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Vlastnosti semen důležité pro jejich predaci

Disertační práce

Ing. Hana Foffová

Školitel: doc. RNDr. Pavel Saska, Ph.D.

Konzultant: doc. Ing. Zdenka Martinková, CSc.,
Ing. Jan Lukáš, Ph.D.

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PROHLÁŠENÍ

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V Praze dne ………………..……………..

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1. Obsah

2. Abstrakt

Predace semen je potravní strategie, kdy predátor požírá během svého života mnoho semen, která následně nejsou schopna vyklíčit. Predace semen přispívá k regulaci půdní zásoby semen plevelů a ovlivňuje tak jejich populační dynamiku. Z bezobratlých se v našich zeměpisných šířkách na predaci semen v polních podmínkách podílejí především střevlíkovití brouci (Coleoptera: Carabidae). Mechanismy, pomocí kterých predátoři semena vyhledávají a které se uplatňují při určení jejich preferencí, nejsou dosud dostatečně popsány. Zejména chybí informace, jaké znaky semen a v jaké míře ovlivňují výběr predátorů semen. Hlavním cílem této disertační práce je tedy zjistit, jaké znaky semen se podílejí na výběru semen střevlíkovitými brouky.

Arénové pokusy identifikovaly morfologické znaky semen (velikost a tvar semen, tvrdost a tloušťku osemení) spolu s fylogenetickou příslušností (čeleď) a životní strategií rostlin (jednoleté, dvouleté, více leté rostliny) jako faktory, které podmiňují preference sledovaných střevlíkovitých brouků. Zároveň nebylo prokázáno, že by na preference střevlíků měly vliv dostupné chemické znaky semen (mastné kyseliny, volatilní látky a látky na povrchu semen) (*Výsledek I.: Foffova et al., 2020, Insects 11:757.*). Tento výsledek nekoresponduje s dosud publikovanou literaturou, kde jsou chemické látky popsány jako potenciální atraktanty / deterenty pro mnoho predátorů semen. Vysvětlení tohoto rozporu může spočívat v designu provedeného arénového experimentu, který kombinoval mnoho různých druhů semen na malém prostoru, což mohlo snížit schopnosti rozlišit tyto látky od látky od sebe, resp. identifikovat jejich zdroj.

Znaky semen (hmotnost, rozměry atd.) se mohou měnit v závislosti na prostředí a čase. Semena nabobtnalá nebo mající poškozené osemení jsou požírána více nežli suchá nepoškozená semena (Výsledek II.: *Foffova et al., 2020, Acta Zoologica Academiae Scientiarum Hungaricae, 66:37 - 48.*). Tento fenomén je možné vysvětlit změnou fyziologické aktivity spojenou s klíčením semen a pozměněnou tvrdostí semene.

Znaky semen se mění i s dobou setrvání v půdě, jež se projevuje zejména na hmotnosti, tvaru a životnosti. Výsledek III.: *Saska et al., 2020, Agronomy, 10:448* navrhuje změnu ve způsobu interpretace dat o dlouhověkosti semen v půdní zásobě, přičemž klade důraz na modelování procentuálního přežití populace semen (PT50 a PT05). V experimentu bylo zjištěno, že některé druhy semen mají dlouhou životnost (např. u druhu *Urtica dioica* L. je PT50 10,5 let). Zatímco jiné druhy např. *Campanula trachelium* L. mají životnost krátkou (PT50 = 1,9 let). Společně s životností semen se mění i morfologické znaky semen.

Změna znaků semen během setrvání v půdní zásobě má odezvu i v predaci semen střevlíkovitými. Výsledky preferenčních arénových pokusů naznačují sníženou konzumaci 6 let starých (zakopaným v půdě oproti čerstvým semenům), u druhů *Pseudoophonus rufipes* (DeGeer, 1774) a *Amara littorea* (C. G. Thomson, 1857). Oproti tomu druh *Harpalus affinis* (Schrank, 1781) preferoval zakopaná před čerstvými semeny (Výsledek IV.: *Saska et al. 2019. EJE, 116:133 - 140*).

Změny znaků semen v půdní zásobě jsou, mimo jiné, způsobeny půdními mikroorganismy. Bylo zjištěno, že diverzita bakterií na povrchu i uvnitř semen je specifická pro různé druhy semen a zároveň podmíněná dobou setrvání v půdě. Bakteriální diverzita na druhové úrovni ovlivňuje preference střevlíkovitých brouků i životnost semen (Výsledek V.: *Saska et al., PLSO – under review*).

Tato dizertační práce přispívá k pochopení zdrojů variability v potravních preferencích střevlíků a vzorců jejich chování. Tato disertační práce se také snaží poukázat na souvislosti mezi fylogenetickými, morfologickými a chemickými znaky semen v návaznosti na čas, který strávila semena v půdě, a prostředí, ve kterém se vyskytují. Poznatky této práce pomáhají s vysvětlením a pochopením složitého fenoménu predace semen, který je v agroenvironmentální praxi podhodnocován či zcela ignorován.

