photoperiod, and predators were allowed to feed for five consecutive days. At the end of the experiment, carabids were removed, and the number of seeds consumed was recorded.

### 4.3. Data Analysis

Seed volatile data were analyzed by using peak area of the chemical compounds identified in the GC-MS as the response variable. Peak areas of the identified chemical compounds were compared using mixed-effects modeling using the function “lmer” (Bates *et al.*, 2015). A maximal model was fitted to the data including weed species, identity of chemical compound, and their possible interactions as main effects. Replicate was used as a random blocking factor as compounds were nested in seed species. Distribution of model residuals showed no violation of model assumptions (Nobre and Singer, 2007). Packages “LmerTest” and “emmeans” were used for comparing compound peak areas among seed species (Lenth, 2019; Schielzeth *et al.*, 2020).

Analysis of variance was used to analyze the data of the seed rinsing experiment (seed non-volatile cues). Mean numbers of seeds consumed between solvent-treated seeds and intact control seeds were compared across the three carabid species. The analysis was initiated by fitting a maximal model including seed treatment, insect species, and their possible interactions as main effects. Model diagnostic plots showed no violation of the normality assumption. Tukey HSD tests were used to perform post-hoc comparisons between the treatments.

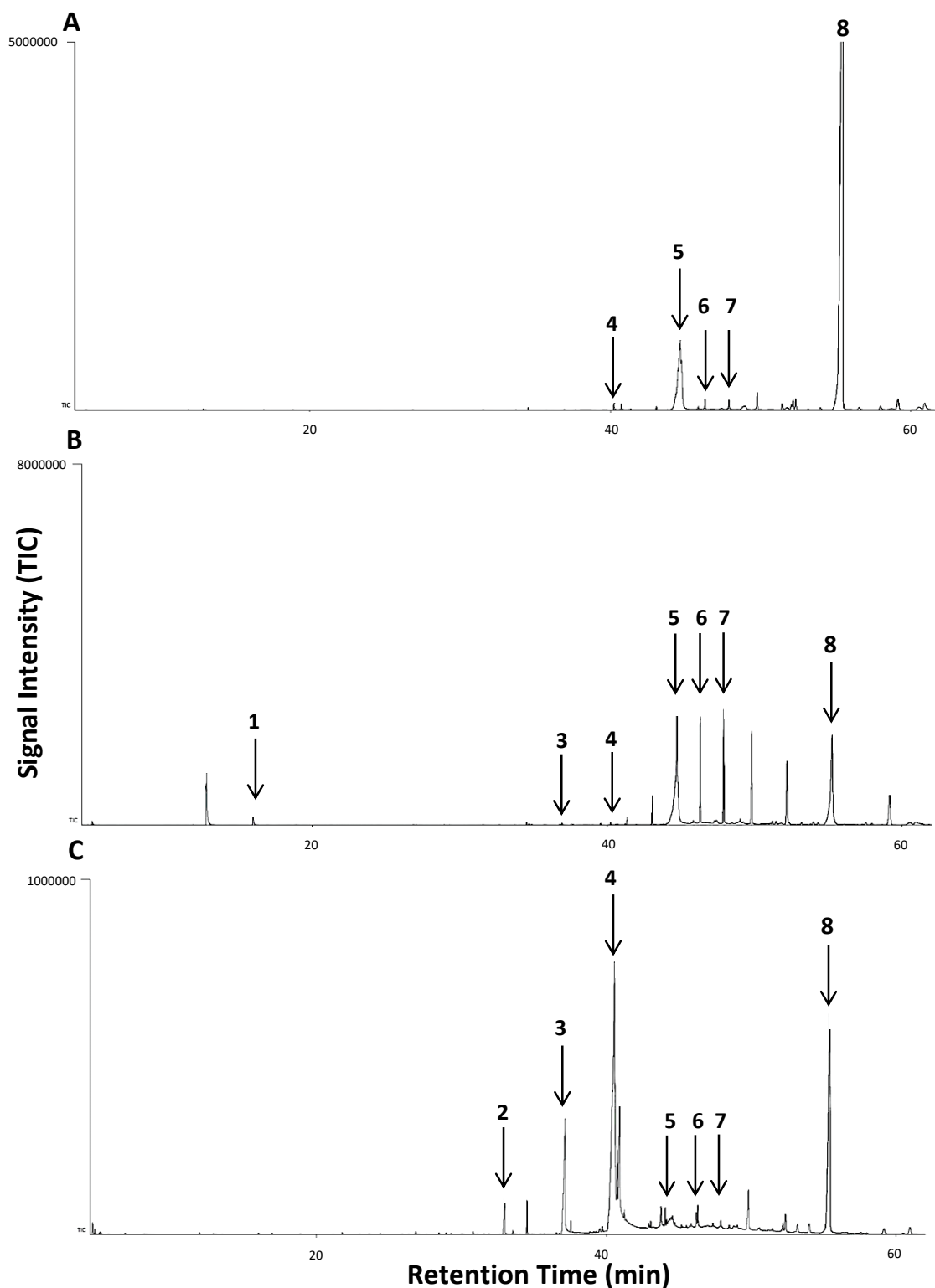
The amount of food (mg) consumed by carabids from the protein pellets offered in the experiment in two-choice or three-choice arenas were compared by mixed-effects models. A maximal model was fitted to the data including pellet treatment, insect species, and their possible interactions as main effects. Replicate was used as a random blocking factor in the model as above. Model assumptions were checked by examining the distribution of model residuals. The “LmerTest” and “emmeans” packages was used to perform post-hoc comparisons on the final models. Similar mixed-effects modeling steps were followed to analyze the data of the seed coating experiment. The number of seeds consumed by carabids from the seed patches offered in the experiment in two-choice arenas were compared by fitting a maximal model to the data, including seed treatment, insect species, and their possible interactions as main effects. Replicate was used as a random blocking factor as above.

The olfactory priming data were analyzed by using the number seeds consumed by each carabid species from each seed species as the response variable. Seed consumption by olfactory-primed and control carabids were compared by mixed effects modeling. The analysis was carried out on the full data set by fitting a maximal model to the data including weed species, olfactory treatment, insect species, and their possible interactions as main effects. Replicate was used as a random blocking factor as above. R v.4.0.3 (R Development Team 2020) was used for all data analysis.

#### 4.4. Results

The dynamic sampling of seed headspace using Porpak Q as an adsorbent material failed to detect species-specific seed volatiles. Only traces of a few long chain alkanes were detected in the headspace of some seed species, and only in a few cases (data not shown). Static sampling of seed headspace with SPME fibers also could not detect any species-specific seed volatile chemicals. The single exception was that traces of allyl isothiocyanate was detected in headspaces of *T. arvense* seeds in some cases (data not shown). No such traces of allyl isothiocyanates were detectable in the headspace of *B. napus* or *S. arvensis* seeds.

The direct extraction of seed surface chemicals with a mixture of *n*-hexanes and dichloromethane (9:1) yielded a wide range of seed volatile chemicals. Seed species revealed significant quantitative differences in some major volatile compounds in their chemical profiles ( $F_{5,72} = 17.6$ ,  $n = 5$ ,  $P < 0.0001$ , Table 4.2). There was also a significant interaction between chemical compounds and seed species ( $F_{8,72} = 4.0$ ,  $n = 5$ ,  $P < 0.0001$ ). The seed volatiles identified through direct extraction of seed surface chemicals were composed of fatty acid derivatives comprising three main groups of long chain aliphatic lipids: alkanes, esters, ketones. Seed species showed significant differences in their profiles of volatile chemicals (Table 4.3). Seeds of *B. napus* featured the simplest profile of surface chemistry, with only two major compounds in their profile (Figure 4.1 A). By contrast, surface chemistries of *S. arvensis* (Figure 4.1 B) and *T. arvense* seeds (Figure 4.1 C) showed more complex profiles of alkanes, ketones, and esters. The structure of the compounds identified in the GC-MS were confirmed via injecting authentic samples in the GC-MS under the same experimental conditions (see Materials and Methods). Fatty acid ethyl esters identified here were not commercially available, so further studies are needed to confirm their chemical structure.



**Figure 4.1.** Seed volatile chemical compounds detected in surface seed extracts of *Brassica napus* (A), *Sinapis arvensis* (B), and *Thlaspi arvense* (C) measured as total ion content TIC (ion abundance) in *mV*. Numbers represent compounds: (1) Nonanal, (2) n-Tetradecanoic acid, (3) Hexadecanoic acid ethyl ester, (4) E-9-Octadecanoic acid ethyl ester, (5) Hexacosane, (6) Hepatacosane, (7) Nonacosane, (8) 15-Nonacosanone.

**Table 4.2.** Mixed effects analysis (P-values) for Seed surface volatile chemicals isolated from the three brassicaceous species used in the experiments showing identity and average percentage of the detected compounds on measurements of peak areas in GC-MS chromatograms.

Effect	ndf	ddf	F-value	P-value	
<i>Weed species</i>	2	72	16.27	< 0.0001	***
<i>Volatile compound</i>	5	72	17.6	< 0.0001	***
<i>Weed species</i> × <i>Volatile compound</i>	10	72	7.0	< 0.0001	***

ndf: numerator degrees of freedom, ddf: denominator degrees of freedom

\* Indicates significant difference  $p < 0.05$

\*\* Indicates strong significant difference  $p < 0.01$

\*\*\* Indicates very strong significant difference  $p < 0.001$

**Table 4.3.** Seed surface volatile chemicals isolated from the three brassicaceous species used in the experiments showing identity and amount of the detected compounds based on peak areas measurements of detected compounds in GC-MS chromatograms.

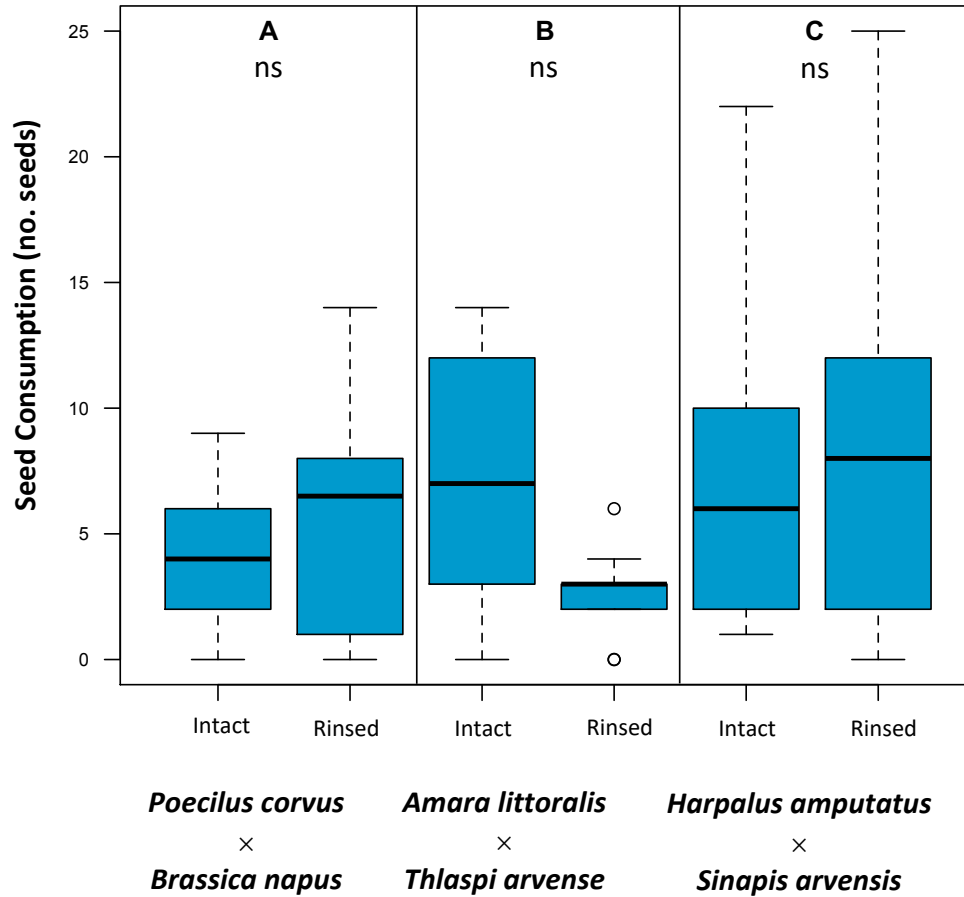
Chemistry	Weed species					
	RT	Formula	CAS #	<i>Brassica napus</i> ( <i>n</i> = 5)	<i>Sinapis arvensis</i> ( <i>n</i> = 5)	<i>Thlaspi arvense</i> ( <i>n</i> = 5)
Nonanal	15.97	C <sub>9</sub> H <sub>18</sub> O	124-19-6	ND	1.97±0.3%	ND
n-Tetradecanoic acid	33	C <sub>14</sub> H <sub>28</sub> O <sub>2</sub>	544-63-8	ND	ND	2.8±0.5%
Hexadecanoic acid ethyl ester	37.23	C <sub>18</sub> H <sub>36</sub> O <sub>2</sub>	626-97-7	ND	< 1%	4.63±1.88% **
E-9-octadecanoic acid ethyl ester	40.73	C <sub>20</sub> H <sub>38</sub> O <sub>2</sub>	6114-18-7	< 1%	< 1%	43.3±7.76% **
Hexacosane	44.64	C <sub>26</sub> H <sub>54</sub>	630-01-3	18±1.45%	11.42±2.87%	< 1% **
Heptacosane	44.87	C <sub>27</sub> H <sub>56</sub>	593-49-7	< 1%	31.47±4.44%	< 1% **
Nonacosane	47.43	C <sub>29</sub> H <sub>60</sub>	630-03-5	< 1%	17.03±2.17%	< 1% **
15-Nonacosanone	55.42	C <sub>29</sub> H <sub>58</sub> O	2764-73-0	79.98±1.73%	32.46±6.29%	45.66±5.3% **

RT: retention time in minutes; CAS #: Chemical Abstracts Service Registry Number in NIST Mass Spectral Library.

ND: not detected; \*\* Indicates significant quantitative differences between volatile chemicals of seed species.

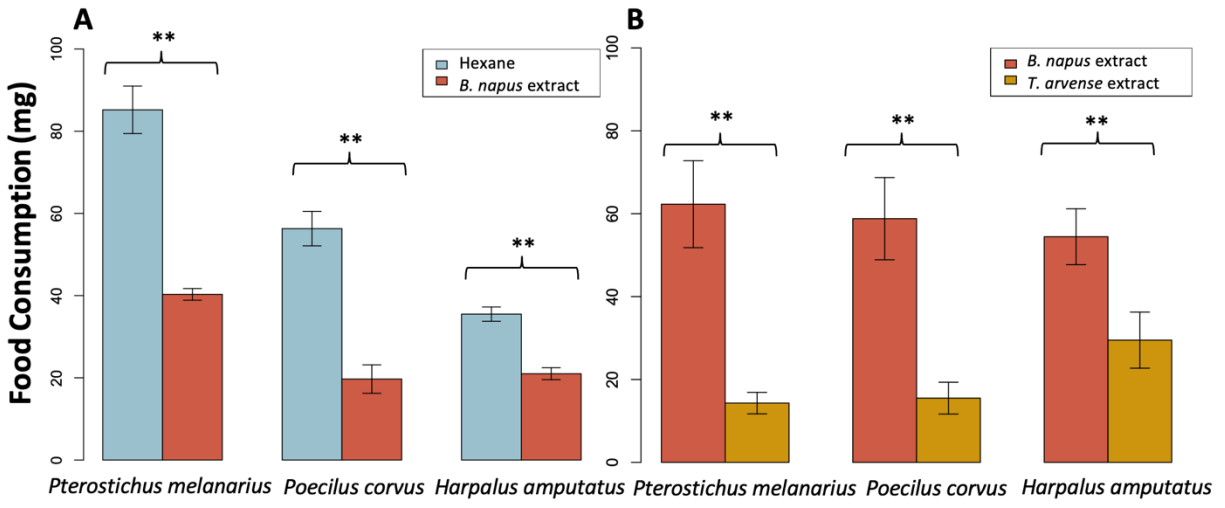
Rinsing the seed specie with a mixture of organic solvents did not bring about any significant differences in seed consumption rates between solvent-treated and intact seeds across the three carabid species ( $F_{2,48} = 2.26$ ,  $P = 0.11$ ,  $n = 10$ , Figure 4.2 A, B and C).



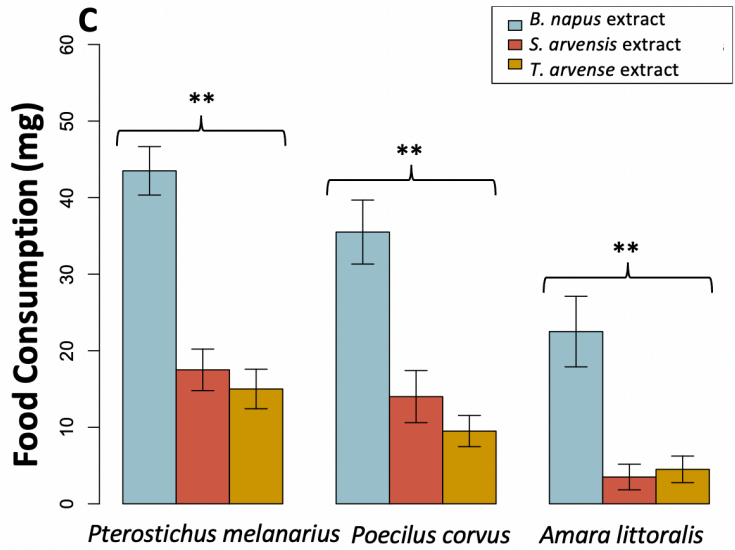


**Figure 4.2.** Comparison of feeding responses of three carabid species offered seeds of three brassicaceous weed species as illustrated in (A), (B), and (C). The offered seeds fell in to two group; one thoroughly rinsed with organic solvents whereas the other was composed of intact seeds. (ns): indicates no significant differences between feeding responses.

Chemical coating of protein pellets with seed surface chemicals revealed that seed surface chemicals affect their preferability to carabid seed predators by influencing their feeding responses ( $F_{3,100} = 15.15$ ,  $P < 0.0001$ ,  $n = 30$ , Table 4.4). Protein pellets coated with hexane only (no seed surface chemicals) were always more preferable to all carabid species than were pellets coated with *B. napus* chemicals (Figure 4.3 A). By contrast, pellets coated with *B. napus* chemicals were more preferable to all species when they were offered against pellets coated with *T. arvense* chemicals (Figure 4.3 B). Furthermore, pellets coated with *B. napus* chemicals were the most preferable for *P. corvus*, *H. amputatus*, and *A. littoralis* when offered against pellets coated with *S. arvensis* and *T. arvense* chemicals in three-choice feeding experiments ( $F_{2,63} = 52.45$ ,  $P < 0.0001$ ,  $n = 30$ , Figure 4.3 C, Table 4.5).



**Carabid Species**



**Carabid Species**

**Figure 4.3.** Comparison of feeding responses (mean food consumption  $\pm$  mean standard error) of three species of carabid seed predators to protein pellets coated with different seed surface chemicals. Pellets treated with surface extracts of *Brassica napus* offered against pellets treated with *n*-hexane (A). Pellets treated with surface extracts of *Brassica napus* offered against pellets treated with surface extracts of *Thlaspi arvense* seeds (B). Pellets treated with surface extracts of *Brassica napus* offered against pellets treated with surface extracts of *Sinapis arvensis* and *Thlaspi arvense* seeds (C). Asterisks (\*\*) indicate significant differences in feeding responses.

**Table 4.4.** Mixed effects analysis (P-values) for measured feeding responses of three carabid species on two types of chemically treated protein pellets as affected by pellet treatment, insect species, and insect sex and their interactions.

Effect	ndf	ddf	F-value	P-value	
<i>Chemical treatment</i>	3	100	16.15	<0.0001	***
<i>Insect species</i>	2	100	8.06	0.00056	***
<i>Insect sex</i>	1	100	2.44	0.121	
<i>Chemical treatment</i> × <i>Insect species</i>	4	100	6.46	0.00011	***
<i>Chemical treatment</i> × <i>Insect sex</i>	3	100	0.42	0.73	
<i>Insect species</i> × <i>Insect sex</i>	4	100	0.25	0.77	
<i>Chemical treatment</i> × <i>Insect species</i> × <i>Insect sex</i>	8	100	0.85	0.85	

ndf: numerator degrees of freedom, ddf: denominator degrees of freedom

\* Indicates significant difference  $p < 0.05$

\*\* Indicates strong significant difference  $p < 0.01$

\*\*\* Indicates very strong significant difference  $p < 0.001$

**Table 4.5.** Mixed effects analysis (P-values) for measured feeding responses of three carabid species on three types of chemically treated protein pellets as affected by pellet treatment, insect species, and insect sex and their interactions.

Effect	ndf	ddf	F-value	P-value	
<i>Chemical treatment</i>	2	63	52.45	<0.0001	***
<i>Insect species</i>	2	68	4.34	0.016	*
<i>Insect sex</i>	1	56	5.1	0.027	*
<i>Chemical treatment</i> × <i>Insect species</i>	4	63	0.2	0.93	
<i>Chemical treatment</i> × <i>Insect sex</i>	2	63	0.45	0.63	
<i>Insect species</i> × <i>Insect sex</i>	4	61	5.8	0.0047	**
<i>Chemical treatment</i> × <i>Insect species</i> × <i>Insect sex</i>	4	63	1.38	0.25	

ndf: numerator degrees of freedom, ddf: denominator degrees of freedom

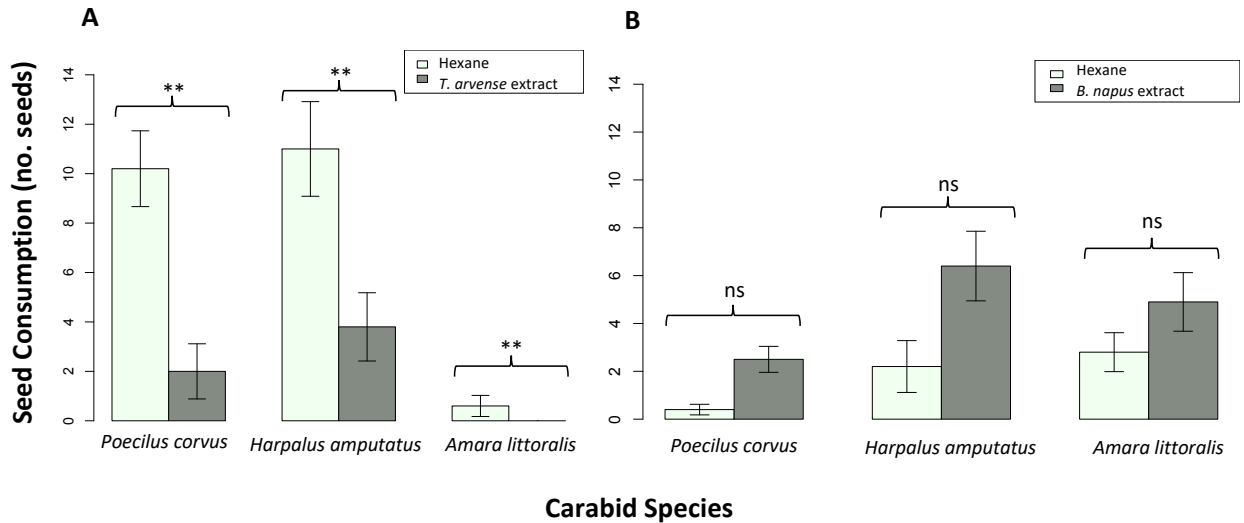
\* Indicates significant difference  $p < 0.05$

\*\* Indicates strong significant difference  $p < 0.01$

\*\*\* Indicates very strong significant difference  $p < 0.001$

Seeds of *B. napus* that were shown in the previous experiment to harbor the kind of chemistry that carabid predators usually prefer became significantly less preferable to all carabids when coated with *T. arvensis* chemicals ( $F_{2,48} = 5.26$ ,  $P < 0.01$ ,  $n = 30$ ; Figure 4.4 A, Table 4.6). On the contrary, coating *T. arvensis* seeds with *B. napus* chemicals made them considerably more acceptable to all species, yet this change in preference could not reach statistical significance ( $F_{2,48} = 0.04$ ,  $P = 0.89$ ,  $n = 30$ , Figure 4.4 B, Table 4.7). Surface chemistry of *T. arvensis* seed was

already complex, and coating with *B. napus* chemicals might have enhanced alkane volatiles, but could not mask the presence of esters. Therefore, no significant shift in seed preference was observed in this case.



**Figure 4.4.** Comparison of feeding responses (mean food consumption  $\pm$  mean standard error) of three species of carabid seed predators to two groups of *Brassica napus* seeds one treated with *n*-hexanes and the other treated with surface extracts of *Thlaspi arvensis* seeds (A). Comparison of feeding responses of three species of carabid seed predators to two groups of *Thlaspi arvensis* seeds one treated with *n*-hexanes and the other treated with surface extracts of seeds *Brassica napus* (B). Asterisks (\*\*) indicate significant differences in feeding responses; (ns): indicate no significant differences in feeding responses.

**Table 4.6.** Mixed effects analysis (P-values) for measured feeding responses of three carabid species on two types of chemically treated canola seeds as affected by seed treatment, insect species, and insect sex and their interactions.

Effect	ndf	ddf	F-value	P-value	
<i>Chemical treatment</i>	1	48	25.74	<0.0001	***
<i>Insect species</i>	2	48	16.74	<0.0001	***
<i>Insect sex</i>	1	48	1.62	0.2	
<i>Chemical treatment</i> $\times$ <i>Insect species</i>	2	48	5.28	0.0084	**
<i>Chemical treatment</i> $\times$ <i>Insect sex</i>	1	48	0.26	0.6	
<i>Insect species</i> $\times$ <i>Insect sex</i>	2	48	2.48	0.093	
<i>Chemical treatment</i> $\times$ <i>Insect species</i> $\times$ <i>Insect sex</i>	2	48	0.45	0.63	

ndf: numerator degrees of freedom, ddf: denominator degrees of freedom

\* Indicates significant difference  $p < 0.05$

\*\* Indicates strong significant difference  $p < 0.01$

\*\*\* Indicates very strong significant difference  $p < 0.001$

**Table 4.7.** Mixed effects analysis (P-values) for measured feeding responses of three carabid species on two types of chemically treated pennycress seeds as affected by seed treatment, insect species, and insect sex and their interactions.

Effect	ndf	ddf	F-value	P-value	
<i>Chemical treatment</i>	1	48	13.62	0.00057	***
<i>Insect species</i>	2	48	5.34	0.008	**
<i>Insect sex</i>	1	48	0.04	0.83	
<i>Chemical treatment</i> × <i>Insect species</i>	2	48	0.93	0.39	
<i>Chemical treatment</i> × <i>Insect sex</i>	1	48	1.12	0.29	
<i>Insect species</i> × <i>Insect sex</i>	2	48	2.45	0.096	
<i>Chemical treatment</i> × <i>Insect species</i> × <i>Insect sex</i>	2	48	0.42	0.65	

ndf: numerator degrees of freedom, ddf: denominator degrees of freedom

\* Indicates significant difference  $p < 0.05$

\*\* Indicates strong significant difference  $p < 0.01$

\*\*\* Indicates very strong significant difference  $p < 0.001$

The different olfactory priming treatments had no significant influence on seed selection responses compared to control across the three tested carabid species ( $F_{3,247} = 0.9$ ,  $P = 0.29$ ). There was a significant interaction between insect species and weed species ( $F_{4,255} = 27.3$ ,  $P < 0.001$ ). This should be expected since the different carabid species used in the experiments had different weed seed preferences.

#### 4.5. Discussion

The experimental studies carried out in this chapter successfully isolated and identified the seed volatile chemicals that guide seed detection and discrimination in carabid seed predators. The seed volatiles identified here are derivatives of long chain fatty acids that comprise three main groups of aliphatic lipids: alkanes, esters, and ketones. Aliphatic lipids (usually termed epicuticular lipids) are an essential constituent of the cuticle layer that covers the surfaces of different plant structures including seeds, and serve wide ecological functions (Moyna and Garcia, 1983; Barthlott *et al.*, 1998; Yeats and Rose, 2013). Plant epicuticular lipids have also been shown to affect important ecological aspects of the interactions between plant species and other organisms such as herbivores (Eigenbrode and Espelie, 1995), pathogens (Ziv *et al.*, 2018), and the natural enemies of plant antagonists (Eigenbrode, 2004). In a similar vein, our data show that epicuticular lipids located on the seed coat surface encode species-specific information that is exploited by

carabid predators to identify suitable seed species with high accuracy. These findings fit into an ample body of evidence documenting plant surface chemicals as interlocutors of feeding and oviposition preferences in insects species, including Diptera (Finch, 1978), Lepidoptera (Mori, 1982; Maloney *et al.*, 1988; Udayagiri and Mason, 1997), Coleoptera (Adati and Matsuda, 2000; Muller and Hilker, 2001), Thysanoptera (Damon *et al.*, 2014), Hymenoptera (Braccini *et al.*, 2013; Braccini *et al.*, 2015; Chen *et al.*, 2018; Fernandez *et al.*, 2019), and Hemiptera (Wojcicka, 2016). Based on that, volatile chemicals derived from the long chain fatty acids located on seed coat surfaces provide the ‘kairomonal’ signals necessary for carabid seed predators to gather information about seed species, and guide their seed selection decisions (Nielsen *et al.*, 2015; Sharma *et al.*, 2019).

The three weed species tested showed qualitative and quantitative differences in their profiles of seed surface chemicals. The quantitative differences in long chain alkanes, ketones, and esters accounted for the majority of species-specific differences among seed species. These findings agree with studies reporting that plant epicuticular lipids encode information specific to plant taxonomic identity, and probably also phylogenetic origins (Medina *et al.*, 2006). The seed chemicals identified here are derived from long chain fatty acids (C<sub>18</sub>-C<sub>29</sub>). Signaling compounds of such high molecular weight are generally low in volatility and can act at close ranges only (Eigenbrode and Espelie, 1995; Heisswolf *et al.*, 2007). This likely explains why carabid species included in this study attempted to ‘touch’ or ‘contact’ the seed surface with their antenna and palps prior to seed selection (Ali, 2019 personal observations). It might also explain why the seed headspace was devoid of any species-specific volatiles in static and dynamic headspace sampling experiments. Carabids are thus more likely to locate seed patches by general habitat-derived cues like whole plant volatiles (Oster *et al.*, 2014), and/or other chemical or physical cues that indicate the presence of plant cover (Blubaugh *et al.*, 2016), or dense seed patches (Honek and Martinkova, 2001). Then, seed surface chemicals can be exploited for identifying suitable seed species.

It was notable that no glucosinolate compounds were detectable in the extracts of seed surface chemicals, even though seeds of brassicaceous species usually harbor considerable amounts of these defensive compounds (Charron and Sams, 2004; Bhandari *et al.*, 2015). Other authors have also reported that glucosinolates and their breakdown products (isocyanates) are not easily detectable in headspaces of brassicaceous species, or in extracts of their epicuticular lipids (Reifenrath *et al.*, 2005; Stadler and Reifnath, 2009). However, these findings seem to

contradict the assumption that glucosinolates may act as deterrents against carabid seed feeding *sensu* Kliebenstein *et al.*, (2002). Therefore, it could be proposed that glucosinolates and their metabolites are unlikely to function as preingestive seed feeding deterrents against carabid seed predators *sensu* Sharma *et al.*, (2019). Hence, seed surface aliphatic lipids emerge as the main preingestive signaling chemical compounds carabid predators exploit to guide their seed foraging behaviors. It remains uncertain, however, whether the same applies for non-brassicaceous seeds.

Rinsing the weed seeds with organic solvents did not bring about significant changes in the ability of carabids to detect seeds. Although solvents are expected to extract considerable amounts of surface waxes, they are not expected to remove them completely since epicuticular lipids are essential constituents of the cuticular layer (Moyna and Garcia, 1983; Kunst and Samules, 2003). This makes seed surface aliphatic lipids that carabids exploit for seed detection and discrimination not only specific but also highly reliable, as there is no way for the seed to be chemically cryptic. The kind of information carabid seed predators extract from seed kairomones remains unknown. Nonetheless, there are well-documented (yet poorly understood) correlations between plant genetics (species and cultivar), cellular fatty acid metabolic-biosynthetic pathways, and the composition of seed surface lipids (Kunst and Samuels, 2003; Jetter and Kunst, 2008). It is thus quite possible that seed surface lipids encode information about the fatty acid composition of seed species, and potentially also of their quantity or quality.

Feeding experiments with protein pellets showed that presence of seed aliphatic lipids alone is enough to induce selective feeding responses in carabid seed predators. The ability for plant surface chemicals alone to drive host plant selection decisions and induce feeding has been reported for other insect species as well (Espelie *et al.*, 1991; Eigenbrode and Pillai, 1998; Damon *et al.*, 2014; Macel *et al.*, 2020). However, all carabids tested preferred uncoated pellets when those were offered against pellets coated with canola extracts. This seems to indicate that prey (uncoated animal protein in this case) is accepted more readily by carabids than seeds (animal protein coated with canola surface extracts) when both are available to the carabid predator. Protein pellets coated with surface extracts of *B. napus* seeds were strongly preferable to all carabid species tested, when no uncoated pellets were offered. The presence of fatty esters among the seed volatiles of *S. arvensis* and *T. arvense* likely rendered them less preferable to carabid seed predators. Fatty acid ethyl esters often indicate the presence of low-quality fatty acids in oils of plant species (Gomez-Coca *et al.*, 2016; Di Serio *et al.*, 2017). Similarly, coating *B. napus* seeds with surface

extracts of *T. arvense* seeds rendered *B. napus* seeds significantly less preferable to all carabid species tested. Seeds of *B. napus* lack fatty esters, but the coating procedure introduced them into *B. napus* seeds, rendering them less preferable. Preferability of *T. arvesne* seeds to carabids did not change significantly when coated with surface extracts of *B. napus* seeds as fatty esters were already present. On the contrary, aliphatic lipids like alkanes, ketones, and alcohols usually act as feeding stimulants for insect herbivores (Mori, 1982; Adati and Matsuda, 1993; Lin *et al.*, 1998). The slight increase in preference towards *T. arvense* seeds coated with surface extracts of *B. napus* seeds could have arisen due to increased levels of aliphatic lipids and alkanes being added to surfaces of coated seeds compared to uncoated ones. Given this, our results provide support for the ‘balance model’ concept for host plant selection in insect species (Renwick, 1989; Schoonhoven *et al.*, 2005), where the balance between positive stimuli (aliphatic lipids here) and negative stimuli (fatty acid esters here) would determine the acceptability of seed species to carabid predators.

Olfactory priming by non-associative learning had no influence on seed selection decisions in the carabid species under study. This lack of effect could be due to the limitations inherent to olfactory priming techniques. In olfactory priming, the experimental insect learns about the availability of a specific food type in the environment through food odor alone, since no feeding is allowed (Huigens and Fatouros, 2013). Extended or repeated exposure to the odor of a specific food type would prime (i.e., sensitize) the sensory apparatus of the insect and enhance its responses towards the odor of that food type by creating an olfactory cognitive bias (Little *et al.*, 2019). This did not turn out to be the case in the three carabids tested here, however. Similarly, olfactory priming of the polyphagous predatory ladybird beetle *Coccinella septempunctata* (L.) with odors of different aphid-infested barley cultivars, without allowing the beetles to feed on aphids (prey), also failed to produce significant changes in feeding preferences (Glinwood *et al.*, 2011). Olfactory priming methodology often lacks the ‘reward’ element essential for associative conditioning and operant learning (Ducas, 2008; Jones and Agrawal, 2017). Therefore, when *C. septempunctata* beetles were allowed to feed on aphids in the experiment, associations between the odor of aphid-infested plants and the presence of prey could be created, leading to significant changes in feeding preferences (Glinwood *et al.*, 2011). It is thus reasonable to propose that seed preferences in omnivorous carabid seed predators may, in some cases, be guided by olfactory search images created by associating seed odors with seed handling parameters.



## CHAPTER 5 Seed Feeding Habits Arise to Address Lipid Limitations in the Diets of Carabid Weed Seed Predators

### 5.1. Introduction

Carabid (ground) beetles are amongst the most important post-dispersal weed seed predators in the temperate arable land (Honek *et al.*, 2003; Lundgren, 2009). Seeds of plants, including weed species, generally contain considerable amounts of nutrients comprising protein, lipids, and carbohydrates (starch) in their tissues at levels that differ between species, genera, and families (Shewry *et al.*, 1995; Bretagnolle *et al.*, 2015). Carabid seed predators seem to seek the consumption of weed seeds to extract specific nutrients to address specific needs relating to survival and reproduction (Frank *et al.*, 2011). Interactions between carabid predators and seeds of weedy plants can thus be considered trophic in their nature (Lundgren *et al.*, 2013). Studying the nutritional basis of the ecological interlinkage between carabids and seed species is still in its nascent stages, however. It is still unclear which nutrients carabid seed predators seek to acquire through feeding on seeds of weed species. Protein (amino acids) and lipids (fatty acids) are generally the two main nutrients arthropod predators, including carabids, are usually in search of in their environments to address the nutritional requirements for survival and development (Jensen *et al.*, 2012; Schmidt *et al.*, 2012; Wilder *et al.*, 2019). It is yet uncertain which of the seed nutrients determines which seed species are more suitable to carabid predators and are therefore more sought after even when alternative foods are available.

There have been some indirect observations in the field (without direct experimental testing) reporting that weed seeds containing an abundance of lipids get chosen more preferably for consumption by carabids than seeds with low lipid content (Petit *et al.*, 2014). Direct testing of these observations through laboratory feeding experiments have shown that, within certain bounds of seed size, lipid-rich weed seeds are more prone to elevated risks of carabid predation (Gaba *et al.*, 2019). Taken together, these pieces of evidence collectively point towards seed lipid content as a potential influential factor in determining seed preferability to carabid seed predators. This finding should be approached with some caution, however. While the laboratory study singled out seed mass and seed lipid content as the only two experimental factors to impact seed preferability to carabids (Gaba *et al.*, 2019), the study ignored other possibly influential factors like seed protein

content, seed chemical defense, and seed physical characteristics. It is also possible that seed lipid content does not act alone in driving seed choice in carabids. Nutrient intake decisions in insects that seek to obtain lipids from plant sources such as pollen seem to be driven not only by the lipid content, but the protein content also (Vaudo *et al.*, 2016; Kraus *et al.*, 2019; Gosh *et al.*, 2020). The nutritional ecology of seed feeding in carabids therefore should be studied based on the interplay between lipid and protein as these two major macronutrients exert substantial influence on carabid feeding ecology (Jensen *et al.*, 2012).

Carabid species usually forage in arable fields where nutrient availability is heterogenous. Prey of different species can often be found in agricultural fields, and numerous species of these prey items usually harbor high levels of protein in their tissues (Wilder *et al.*, 2013). This makes carabids highly unlikely to suffer harmful limitations in their dietary protein under the conditions of agricultural environments (Denno and Fagan, 2003; Wilder, 2019). By contrast, lipids are usually scarce in prey tissues and this scarcity tends to increase at higher trophic levels (Wilder *et al.*, 2013). Scarcity of lipids in prey species makes carabid predators more prone to suffer limitations in dietary lipids when they forage for nutrients in agricultural fields (Jensen *et al.*, 2012; Toft *et al.*, 2019). It has been shown in the previous chapter that volatile chemicals derived from seed surface lipids mediate seed discrimination in carabid seed predators. Seed volatile fatty acid derivatives potentially encode information about the fatty acid composition of the seed and potentially also the quantity or quality of seed fatty acids (Kunst and Samuels, 2003; Jetter and Kunst, 2008). This might be another indication that carabids, through odor-mediated selection of seed species, could be seeking the acquisition of certain essential fatty acids from the seed. It can be hypothesized along these lines that omnivorous carabids may seek to consume seeds of weed species to obtain lipids that are not sufficiently available through prey hunting alone. This would likely manifest as carabid seed predators exhibiting a tight regulation of their lipid intake relative to protein when they face unbalanced protein-to-lipid conditions in their environments (Simpson and Raubenheimer, 1993a). It is difficult to test this assumption given the confounding effects of seed non-nutritional chemicals (defensive chemicals) and physical characteristics, however. Detailed mechanistic studies conducted outside the confinements of the physical characteristics of seeds are needed to clarify whether the scarcity of lipids in agricultural environments drive seed feeding habits in carabid seed predators, and how the non-nutritional seed chemistry might affect the availability of seed nutrients to carabid predators.

Seed nutrients are potentially among the core factors that determine seed suitability to carabid predators and thus, predispose seed species to carabid predation risks especially when their levels in seed tissues are high (Dalling *et al.*, 2011; Wallinger *et al.*, 2015). Due to this vulnerability, seed species are generally defended by a wide array of secondary metabolites to protect the seed against seed eaters (Ibanez *et al.*, 2012; Kessler and Kalske, 2018). Seed secondary metabolites (i.e., seed toxins) may act preingestively against carabid seed predators by deterring carabids from feeding on the seed altogether (Wang and Chen, 2012; Pascacio-Villafan *et al.*, 2016). Alternatively, seed toxins may act postingestively against carabids through direct toxicity or by rendering seed nutrients less digestible (Behmer *et al.*, 2002; Stevenson *et al.*, 2017). Seed suitability to carabid predators is thus more likely determined by the interplay between seed nutrients and defensive chemicals (Agrawal and Fishbein, 2006). In addition to secondary metabolites, protein quality in terms of amino acid composition can also affect the nutritional suitability of plant proteins to insect species (Felton, 1996; Lee, 2007). Seed protein quality may, therefore, be another potential defensive mechanism through which seed species can be protected against seed predators such as carabid beetles. Still, the ways in which seed non-nutritional chemicals (i.e., chemical defenses) interact with seed nutritional chemicals (i.e., nutrients) and influence seed selection decisions in carabid seed predators remain poorly studied.

I carried out an experimental study to explore some of the nutritional aspects of seed preference in carabid seed predators outside the confounding effects of seed physical characteristics. I prepared synthetic diets of different lipid-to-protein ratios to test which of these two macronutrients was more limiting to nutrient foraging in carabids. Based on the above line of reasoning, I hypothesized that lipid would be more limiting to nutrient foraging in carabid seed predators than protein. Carabids should therefore tightly regulate their lipid ingestion when they encounter unbalanced protein-to-lipid ratios in the experiment. I was also interested in elucidating the influence of seed defensive chemicals such as low-quality protein and secondary metabolites on nutrient foraging in carabid seed predators. For this purpose, I manipulated diet quality either by reducing the quality of protein or by adding the seed secondary metabolite allyl isothiocyanate to the diet.