Klíčová slova: Střevlíkovití, půdní zásoba semen, plevel, preference, semenožravost

3. Abstract

Seed predation is a feeding strategy in which a predator consumes many seeds in its lifetime. After the attack, seeds are killed and not able to germinate anymore. Seed predation contributes to the regulation of the weed seed bank and thus may affect weed dynamics. In our latitudes, the ground beetles (Coleoptera: Carabidae) are the main seed predators in field conditions. The mechanisms which predators use when searching for seeds and determining their preferences have not been sufficiently described. In particular, information is lacking on which seed traits are involved during the seed selection process by carabid seed predators. The main aim of this PhD. thesis is, therefore, to ascertain which seed traits are involved in choosing seeds by ground beetles.

Arena experiments identified morphological traits of seeds (seed size and shape, seed hardness and thickness) along with phylogenetic affiliation (family) and plant life strategy (annual, biennial, perennial plants) as main factors determining the preferences of carabids. At the same time, seed chemical traits (fatty acids, volatile compounds and chemical compounds on the surface of seeds) have not affected ground beetle preferences (*Paper I.: Foffova et al., 2020a, Insects 11: 757*). This result does not correspond with the literature, where chemical traits were found to be potential attractants/deterrents for seed predators. This disagreement can be explained by the design of the cafeteria test, which combined many different species of seeds in a small space and could reduce the ability of the beetles to distinguish the source of the volatile compounds.

Seed traits (weight, dimensions, etc.) may vary in different environments and times. Imbibed seeds or seeds with damaged seed coats were eaten more than dry and undamaged seeds (*Paper II.: Foffova et al., 2020b, Acta Zoologica Academiae Scientiarum Hungaricae, 66:37-48).* This result can be explained by the change in physiological activity associated with seed germination and changes in seed coat hardness.

The seed traits may change with the time spent in the soil seed bank, which is reflected mainly in the case of mass, shape and longevity. *Paper III .: Saska et al., 2020, Agronomy, 10: 448* suggests that interpretation of data related to seed longevity in soil bank should be based on modelling the percentage survival of the seed population, based on which persistence times can be estimated (PT$_{50}$ and PT$_{05}$). It was found that the persistence curve is different among the species (e.g. PT$_{50}$ of *Urtica dioica* L. and *Campanula trachelium* L. was estimated to be 10.5 and 1.9 years, respectively). Along with seed viability, the morphological traits of the seeds also change due to time spent in soil.

The change in the seed traits during the years in the soil seed bank affected seed preferences of ground beetles. *Pseudoorphanus rufipes* DeGeer, 1774) and *Amara littorea* (C. G. Thomson, 1857) preferred fresh seeds before buried seeds. In contrast, *Harpalus affinis* (Schrank, 1781) preferred buried seeds over fresh seeds (*Result IV.: Saska et al. 2019. EJE, 116: 133-140*).

Soil microorganisms are another factor that interfere with seeds stored in the soil bank, and possibly also with seed preferences by the carabids. It was found that the diversity of bacteria on the surface and inside the seeds is species-specific and it changes with the time spent in the soil. Bacterial diversity affected the preferences of ground beetles for particular seeds and also the viability of the seeds (*Paper V.: Saska et al., PLSO - under review*).

This Ph.D. thesis contributes to our understanding the sources of variability in carabid preferences, food webs and behavioural patterns. It also tries to indicate the connections between phylogenetic, morphological and chemical traits of seeds in interaction with time when the seeds stayed in soil seed bank and dynamics and the environment in which they occur. Findings included in this thesis help to explain the complex phenomenon of seed predation, the practical aspect of which is currently underestimated or completely ignored in agri-environmental practice.

Keywords: Ground beetles, soil seed bank, weeds, preferences, granivory

4. Úvod

Z ekologického hlediska se za pravého predátora považuje organismus, který svou kořist (živý organismus) usmrtí víceméně bezprostředně po napadení, a zároveň během svého života usmrtí více jedinců kořisti (Begon et al., 1997). Za pravé predátory tak považujeme i druhy, které napadají a požírají semena rostlin, a tím je vyřadí z populace. Požírání semen se pak označuje termínem „predace semen“, který byl poprvé použit Danielem H. Janzenem (1971). Skupin živočichů, kteří se živí semeny, je celá řada. Patří mezi ně obratlovci (savci a ptáci) i bezobratlí (především hmyz, ale i měkkýši, korýši, mnohonožky, kroužkovci a mnoho dalších). K semenožravosti nedochází jen na souši, ale i ve vodních habitatech (Wigand and Churchill, 1988), kde jsou semena požírána vodními korýši, měkkýši a rybami. Semena jsou požírána nejen s ohledem na jejich velikost, (ne)hybnost (setrvávají na jednom místě po velmi dlouhou dobu), ale především kvůli vysoké nutriční hodnotě na jednotku hmotnosti (Janzen, 1971, Wallinger et al., 2015).