## 5.2. Materials and Methods

### 5.2.1. Carabids

Adults of the omnivorous carabid species *Poecilus corvus* (Leconte), *Pterostichus melanarius* (Illiger), and *Harpalus amputatus* Say, which are known to consume weed seeds, were used in this study. Live adults of those carabids were collected from different field sites at the Kernen Crop Research Farm near Saskatoon, SK, Canada (52°09'10.3" N 106°32'41.5" W) summers of 2019-20 via dry pitfall trapping, as described in the previous chapters.

### 5.2.2. Preparation of Synthetic Diets

Synthetic diets were used to study how the dynamics of lipid and protein intake in carabid seed predators may impact seed preferences. Synthetic food allowed for the investigation of changes in carabid nutritional intake decisions in response to food chemistry alone without the potential confounding effects of seed physical characteristics. Synthetic diets of three different protein-to-lipid (P:L) ratios (*viz.* 35:7, 21:21, 7:35) were prepared as described by Simpson and Abisgold (1985). Protein and lipid were chosen as they generally explain a large proportion of variation in performance as well as physiological and behavioral responses of omnivorous insects, including carabid predators (Jensen *et al.*, 2012; Csata and Dussutour, 2019). In brief, diets represented a complete mix of nutrients, containing fixed levels of proteins and lipid (42% dry weight), amended with micronutrients, salts, and vitamins (4%). The remaining 54% of the diets were filled with cellulose as a non-nutritive bulking agent in order to maintain a constant bulk. The protein sources in each diet represented a 3:1:1 mixture of casein, bacteriological peptone and egg albumen, whereas lard (pure fat) was used as the main source of lipid (Lee *et al.*, 2006; Jensen *et al.*, 2012). The prepared dry dietary mixtures, before being presented to the carabids, were suspended in 2% agar as a 5:1 agar: dry food formula resulting in *ca.*86% water content (Lee, 2007).

### 5.2.3. The Impact of Protein-to-Lipid Ratio on Dietary Intake Regulation

The impact of P:L ratio on dietary intake regulation was studied via offering three different combinations of the P:L diets described above in two-choice feeding bioassays. In short, the feeding bioassays were set up in Petri dishes ( $\varnothing = 25$  cm, 5 cm depth) by dividing each petri dish into two arenas (two halves). In each arena, a block of synthetic food (400 - 500 mg diet cubes) was placed near the perimeter of the dish. Each food block represented a food source containing a known P:L ratio, and the placement of the food blocks was randomized in order to avoid bias. Treatment groups were established as three different pairings of the P:L diet blocks: 35:7 + 7:35; 21:21 + 7:35; 21:21+ 35:7 (Table 5.1). The P:L pairings created experimental bi-dimensional nutritional landscapes that covered protein-to-lipid values ranging between P:L= 5:1 and P:L= 1:5. These ratios were not based on estimates of protein and fatty acids in brassicaceous seeds. Rather, diet pairings represented a standard laboratory protocol adopted for testing experimental hypotheses, and determining which nutrient is limiting to carabid nutrient foraging decisions. I thought that imposing such strong nutritional imbalances on carabids would better capture the changes in their nutrient intake decisions.

Prior to feeding experiments, food blocks were weighed to the nearest 0.1 mg of fresh mass, and then each food block was placed into one side of a Petri dish. After that, a single adult predatory beetle was released into the Petri dish following 73 hr of starvation. Petri dishes were then incubated in a growth chamber at  $21 \pm 1$  °C and 16:8 L:D photoperiod. Beetles were left to feed on the synthetic food for 24 hours. After 24 hours, food blocks were replaced with fresh ones, and the food remnants (i.e., removed blocks) were dried to a constant mass in a desiccation oven (at 50°C for 24 hours). After drying, food remnants were weighed to the nearest 0.1 mg of dry mass. This daily protocol was repeated for five consecutive days of feeding. Throughout the experiments, each beetle was used only once, and treatments were replicated 14 times for *P. corvus*, and *P. melanarius*, and 12 times for *H. amputatus*. Carabids were removed from the feeding arena at the end of the experiment and food consumption was calculated. The fresh mass (mg) for every carabid used in the experiments were recorded by weighing the carabid to the nearest 0.1 mg after starvation and prior to its release in the Petri dishes.

**Table 5.1.** Treatment list for laboratory feeding trials testing the impact of P:L ratio on dietary intake regulation for three carabid species associated treatment descriptions.

Treatment number	Diet pairing	Treatment description
1	21:21 + 35:7 (P:L = 1:2)	Protein-biased bi-dimensional nutritional landscapes
2	21:21 + 7:35 (P:L = 2:1)	Lipid-biased bi-dimensional nutritional landscapes
3	35:7 + 7:35 (P:L = 1:1)	Balanced bi-dimensional nutritional landscapes

#### 5.2.4. *The Impact of Protein Quality on Dietary Intake Regulation*

Protein quality in the synthetic diets was manipulated to create two levels of protein quality in the food blocks offered to carabid predators. The level of protein quality was changed by replacing 50% or 100% of casein in the normal diet with zein, a low-quality protein (Lee, 2007). The 50% casein substitutions represented protein of moderate quality in the experiment, whereas the 100% casein substituents represented protein of low quality. The main treatments here represent three P:L pairings each with a specific proportion of casein substitution ( $3 \times 2 = 6$  treatments, Table 5.2). Data from experimentation with the normal diet served as the control here.

**Table 5.2.** Treatment list for laboratory feeding trials testing the impact of protein quality on dietary intake regulation for three carabid species associated treatment descriptions.

Treatment number	Diet pairing	Treatment description
1	21:21 + 35:7 (P:L = 1:2)	Protein-biased bi-dimensional nutritional landscapes
	50% casein substitution	Protein quality is moderate
2	21:21 + 7:35 (P:L = 2:1)	Lipid-biased bi-dimensional nutritional landscapes
	50% casein substitution	Protein quality is moderate
3	35:7 + 7:35 (P:L = 1:1)	Balanced bi-dimensional nutritional landscapes
	50% casein substitution	Protein quality is moderate
4	21:21 + 35:7 (P:L = 1:2)	Protein-biased bi-dimensional nutritional landscapes
	100% casein substitution	Protein quality is low
5	21:21 + 7:35 (P:L = 2:1)	Lipid-biased bi-dimensional nutritional landscapes
	100% casein substitution	Protein quality is low
6	35:7 + 7:35 (P:L = 1:1)	Balanced bi-dimensional nutritional landscapes
	100% casein substitution	Protein quality is low

### 5.2.5. The Impact of Allyl Isothiocyanate Level on Dietary Intake Regulation

The normal diet (i.e., no protein quality manipulation) was augmented with three doses of allyl isothiocyanate (0.5%, 2.5%, and 10% v/v). Allyl isothiocyanate is among the major glucosinolate-derived defensive compounds commonly found in seeds of brassicaceous species (Tsao *et al.*, 2002; Sharma *et al.*, 2018). The aim here was to investigate the impact of allyl isothiocyanate on nutrient intake regulation in the carabid species under study. The main treatments here were three P:L pairings each with a specific concentration of allyl isothiocyanate (3×3 = 9 treatments, Table 5.3). Data from experimentation with the normal diet served as the control for this experiment.

**Table 5.3.** Treatment list for laboratory feeding trials testing the impact of allyl isothiocyanate levels on dietary intake regulation for three carabid species associated treatment descriptions.

Treatment number	Diet pairing	Treatment description
1	21:21 + 35:7 (P:L = 1:2)	Protein-biased bi-dimensional nutritional landscapes
	0.5% allyl isothiocyanate dosage (v/v)	Low dose of seed chemical defense
2	21:21 + 7:35 (P:L = 2:1)	Lipid-biased bi-dimensional nutritional landscapes
	0.5% allyl isothiocyanate dosage (v/v)	Low dose of seed chemical defense
3	35:7 + 7:35 (P:L = 1:1)	Balanced bi-dimensional nutritional landscapes
	0.5% allyl isothiocyanate dosage (v/v)	Low dose of seed chemical defense
4	21:21 + 35:7 (P:L = 1:2)	Protein-biased bi-dimensional nutritional landscapes
	2.5% allyl isothiocyanate dosage (v/v)	Moderate dose of seed chemical defense
5	21:21 + 7:35 (P:L = 2:1)	Lipid-biased bi-dimensional nutritional landscapes
	2.5% allyl isothiocyanate dosage (v/v)	Moderate dose of seed chemical defense
6	35:7 + 7:35 (P:L = 1:1)	Balanced bi-dimensional nutritional landscapes
	2.5% allyl isothiocyanate dosage (v/v)	Moderate dose of seed chemical defense
7	21:21 + 35:7 (P:L = 1:2)	Protein-biased bi-dimensional nutritional landscapes
	10% allyl isothiocyanate dosage (v/v)	High dose of seed chemical defense
8	21:21 + 7:35 (P:L = 2:1)	Lipid-biased bi-dimensional nutritional landscapes
	10% allyl isothiocyanate dosage (v/v)	High dose of seed chemical defense
9	35:7 + 7:35 (P:L = 1:1)	Balanced bi-dimensional nutritional landscapes
	10% allyl isothiocyanate dosage (v/v)	High dose of seed chemical defense

### 5.2.6. Measurements of Dietary Intake

The dietary intake measurements were based on dry mass values of the diet blocks. This required estimating the relationship between fresh mass and dry mass of the food blocks. For this purpose, the exact same experimental protocols described above were repeated but without releasing any carabid beetles into the Petri dishes ( $n= 20$  blocks per each P:L diet). The data collected here served as technical controls, and were used to establish the relationship between fresh and dry masses of the food blocks via regression analyses. Regression equations were used to convert fresh mass values to dry mass values. Daily food consumption was then calculated as the difference between initial and final dry masses of the food blocks offered to the beetles in each Petri dish (mg food dry mass). The values of daily food intake were added up to calculate the cumulative food intake for each beetle. Finally, the amount of protein and lipid (mg dry mass) ingested by each beetle over the five days was calculated by multiplying total food consumption by the corresponding P:L ratio of the diet block.

### 5.3. Data Analysis

The amount of protein and lipid consumed by carabids (dry mg) over five days under the three different experimental P:L conditions were compared via mixed effects modeling (Bates *et al.*, 2015). The initial data analysis was carried out by pooling data from all three species and all three experiments together in one massive data set. Collating the data created a factorial experimental design, allowing nutrient intake responses to be compared under different nutritional P:L conditions as well as different diet quality parameters. The analysis was initiated by fitting a maximal model to the data including macronutrient (protein or lipid), nutritional landscape (P:L ratio), protein quality (percentage of casein substitution), diet toxicity (allyl isothiocyanate dosage), insect species, insect sex, insect body mass, and their possible interactions as main effects. Replicate was used as a random blocking factor in the model to account for the spatial structure in the design (i.e., two diet blocks nested in each Petri dish). Protein-to-lipid ratio in the food blocks was included in the random term of the model.

The maximal model revealed significant differences in the nutritional intake responses among the carabid species tested under the different experimental conditions. Data were thus



reanalyzed for each species and each experiment separately. Here, the analysis was initiated by fitting a maximal model including macronutrient, nutritional landscape, insect sex, body mass, and their possible interactions as the main effects. Protein-to-lipid ratio in the food blocks was included in the random term, and replicate was used as a random blocking factor as above. Models were simplified, where applicable, by removing insignificant interaction terms in a step-wise manner. Simplified models were compared to the maximal model to check for any significant loss in predictive power. Akaike Information Criterion (AIC) comparisons were carried out to select the model of best fit. Model validity was checked by examining the distribution of model residuals (Nobre and Singer, 2007). The R packages “LmerTest” and “emmeans” packages were used to perform post-hoc comparisons on the final models (Kuznetsova *et al.*, 2007; Lenth, 2019; Schielzeth *et al.*, 2020).

Geometric Framework (GF) bivariate analysis was carried out to estimate the dietary intake targets of carabid species under different protein-to-lipid ratios and track their changes under different diet quality parameters (Behmer *et al.*, 2002; Behmer, 2009). The GF approach offered an informative tool to elucidate the patterns in of nutrient intake regulation in carabids, and how intake targets would change in repose to suboptimal nutritional (Simpson and Raubenheimer, 1993a). The R Package v.4.0.3 (R Development Team 2020) was used for all data analysis

#### **5.4. Results**

The dietary intake analysis for the full data set revealed that nutrient intake decisions in carabid seed predators were driven by a complexity of factors, including: the protein-to-lipid balance in the experiment ( $F_{2,970} = 17.98$ ,  $P < 0.001$ ; Table 5.4), the quality of dietary protein ( $F_{2,972} = 70.02$ ,  $P < 0.001$ ), and the dose of seed defensive chemical added ( $F_{1,985} = 24.93$ ,  $P < 0.001$ ). Body mass of the carabid predators did not influence their feeding responses, so body mass was removed from the model. None of the influential factors listed above appeared to act alone in influencing nutritional intake decisions in carabids. Instead, nutrient intake decisions were the outcome of complex interactions between multiple nutritional factors, including protein-to-lipid ratio in the diet, protein quality, and the level of seed toxins in the diet (see Table 5.4).

**Table 5.4.** Mixed effects analysis (P-values) for measured feeding responses of three carabid species on different synthetic diets of a moderate allyl isothiocyanate dosage as affected by protein-lipid P:L ratio, insect sex, and their interactions.

<b>Statistical term</b>	<b>ndf</b>	<b>ddf</b>	<b>F-value</b>	<b>P-value</b>
<i>Macronutrient</i>	2	976	1.79	<i>P</i> = 0.16
<i>Nutritional landscape</i>	2	970	17.98	<b><i>P</i> &lt; 0.001</b>
<i>Toxicity</i>	1	985	24.93	<b><i>P</i> &lt; 0.001</b>
<i>Diet quality</i>	2	972	70.02	<b><i>P</i> &lt; 0.001</b>
<i>Insect species</i>	2	974	23.67	<b><i>P</i> &lt; 0.001</b>
<i>Insect sex</i>	1	976	20.83	<b><i>P</i> &lt; 0.001</b>
<i>Macronutrient</i> × <i>Nutritional landscape</i>	2	969	55.18	<b><i>P</i> = 0.001</b>
<i>Macronutrient</i> × <i>Toxicity</i>	1	969	25.46	<b><i>P</i> &lt; 0.001</b>
<i>Nutritional landscape</i> × <i>Toxicity</i>	2	969	33.09	<b><i>P</i> &lt; 0.001</b>
<i>Macronutrient</i> × <i>Diet quality</i>	4	969	3.18	<b><i>P</i> = 0.013</b>
<i>Macronutrient</i> × <i>Insect species</i>	3	969	2.23	<i>P</i> = 0.08
<i>Nutritional landscape</i> × <i>Diet quality</i>	4	970	8.1	<b><i>P</i> &lt; 0.001</b>
<i>Nutritional landscape</i> × <i>Insect species</i>	4	970	5.92	<b><i>P</i> = 0.0001</b>
<i>Toxicity</i> × <i>Insect species</i>	2	971	24.28	<b><i>P</i> &lt; 0.001</b>
<i>Diet quality</i> × <i>Insect species</i>	4	972	32.49	<b><i>P</i> &lt; 0.001</b>
<i>Macronutrient</i> × <i>Insect sex</i>	2	969	0.37	<i>P</i> = 0.53
<i>Nutritional landscape</i> × <i>Insect sex</i>	2	978	2.94	<i>P</i> = 0.053
<i>Toxicity</i> × <i>Insect sex</i>	1	986	28.08	<b><i>P</i> &lt; 0.001</b>
<i>Insect species</i> × <i>Insect sex</i>	2	984	1.72	<i>P</i> = 0.17
<i>Macronutrient</i> × <i>Nutritional landscape</i> × <i>Toxicity</i>	2	969	1.24	<i>P</i> = 0.28
<i>Macronutrient</i> × <i>Nutritional landscape</i> × <i>Insect species</i>	4	969	2.95	<b><i>P</i> = 0.019</b>
<i>Macronutrient</i> × <i>Nutritional landscape</i> × <i>Diet quality</i>	4	969	15.88	<b><i>P</i> &lt; 0.001</b>
<i>Macronutrient</i> × <i>Toxicity</i> × <i>Insect species</i>	2	969	4.01	<b><i>P</i> = 0.018</b>
<i>Nutritional landscape</i> × <i>Toxicity</i> × <i>Insect species</i>	4	969	5.75	<b><i>P</i> = 0.001</b>
<i>Macronutrient</i> × <i>Toxicity</i> × <i>Insect sex</i>	2	969	0.16	<i>P</i> = 0.68
<i>Macronutrient</i> × <i>Diet quality</i> × <i>Insect species</i>	4	969	0.77	<i>P</i> = 0.54
<i>Nutritional landscape</i> × <i>Toxicity</i> × <i>Insect sex</i>	1	969	0.57	<i>P</i> = 0.56
<i>Nutritional landscape</i> × <i>Diet quality</i> × <i>Insect species</i>	8	970	2.52	<b><i>P</i> = 0.01</b>
<i>Macronutrient</i> × <i>Insect species</i> × <i>Insect sex</i>	2	969	0.1	<i>P</i> = 0.9
<b><i>Macronutrient</i> × <i>Nutritional landscape</i> × <i>Diet quality</i> × <i>Insect species</i></b>	8	969	2.68	<b><i>P</i> = 0.006</b>
<i>Macronutrient</i> × <i>Nutritional landscape</i> × <i>Toxicity</i> × <i>Insect species</i>	4	969	2.18	<i>P</i> = 0.06
<i>Nutritional landscape</i> × <i>Insect species</i> × <i>Toxicity</i> × <i>Insect sex</i>	2	969	0.58	<i>P</i> = 0.67
<b><i>Nutritional landscape</i> × <i>Toxicity</i> × <i>Insect species</i> × <i>Insect sex</i></b>	4	973	3.3	<b><i>P</i> = 0.01</b>
<i>Macronutrient</i> × <i>Toxicity</i> × <i>Insect species</i> × <i>Insect sex</i>	2	969	0.37	<i>P</i> = 0.68

The three carabid species under study showed a tight regulation of their protein and lipid intake. These regulatory responses were driven mainly by the protein-to-lipid balance in the diet rather than the mere presence of either protein or lipid ( $F_{2,976} = 1.79$ ,  $P = 0.16$ ). The dynamics of nutrient intake regulation under the different protein-to-lipid ratios emerged as species-specific ( $F_{4,970} = 5.92$ ,  $P = 0.0001$ ). The presence of low-quality protein in carabid diets triggered species-specific changes in nutrient intake decisions as well ( $F_{8,969} = 2.68$ ,  $P = 0.006$ ). By contrast, the carabids under study appeared to respond to the presence of allyl isothiocyanate in their diets in somewhat similar fashions ( $F_{4,969} = 0.58$ ,  $P = 0.67$ ). The overall model did not show any significant effect of sex on nutrient intake responses across the carabid species tested ( $F_{2,969} = 0.53$ ,  $P = 0.58$ ). To drill down through this complexity and clarify the responses of carabids to dietary P:L conditions and the different aspect of diet quality, data were analyzed and presented for each species and each experiment separately.

#### 5.4.1. *The Impact of Protein-to-Lipid Ratio on Dietary Intake Regulation*

*Poecilus corvus* carabids significantly changed its lipid and protein intake in response to the three different P:L conditions in the experiment ( $F_{2,72} = 15.16$ ,  $P < 0.001$ ,  $n = 14$ , Table 5.5). These dietary changes were strongly linked to the P:L ratio in the experiment ( $F_{2,72} = 25.14$ ,  $P < 0.001$ ,  $n = 14$ , Figure 5.1 A and B). Intake targets were strongly protein-biased in the protein-biased landscape and remained protein-biased in the balanced landscape, although to a lesser extent. By contrast, intake targets shifted towards lipid-bias in the lipid-biased landscape. Lipid ingestion was tightly regulated by this species as the lipid intake targets remained relatively stable without significant differences across the different landscapes. Protein ingestion showed significant and strong changes across the nutritional landscapes. Over-ingestion of protein was the mechanism through which this species reached its lipid intake targets when lipid was scarce. Males and females of this species showed significant differences in their nutrient intake responses to the dietary P:L ratios used in this experiment. Females of this species ingested significantly more protein and lipid than males ( $F_{2,72} = 10.44$ ,  $P = 0.0001$ ,  $n = 14$ ).

*Pterostichus melanrius* carabids showed significant adjustments in their intake targets of lipid and protein in response to the changes in the P:L ratio in the experiment ( $F_{2,59} = 27.45$ ,  $P < 0.001$ ,  $n = 14$ , Table 5.5). Similar to the previous species, nutrient intake responses were driven by the P:L ratio of the nutritional landscape ( $F_{2,60} = 53.76$ ,  $P < 0.001$ ,  $n = 14$ , Figure 5.1 C and D). This species also showed a tight regulation of its lipid intake as lipid intake targets did not show significant changes across the different nutritional landscapes. Over-ingesting protein was the mechanism through which lipid intake targets were reached as shown by the significant interaction between macronutrient intake and the dietary P:L ratio ( $F_{2,59} = 48.04$ ,  $P < 0.001$ ,  $n = 14$ ). Females of this carabid species also ingested more protein and lipid than males ( $F_{2,68} = 8.85$ ,  $P < 0.001$ ,  $n = 14$ ).

*Harplaus amputatus* carabids significantly changed their intake targets of lipid and protein in response to changes in the dietary P:L ratio in the experiment ( $F_{2,40} = 4.24$ ,  $P < 0.01$ ,  $n = 12$ , Table 5.5). Lipid intake was also tightly regulated by this species under the different P:L ratios ( $F_{2,40} = 13.65$ ,  $P < 0.01$ ,  $n = 12$ , Figure 5.1 E and F). Interestingly, lipid intake targets in this species were significantly higher under unbalanced P:L conditions compared to balanced diets. Protein over-consumption was also observed when lipid in the diet was low, but protein over-ingestion was not strongly excessive, as was observed for the other two species. There were no differences in feeding responses between males and females of this species ( $F_{1,29} = 3.4$ ,  $P = 0.34$ ,  $n = 12$ ).

**Table 5.5.** Mixed effects analysis (P-values) for measured feeding responses of three carabid species on different synthetic diets as affected by protein-lipid P:L ratio, insect sex, and their interactions.

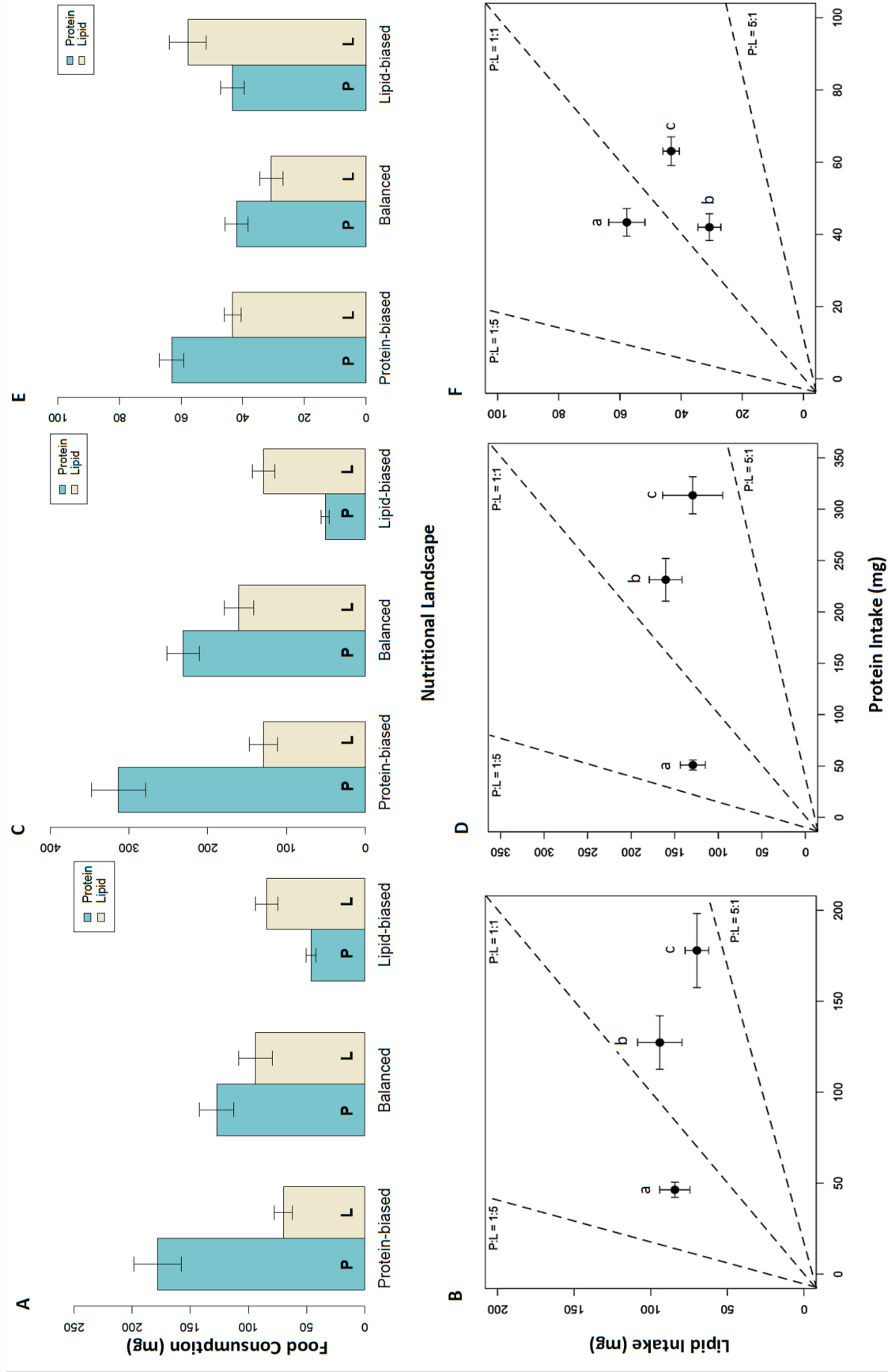
Effect	Carabid species														
	<i>Pterostichus melanarius</i> (n = 14)				<i>Poecilus corvus</i> (n = 14)				<i>Harpalus amputatus</i> (n = 12)						
	ndf	ddf	F-value	P-value	ndf	ddf	F-value	P-value	ndf	ddf	F-value	P-value			
<i>Macronutrient</i>	1	59	27.45	<0.0001	***	1	72	17.73	<0.0001	***	1	40	4.24	0.045	*
<i>Nutritional landscape</i>	2	60	53.76	<0.0001	***	2	72	15.16	<0.0001	***	2	42	14.87	<0.0001	***
<i>Insect sex</i>	1	63	40.79	<0.0001	***	1	72	123.14	<0.0001	***	1	29	3.4	0.074	1
<i>Macronutrient × Nutritional landscape</i>	2	59	48.04	<0.0001	***	2	72	25.14	<0.0001	***	2	40	13.65	<0.0001	***
<i>Macronutrient × Insect sex</i>	1	59	0.19	0.66		1	72	4.46	0.038	*	1	40	0.05	0.8	1
<i>Nutritional landscape × Insect sex</i>	2	68	8.85	0.00037	***	2	72	10.44	0.00014	***	2	44	2.58	0.086	1
<i>Macronutrient × Nutritional landscape × Insect sex</i>	2	59	2.51	0.089		2	72	8.51	0.00048	***	2	40	1.1	0.34	1

ndf: numerator degree of freedom, ddf: denominator degree of freedom

\* Indicates significant difference p<0.05

\*\* Indicates strong significant difference p<0.01

\*\*\* Indicates very strong significant difference p<0.001



**Figure 5.1.** Protein (P) and lipid (L) ingestion rates and bivariate intake points showing estimates nutrient ingestion rates of *Poecilus corvius* (A and B respectively), *Pterostichus melanarius* (C and D respectively), and *Harpalus amputatus* (E and F respectively) beetles under balanced and unbalanced protein-to-lipid dietary conditions. (a): lipid-biased conditions; (b): balanced conditions; (c): protein-biased conditions. Error bars: mean nutritional intake  $\pm$  standard error.

#### 5.4.2. The Impact of Protein Quality on Dietary Intake Regulation

Partial replacement of casein by zein (50% substitutions) caused all carabids tested to lose the ability to regulate protein and lipid intake under the different P:L conditions in the experiment (see Table 5.6). By contrast, complete replacement of casein by zein (100% substitutions) did not severely damage the ability of carabids to regulate protein and lipid intake.

*Poecilus corvus* consumed diets of significantly different protein-to-lipid ratios (intake targets) when casein was completely replaced by zein ( $F_{2,53} = 31.61$ ,  $P < 0.001$ ,  $n = 14$ , Table 5.7). This carabid species reached its lipid targets under balanced and lipid-biased P:L conditions, but failed to do so under protein-biased conditions (Figure 5.2 A and B). The overall intake rates of protein and lipid here were significantly lower compared to the normal diet (ca. 70% and ca. 52%, respectively). Lowering protein quality in the diet caused this species to avoid protein consumption. Protein avoidance was strongest and lipid ingestion was the lowest when protein ratio in the diet was the highest. The strength of protein avoidance progressively extenuated and lipid ingestion progressively increased as the ratio of protein in the diet started to fall. There were no significant differences in nutrient intake responses between males and females of this species ( $F_{1,53} = 1.38$ ,  $P = 0.24$ ,  $n = 14$ ).

*Pterostichus melanarius* was able to regulate its dietary intake when casein was completely replaced by zein in the diet ( $F_{2,40} = 3.61$ ,  $P = 0.035$ ,  $n = 14$ , Table 6.7). This species reached its lipid targets under the balanced and lipid-biased P:L conditions, but not under protein-biased conditions (Figure 5.2 C and D). This carabid also seemed to avoid protein of low quality in the diet, and the overall rates of protein and lipid intake were significantly reduced compared to the normal diet (ca. 65% and ca. 50%, respectively). Males and females of this species showed no significant differences in their dietary intake responses ( $F_{1,48} = 2.02$ ,  $P = 0.87$ ,  $n = 14$ ).

*Harpalus amputatus* did not lose the ability to regulate its protein and lipid intake when casein was completely substituted with zein in the diet ( $F_{2,43} = 36.19$ ,  $P < 0.001$ ,  $n = 12$ , Table 5.7). Protein intake rates here dropped by almost 25% compared to the normal diet. Protein intake was stabilized around an average of 40 mg across the different P:L conditions, which might represent the maximum tolerance threshold for low-quality protein in this species (Figure 5.2 E and F). There were no significant differences in nutrient intake responses between males and females of this species ( $F_{1,43} = 0.13$ ,  $P = 0.71$ ,  $n = 12$ ).

**Table 5.6.** Mixed effects analysis (P-values) for measured feeding responses of three carabid species on different synthetic diets of moderate protein as affected by protein-lipid P:L ratio, insect sex, and their interactions.

Effect	Carabid species													
	<i>Pterostichus melanarius</i> (n = 14)				<i>Poecilus corvus</i> (n = 14)				<i>Harpalus amputatus</i> (n = 12)					
	ndf	ddf	F-value	P-value	ndf	ddf	F-value	P-value	ndf	ddf	F-value	P-value		
<i>Macronutrient</i>	1	54	1.22	0.27	1	53	1.33	0.025	1	43	5.65	0.021	*	
<i>Nutritional landscape</i>	2	54	1.21	0.3	2	54	8.97	<0.00042	2	44	7.96	0.0011	**	
<i>Insect sex</i>	1	51	1.73	0.19	1	22	0.67	0.41	1	48	2.57	0.11		
<i>Macronutrient</i> × <i>Nutritional landscape</i>	2	54	29.13	<0.0001	***	2	53	21.61	<0.0001	***	2	43	3.09	0.055
<i>Macronutrient</i> × <i>Insect sex</i>	1	54	6.49	0.013	*	1	53	1.38	0.24	1	43	0.02	0.86	
<i>Nutritional landscape</i> × <i>Insect sex</i>	2	60	2.7	0.07		2	57	4.04	0.022	*	2	53	0.9	0.41
<i>Macronutrient</i> × <i>Nutritional landscape</i> × <i>Insect sex</i>	2	54	2.02	0.14		2	53	0.84	0.43	2	43	0.09	0.9	

ndf: numerator degree of freedom, ddf: denominator degree of freedom

\* Indicates significant difference p<0.05

\*\* Indicates strong significant difference p<0.01

\*\*\* Indicates very strong significant difference p<0.001



**Table 5.7.** Mixed effects analysis (P-values) for measured feeding responses of three carabid species on different synthetic diets of low protein as affected by protein-lipid P:L ratio, insect sex, and their interactions.

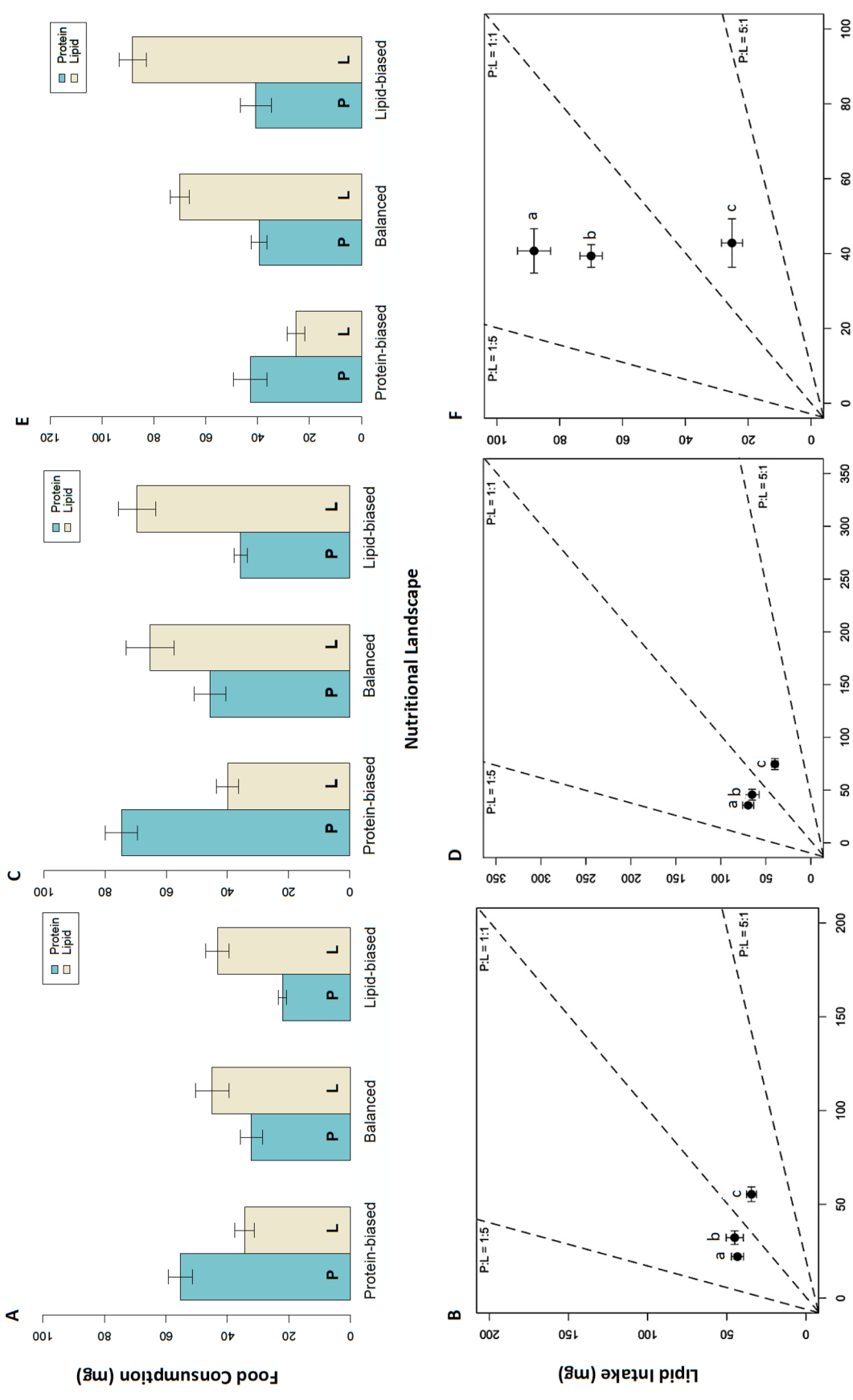
Effect	Carabid species														
	<i>Pterostichus melanarius</i> (n = 14)				<i>Poecilus corvus</i> (n = 14)				<i>Harpalus amputatus</i> (n = 12)						
	ndf	ddf	F-value	P-value	ndf	ddf	F-value	P-value	ndf	ddf	F-value	P-value			
<i>Macronutrient</i>	1	33	10.62	0.0025	**	1	53	1.33	0.25	1	43	39.42	<0.0001	***	
<i>Nutritional landscape</i>	2	54	15.2	<0.0001	***	2	54	8.97	0.00042	***	2	43	28.08	<0.0001	***
<i>Insect sex</i>	1	50	1.08	0.3		1	22	0.67	0.41		1	53	4.72	0.034	*
<i>Macronutrient × Nutritional landscape</i>	2	40	3.61	0.035	*	2	53	31.61	<0.0001	***	2	43	36.19	<0.0001	***
<i>Macronutrient × Insect sex</i>	1	48	0.02	0.87		1	53	1.38	0.24		1	43	0.13	0.71	
<i>Nutritional landscape × Insect sex</i>	2	51	0.06	0.93		2	57	4.04	0.022	*	2	45	3.91	0.027	*
<i>Macronutrient × Nutritional landscape × Insect sex</i>	2	48	0.17	0.83		2	53	0.84	0.43		2	43	0.5	0.6	

ndf: numerator degree of freedom, ddf: denominator degree of freedom

\* Indicates significant difference p<0.05

\*\* Indicates strong significant difference p<0.01

\*\*\* Indicates very strong significant difference p<0.001



**Figure 5.2.** Protein (P) and lipid (L) ingestion rates and bivariate intake points showing estimates nutrient ingestion rates of *Poecilus cornutus* (A and B, respectively), *Pterostichus melanarius* (C and D, respectively), and *Harpalus amputatus* (E and F, respectively) beetles under balanced and unbalanced protein-to-lipid dietary conditions and low-quality protein. (a): lipid-biased condition; (b): balanced condition; (c): protein-biased conditions. Error bars: mean nutritional intake  $\pm$  mean standard error.

### 5.4.3. The Impact of Allyl Isothiocyanate Level on Dietary Intake Regulation

Carabids were able to regulate their protein and lipid intake when allyl isothiocyanate was added to their synthetic food at 0.5% v/v, as intake targets showed significant differences among the different P:L conditions (see Table 5.8). By contrast, the 2.5% v/v dosage of allyl isothiocyanate was detrimental to the ability of carabids to regulate their dietary intake and reach nutrient intake targets (see Table 5.9). Adding allyl isothiocyanate to the synthetic food at 10% v/v was lethal to all carabid species under study.

*Poecilus corvus* was able to regulate its dietary intake when the synthetic food was augmented with 0.5% of allyl isothiocyanate; protein and lipid intake targets were significantly different among the different P:L conditions ( $F_{2,60} = 23.4$ ,  $P < 0.001$ ,  $n = 14$ , Table 5.8). Protein ingestion was significantly lower (ca. 40%) compared to the normal diet (Figure 5.3 A and B). By contrast, lipid ingestion did not show a significant reduction when compared to the normal diet. These differences caused intake targets to shift toward lipid bias when compared to the normal diet. Lipid ingestion increased as the ratio of protein in the diet fell and moved away from protein-bias. Males and females of this species showed no significant differences in their nutrient intake responses in this experiment ( $F_{1,60} = 0.26$ ,  $P = 0.6$ ,  $n = 14$ ).

*Pterostichus melanarius* was also able to regulate its dietary intake when the synthetic food was augmented with 0.5% of allyl isothiocyanate ( $F_{2,60} = 9.43$ ,  $P < 0.001$ ,  $n = 14$ , Table 5.8). Protein consumption across the dietary P:L ratios was significantly lower (ca. 50%) compared to the normal diet. Lipid intake did not show a significant drop when compared to the normal diet, which brought about a general shift in intake target toward lipid bias across the three different dietary P:L ratios (Figure 5.3 C and D). Males and females of this species showed no significant differences in their dietary intake responses in this experiment ( $F_{1,58} = 2.5$ ,  $P = 0.11$ ,  $n = 14$ ).

*Harpalus amputatus* was able to regulate its intake of protein and lipid when the synthetic food was augmented with 0.5% of allyl isothiocyanate ( $F_{2,47} = 18.39$ ,  $P < 0.001$ ,  $n = 12$ , Table 5.8). Intriguingly, the 0.5% dose caused this species to increase its protein consumption by almost 15% while lipid intake increased by almost two-fold (ca. 40% increase) when compared to the normal diet (Figure 5.3 E and F). Lipid intake increased as the ratio of protein in the diet fell, shifting intake targets towards lipid-bias. The dietary intake responses of this species were not affected by sex in this experiment ( $F_{1,47} = 0.05$ ,  $P = 0.94$ ,  $n = 12$ ).

**Table 5.8.** Mixed effects analysis (P-values) for measured feeding responses of three carabid species on different synthetic diets of a low allyl isothiocyanate dosage as affected by protein-lipid P:L ratio, insect sex, and their interactions.