Predaci semen je žádoucí odlišovat od endozoochorie, tj. disperze semen za pomoci živočichů. Endozoochorie je nejvýznamnější u druhů rostlin, které mají dužnaté plody. Právě dužnina plodu je velmi atraktivní pro konzumenty, kteří spolu s dužninou sežerou i semena. Pozřená semena nemusejí být vždy plně strávena, zvlášť pokud byla spolknuta celá, a díky tomu mohou semena vyklíčit i po projití trávicím traktem. U některých druhů semen (např. bezu černého (*Sambucus nigra* L.)) mají semena zvýšenou klíčivost v důsledku projití trávicím traktem. U strávených semen dochází k narušení jejich osemení, které jinak mechanicky brání klíčení (Atkinson and Atkinson, 2002). Fenomén endozoochorie je mnohem složitější a mnohdy se nedá spolehlivě rozhodnout, zda živočich způsobuje disperzi nebo predaci semen. U některých rostlinných druhů se stává, že semena stejného druhu jsou sežrána jedním predátorem semen a částečně projdou jeho tělem nepoškozena, avšak zbylá část semen není schopná vyklíčit, neboť je strávena. Příkladem z polních podmínek je plzák španělský (*Arion vulgaris* Moquin-Tandon, 1855), který běžně požírá semena pampelišky *Taraxacum* agg. U tohoto druhu je popsáno, že až 70 % pozřených semen projde jeho trávicím traktem bez porušení a ztráty klíčivosti, avšak zbylých 30 % zcela ztratí svou klíčivost (Honek et al., 2009).

V případě predace semen rozlišujeme dva hlavní typy - predisperzní a postdisperzní predaci semen. Predisperzní predace probíhá ještě na mateřské rostlině. Příkladem predátorů tohoto typu jsou larvy bejlomorky lipové (*Didymomyia tiliacea* (Bremi, 1847)), které konzumují semena chrpy (*Centaurea cyanus* L.) (Koprdova et al., 2015), nebo nosatcovití brouci (např. *Rhinocyllus conicus* (Frölich, 1792), *Larinus planus* (Fabricius, 1792) a *Larinus turbinatus* Gyllenhal, 1835) konzumující vyvíjející se semena rodu *Cirsium*. Někteří predisperzní predátoři požírají i květní lůžka rostlin, ve kterých se vyskytují. Požíráním květních lůžek snižují fitness semen, která se na květních lůžkách vyvíjejí (Gijsman and Vitt, 2021). Predisperzní predaci semen se v této práci nebudeme nadále věnovat.

Pojem postdisperzní predace semen popisuje predační aktivitu na semenech po jejich dozrání a uvolnění z mateřské rostliny. Právě postdisperzní predace semen je jednou z ekosystémových služeb, která pomáhá regulovat plevele v polních podmínkách, a to jak na povrchu půdy, tak v půdě. Uvádí se, že díky fenoménu postdisperzní predace může dojít ke zničení až 70 % roční produkce semen plevelů v porostech obilnin (Westerman et al., 2003). Přestože existuje mnoho dokladů o tom, jaké skupiny živočichů se na ni podílejí, např. hmyz (mravenci, brouci, cvrčci aj.), kroužkovci, měkkýši, korýši, savci a ptáci (Lambert et al., 2005), není zcela objasněno, jaké faktory ovlivňují preference predátorů semen. Z tohoto důvodu jsme schopni modelovat populační dynamiku plevelů jen velmi omezeně (Daouti et al., 2022).

V našich zeměpisných šířkách jsou v porostech plodin dominantními predátory semen střevlíkovití brouci (Coleptera: Carabidae). Ti se živí širokým spektrem dostupných druhů semen. Bylo zjištěno, že preference semen jsou silně ovlivněny vztahem mezi velikostí semen a velikostí jejich potenciálního predátora (Honek et al., 2007). Existují i další znaky semen, které jsou ve vzájemné interakci a mohou tak podmiňovat jejich predaci střevlíkovitými, např. tvar, hustota, tvrdost a tloušťka osemení nebo obsah chemických látek. Tyto znaky semen a jejich změny jsou testovány v této disertační práci po různé době setrvání semen v půdě nebo v souvislosti s jejich fyziologickým stavem (nabobtnáním nebo poškozením).