Effect	Carabid species													
	<i>Pterostichus melanarius</i> (n=14)				<i>Poecilus corvus</i> (n=14)				<i>Harpalus amputatus</i> (n=12)					
	ndf	ddf	F-value	P-value	ndf	ddf	F-value	P-value	ndf	ddf	F-value	P-value		
<i>Macronutrient</i>	1	60	6.41	0.013	*	1	37	6.4	0.015	1	49	2.09	0.15	
<i>Nutritional landscape</i>	2	60	2.19	0.12		2	44	3.0	0.059	2	50	3.53	0.036	
<i>Insect sex</i>	1	59	0.15	0.69		1	22	0.11	0.73	1	28	0.02	0.88	
<i>Macronutrient × Nutritional landscape</i>	2	60	26.52	<0.001	***	2	41	27.96	<0.001	***	2	49	11.15	<0.001
<i>Macronutrient × Insect sex</i>	1	60	0.23	0.62		1	40	0.61	0.68	1	49	0.13	0.71	
<i>Nutritional landscape × Insect sex</i>	2	60	0.24	0.78		2	51	0.14	0.86	2	55	0.71	0.49	
<i>Macronutrient × Nutritional landscape × Insect sex</i>	2	60	0.33	0.71		2	46	0.19	0.82	2	49	0.4	0.66	

ndf: numerator degree of freedom, ddf: denominator degree of freedom

\* Indicates significant difference p<0.05

\*\* Indicates strong significant difference p<0.01

\*\*\* Indicates very strong significant difference p<0.001

**Table 5.9.** Mixed effects analysis (P-values) for measured feeding responses of three carabid species on different synthetic diets of a moderate allyl isothiocyanate dosage as affected by protein-lipid P:L ratio, insect sex, and their interactions.

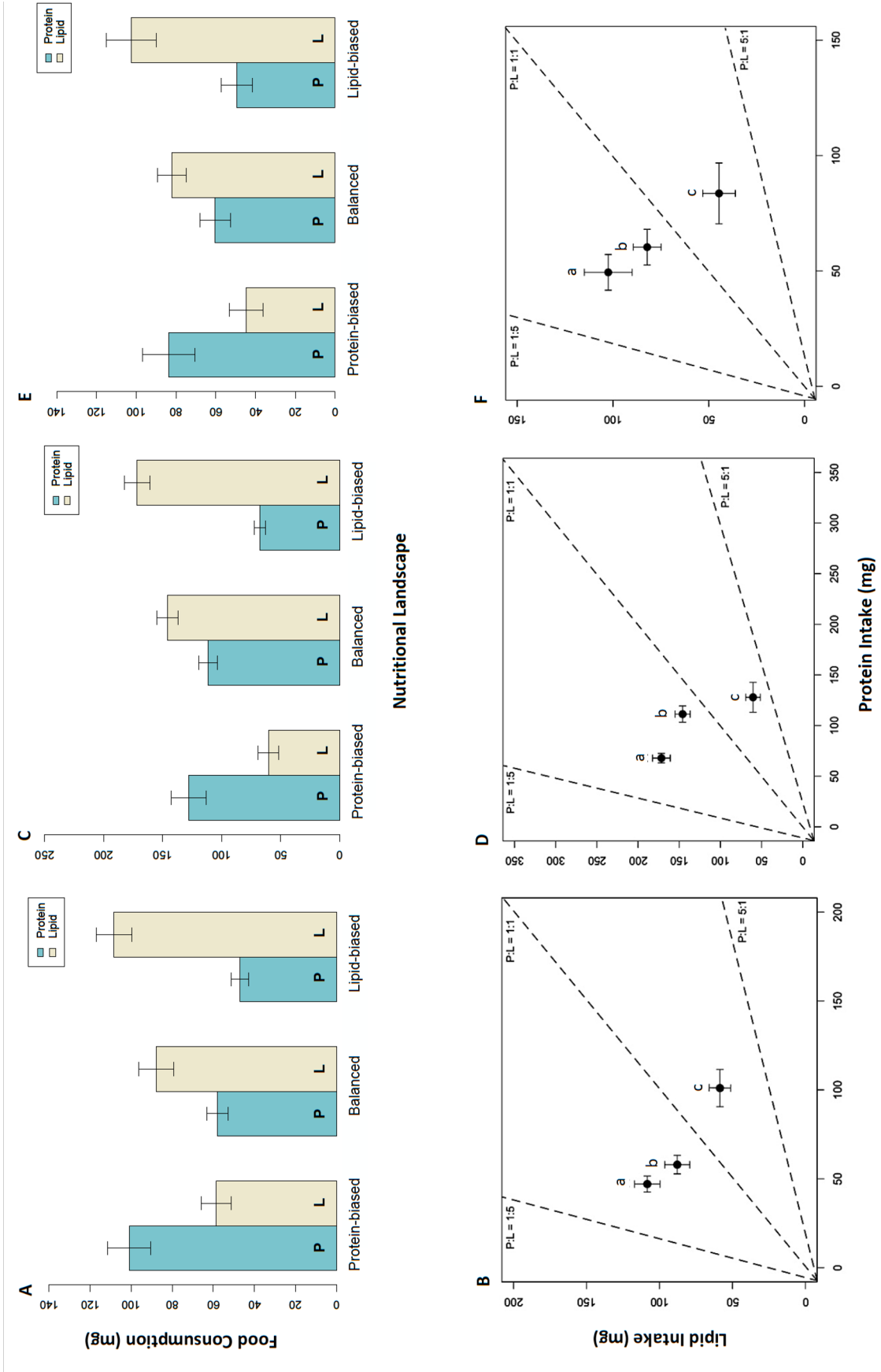
Effect	Carabid species													
	<i>Pterostichus melanarius</i> (n = 14)				<i>Poecilus corvus</i> (n = 14)				<i>Harpalus amputatus</i> (n = 12)					
	ndf	ddf	F-value	P-value	ndf	ddf	F-value	P-value	ndf	ddf	F-value	P-value		
<i>Macronutrient</i>	1	60	12.12	0.00093	***	1	60	6.42	0.013	*	1	47	3.97	0.051
<i>Nutritional landscape</i>	2	60	11.89	<0.0001	***	2	60	1.43	0.24		2	48	1.58	0.21
<i>Insect sex</i>	1	59	2.39	0.12		1	60	4.16	0.045	*	1	12	5.43	0.037
<i>Macronutrient × Nutritional landscape</i>	2	60	9.43	<0.0001	***	2	60	23.4	<0.0001	***	2	47	18.39	<0.0001
<i>Macronutrient × Insect sex</i>	1	59	2.5	0.11		1	60	0.26	0.6		1	47	0.05	0.94
<i>Nutritional landscape × Insect sex</i>	2	60	6.76	0.0022	**	2	60	4.38	0.016	*	2	49	5.18	0.009
<i>Macronutrient × Nutritional landscape × Insect sex</i>	2	60	0.24	0.78		2	60	0.3	0.73		2	47	0.61	0.54

ndf: numerator degree of freedom, ddf: denominator degree of freedom

\* Indicates significant difference p<0.05

\*\* Indicates strong significant difference p<0.01

\*\*\* Indicates very strong significant difference p<0.001



**Figure 5.3.** Protein (P) and lipid (L) ingestion rates and bivariate intake points showing estimated nutrient ingestion rates *Poecilus corvius* (A and B, respectively), *Pterostichus melanarius* (C and D, respectively), and *Harpalus amputatus* (E and F, respectively) beetles under balanced and unbalanced protein-to-lipid dietary conditions with protein quality high and allyl isothiocyanate added at 0.5% (v/v). (a): lipid-biased conditions; (b): balanced conditions; (c): protein-biased conditions. Error bars: mean nutritional intake ± mean standard error.

## 5.5. Discussion

The nutritional ecology data presented in this study demonstrate that carabid seed predators forage selectively for protein and lipids, and regulate their protein and lipid intake depending on the protein-to-lipid balance of the food available in the environment. Therefore, optimal foraging in carabid seed predators appears to be driven by acquiring specific nutrients rather than maximizing energy intake *per se* (Raubenheimer *et al.*, 2007; Jensen *et al.*, 2012). These results show carabid predators as selective foragers that seek out specific nutrients to address the nutritional needs for survival and development during their food searching efforts. These findings agree with other lines of evidence suggesting that obtaining specific nutrients is central to the optimal foraging of invertebrate predators in general (Mayntz *et al.*, 2005; Jensen *et al.*, 2012; Shareefi and Cotter, 2019). Based on that, the classic view depicting carabid seed predators as merely opportunistic feeders seeking to maximize their energy intake and choosing their foods randomly seems incorrect.

The nutrient-based foraging decisions observed for carabids in this study showed complex dynamics. The carabids under study selectively foraged for protein and lipids under both balanced and unbalanced protein-to-lipid conditions in the experiments. Lipid intake targets could be reached only by the excessive over-ingestion of protein under protein-biased conditions. Both *P. corvus* and *P. melanarius* exhibited protein over-ingestion when lipid in the diet was scarce. Protein over-compensation is the feeding mechanism that generalist predators often employ to acquire specific limiting nutrients when the nutritional dietary conditions are out of balance (Raubenheimer and Simpson, 2003; Lee *et al.*, 2003). Similarly, *Harpalus amputatus* showed protein compensation responses when the dietary P:L ratio was imbalanced, but the increase in protein ingestion under protein-biased conditions was not very sharp. A lack of sharp compensatory feeding responses under nutritional imbalance is the hallmark of specialist feeders, as their ability for nutrient utilization is usually enhanced compared to generalist feeders (Simpson *et al.*, 2002; Lee *et al.*, 2003, 2006). *Harpalus* sp. carabids are usually more specialized towards seed feeding (Harrison and Gallant, 2012; Talarico *et al.*, 2016), which may explain why no sharp compensatory feeding responses were observed for this carabid when the dietary P:L was protein-biased. Curiously however, *H. amputatus* consumed significantly more lipids when the P:L ratio in the experiment became unbalanced in contrast to when it was balanced. It is unclear why this

was the case. Perhaps the carabid species tested employ different rules of compromise for nutrient intake regulation under unbalanced nutritional conditions based on the breadth of their dietary specialization (Raubenheimer and Simpson, 1999). Further research is needed to elucidate the relationships that may exist between carabid dietary breadth, nutritional physiology, and the rules of optimal nutrient foraging.

Carabids tested in this study, besides their dietary breadth, showed a tight regulation of their lipid intake when dietary protein-to-lipid ratios were out of balance. Lipids thus appear likely to be limiting to nutrient foraging in carabid seed predators probably because fatty acids are less accessible or more difficult to obtain compared to amino acids during the foraging bouts of carabids. This is reasonable given that prey species in agricultural fields are often deficient in essential fatty acids (Wilder *et al.*, 2013). If carabid predators feed on prey alone, they need to consume excessive amounts of prey to extract sufficient amounts of lipids for survival and development (Potter *et al.*, 2018). However, the excessive intake of protein is usually deleterious to the survival of insect predators (Dussutour and Simpson, 2012; Csata and Dussutour, 2019), and so feeding on prey alone would be suboptimal for carabid feeding ecology. Carabid seed feeding habits may thus arise to address the scarcity of some essential fatty acids in alternative food sources. This conclusion is plausible given that species of carabid predators that inhabit agricultural fields tend to generally suffer lipid limitations in their diets (Jensesn *et al.*, 2012; Toft *et al.*, 2019, 2021). There is also evidence suggesting that nutrient foraging in arthropod predators, including both insects and spiders, tends to be generally profoundly influenced by the scarcity of lipids in their habitats (Wilder and Eubanks, 2010; Wilder *et al.*, 2013; Al Shareefi and Cotter, 2019). Therefore, the lipid scarcity is probably one of the major nutritional challenges that carabid predators, akin to other arthropod predators, need to surmount in order to survive and reproduce. It is tempting to conclude, therefore, that seed feeding habits in omnivorous carabids are likely driven by the general scarcity of fatty acids in agri-environments. However, generalizing this conclusion to most omnivorous carabids that inhabit agricultural fields remains untenable at this point since the number of carabid species tested in this regard is still limited.

Gender-based differences in nutrient intake responses were detected for some of the carabid species under study in certain cases. Sex ratio was skewed (male- or female-biased) for some carabid species in a few of the experiments described above. This might explain the conflicting results regarding the effect of carabid sex on dietary intake between the full model and



species-specific models. The unbalanced sex ratios (male- or female-biased) in some experiments might have given way to statistically-biased differences between carabid males and females to arise. Thus, the sex-specific differences detected in some cases of the current study were possibly due to a statistical artifact and cannot be treated as a biological phenomenon. The impact of carabid sex on nutrient intake decisions remains uncertain at this point, and further research is needed to clarify its effects in this regard.

Carabid species tested also adjusted their nutrient foraging decisions in response to different diet quality parameters. The presence of low-quality protein in the diet brought about a significant drop in consumption rates of both protein and lipid by all carabid species tested in contrast to the normal diet. The presence of low-quality protein triggered strong shifts in nutrient intake targets toward lipid bias. This was generally observed across all three carabid species tested in the experiment even though their responses to protein of low quality were species-specific. Lowering protein quality in the diet did not trigger protein over-compensation responses, as would be expected. Alternatively, all carabid species under study exhibited an avoidance of low-quality protein, which grew stronger as the protein ratio in the synthetic food increased. Similar protein avoidance responses were observed when protein quality was made low in the synthetic food offered to caterpillars of a generalist lepidopteran herbivore (Lee *et al.*, 2007). Protein of low quality was deleterious to caterpillar survival, so the strength of protein avoidance also progressively increased as the proportion of low-quality protein in the diet increased. These findings agree with some authors proposing that protein quality could be an important factor in determining the suitability of plant species to insect herbivores (Broadway and Duffy, 1988; Felton, 1996, Barbehenn *et al.*, 2013). Based on our data, protein quality strongly obstructed carabids from reaching their lipid intake targets, especially when the ratio of low-quality protein in the diet was elevated.

Adding the seed secondary metabolite allyl isothiocyanate to the diet also caused carabids to adjust their nutrient intake targets toward lipid bias. The effects arising from adding allyl isothiocyanate to the synthetic food of carabids were more complex, however. Secondary metabolites in plants usually engage in different and complex interactions with primary metabolites (Slansky, 1992). These interactions can bring about drastic changes in the feeding responses and nutrient intake decisions of insects due to nutrient complexing, intoxication, or feeding deterrence (Broadway and Duffy, 1988; Slansky, 1992; Glendinning and Slansky, 1994).

Adding allyl isothiocyanate at tolerable levels (non-lethal) to the food sources of carabids did not deter feeding altogether, as carabids still accepted the food blocks laced with allyl isothiocyanate. The impact of allyl isothiocyanate on carabids was instead postingestive, bringing about significant changes in nutrient intake dynamics. At the 0.5% dosage, allyl isothiocyanate did not bring about any significant reductions in lipid consumption by *P. corvus* or *P. melanarius*. On the contrary, this chemical caused *P. corvus* and *P. melanarius* to significantly reduce protein ingestion compared to the normal diet. These observations could be attributed to potential interactions between allyl isothiocyanates and protein. Allyl isothiocyanate is among the plant secondary metabolites that can bind to amino acids (at the N-terminus) and render them less digestible (i.e., less utilizable) by insect species (Simpson and Raubenheimer, 1993b; Felton, 1996). The presence of allyl isothiocyanate in the synthetic food of *P. corvus* and *P. melanarius* had likely rendered the protein less digestible due to protein complexing effects, which induced these carabids to change their nutrient foraging mode towards protein avoidance. Protein avoidance in this case was less severe compared to zein replacements, as lipid ingestion rates here were almost similar to ones observed when carabids were fed the normal diet.

The physiological effects of the protein binding by allyl isothiocyanate seem to vary between carabid species. Allyl isothiocyanate caused *H. amputatus* to increase its lipid intake by almost two-fold compared to non-toxic synthetic food. Yet, this chemical did not induce this carabid to avoid allyl-isothiocyanate-bound protein since protein ingestion did not fall in this case. The differences observed between carabid species must be related to different species-specific nutritional physiologies, as all species were subject to the same starvation procedure prior to experimentation (Simpson and Raubenheimer, 1996; Toft *et al.*, 2021, Le Gall *et al.*, 2021). It is possible that there may be some correlations between the dietary breadth of carabid species and their nutritional physiology (Ali and Agrawal, 2012). *Harpalus* sp. carabids are usually more specialized towards weed seed feeding (stenotopic dietary breadth), and could thus be more able to neutralize the effects allyl isothiocyanate through detoxification (Dowd *et al.*, 1983). Detoxification usually incurs high metabolic costs, which could explain the elevated lipid ingestion shown by *H. amputatus* when allyl isothiocyanate was added to its food (Illius and Jessop, 1995; Berenbaum *et al.*, 2015). By contrast, *P. corvus* and *P. melanarius* are more generalist in their feeding habits (eurytopic dietary breadth), and both appeared to avoid consumption of the protein bound by allyl isothiocyanate perhaps to reduce detoxification costs since detoxification is often

less efficient and more costly in generalist feeders compared to specialized feeders (Ibanez *et al.*, 2012; Jeschke *et al.*, 2017). Overall, allyl isothiocyanate at tolerable levels did not constrain carabids from reaching their lipid intake targets. Therefore, seed defensive chemicals may not always confer protection against carabid predation since carabids seem to prioritize lipid ingestion when seeds are chemically defended.

The findings of this study suggest that mixed feeding habits that combine seed and prey items are likely more optimal for carabids to overcome the dietary challenges they encounter in their environments (Westboy, 1978; Le Gall and Behmer, 2014). In this way, carabids can obtain scarce lipids, extract nutritious proteins, eschew harmful proteins, and avoid the detriment of protein over-consumption. Mixed feeding could also dilute the effects of seed defensive chemicals, potentially mitigating their harmful effects (Hagele and Rowell-Rahier, 1999; Singer *et al.*, 2002). This could explain why such a large proportion of carabid species inhabiting agri-environments tend to be omnivorous and include substantial amounts of seeds into their diets. The findings also help to explain why lipid-rich seed species are chosen more preferably for consumption by carabid predators (Petti *et al.*, 2014; Gaba *et al.*, 2019), and why carabids maintain their strong preference for weed seeds even when prey is available as an alternative food source (Klimes and Saska, 2010; Frank *et al.*, 2011; Blubaugh *et al.*, 2016). Furthermore, these findings may also help to explain why true granivory (i.e., strictly specialized seed feeding) remains rare and restricted to certain environments, where an abundance of preferable seeds are shed frequently enough to allow physiological adaptation (Futuyma and Moreno, 1988; Klimes and Saska, 2010; Forister *et al.*, 2012). Given the data, I can conclude that within certain limits of protein quality, seed toxicity, and physical seed characteristics, seed species that are rich in lipids are more likely to suffer intense carabid attacks in the field. In other words, the active selection of nutritious seed species is a luxury that carabids do not always have. Physical seed characteristics such as size, mass, volume, or coat hardness can act as physical barriers that constrain carabid predators from actively selecting nutritious seeds species by rendering seed nutrients difficult to obtain or costly to exploit (Cardina *et al.*, 1996; Honek *et al.*, 2007; Lundgren and Rosentrater, 2007). Therefore, seed nutrients and defensive seed chemicals should not be treated as the sole drivers of seed selection decisions. Instead, seed chemistry is likely to influence or drive seed selection decisions in carabids only when the seed traits associated with seed handling costs are not widely variable among the seed species available to carabid seed predators (Saska *et al.*, 2019a,b; Foffova *et al.*, 2020a,b).

## CHAPTER 6 Predator-Prey Mass-Ratio Scaling Relationships Underlie Seed Choice in Carabid Weed Seed Predators

### 6.1. Introduction

Seed-feeding carabid beetles are thought to seek seed species to obtain nutrients that are essential for their survival and development (Saska, 2015; Wallinger *et al.*, 2015). Seed nutrients and perhaps defensive chemicals, should therefore be among the important factors that shape the nutritional value of seed species and thus, influence nutrient foraging decisions in carabid seed predators. It turns out, however, that seed chemistry is central to seed preferability in carabids only if physical characteristics of seed species are invariable (i.e., constant) or vary within certain limits (Gaba *et al.*, 2019; Foffova *et al.*, 2020a,b). In other words, the study of seed choice in carabid seed predators based on seed-chemistry-centric approaches tends to be reductionist as seed nutrients and non-nutritional seed chemicals (toxins or anti-feedants) are treated as the sole factors influencing seed selection decisions (Behmer *et al.*, 2002; Leonhardt *et al.*, 2020). Neglecting or negating the physical properties or traits of seed species leaves important and unbridged gaps in the knowledge surrounding seed preference biology in carabid seed predators. Physical and chemical properties of seed species should instead be integrated in the study of seed predation interactions to further and deepen our understanding of the biology and ecology of seed feeding and seed preferences in carabid seed predators.

The physical and chemical characteristics of seed species are tightly entwined through complex matrices of interactions, which vary widely among plant lineages (Dalling *et al.*, 2011; Tiansawat *et al.*, 2014). The interactions between physical and chemical traits of seed species remain poorly understood, but they tend to serve defense functions against the mortality pressures imposed on the seed by natural agents like pathogens or seed predators, such as carabid beetles (Wang and Chen, 2012; Dalling *et al.*, 2020). Seed physical characteristics or traits can thus act as physical barriers that may restrain carabid predators from feeding on seed species by rendering seed nutrients difficult to obtain or costly to exploit; they may even block carabids from feeding on the seed altogether by making seed nutrients inaccessible (Cardina *et al.*, 1996). Physical attributes of seed species therefore could defend the seed against carabid predation, and should be considered among the factors that affect seed selection decisions in carabid seed predators

(McArthur and Pianka, 1966; Ydenberg *et al.*, 1994). Seed traits that relate to the physical defense against carabid seed predators are supposed to be numerous, yet it remains unclear which of these traits actually carries sufficient ecological power to deter or restrain carabids from feeding on seed species, hence swaying seed selection decisions in ways that may not be predictable or explicable through seed chemistry *per se*.

Seed size is one physical trait of special interest to the ecology of seeds in general (Baskin and Baskin, 1998). Seed size as a functional physical trait may also capture other ecologically important seed characteristics like nutritional content, chemical defenses, or seed coat hardness (Moles *et al.*, 2003; Petit *et al.*, 2014; Gong *et al.*, 2015). It should not be unexpected, therefore, for seed size as a trait associated with other seed physical and chemical characteristics to exert some influence on seed selection decisions in carabid seed predators (Foffova *et al.*, 2020a,b). However, the associations linking seed size to other ecological seed traits follow complex patterns that are not easy to capture and account for (Davis *et al.*, 2008, 2016). Based on that, the impact of seed size on seed choice responses in carabid seed predators tend to vary depending on how seed size parameters are measured in experimental studies. Seed length measurements, for instance, have not detected any significant influence on seed selection decisions in some species of carabid seed predators (Kulkarni *et al.*, 2016). By contrast, measurements of seed volume (cm<sup>3</sup>) have revealed conflicting results as seed volume significantly affected seed choice responses in some species of carabid seed predators but not in others (Lundgren and Rosentrater, 2007). Consequently, seed length and seed volume do not always seem to capture the seed physical characteristics that carabids would assess to identify suitable seeds. Alternatively, seed mass measurements appear to better capture seed size and its associations with other seed properties of influence for carabid seed selection decisions (Martinkova *et al.*, 2019). Using dry mass of 25 different weed species and the body mass of 30 carabid seed predators in laboratory experiments, Honek *et al.* (2007) established an allometric mass scaling relationship between carabids and seed species. That is, the size of preferable seeds increase as the body mass of the carabid predator increases. Other laboratory carabid seed predation studies also reported similar mass allometry observations (Martinkova *et al.*, 2019; Saska *et al.*, 2019b). The mass allometry findings indicate that seed selection decisions in carabid seed predators could be constrained by their body mass (Vucic-Pestic *et al.*, 2010). Still, mass allometry does not capture the ratio between the body mass of carabid predators and the seed species they select most frequently for consumption. Further

studies are needed in this respect to clarify whether mass-ratio scaling relationships between carabid and seed species underlie the well-evidenced carabid-to-seed mass allometry observations.

Body mass in carabid predators shows positive scaling relationships with other functionally important traits such as metabolic rate and food handling efficiency (Evans and Forsythe, 1985; Gudowska *et al.*, 2017). Diagnosis of the allometry between body mass and metabolic rates in carabid predators have shown that metabolic rates can affect the interaction strength between carabids and their chosen food types only partially (Brose *et al.*, 2006, 2008; Reum, 2019). Thus, size-dependent metabolic rates should not be expected to set strict limits on seed selection decisions in carabids. Instead, the relationship between carabid body mass and seed handling efficiency is more likely to mediate the allometry between body mass of carabid predators and the mass of seed species they choose more preferably for consumption (Erikstad *et al.*, 1989; Brose, 2010). Unlike simple mass allometries, the mass-ratio scaling relationships used for studying predator-prey dynamics generally capture the functional linkage between the body mass of the consumer (carabid) and the mass spectrum of suitable resources (seed or prey) that can be exploited at reasonable costs (Nakazawa *et al.*, 2011; Brousseau *et al.*, 2018). Mass-ratio scaling relationships are, therefore, a better and more informative approach than mass allometry for studying the seed feeding ecology in carabid predators.

Mass-ratio scaling relationships can capture core aspects of predator-prey dynamics in terms of prey selection decisions and interaction strength (i.e., magnitude of predation pressure) in both vertebrate and invertebrate predator-prey systems (Leaper and Huxham, 2002; Sanders *et al.*, 2015; Eilersen and Sneppen, 2019). In accordance with this, functional ecological studies on carnivorous carabid predators have revealed that mass-ratio scaling relationships are among the key predictors of prey selection and the magnitude of population suppression carabid predators impose on different prey species (Roubah *et al.*, 2014; Rusch *et al.*, 2015; Brousseau *et al.*, 2018). Studies of this sort remain lacking in the carabid seed predation literature, however. This highlights the need for detailed research that studies how mass-ratio scaling relationships may influence seed selection decisions in carabid seed predators. This knowledge can then be utilized to produce predictions about the strength of seed predating interactions based on mass characteristics of both seeds (source) and carabid predators (consumers).

The current study was conducted to study the mass-ratio scaling relationships in carabid seed predation systems to explore the ways by which mass-ratio scaling relationships might

influence or drive seed selection decisions. I hypothesized that mass-ratio scaling relationships rule the interactions between carabid predators and seeds of weedy species when carabids of different body mass are offered seed species of variable mass. Carabid seed predators of different body mass should, therefore, exhibit mass-driven seed selection responses when seed species of variable mass are accessible. I was also interested in testing whether mass-ratio scaling relationships in seed predation systems might be sensitive to sex-related differences between males and females of the carabid species under study.

## **6.2. Materials and Methods**

### *6.2.1. Seed material*

Seeds of the same three brassicaceous weed species (Brassicaceae) mentioned in previous chapters were used in this study. These seeds had characteristics that offered a good model for studying the influence of predator-to-seed mass-ratio scaling relationships on seed selection decisions. The mass of individual seeds was estimated by measuring the mass of 50 seeds per each weed species. Seed mass measurements (to the nearest mg) were repeated 10 times for each weed species, and an independent seed sample (50 seeds) was used for each measurement (replicate). Seed mass measurements and carabid feeding experiments were carried out using dry seeds to avoid the potential influence of seed water content on seed mass and volume. Also, seed water content can, in certain cases, alter seed preferability to carabid predators (Hurst and Doberski, 2003; Foffova *et al.*, 2020b). Therefore, the confounding effects of seed water content on seed characteristics and/or preferability to the carabids tested in the current study were minimal. Carabids also were shown to rely on seed surface waxes to guide their seed selection decisions in the previous chapters. Seed surface waxes usually do not exhibit considerable changes in association with water content of seed species (Paulsen *et al.*, 2013). Thus, seed water content was not expected to influence the volatile chemistries of seed species under study.

### 6.2.2. Carabids

Adults of the following eight omnivorous carabid species were captured and used in laboratory feeding experiments: *Pterostichus melanarius* Illiger, *Poecilus corvus* Leconte, *Harpalus rufipes* Degeer, *Harpalus amputatus* Say, *Amara familiaris* Duftschmidt, *Amara littoralis* Dejean, *Harpallelus basilaris* Kirby, and *Bembidion nitidum* Kirby. These eight carabids represented the dominant carabid taxa in the carabid community. All were collected from different field sites at the Kernen Crop Research Farm near Saskatoon, SK, Canada (52°09'10.3" N 106°32'41.5" W) during summers of 2018-20 via dry pitfall trapping, as describe in previous chapters. The carabids listed above also showed considerable differences in their size and species-averaged body mass (see below). These differences in carabid mass offered a good model for studying the effects of mass-ratio scaling relationships between carabid predators and seeds of weed species.

### 6.2.3. Cafeteria Multiple-Choice Feeding Bioassays

Cafeteria feeding bioassays were carried out to elucidate the impact of mass-based interactions between carabid predators (consumers) and weed seeds (resources) on seed selection decisions in carabid seed predators (Barrios-O'Neill *et al.*, 2016). For this purpose, seeds of the three brassicaceous weed species were offered to eight species of carabids in multiple-choice feeding bioassays. The feeding arenas were laid out in large Petri dishes ( $\varnothing = 25$  cm, 5 cm depth) lined with a 2-cm layer of moist and sterilized sand (Saska *et al.*, 2014). Seeds were offered in plastic tray rings ( $\varnothing = 28$  mm, 6 mm depth) filled with white plasticine. Plasticine has been shown not to interfere with seed choice in carabid seed predators (Honek *et al.*, 2007). In each ring, 25 seeds of one weed species were pressed halfway into the plasticine layer and seed trays were then placed near the perimeter of the Petri dishes. A total of three trays (one per each seed species) were placed into each Petri dish so that the seed patch was at the same level with sand layer. Seed trays in this design offered seeds of different weed species in the same abundance and in patches of the same size. The impact of seed availability on mass-ratio relationships could thus be minimized (Tsai *et al.*, 2016). Carabids were released into the feeding arenas (one carabid per each Petri dish)



after their level of hunger was standardized by starving the beetles for 72 h (Law and Gallagher, 2015; see Table 6.1).

**Table 6.1.** Treatment list for predator-to-seed mass-ratio scaling experiment with associated treatment descriptions.

Treatment number		Treatment description
1	<i>Pterostichus melanarius</i> (Body mass = 137 ± 4.5 mg)	Seeds of <i>Brassica napus</i> (Seed mass = 4 ± 0.06 mg)*
		Seeds of <i>Sinapis arvensis</i> (Seed mass = 2.38 ± 0.09 mg)*
		Seeds of <i>Thlaspi arvense</i> (Seed mass = 0.95 ± 0.01 mg)*
2	<i>Poecilus corvus</i> (Body mass = 81 ± 1.7 mg)	Seeds of <i>Brassica napus</i>
		Seeds of <i>Sinapis arvensis</i>
		Seeds of <i>Thlaspi arvense</i>
3	<i>Harpalus rufipes</i> (Body mass = 73 ± 2.1 mg)	Seeds of <i>Brassica napus</i>
		Seeds of <i>Sinapis arvensis</i>
		Seeds of <i>Thlaspi arvense</i>
4	<i>Harpalus amputatus</i> (Body mass = 50.57 ± 2.1 mg)	Seeds of <i>Brassica napus</i>
		Seeds of <i>Sinapis arvensis</i>
		Seeds of <i>Thlaspi arvense</i>
5	<i>Amara littoralis</i> (Body mass = 31.21 ± 0.39 mg)	Seeds of <i>Brassica napus</i>
		Seeds of <i>Sinapis arvensis</i>
		Seeds of <i>Thlaspi arvense</i>
6	<i>Harpallelus basilaris</i> (Body mass = 30.89 ± 1.35 mg)	Seeds of <i>Brassica napus</i>
		Seeds of <i>Sinapis arvensis</i>
		Seeds of <i>Thlaspi arvense</i>
7	<i>Amara familiaris</i> (Body mass = 17.03 ± 0.78 mg)	Seeds of <i>Brassica napus</i>
		Seeds of <i>Sinapis arvensis</i>
		Seeds of <i>Thlaspi arvense</i>
8	<i>Bembidion nitidum</i> (Body mass = 4.82 ± 0.33 mg)	Seeds of <i>Brassica napus</i>
		Seeds of <i>Sinapis arvensis</i>
		Seeds of <i>Thlaspi arvense</i>

\*The mean mass values of individual seeds were estimated based on ten independent bulk mass measurements of fifty seeds per each seed species.

The beetles were starved by placing a single beetle into a clean and sterile Petri dish ( $\emptyset = 6$  cm, 2 cm depth) lined with a moist filter paper, then incubating the Petri dishes in a growth chamber at  $21 \pm 1$  °C and 16:8 L:D photoperiod. This procedure prevented cannibalism and allowed for any olfactory memory that might have formed while carabids had been foraging in the field to decay before the commencement of feeding experiments (Glinwood *et al.*, 2011). After 72 hours of starvation, carabid predators were weighed to the nearest 0.1 mg (fresh body mass) and then released into the Petri-dish feeding setup. Petri dishes were then incubated in a growth chamber at  $21 \pm 1$  °C and 16:8 L:D photoperiod (White *et al.*, 2007). Beetles were left to feed for five consecutive days without replacing the consumed seeds (Petit *et al.*, 2014). Feeding experiments were replicated 20 times for each carabid species, and each carabid beetle was used only once. The sex ratio remained around 50♂:50♀ for some but not all carabid species under study. At the end of the experiment, carabids were removed from the Petri dishes and the number of seeds consumed from each seed patch was recorded. Seeds were considered consumed if >50% of the seed was eaten since such half-eaten seeds are unlikely to germinate (Honek *et al.*, 2005).

#### 6.2.4. Predator-to-Seed Mass-Ratio Calculations

Predator-to-seed mass-ratios (PSMRs) are usually calculated based on the proportions of seeds that carabid predators consume (total number of seeds) per each seed species offered in the cafeteria feeding experiments (Nakazawa *et al.*, 2011, 2013; see also equation 6.1 below). Comparing of the PSMR values among different carabids provides an informative tool to better understand how seed-eating carabids in a given community might compete, interfere, or even coexist in relation to the distribution of seed mass in the weed community. The experimental design did not allow detection of any significant differences in body mass values within carabid or seed species (see below). Thus, PSMR values were calculated based on species-averaged mass for carabid and seed species. The mathematical equation 6.1 was used for calculating PSMR values. The equation and its annotations are given below.

$$\text{PSMR} = \sum \frac{M_i}{m_j} \times q_{ij} \text{ (calculation based on species-averaged carabid body mass) } \dots\dots\dots (6.1)$$

Where  $m_i$  species-averaged body mass of the predator species  $i$ ,  $m_j$  species-averaged mass value of the seed species  $j$ , and  $q_{ij}$  is relative consumption of seed species  $j$  by predator species  $i$ .

### 6.3. Data Analysis

Interspecific weed seed mass values were compared via analysis of variance. Data in this section followed the normality assumption of ANOVA as shown by model diagnostic plots, raising no concern about model validity. Tukey HSD test was used to compare interspecific seed mass values.

Body mass values of the carabid species (mg of fresh body mass) were compared between carabid species and within species, and also between males and females within each species through three-way analysis of variance. For this purpose, each individual carabid beetle was assigned a unique individual code and insect species, insect individual code, insect sex, and their possible interactions were used as the main factors in the analysis. Individual code was used as a factor to check for any significant differences in intraspecific body mass values. Model diagnostic plots were used to examine the distribution of model residuals and check for any violations of model assumptions. Tukey HSD test was used to compare the body mass values of carabid predators inter- and intra-specifically.

The number of seeds consumed by each carabid beetle from each seed species was used as the response variable to compare seed feeding responses among carabid species via mixed effects models using the function “lmer” (Bates *et al.*, 2015). The initial analysis was carried out by fitting a maximal model to the data including seed species, insect species, predator body mass, insect sex, and their possible interactions as fixed effects. Replicate was used as a random blocking factor in the model to account for the spatial structure in the design (three weed species nested in each Petri dish). Model validity was checked by diagnosing the distribution of residuals (Nobre and Singer, 2007). The packages “LmerTest” and “emmeans” were used to compare seed selection responses among the carabid species under study (Lenth, 2019; Schielzeth *et al.*, 2020).

The species-specific PSMR values were used as the response variable to compare the size of seed feeding niches among the carabid species under study using Generalized Linear Models (GLMs). Data analysis was initiated by fitting a maximal model to the data including insect species, insect sex, and their possible interactions as the main effects. Poisson distribution with a log-link function was used to account for the heteroscedasticity in the count-based seed consumption data (Sroka and Nagaraja, 2018). The overdispersion criterion was used to check for any violation of model assumptions, and when violations occurred (overdispersion criterion > 1.5),

modeling procedures were switched to quasi-likelihood estimations (Fitzmaurice, 1997, Kim *et al.*, 2014). Estimates of model parameters were then used to assess the relationship between species-averaged carabid body mass (categorical variable) and breadth (lower and upper bounds) of the mass-based seed preference (numerical variable) for each carabid species based on PSMR calculations (Faraway, 2016). Package “car” was used for diagnosing model parameters (Fox and Weisberg, 2019). The R Package v.4.0.3 (R Development Team 2020) was used for all data analysis

#### 6.4. Results

The mean mass of individual seeds showed significant differences between the three weed species ( $F_{2,22} = 41.59$ ,  $P < 0.001$ ). The seed mass values for *B. napus* were the largest among the three species ( $4 \pm 0.06$  mg), followed by *S. arvensis* ( $2.38 \pm 0.09$  mg) and then *T. arvense* ( $0.95 \pm 0.01$  mg). The experimental design did not allow any comparisons of intraspecific seed mass values. For this reason, species-averaged seed mass values were adopted as the basis for studying mass-based seed selection responses (seed choice) in the carabid species under study.

The mean body mass (interspecific body mass) showed significant differences among the eight carabid seed species tested ( $F_{7,160} = 146.04$ ,  $P < 0.001$ , Figure 6.1). By contrast, none of the carabid species tested showed statistically significant differences in body mass between males and females ( $F_{4,160} = 0.71$ ,  $P = 0.58$ ). Within species, comparisons of individual body mass values revealed no significant differences ( $F_{6,160} = 0.25$ ,  $P = 0.95$ ). For this reason, species-averaged body mass values were adopted as the basis for studying mass-based carabid seed selection responses (seed choice) in our study.

Seed choice responses showed significant differences among the carabid species under study ( $F_{7,455} = 7.37$ ,  $n = 20 \times 8$ ,  $P < 0.001$ , Table 6.2). Size (species-averaged mass) of the preferable seed species changed as a function of carabid body mass. Carabid species smaller in mass showed strong preferences toward the smaller seed species offered in the experiment, and vice versa for larger carabid species (Figure 6.2). More intriguingly, choice of the highly preferable seed species appeared to be dependent on species identity of the carabid predator ( $F_{12,449} = 10.76$ ,  $n = 20 \times 8$ ,  $P < 0.001$ ), as well as the average body mass specific to species identity of the carabid ( $F_{7,459} = 59.42$ ,  $n = 20 \times 8$ ,  $P < 0.001$ ). Seed preference was not influenced by the sex of carabid

predators as males and females of the same species showed similar seed preferences ( $F_{2,484} = 0.01$ ,  $n = 20 \times 8$ ,  $P = 0.98$ ).

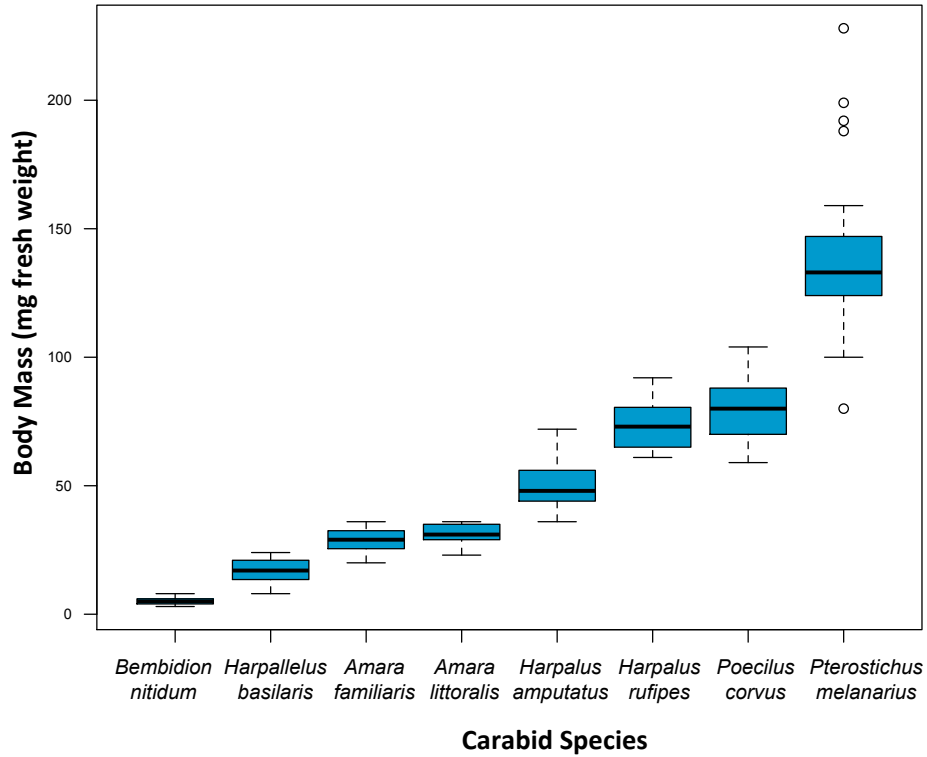
Nonetheless, there was a significant three-way interaction between weed species, body mass, and insect sex ( $F_{2,478} = 3.78$ ,  $n = 20 \times 8$ ,  $P = 0.023$ ). These sex-related effects arose outside the realm of carabid species identity as the three-way interaction between weed species, insect species, and insect sex was not statistically significant ( $F_{2,483} = 1.23$ ,  $n = 20 \times 8$ ,  $P = 0.25$ ). Males and females within some of the carabid species under study showed differences in the number of seeds they consumed, but not in their choice of the preferable seed species. Females consumed almost 40% more of the preferable seed species compared to males in cases where sex-related differences in seed feeding responses were detectable.

**Table 6.2.** Mixed effects analysis (P-values) for measured feeding responses of eight carabid species on three different brassicaceous weed species as affected by weed species, insect species, predator body mass, insect sex, and their interactions.