V této práci je používán termín semeno i pro plody typu nažka, obilka nebo tvrdka. Toto vychází z anglického slova „seed predation“ a související literatury, která zpravidla používá termín „seed“, tj. semeno, i pro plody bylin typu nažka, obilka a podobně (např. práce Honek et al., 2009, kde jsou použity nažky pampelišky (*Taraxacum officinale* L.) nebo Daouti et al., 2022, kde jsou použity obilky psárky polní (*Alopecurus myosuroides* Huds). Z tohoto důvodu bude v následujícím textu použit termín osemení, i když se u některých zkoumaných druhů jedná o oplodí. V anglických odborných článcích je pro osemení/oplodí používán společný termín „seed coat“.

5. Cíle disertační práce

Hlavním cílem této disertační práce je přispět k poznání, jaké znaky semen mají vliv na preferenci semen střevlíkovitými brouky. V literatuře se uvádí, že mezi hlavní znaky semen, které mohou ovlivňovat preference semen, patří velikost, hmotnost, tvar, fyzikální znaky osemení a chemické látky na povrchu i uvnitř semen. Stav znaků semen se liší mezi jednotlivými druhy na základě jejich fylogenetické příbuznosti a na momentálním stavu semen. Momentálním stavem je myšleno, zda jsou semena nabotnalá, poškozená, nebo naopak suchá. Dále můžou být znaky semen ovlivněny dobou setrvání v půdě.

Vliv různých znaků semen (velikost, hmotnost, tvar, fyzikální znaky osemení a obsah chemických látek) na výběr semen střevlíky nebyl nikdy souborně testován v jedné analýze. V dostupné literatuře byly jednotlivé znaky zkoumány odděleně, avšak mezi jednotlivými znaky nejspíše dochází k interakcím. Proto byly znaky semen změřeny a následně analyzovány společně v jedné analýze. Další výzkumnou otázkou bylo, jestli se preference zástupců dvou tribů (Zabrini a Harpalini) liší, a pokud ano, jaké znaky semen tyto preference ovlivňují (*Foffová et al. 2020, Insects 11:757*).

Jednotlivé stavy znaků semen se mění s momentálním stavem semen, např. nabobtnáním nebo poškozením. Cílem práce *Foffová et al. 2020, Acta Zoologica Academiae Scientiarum Hungaricae 66:37 - 48* je zjistit, jaký vliv na preference semen má momentální stav semen. Práce také zjišťuje, zda na preference semen střevlíkovitými brouky má vliv jejich geografický původ, tj. jestli střevlíci preferují semena ze shodné geografické oblasti před semeny pocházejícími z jiných klimatických podmínek. Posledním dílčím cílem této práce bylo odhalit efekt pohlaví na preference.

Znaky semen se mění v průběhu doby, po kterou setrvávají v půdě. Je možné, že tyto změny ovlivňují i preference predátorů. Práce *Saska et al. 2020, Agronomy 10:448* měla za cíl detekovat změny v morfologii (hmotnost, velikost, tvar a hustota) a životnosti semen, která setrvala v uměle vytvořené půdní zásobě semen po dobu 1 - 8 let. Dalším cílem této práce bylo za pomoci logistického modelu popisujícího změny v přežívání v čase předpovědět 5% a 50% přežití populace semen (PT05 a PT50). Po zjištění stavu znaků semen v půdní bance byly tyto poznatky použity ve výsledku *Saska et al. 2019, EJE 116:133 - 140,* který měl za cíl porovnat preference střevlíkovitých brouků mezi semeny čerstvými a zakopanými po dobu 6 let v půdní zásobě semen. Jako modelové druhy predátorů byly zvoleny *Pseudoophonus rufipes* (De Geer, 1774), *Amara littorea* C. G. Thomson, 1857 a *Harpalus affinis* (Schrank, 1781). Tyto tři druhy byly vybrány, protože se liší v míře potravní specializace. Jednou z hypotéz bylo, že generalisté budou požírat zakopaná i čerstvá semena stejně a některá zakopaná semena dokonce více, neboť zakopaná semena ztratí některé prvky své ochrany před predátory. Předpokládáme, že specialisté budou požírat jen některé druhy semen a jejich specializace se zúží na čerstvá semena, neboť zakopaná semena mohou ztratit atraktivitu. Poslední otázkou této práce bylo, zdali ke změnám preferencí predátorů semen mezi zakopanými a čerstvými semeny dochází z důvodu morfologických změn v semenech.

Na predaci a preference střevlíků mohou mít také vliv mikroorganismy, které se vyskytují na semenech a uvnitř semen. Práce *Saska et al., PLSO-under review* měla za cíl najít změny v zastoupení druhů bakterií v čerstvých semenech oproti semenům, která setrvala v uměle vytvořené půdní zásobě po dobu dvou let. Práce dále ukazuje na změnu znaků zakopaných semen (hmotnost, životaschopnost) v interakci s atraktivitou pro modelového predátora, kterým byl *Pseudoophonus rufipes* (DeGeer).