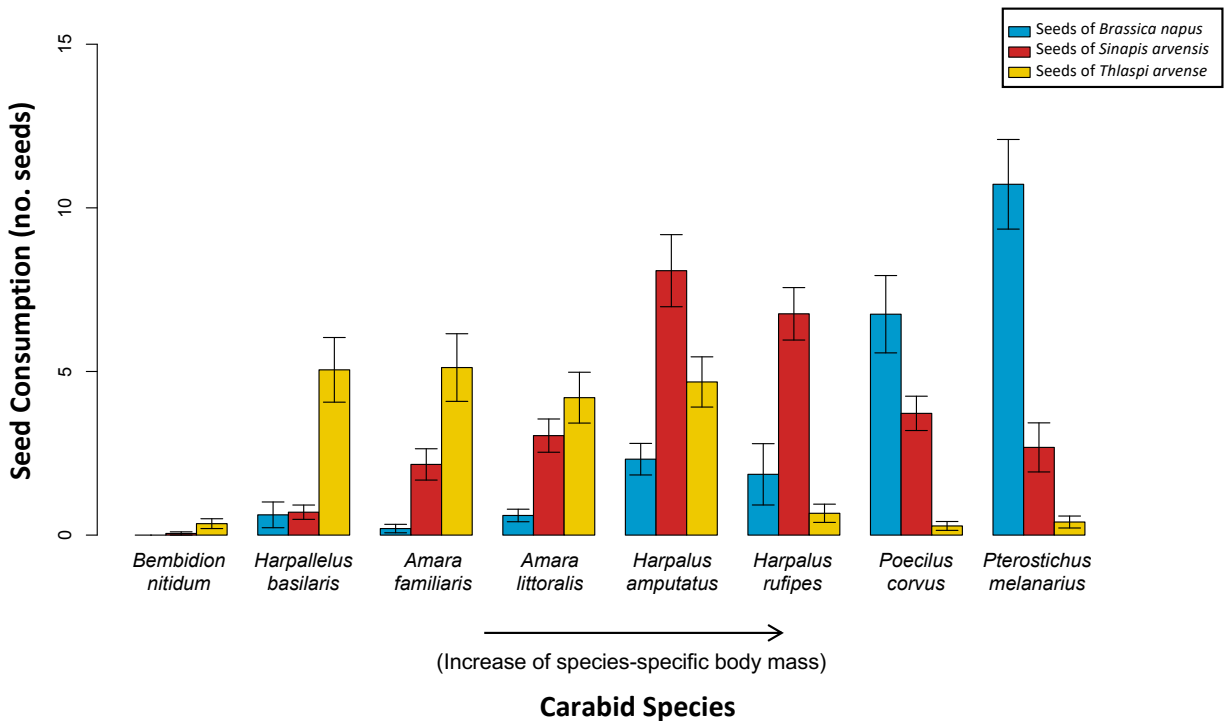
Statistical term	ndf	ddf	F-value	P-value
<i>Weed species</i>	2	468	0.12	$P = 0.88$
<i>Predator body mass</i>	1	502	0.24	$P = 0.61$
<b><i>Insect species</i></b>	7	455	7.37	<b><math>P &lt; 0.0001</math></b>
<i>Insect sex</i>	1	444	0.01	$P = 0.88$
<b><i>Weed Species</i> × <i>Predator body mass</i></b>	2	459	59.42	<b><math>P &lt; 0.0001</math></b>
<b><i>Weed Species</i> × <i>Insect species</i></b>	12	449	10.76	<b><math>P &lt; 0.0001</math></b>
<i>Weed Species</i> × <i>Insect sex</i>	2	484	0.01	$P = 0.98$
<i>Predator body mass</i> × <i>Insect sex</i>	1	413	1.34	$P = 0.24$
<i>Insect species</i> × <i>Insect sex</i>	6	439	1.57	$P = 0.15$
<b><i>Weed species</i> × <i>Predator body mass</i> × <i>Insect sex</i></b>	2	478	3.78	<b><math>P = 0.023</math></b>
<i>Weed species</i> × <i>Insect species</i> × <i>Insect sex</i>	12	483	1.23	$P = 0.25$

ndf: numerator degrees of freedom.

ddf: denominator degrees of freedom.



**Figure 6.1.** Comparisons of mean body mass measurements among the eight species of carabid predators used in the experiment showing some overlaps and also significant differences in fresh weight (mg) following three days of starvation.



**Figure 6.2.** Seed feeding responses (mean number of seeds consumed  $\pm$  mean standard error) of eight species of carabid predators offered seeds of three weed species in multiple-choice seed feeding bioassays. The hierarchy of seed mass in the experiment was as follows: *Brassica napus* (blue) > *Sinapis arvensis* (red) > *Thlaspi arvense* (yellow).

Generalized linear modeling revealed significant differences between PSMR values when calculations were based on species-averaged carabid mass ( $X^2= 642.8$ ,  $df= 7$ ,  $P < 0.001$ , Table 6.3). The PSMR values at the species level increased exponentially as the body mass of carabid species increased (Figure 6.3). The bounds of the mass-based seed preference (i.e., breadth of the seed feeding niche within which carabid species can function), as captured by species-specific PSMR calculations, showed statistically significant differences when estimates of model parameters were compared (see Table 6.3).

**Table 6.3.** Mixed effects analysis (P-values) for Predator-Seed Mass-Ratio PSMR of eight carabid species feeding on three different brassicaceous weed species as affected by insect species, insect sex, and their interactions.

Effect	ndf	ddf	X <sup>2</sup> -value	Pr(> Chi )
<i>Insect species</i>	7	192	642.8	$P < 0.001$ ***
<i>Insect sex</i>	1	191	0.59	$P = 0.44$
<i>Insect species</i> × <i>Insect sex</i>	7	184	20.65	$P = 0.0043$ **

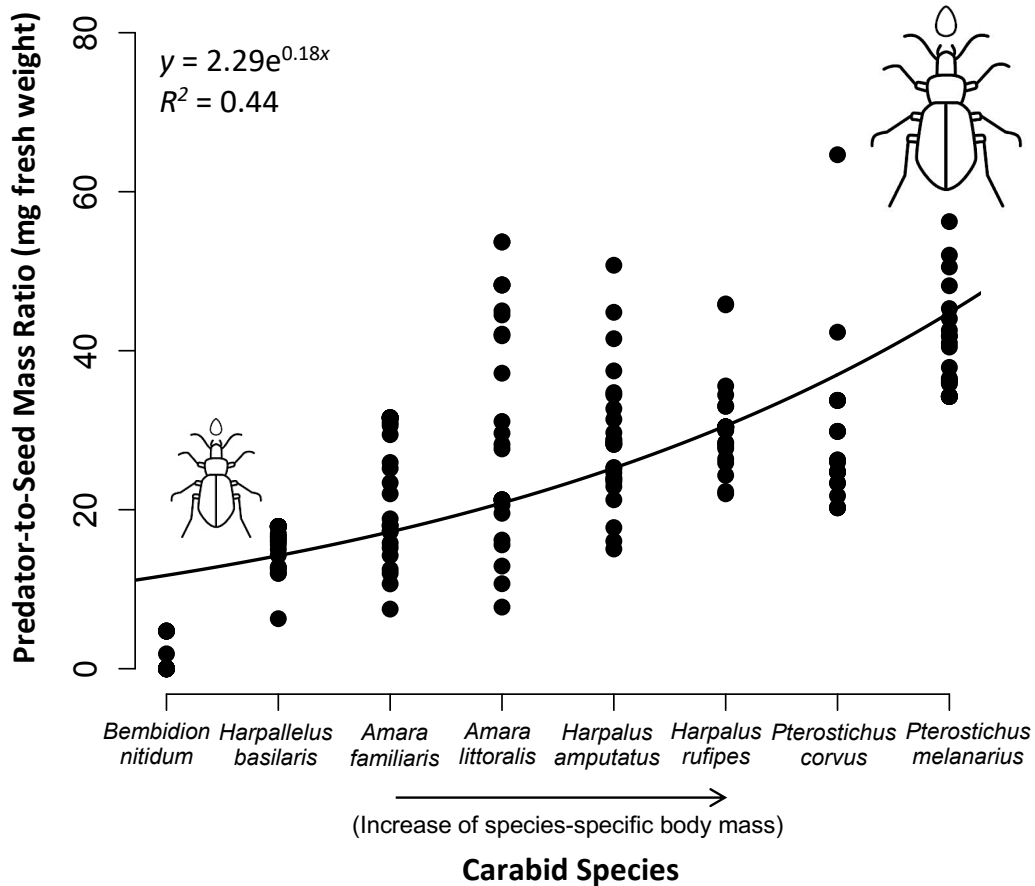
  

Carabid species	PSMR			
	Estimate	St. Error	Z-value	Pr (> z )
1. <i>Bembidion nitidum</i>	0.577	0.1499	3.85	$P = 0.000118$ ***
2. <i>Harpallelus basilaris</i>	2.176	0.1581	13.76	$P < 0.001$ ***
3. <i>Amara familiaris</i>	2.522	0.1558	16.19	$P < 0.001$ ***
4. <i>Amara littoralis</i>	2.847	0.1542	18.47	$P < 0.001$ ***
5. <i>Harpalus amputatus</i>	2.782	0.1544	18.02	$P < 0.001$ ***
6. <i>Harpalus rufipes</i>	2.843	0.1542	18.44	$P < 0.001$ ***
7. <i>Poecilus corvus</i>	2.768	0.1545	17.92	$P < 0.001$ ***
8. <i>Pterostichus melanarius</i>	3.213	0.2133	21.02	$P < 0.001$ ***

\* Indicates significant difference  $p < 0.05$

\*\* Indicates strong significant difference  $p < 0.01$

\*\*\* Indicates very strong significant difference  $p < 0.001$



**Figure 6.3.** The mass-ratio scaling relationships between eight species of carabid predators and seeds of three brassicaceous weed species based on species-averaged values of carabid body mass and seed mass.

Carabid sex, by contrast, did not have any significant bearing on the PSMR values at the species level as both males and females of any given carabid species exhibited similar seed preferences ( $\chi^2 = 0.59$ ,  $df = 1$ ,  $P = 0.44$ ). This was generally the case at the species level. However, the model detected some significant differences in PSMR values between males and females of the carabid species tested ( $\chi^2 = 20.65$ ,  $df = 7$ ,  $P = 0.0043$ ).

## 6.5. Discussion

Body mass of carabid species in the current study was among the important functional traits that influenced which seed species was more prone to elevated attack rates by carabid seed predators. Seed species that carabids attacked at higher frequency was determined by seed species identity, carabid species identity, and carabid body mass. This means that carabid-to-seed mass



relationships, among other things, significantly influence seed selection decisions when both carabids and seed species show considerable differences in their mass characteristics. These findings resonate with a large body of evidence showing that body mass in predatory species is an important ecological and functional trait that can shape fundamental aspects of trophic interactions, which in turn can affect the structure and dynamics of food webs (Troost *et al.*, 2008; Riede *et al.*, 2011). The design of the current study did not allow mass as a functional trait to be separated from species identity for either carabids or seed species. Therefore, the discussion of mass traits here should be kept within the boundaries of species phylogeny (Naisbit *et al.*, 2011). Despite the correlation between species identity and body mass of carabid predators, the impact of body mass on seed selection was significant in the statistical model. This seems to suggest that body mass is a strong predictor of seed selection decisions in carabids when there is a mass-based structure or hierarchy in both the seed and carabid communities.

Carabid species in our study were given five days to feed on the seed species in the feeding arenas. This is an extended period of time, and hence one might assume that hunger might have affected their seed feeding responses. Although hunger is among the factors that can affect nutrient intake decisions in carabids (Toft *et al.*, 2021), hunger was unlikely a factor of major influence on seed feeding responses in the carabids under study as the level of hunger was standardized by starvation for all carabid beetles prior to feeding experiments. Moreover, all carabids tested showed a clear and statistically significant tendency to mainly consume seeds of a specific species among the three seed species offered in the experiment. Therefore, the seed feeding responses exhibited by the carabids under study were not random or passively driven by hunger *per se* (Deroulers and Bretangnolle, 2018). Instead, seed feeding responses were most likely driven by carabids assessing the different suitability aspects of seed species offered in the experiment, including seed mass parameters as shown above.

The current study shows that suitable seeds were selected by carabids based on species identity interactions as well as mass-based interactions between carabids and seeds species. By and large, smaller carabid species tended to feed more preferably on seeds of smaller seed species whereas larger carabids preferred feeding on larger seed species. These findings add to other lines of evidence suggesting that seed selection decisions in carabid seed predators are likely driven by the interplay between body mass of carabid predators and mass of seed species (Honek *et al.*, 2007; Martinkova *et al.*, 2019). These mass-based rules have been found to drive prey choice decisions

in a similar fashion within carnivorous carabids when the prey of different species was accessible in the environment (Bell *et al.*, 2008; Schmitz 2009; Seric-Jelaska *et al.*, 2014; Rusch *et al.*, 2015). It could be concluded that size-structured predator-prey dynamics are likely to mediate the predation pressures carabid species impose on the prey or seed species they target in agricultural fields (Brousseau *et al.*, 2018; Fischer *et al.*, 2021). Given this, it might be expected that carabid seed predation pressures would be high when the distribution of seed mass in the weed community overlaps with the structure of functional body mass in the carabid community (Rall *et al.*, 2012; Reum *et al.*, 2019). Beyond that, predation pressures imposed by carabids on seed species would progressively wane as the divergence in mass structures between carabid and seed communities grows wider.

The mass-based seed selection rules that govern seed preference in carabid seed predators are not as simple and straightforward as they may appear. Body mass is an important biophysical characteristic of carabid predators that features strong correlations with other functional traits of influence on seed handling efficiency (Seric-Jelaska and Durbesic, 2009; Brose, 2010; Kalinkat *et al.*, 2013). Seed choice decisions in carabids are therefore not driven by simple allometric scaling between carabid mass and mass of seed species. Instead, carabids appear somehow able to accurately assess the physical characteristics of different seed species based on their mass, and then use this information to select the seed species they can handle with utmost efficiency (Harrison and Gallandt, 2012; Rall *et al.*, 2012). Carabid-to-seed mass-ratio scaling relationships are probably the mechanism through which carabids gauge the physical properties of different seed species and relate that to their inherent ability for efficient seed handling, as size-structured predator-prey dynamics posit (Emmerson and Raffelli, 2004; Guzman and Sirvastava, 2019). As such, models based on mass-ratio scaling relationships seem to capture the functional linkage between seed mass and carabid seed handling efficiency much better than simple allometric mass scaling models. This could be one reason why simple mass allometry between carabid seed predators and seed species in other studies failed to generate robust predictions about seed choice responses (e.g., Honek *et al.*, 2007). By contrast, mass-ratio scaling relationships generated robust predictions about seed selection decisions in our study, even though the phylogenetic signal in the model was statistically significant.

The functional link between body mass characteristics of carabid seed predators and their innate seed handling efficiency remains poorly understood. Nonetheless, the general trend in

associations between carabid body mass and their seed handling efficiency points towards larger carabids being less efficient at handling smaller seed species (Wilson, 1975; Kalinkat *et al.*, 2013; Reum *et al.*, 2019). Smaller seeds as such become more laborious and less rewarding (more costly) to handle for larger carabids, and seed handling costs become higher as the divergence in mass-ratio between carabids and weed seeds grows wider (Ball *et al.*, 2015). The lower and upper mass thresholds for efficient prey handling are known to change as a function of the body mass of the predator based on predator-prey models (Hirvonen and Ranat, 1996; Klecka, 2014). Taking this in concert with the results of our study, we postulate that the only seed species of mass values falling within the upper and lower thresholds for efficient seed handling would be selected most frequently for consumption by carabid predators. If this is proven correct, it might offer an explanation as to why the variation in interspecific seed mass was reported to strongly influence carabid seed preferences in certain cases, but not in others (Saska *et al.*, 2019a,b).

Species-specific body mass of carabid seed predators emerged as an important functional trait that shape core aspects of seed selection decisions in the current study. Comparisons of species-specific PSMR values among the carabid species under study showed significant statistical differences despite the overlaps. Similar patterns have been observed in other predator-prey systems where feeding niches may show considerable overlaps, but remain ecologically distinctive (Tsafack *et al.*, 2021). These differences in the properties of the carabid seed feeding niche, even though subtle, could allow different carabid species in the community to coexist without elevated interspecific competition (Basset, 1995; Hartvig and Anderson, 2013; Zalewski *et al.*, 2014). By contrast, intraspecific differences in carabid body mass values were very minor in our study, and probably lacked the ecological power to exert considerable influence on seed selection decisions. These findings should be approached with caution, however, as some lines of evidence have reported that considerable intraspecific variation in body size can be detected for some carabid species in response to certain environmental conditions (Juliano, 1983; Sota *et al.*, 2000). Intraspecific variability in carabid body mass due to environmental factors tends to be most pronounced when carabid populations are exposed to wide climatic or habitat gradients (Beckers *et al.*, 2020), which clearly was not the case in the current study. Intraspecific variation in carabid body mass can also arise from intense intraspecific competitive interactions between carabid populations on some occasions (Sota *et al.*, 2000). This did not seem to be the case in the current study either. Given these observations, seed feeding ecology in carabids might be less sensitive to

intraspecific variability in body mass under the conditions of local environments as shown by data of the current study. Still, further studies are needed to test whether this observation holds true when carabids are sampled from populations that span a wide range of environmental gradients, especially that intraspecific variability in body mass is generally an influential factor in predator-prey systems (Tsai *et al.*, 2016; Jerde *et al.*, 2019).

Sex-driven differences in carabid body mass (males and females) exerted no considerable influences on seed choice decisions in the carabids tested. Carabid females tend to be heavier than carabid males in general (Sota *et al.*, 2000). Sexual-dimorphism in carabid body mass, however, turned out to be mainly derived from differences in size and morphology of the genitalia and sometimes also from differences in mating status (Juliano, 1985; Benitez *et al.*, 2013). Thus, sex-related differences in carabid body mass are not expected to be involved in strong associations with feeding ecology. Body mass also did not affect the total number of seeds consumed by carabid predators in the experiment (data not shown).

I have thus far established that mass-ratio scaling relationships rule seed selection decisions when carabid species and seeds of weed species show considerable differences in their size characteristics. Carabid seed selection decisions in this case were inexplicable based on seed nutritional chemistry. Seeds of *B. napus*, for example, seem to harbor the kind of seed chemistry that carabid species tend to favor as shown by other studies (Haq *et al.*, 2009; Kulkarni *et al.*, 2016). If seed selection decisions were driven by seed chemistry alone, all of the carabid species tested here should have favored *B. napus* seeds. Seed nutritional chemistry seem thus to drive seed selection in carabids only when all else is physically equal (Toft, 2005). Data here show that outside the physical equality assumption, seed selection decisions become driven by carabid-to-seed mass-ratio scaling relationships and seed chemistry fails to produce robust predictions about seed preference in carabids, which agrees with Reum *et al.* (2019) and Foffova *et al.* (2020a,b). Nevertheless, the inner workings of carabid-to-seed mass-ratio scaling relationships remain mostly unknown despite the high predictive power they exhibit with regard to seed selection decisions.

Although the inner workings of mass-ratio scaling effects still await further exploration, my findings suggest that knowledge of carabid and seed masses can suffice to produce relatively accurate predictions about the interaction strength (Vucic-Pestic *et al.*, 2010). Weed and carabid communities in agroecosystems are often composed of multiple species with various functional traits, including mass characteristics (Booth and Swanton, 2002; Pufal and Klein, 2013).

Presumably, complexity of such sort would hinder accurate predictions to be made about seed predation pressures under realistic situations. It turns out, however, that seed predation pressures in arable fields are particularly sensitive to composition of the carabid community, rather than the diversity of weed community (Schumacher *et al.*, 2020). This may indicate that structure of the functional body mass in the carabid community is fundamentally important to the determination of predation pressures carabids exert on seed species in the weed community (Fischer *et al.*, 2021). If the carabid community is dominated by large species, large seed species would suffer intense predation and a strong population suppression as a result (Lami *et al.*, 2020). By contrast, small seed species would escape intense predation and thus, could dominate the weed community. Therefore, the ideal scenario could likely unfold when the carabid community is widely diverse and composed of species that span a wide range of functional body mass, so that species niche complementarity could increase and population suppression would be distributed more evenly among seed species in the weed community (Schumacher *et al.*, 2020; Lami *et al.*, 2020).

In addition to predicting predation pressures (interaction strength), mass-ratio scaling can give good predictions about the type of functional responses (type II or III functions) that carabids employ in their seed foraging strategies (Rudolf, 2008; Kalinkat *et al.*, 2013). When distribution of the functional body mass in the carabid community overlaps greatly with seed mass distribution in the weed community, carabids would spend less time handling the seeds, and type II functional responses would rule foraging strategies (Kalinkat *et al.*, 2013). Suppression of weed populations in this case would reach high intensities at low seed densities, and could lead to local population extinctions as such, giving rise to destabilizing inverse-density dependent effects on the weed community (Holling, 1959; Vucic-Pestic *et al.*, 2010). Beyond that, if mass structures in carabid and seed communities are widely divergent, carabids would spend more time handling the seeds and their functional responses would therefore shift towards type III functions (Kalinkat *et al.*, 2013). Population suppression in such a case would be rather weak at low seed densities, allowing seed species to escape local extinctions since the density-dependent seed foraging strategies here would stabilize the weed community (Holling, 1959; Barrios-O'Neill *et al.*, 2016). It remains to be investigated whether the mass-ratio effects in seed predation systems may be sensitive to the intraspecific (individual) mass variability in body mass of carabid predators and/or seed species. If this turns out to be the case, it could hold important implications for the ecological studies of seed preferences in carabid seed predators.