6. Literární přehled

6.1. Střevlíkovití brouci

Střevlíci jsou brouci (Coleoptera: Carabidae), jejichž velikost těla dosahuje od několika milimetrů až po několik centimetrů. Žijí v přirozených i člověkem obhospodařovaných habitatech, jako jsou např. pole, louky, lesy (Frank et al., 2011, Kromp, 1999). Do této čeledi řadíme cca 30 tisíc druhů. V České republice je dokumentováno více než 500 druhů (Hůrka, 1996). Střevlíci jsou systematicky řazeni do podřádu masožraví (Adephaga), přesto ne všechny druhy požírají pouze živočišnou potravu (Bilde and Toft, 1998). Mezi střevlíky můžeme najít druhy, které jsou omnivorní, karnivorní i čistě herbivorní. Herbivorní druhy střevlíků konzumují semena, plody, pyl nebo listy (Hengeveld, 1980, Lundgren, 2009). Některé druhy střevlíků mají velmi specifickou potravu, např. jen semena jedné čeledi rostlin, ačkoliv většina střevlíků je poměrně polyfágní (Kromp, 1999, Thiele, 1977). U mnoha i zcela běžných druhů není složení jejich potravy ani preference dosud popsáno, což je vzhledem k významu této čeledi v ekosystémech překvapivé.

Z hlediska funkce v zemědělství jsou střevlíkovití brouci zdrojem přirozené regulace škodlivých organismů v polních porostech, kde požírají vajíčka, larvy, kukly i dospělce škůdců (Kromp, 1999). Dále jsou to jedni z mála organismů, kteří jsou schopni regulovat suchozemské plže (Bohan et al., 2000). Především však pomáhají regulovat půdní zásobu semen (anglicky „seed bank“) (Carbonne et al., 2020, Bohan et al., 2011). Na rozdíl od jiných predátorů semen nevykazují tzv. „ecosystem disservises“ (v češtině navrhujeme termínem „ekosystémové škody“ (Foffová et al. – 2021)), tj. situace, kdy některé druhy živočichů při poskytování ekosystémové služby zároveň škodí (např. hraboši v porostech obilnin) (Tschumi et al., 2018). Střevlíkovití, na rozdíl od jiných živočichů, většinou požírají semena z povrchu půdy (Kulkarni et al., 2015) ještě před tím, než se semena dostanou do půdní zásoby.

6.2. Co se děje se semeny v půdní zásobě?

Po dozrání semen na mateřské rostlině a následné disperzi se většina uvolněných semen dostane na povrch půdy. Mnoho semen je schopno vyklíčit okamžitě. Jiná se za pomoci vnějších vektorů (např. orba, eroze) (Mayer et al., 2002) dostávají do tzv. půdní zásoby semen (Baskin and Baskin, 1998), kde čelí nepříznivým abiotickým vlivům a antagonistům. Množství semen alokovaných v půdní zásobě se liší mezi stanovišti a hloubkou půdního profilu. Nejvíce semen se nachází v hloubce do 30 cm od povrchu půdy (Harrison and Law, 2012, Reuss et al., 2001).

Semena mohou setrvávat v půdní zásobě po celou řadu let (Thompson and Grime, 1979, Burnside et al., 1996, Conn et al., 2006). Existují dva hlavní způsoby, jak klasifikovat druhy semen podle doby setrvání v půdní zásobě. První klasifikací je dělení na tzv. sezónní a trvalou dlouhověkost semen v půdní zásobě (např. Thompson et al., 1998). Sezónní dlouhověkost semen v půdní zásobě je zaznamenaná u semen na dané lokalitě jen po krátkou sezonu (např. letní sezonu). Následně semena ztratí svou životnost. Trvalá dlouhověkost semen je zjištěna u semen, která v půdní zásobě přetrvávají po celou řadu let (Thompson, 1987). Druhým možným dělením půdní zásoby semen podle dlouhověkosti je rozdělení do tří kategorií - krátkodobá, střednědobá a dlouhodobá zásoba semen (Mikulka et al., 2005, Grime et al., 1990, Thompson et al., 1997). Krátkodobou životnost mají semena, která klíčí během krátkého období do jednoho roku od uvolnění z mateřské rostliny, např. podběl lékařský (*Tussilago farfara* L.). Střednědobou životnost mají semena, která klíčí do 5 let od vstupu do půdní zásoby, např. pampeliška lékařská (*Taraxacum officinale* L.). Semena s dlouhodobou životností jsou schopna přežít v půdě více než 5 let od vstupu do půdní zásoby, např. konopice rolní (*Galeopsis tetrahit* L.) (Grime, 1979, Thompson et al., 1997, Mikulka et al., 2005). Doba životnosti v půdě se liší i u různých morf semen heterokarpických druhů (např. *Atriplex sagittata* Borkh.) (Mandak and Holmanova, 2004).