## CHAPTER 7 General Discussion

### 7.1. General Discussion

My work has shown that chemoperception of seed-derived volatile chemical cues enables carabid seed predators to detect and discriminate among seeds of different species. By contrast, visual perception does not seem to elicit the sensory response necessary for carabid predators to detect and distinguish between seeds of different species. These observations were consistent across all carabid species tested in my work, which lends good support to my hypothesis that seed detection and discrimination in carabid seed predators is mediated by chemoperception rather than vision. Chemoreceptors previously have been found to enable generalist (omnivorous) as well as specialist carabid predators to identify prey of suitable species (McKemey *et al.*, 2004; Thomas *et al.*, 2008; Talarico *et al.*, 2010; Giglio *et al.*, 2012). Visual receptors in carabids seem more attuned towards the detection of prey movement, and should thus be more useful for hunting down highly mobile prey rather than detecting seed species or sessile prey (Wheater, 1989; Oster *et al.*, 2014). Based on that, perception of olfactory cues is probably the universal mechanism by which carabids detect and identify seed and prey species. That is, the olfactory-guided seed and prey foraging behaviors in carabids are likely to transcend the taxonomic borderlines between species and genera, and are unlikely, therefore, to reveal fundamental differences among specialized and unspecialized feeders or between diurnal and nocturnal species. These conclusions remain tentative at this point, however, as the sensory biology of food perception has been studied only in a small number of carabid species. Moreover, the carabids tested in my work and the work of others were generally nocturnal, omnivorous feeders. Thus, further research is needed to clarify if the differences in ecology of carabid species (e.g., feeding specialization, seasonal rhythms, diel rhythms, etc.) are also associated with some differences in the sensory biology of food perception.

Carabid predators, as follows from the argument above, have capacity to actively select favorable prey or seed species, and can potentially employ olfactory templates or search images to guide their prey and seed foraging behaviors. Olfactory templates or search images act as cognitive benchmarks that enable olfactory-oriented predators to interpret the olfactory information collected from the environment, so that suitable prey can be identified (Cross and Jackson, 2010). Carabid predators, as olfactory-guided predators, are expected to rely on similar cognitive

mechanisms to identify suitable species of prey and seeds. The chemical ecology study I conducted shows that carabids exploit the volatile chemicals derived from long chain aliphatic fatty acids located on the seed coat surface (epicuticular waxes) as kairomones to guide their seed foraging behaviors. Surface waxes that cover somatic and reproductive tissues of plant species generally encode specific and accurate information about plant species and thus, are among the main interlocutors of feeding and oviposition preferences in plant-insect interactions (Eigenbrode and Espelie, 1995). The seed foraging kairomones exploited by carabids as such fall among the reliable, but hard to detect, plant-derived chemical signals (Bruce *et al.*, 2005). This corroborates my hypothesis that carabids, in their interactions with plant seeds, seek sensory cues that encode accurate and specific information about seed species. This finding may hold important implications for the study of carabid chemical ecology as it shows carabids, which were previously thought to be opportunistic seed feeders, search for accurate and reliable seed cues to guide their seed foraging efforts. The seed volatile cues identified in my work can act at short ranges only, which may force carabids to rely on other, more detectable habitat-derived cues to locate seed patches, before seed volatiles can be exploited for guiding seed selection decisions. The sensory cues that can facilitate seed patch location by carabids within the habitat remain unknown at this point. Such cues could be derived from whole plant volatile chemicals (Oster *et al.*, 2014), and/or other chemical or physical cues that indicate the presence of plant cover (Blubaugh *et al.*, 2016), or dense seed patches (Honek and Martinkova, 2001).

By contrast, the kairomones that carabids seek to guide their prey foraging behavior are often derived from highly volatile and chemically less sophisticated compounds, representing odors that emanate naturally from prey species (e.g., pheromones) or their habitat (e.g., host plant volatiles) (McKemey *et al.*, 2004; Munday *et al.*, 2000; Tréfás *et al.*, 2011). These differences between prey and seed foraging kairomones in carabids may indicate that seed perception is potentially more cognitively demanding than prey perception (Hansson and Stensmyr, 2011). It may also indicate that interactions between carabids and seed species are probably more directed than random or opportunistic as previously assumed. Taken together, these lines of thought seem to uphold my argument that seed feeding habits in omnivorous carabids have potentially evolved due to specific biological needs that may not be satisfied through prey hunting alone.

Chemical sensory information as above emerges as a crucial requirement for the ecological functioning of carabid species as potent, polyphagous biocontrol agents of pests in

agroecosystems. This adds further emphasis to the role of informational chemicals as the key mediators of plant-insect and insect-insect interactions in agroecosystems (Dicke, 2000). Still, detailed studies are needed to investigate whether synthetic chemicals can be used in the future to manipulate the foraging behaviors of carabid predators in ways that can enhance their contributions to pest and weed biocontrol in agroecosystems (Baker and Longhurst, 1981, Foster and Harris, 1997; Reisenman *et al.*, 2016).

The kind of information encoded in the chemical seed cues that I managed to isolate and identify in this work remain unknown. Given that seed cues are derived from long chain fatty acid signaling compounds, they may encode information about the nutritional quality of seed species (Kunst and Samuels, 2003; Medina *et al.*, 2006). And given the fact that agri-environments are often lipid-limited (Jensen *et al.*, 2012; Toft *et al.*, 2019), I hypothesized that carabids may be seeking the acquisition of fatty acids through seed feeding. The nutritional ecology experiments I conducted using synthetic diets seem to lend support to this hypothesis. Carabids in my work selectively foraged for protein and lipids and regulated their intake of these two macronutrients depending on the nutritional conditions in the experiment. These findings agree with other studies documenting that protein and lipids are both essential for the survival and reproduction in carabid predators and, therefore, drive key aspects of their feeding ecology (Mayntz *et al.*, 2005; Jensen *et al.*, 2012). The carabid species under study showed a tight regulation of their lipid intake relative to protein under the different nutritional conditions in the experiment. This may indicate that carabids are likely to forage for seeds to acquire fatty acids that are scarce or hard to obtain in agri-environments.

Based on the nutritional ecology data, carabid predators seem to prioritize protein consumption (protein-biased intake targets), and over-ingest protein-rich foods to acquire essential lipids whenever protein of high quality is abundant. By contrast, when protein is scarce or low in quality, carabid predators tend to avoid excessive protein ingestion and shift their nutrient foraging behaviors towards enhanced lipid acquisition (lipid-biased intake targets). Therefore, if prey (protein-rich) is abundant in the environment, and seeds (lipid-rich) are scarce, carabid predators would feed mainly on prey and thus, kill more prey individuals to satisfy their lipid needs (see Table 7.1). The abundant prey species would suffer intense suppression in a such case, while seed species would largely escape strong carabid predation. If prey is scarce and seeds are abundant, carabids would prioritize lipid acquisition, and seed species containing desirable lipids would



suffer the intense carabid predation (see Table 7.1). When both prey and seeds are available, carabids would more likely mix prey feeding with seed feeding to balance the protein and lipid ingestion, without much compensatory feeding (over- or under-ingestion) of either micronutrient. Prey and seeds of suitable species are both expected to suffer strong carabid predation in such a case. Still, prey is expected to suffer stronger predation pressure compared to seeds here since omnivorous carabids tend to often self-compose protein-rich optimal diets when given the chance to mix different nutrient-deficient food types (Jensen *et al.*, 2012; also see Table 7.1). Data from some field studies seem to align with the predictions outlined above based on my nutritional ecology studies (e.g., Blubaugh *et al.*, 2016; Carbonne *et al.*, 2020a). Overall, lipid acquisition seems to underlie the trophic linkage between carabids and seed species. This agrees with speculation of Gaba *et al.* (2009) and may explain why carabid seed consumption reaches its highest level late in the summer, when weedy plants start to shed their seeds (Blubaugh and Kaplan, 2016). Lipid acquisition late in the season enhances egg production in fall-breeding carabids, and also enhances the lipid storage necessary for hibernation in spring-breeding carabids (Jensen *et al.*, 2012).

**Table 7.1.** Predictions about the feeding habits of omnivorous carabid predators under different scenarios of prey and seed abundance based on the nutritional ecology data produced in this thesis.

Nutrient-driven feeding habits in omnivorous carabid predators					
Prey (Protein-rich)	Seed (Lipid-rich)	Nutritional intake targets	Predominant feeding habit	Predation pressure	
				Prey	Seed
Abundant	Scarce	Protein-biased (P:L = 1: 0.5)	Prey hunting	High (Prey overkill)	Weak
Scarce	Abundant	Lipid-biased (P:L = 1: 1.5-2)	Seed feeding	Weak	Intense seed predation
Abundant	Abundant	Protein-biased (P:L = 1: 0.75)	Mixed feeding	Predation pressure is distributed among prey and seed specie, but potentially stronger against prey	

The dietary mixing of seeds and prey enables carabid predators to satisfy the nutritional needs of survival and reproduction and avoid the costs of compensatory feeding as well as the detriment of defensive chemicals (Hagele and Rowell-Rahier, 1999; Singer *et al.*, 2002; Le Gall and Behmer, 2014). This may explain why omnivory tends to predominate the feeding habits in

most carabids inhabiting agri-environments, where the availability of suitable prey or seeds is heterogenous (Frei *et al.*, 2019; De Heij and Willenborg, 2020; Carbonne *et al.*, 2020a). Mixed, unspecialized feeding is expected to improve the fitness functions of the carabids foraging for food under the conditions of agri-environments, precluding more specialized feeding habits from evolving and taking hold in the carabid community (Krivan, 2010; Cressman *et al.*, 2014). Specialized feeding habits towards carnivory or granivory can only evolve in certain habitats where the abundance of specific seed or prey species is stable enough to allow morphological and/or physiological adaptations to evolve and take hold (Forsythe, 1982, 1987; Klimes and Saska, 2010). Still, the nutritional ecological findings and their implication (as above) were based on experimenting with only two macronutrients (protein and lipid) and only one defensive chemical (allyl isothiocyanate). The actual chemistry of seed species is more complex than the reductionist approach adopted for my experiments. Therefore, the arguments given above offer somewhat simplistic explanations of what may be happening under realistic situations, and for this reason should be considered within the limits of the study.

Seed chemistry in terms of volatile, nutritional, and non-nutritional (defensive) chemicals emerged as the central pillar of seed perception and seed preference in carabid seed predators. The seed species used in my chemical ecology experiments showed distinctive volatile chemistries which potentially reflected the nutritional suitability of the seed. In addition to volatile chemistry, seed species in my experiments showed significant differences in their size characteristics as measured by species-averaged mass. Size is an ecologically important seed physical trait and should be considered in the study of seed preferences in carabid seed predators (Foffova *et al.*, 2020a). My work in this respect has revealed that predator-to-seed mass-ratio scaling relationships can drive seed selection decisions when carabids of different body mass are offered seed species of various mass. Seed size characteristics can even overpower seed volatile chemistry (and potentially also the seed nutritional chemistry as encoded by the seed odor), and sway seed selection responses away from the predictions of seed chemistry models (see above). Seed mass, based on the current data, turns out to be the most influential seed trait in determining seed preferability to carabid predators when there is a seed-mass structure (i.e., hierarchy) in the weed community. If mass structures in the carabid and seed communities overlap, then the intensity of seed predation pressures and the suppression against weed community are expected to be rather high (Kalinkat *et al.*, 2013; Pocok *et al.*, 2021). Beyond that, seed predation pressures would be

weak and insufficient for effective suppression to be imposed against the weed community. By the same token, mass-ratio scaling relationships have been found to also drive prey selection in carabids, and shape the intensity of suppression they exert on prey species in agroecosystems (McKemey *et al.*, 2003; Rusch *et al.*, 2015). One may interpret these lines of evidence as seed and prey foraging in carabid predators being ruled by behavioral mechanisms that are not fundamentally different (Talarico *et al.*, 2016).

The ways by which mass-ratio scaling relationships drive seed selection decisions in carabid seed predators remain to be determined. Seed handling costs seem to differ significantly among seed species of different mass (Honek *et al.*, 2007; Foffova *et al.*, 2020a,b). Seed handling efficiency is also expected to vary among carabid species depending on their body mass (Kalinkat *et al.*, 2013). Therefore, the active selection of suitable (nutritionally-desirable) seed species in carabid seed predators can be constrained by seed handling costs in some cases. This is plausible given that prey handling costs were found to constrain prey selection in *Poecilus cupreus* (L.), which made this carabid species select less attractive prey species (lower in quality) because they were easier to handle (Lang and Gsodl, 2001). Furthermore, functional trait analysis research has revealed that the carabid predators select suitable prey species based on the ability of their mandibles to break through the cuticle of different prey species (Brousseau *et al.*, 2018). Body mass is intimately correlated with the jaw musculature and biting force in carabid predators (Evans and Forsythe, 1985; Wheater and Evans, 1989). Therefore, it is possible for the relationship between carabid body mass, jaw musculature, and the bite force to also determine which species of seeds that can be handled efficiently and should, therefore, be favorably chosen for consumption. If this turns out to be the case, the physical properties of seed and prey species would emerge as the key barrier against the evolution of specialized feeding habits in carabid predators *sensu* Goodman and Ewald (2021). Specialized feeding towards certain seed species in such case would arise by carabids evolving the ability to break the defenses of the abundant prey or seed species in their habitats (Fawki *et al.*, 2003). Beyond that, handling costs are likely to always constrain the choice of nutritionally-desirable seed species in unspecialized carabid predators. Based on that, seed predation dynamics should be powered by the functional traits (i.e., species identity) on each side of the interaction (Brousseau *et al.*, 2018).

Given the above, carabid seed predators tend to assess both the chemical and physical characteristics of seed species before they decide which species should be selected for

consumption. Chemical and physical characteristics of plant species are often both evaluated when insects select the suitable host plant in plant-insect interactions (Calatayud *et al.*, 2008; Pontes *et al.*, 2010). This falls nicely in line with my argument that interactions between carabids and seed species are a special case of genuine plant-insect interactions. Seed chemical properties are assessed through the olfactory perception of seed volatiles as shown in this work. On the other hand, it remains unclear how carabid seed predators are able to assess or gauge the mass or size parameters of different seed species, and identify species that are physically suitable for consumption. Perhaps the ratio between mandible length and labrum width may help carabids gauge the dimensions of different seed species and thus, their physical suitability (Deroulers and Bretangnolle, 2018). Or perhaps, mechanisms more sophisticated than gape size *per se* underlie the process of picking out physically suitable seed species given that carabids are olfactory-guided foragers. Learning and experience play crucial roles in guiding the choice of suitable prey or host in olfactory-oriented polyphagous (unspecialized) insect predators and parasitoids that tend to assess both the chemical and physical aspects of prey or host suitability (Barobsa, 1988; Vinson, 1998; Huigens and Fatouros, 2013). Learning is also essential for insects to overcome the reliability-detectability problem imposed by certain types of food foraging kairomones (seed epicuticular lipids in case of carabid seed predators) (Schoonhoven *et al.*, 2005; Kather and Martin, 2012). Together, these factors have probably placed carabids under strong selection pressures to evolve some capacity for olfactory learning (Hassell and Southwood, 1978). Olfactory priming of carabid predators with odors of specific seed species (no seed handling was allowed) did not alter seed selection responses in our cafeteria multiple-choice seed feeding experiments. Thus, non-associative learning does not seem to underlie the formation of the olfactory templates or search images necessary for guiding seed foraging behaviors in carabids. This agrees with Plotkin (1979) who found that *Pterostichus melanarius* (Illiger) could learn by detecting and memorizing olfactory cues from the environment, but odor cues alone were insufficient to account for the olfactory learning capacity in this carabid species. Therefore, more sophisticated mechanisms seem to mediate the formation of the olfactory memory necessary for guiding seed and prey searching behaviors in carabid predators.

If carabids learn by associative conditioning or operant conditioning (trial and error), seed or prey handling would be an essential requirement for olfactory templates to form and then guide foraging behaviors (Ducas, 2008; Huigens and Fatouros, 2013). Carabid seed predators, through

associative or operant conditioning, are expected to associate the smell of different seed species with their handling costs (Solowski *et al.*, 2010). Odor of the seed species that is not too costly to handle should be memorized, and this olfactory memory would then guide the seed selection decisions during future encounters between carabid predators and seed species (Chapman and Bernays, 2005; Liu *et al.*, 2006; Garay *et al.*, 2018). Disabling chemoperception in carabid species in my work caused the whole process of seed detection and discrimination to be disabled. Carabids thus need to smell and also physically probe or handle seed species before seed selection can take place. Linking the chemical and physical properties of seed species is, therefore, the likely mechanism by which an olfactory memory can form and then, guide the search for suitable seeds in carabid seed predators *sensu* Hollis and Guillette (2011).

The ideas above regarding associative learning in carabids remain mostly untested and thus, are ripe for future empirical investigation. If proven correct, seed feeding and seed preferences in carabids should be treated as a phenomenon of local rather than universal applications (Barney and Pass, 1986). Seed preference and the intense weed suppression they entail would thus be determined by the identity (i.e., functional traits) of the carabid species that dominate the carabid community in terms of efficiency for handling different seed species in the weed community. Data from some field studies seem to support the logic of this argument (e.g., Carbonne *et al.*, 2020b). This highlights the importance of local species surveys along with laboratory and field seed feeding experiments for identifying carabid species that are likely to impose strong suppression against the local weed communities. If the dominant carabids can handle seeds of different species in the weed community without considerable costs, seed chemistry should rule seed selection and nutritious seed species (lipid-rich) would actively be selected and thus, incur intense population suppression. On the contrary, if seed handling by the dominant carabids varies widely among seed species, seed handling would constrain the active selection of the nutritionally-desirable seeds species. Therefore, seed species that can be handled without high costs would be selected more frequently by carabids, irrespective of their chemical suitability. Finally, if functional traits in the carabid and seed communities are largely mismatched, seed feeding would become more opportunistic or random than selective. These lines of thought may help explain why preferences to specific seed species could be observed in certain seed predation studies (e.g., Kulkarni *et al.*, 2016), while opportunistic or random seed feeding that lack

any signs of active seed selection were observed in other studies (e.g., Cardina *et al.*, 1996; Hurst and Doberski, 2003).

It is worth mentioning that in addition to functional traits, seed selection decisions in carabids can also be sensitive to the biotic and abiotic conditions of local environments. Habitat properties of carabid predators (Petit *et al.*, 2017; Carbonne *et al.*, 2022), interference and competition among and within carabid species (Charalabidis *et al.*, 2019), in addition to inter- and intra-guild predation risks (Charalabidis *et al.*, 2017) can all bring about profound changes in seed selection decisions. Such changes may not be explicable or predicable through the trait mismatch models discussed above. Again, this places more emphasis on the complexity of carabid seed predation systems, and highlights the dangers of making broad generalizations. In closing, seed ‘preferences’ in carabid seed predators emerge as an equivocal concept since seed selection is not always driven by the active selection of desirable, nutritious seed species. This should be considered in future studies of seed predation ecology, and perhaps more ecologically appropriate terminology should be adopted to better describe the phenomenon of seed choice in carabid seed predators.

## **7.2. Recommendations for Future Studies**

The work presented throughout this thesis has successfully achieved the research objectives outlined in the first chapter. Data presented and discussed in the thesis chapters bridge some important gaps in the knowledge around carabid seed predation ecology. Still, much remains to be discovered about the ecology of seed feeding habits in carabid beetles, and we recommend the following lines of inquiry for future studies:

1. Conduct more sensory studies and test both diurnal and nocturnal carabid species to check if the activity period of carabid species may be correlated to differences in the sensory biology of seed perception. Diurnal and nocturnal carabids often have distinct packages of sensory receptors, which may entail subtle differences in the sensory mechanisms of food perception. It is thus important to test if visual cues may become more valuable for discrimination among seed species when carabid species are of diurnal activities.

2. Isolate and identify seed foraging cues from other brassicaceous and non-brassicaceous seed species to explore how the chemical language that carabids exploit to identify suitable seed species may vary across plant lineages. The volatile chemicals derived from plant epicuticular lipids (seeds included) are highly variable among and within plant lineages. Therefore, it is important to investigate how such variability may affect seed choice, and whether volatile secondary seed metabolites may be detectable among the volatiles of non-brassicaceous seed species.
3. Develop synthetic seeds that could be chemically and physically manipulated to study how the interplay between chemical and physical seed characteristics may influence seed selection decisions in carabid seed predators. If these seed mimics can be developed, researchers will be able to study the interactions between different seed nutrients, defensive chemicals, and physical characteristics such as size, shape, texture, and hardness in more depth and with less statistical noise.
4. Test if seed-mass-ratio scaling relationships may be sensitive to the intraspecific variability in seed mass. Ideally, this could be done by choosing one or a few seed species that show high variability in their intraspecific seed mass parameters. In this way, the species identity of the seed could be kept constant and the effects of seed size could be studied by creating seed-size cohorts within the seed species under study.
5. Explore the relationship between carabid body mass and their biting force and test if the interaction between bite force and seed coat hardness may better predict seed selection decisions in carabids. These studies require specialized instruments for measuring the coat hardness of seed species and the bite force of carabid species. Such instruments may not be easily accessible, but they are essential for establishing the relationship between seed mass (both intra- and inter-specific) and seed coat hardness as well as the relationship between the body mass of carabid predators (both intra- and inter-specific) and the bite force they exert on seed coats. If these relationships are successfully established, the exact biophysical factors that constrain the active selection of nutritious seed species may finally be unmasked.

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