Údaje o dlouhověkosti semen jsou mezi jednotlivými pracemi velmi variabilní. Ilustrovat to můžeme na příkladu laskavce ohnutého (*Amaranthus retroflexus* L.). Mikulka et al. (2005) uvádí, že semena laskavce ohnutého vydrží v půdě 3-10 let. Oproti tomu Kohout (1997) uvádí, že semena přežívají od 1 do 10 let, avšak většina semen ztratí životnost v prvních třech letech (Kohout, 1997). Nejdelší dobu přežívání semen laskavce ohnutého (40 let) uvádí Priestley (1986). Tato variabilita může být vysvětlena stanovištěm a specifickými vlastnostmi sledované populace (Thompson et al., 1997, Honek et al., 2011), ale i metodami, které byly pro detekci životnosti semen použity (hloubka zakopání, způsob uložení semen, promíchání se substrátem nebo počet použitých semen) (Borza et al., 2007, Baskin and Baskin, 1998) .

Variabilita dlouhověkosti semen se vyskytuje nejen mezi jednotlivými druhy stejné čeledi, ale i mezi jedinci stejného druhu. Při kategorizaci dlouhověkosti semen se používá striktní rozdělení do několika skupin, které není přesné. Část populace žije déle nežli zbylá část populace. Nelze proto na problematiku životnosti semen v půdě nahlížet skrz diskrétní kategorie, neboť podíl životaschopných semen zřejmě klesá nelineárně s dobou strávenou v půdní zásobě (např. Burnside et al., 1996, přičemž u některých druhů semen zůstává životaschopný malý zlomek původní populace semen ještě po dlouhou dobu. V literatuře se začalo používat exponenciálního modelování přežívání semen (např. Conn et al., 2006, Mahajan and Chauhan, 2021, Lutman et al., 2002), nicméně exponenciální křivka (Conn et al., 2006) není zpravidla schopna zachytit nerovnoměrné tempo poklesu životnosti v populaci v čase. Problematika pravděpodobnosti přežívání semen v půdní zásobě byla studována v práci *Saska et al. 2020, Agronomy, 10:448*. Tato práce popisuje a kvantifikuje změnu v morfologii a životaschopnosti semen 26 druhů bylin. Dále dokládá, že životnost semen neklesá lineárně s dobou setrvání v půdě. U semen zakopaných v uměle vytvořené půdní zásobě následně stanovuje odhad doby přežití pro 50 a 5 % populace semen (PT50 a PT05) na základě modelování za pomoci logistické regrese.

6.3. Životnost, stárnutí semen a působení mikroorganismů na semena

Semena, která nevyklíčí, zůstávají v půdě, podléhají stárnutí nebo svým antagonistům. Postupně dochází k vyčerpání zásobních látek a k nevratným defektům (morfologickým, metabolickým a chemickým změnám; včetně peroxidace lipidů nebo poškození DNA) (Jyoti and Malik, 2013). Během doby setrvání v půdě semena mohou měnit svůj objem, velikost, hmotnost i klíčivost. Tyto změny jsou v literatuře popsány jen částečně, přestože by tyto změny semen mohly napomoci pochopení mezidruhové a mezipopulační variability v životnosti a umožnit předpovědět životnost semen jednotlivých druhů.

O životnosti a dlouhověkosti semen rozhodují faktory, které dělíme na vnitřní a vnější. Mezi vnitřní faktory řadíme genetickou výbavu (Mondoni et al., 2014), který reflektuje fylogenetické zařazení do čeledí a druhů (Walters et al., 2005). Dále mezi vnitřní faktory řadíme fyziologické, chemické a metabolické znaky semen (Shelar, 2008). Mezi vnější faktory řadíme podmínky prostředí v daném roce (teplota, množství vody substrátu atd.) (Kremer, 1993), utužení půdy spojené se změnou koncentrace CO_2 (Kohout, 1997) a přítomnost různých antagonistů semen v půdě. Mezi nejčastější antagonisty semen řadíme predátory semen nebo mikroorganismy.

Predátoři semen jsou schopni najít semena i v půdě (Vander Wall, 1998, Dalling et al., 2011). Nicméně je pro ně obtížnější najít semena, která jsou pod povrchem půdy (Gallandt et al., 2005). Ve většině vědeckých studií se setkáváme s predací čerstvých semen. Čerstvými semeny myslíme ta semena, která dozrála v daném roce (např. Westerman et al., 2003, Honek et al., 2007, Chauhan et al., 2010). V literatuře je jenom malé množství studií zabývajících se predací semen, která se z půdní zásoby semen dostala zpět na povrch půdy (za pomoci orby nebo eroze). Práce Martinkova et al. (2006) se zabývá predací 6 druhů semen z různých čeledí, které setrvaly v půdní zásobě po dobu 6 měsíců. V práci byla zjištěna změna v preferencích střevlíků jen u 2 druhů semen (*Taraxacum officinale* Weber a *Tripleurospermum inodorum* (L.) Sch. Bip.). Je možné, že po 6 měsících v půdě nedochází u zbylých semen k takovým změnám ve znacích semen, které by ovlivnily preference střevlíků. Bohužel neexistují dlouhodobé studie, které by popisovaly tento fenomén u semen, která setrvala v půdní zásobě po řadu let. Pro pochopení změn v preferencích predátorů vznikla práce *Saska et al. 2019, EJE, 116:133 – 140.*, která se zabývá rozdílem v preferencích střevlíků mezi čerstvými a zakopanými semeny (po dobu 6 let), díky čemuž je možné sledovat změnu v preferencích střevlíků na semena, která setrvala v půdní zásobě po řadu let. Tato práce také zaznamenává proběhlé změny znaků semen a jejich vliv na výběr semen střevlíky.

V půdě jsou semena napadána mikrobiálními antagonisty (houbami a bakteriemi). Semena jsou nejčastěji kolonizována houbami z rodu *Aspergillus, Penicillium* nebo *Fusarium* (Kremer and Schulte, 1989). Tyto houby produkují celou škálu mykotoxinů (Jyoti and Malik, 2013). Ty mohou následně zabránit klíčení či způsobit odumření semena. Z bakterií se na semenech nejčastěji vyskytují bakterie z kmene *Proteobacteria* (Jang and Kikuchi 2020) a *Firmicutes* (Truyens et al., 2015). Druhové zastoupení jednotlivých skupin mikroorganismů a jejich početnost je v půdě velmi variabilní. Variabilita je způsobena vlastnostmi půdy, jako je pH nebo chemické složení (Long et al., 2009). Dále mohou druhovou variabilitu mikroorganismů v půdě ovlivnit i rostliny kořenící v blízkosti semen, kde jsou mikroorganismy ovlivněny kořenovými exudáty (Chee-Sanford et al., 2006). Tyto exudáty mají různé chemické složení v závislosti na druhové skladbě daného společenstva (Klironomos, 2002). Svůj vliv na složení mikroorganismů a jejich na kolonizaci semen v půdní zásobě má i člověk a jeho systém hospodaření na dané lokalitě (Bekker et al., 1998, Davis et al., 2005). Většina mikroorganismů žijící na semenech nebo uvnitř semen má vliv na fyziologii i morfologii kolonizovaných semen (Dalling et al., 2011, Long et al., 2015), kdy mohou deformovat osemení nebo snižovat klíčivost. Mikroorganismy mohou být pro semena i prospěšné, neboť mohou narušit osemení a tím semeno může snáze vyklíčit (Delgado-Sanchez et al., 2011). Nicméně pro druhy semen (např. *Abutilon theophrasti* Medik., nebo *Chenopodium album* L.), které se postdisperzní mortalitě brání především tvrdým oplodím nebo osemením, může být narušení osemení fatální (Dalling et al., 2011).

Mikroorganismy žijící na povrchu nebo uvnitř semen mohou spoluvytvářet mikrobiom trávicího traktu predátorů semen, včetně střevlíků (Lundgren and Rosentrater, 2007). Tyto mikroorganismy se podílejí na trávení semen. Pomáhají především při trávení těžko stravitelných látek (např. celulóza nebo lignin), které se v semenech vyskytují (Schmid et al., 2015, Lundgren, 2009). Studium mikrobiomu jednotlivých druhů (nejen predátorů semen, ale i semen samotných) a jeho vztah k potravní ekologii je však stále na počátku. Zdá se, že jsou to právě mikroorganismy, které mohou měnit preference v dietě mezi jedinci stejného druhu (Bredon et al., 2021). Abychom byly schopni lépe pochopit mikrobiom semen a jeho vliv na preference střevlíků vznikla práce *Saska et al., PLSO – under review*. Ta se zabývá změnami v bakteriálním mikrobiomu semen čerstvých oproti semenům, která setrvala v půdě. Práce se dále snaží zjistit, zda lze změny v bakteriálním mikrobiomu semen v souvislosti se setrváním v půdě asociovat se změnami v preferencích střevlíků.

6.4. Predace semen a preference střevlíků

Semenožravost se nejhojněji vyskytuje u druhů z tribů Zabrini a Harpalini (Goldschmidt and Toft, 1997), avšak na predaci semen se podílí i celá řada dalších taxonomických skupin (např. Honek et al., 2003, Saska et al., 2019, Lundgren, 2009). Některé druhy střevlíků požírají semena jen příležitostně (Kamenova et al., 2015). Nedávné výsledky molekulárních testů regurgitátů střevlíků ukazují, že velké množství omnivorních druhů střevlíků (např. *Poecilus cupreus* (Linnaeus, 1758)) konzumuje semena mnohem častěji, než jsme se doposud domnívali (Frei et al., 2019). Každý predátor semen má určité preference při výběru semen (např. Honek et al., 2007, Lundgren, 2009, Saska et al., 2019). Preference jsou mezi jednotlivými druhy střevlíků variabilní. Mezi střevlíky existují druhy, které je možné zařadit podle jejich potravy mezi generalisty (např. *Pterostichus melanarius* (Illiger, 1798)), ale najdeme mezi střevlíky i potravní specialisty (např. rod *Ophonus* nebo některé druhy rodu *Amara*), kteří se zaměřují pouze na semena jedné čeledi. Thiele (1977) shrnul, že rod *Amara* spp. preferuje semena čeledi *Brassicaceae*, zatímco rod *Ophonus* se zaměřuje na semena čeledi *Apiaceae*. Je zřejmé, že preference predátorů jsou úzce spojeny se znaky semen a se schopností predátora tato semena nalézt a sežrat. Někteří predátoři synchronizovali dobu svého rozmnožování nebo dobu svojí nejvyšší abundance s dobou, kdy je výskyt preferovaných semen nejvyšší. Příkladem může být druh *Harpalus pensylvanicus* (DeGeer, 1774), který má synchronizovanou dobu rozmnožování s dobou dozrávání semen trav (Tooley and Brust, 2002), nebo dospělci druhu *Amara montivaga* Sturm, 1825, kteří byli nejvíce odchytáváni do zemních pastí v době po dozrávání semen rodu *Taraxacum* (Honek et al., 2005), na něž se specializují (Saska, 2015).

Střevlíci nejčastěji preferují semena z čeledi *Asteraceae, Brassicaceae, Caryophylaceae*, *Violaceae* nebo *Urticaceae* (např. Honek et al., 2003, Saska et al., 2019). Larvy jsou potravně specializovanější nežli dospělci (Lovei and Sunderland, 1996, Allen, 1979). U mnoha druhů střevlíků není potrava larev plně známa. Je tomu především kvůli obtížnému chovu larev mimo přirozené podmínky. Některé druhy larev střevlíků jsou dokonce natolik vázány na specifickou složku potravy, že pokud ji nedostávají, nejsou schopny dalšího vývoje (Saska, 2005).

Preference predátorů semen byly sledovány v mnoha experimentech, ve kterých bylo testováno mnoho druhů semen s různou kombinací druhů střevlíků. Bohužel u některých druhů (jak semen, tak střevlíků) byly experimenty s danou kombinací semen a predátorů provedeny jen jednou. Mezi nejčastěji používaná patří semena druhů *Viola arvensis* (Murray) (např. Petit et al., 2014, Tooley et al., 1999), *Capsella bursa-pastoris* (L.) Medicus (Petit et al., 2014), *Taraxacum officinale* Weber (např. Saska et al., 2010, Honek et al., 2011), *Chenopodium album* L. (např. Swanton et al., 1999, Navntoft et al., 2009). Velmi dobře jsou preference jednotlivých druhů střevlíků zpracovány v tabulce 9.1 v práci Lundgren (2009).

Množství sežraných semen je však ovlivněno vnějšími podmínkami, např. systémem hospodaření, geografickou lokalitou, časovým obdobím (např. Davis et al., 2013, Sarabi, 2019). Přesto Honek et al. (2007) odhadli, že střevlíci jsou schopni sežrat až 1000 semen na jeden metr čtvereční denně.

6.5. Proces nalézání semen a obrana semen v jednotlivých krocích

Díky své imobilitě si rostliny musely vytvořit velké množství obranných mechanismů pro své přežití a rozšiřování. Semena na rozdíl do zelených rostlin nemají takové možnosti fyziologicky indukované obrany (Bennett and Wallsgrove, 1994, Bari and Jones, 2009). Z tohoto důvodu je nezbytné, aby mateřské rostliny vedle strategie množstevní nadprodukce semen alokovaly část energetických zdrojů preventivně do pasivních obranných mechanismů semen (Baskin and Baskin, 1998). Jejich povaha je jak mechanická (např. tvar, tvrdost semenných obalů a mnoho dalších) (Lundgren and Rosentrater, 2007), tak chemická (především sekundární metabolity) (Tiansawat et al., 2014). Výhodou mechanické obrany proti chemické obraně semen je to, že není tak energeticky náročná jako chemická obrana (Davis et al., 2008). Přes tyto investice je antagonisty semen zničeno až 90 % z každoroční produkce semen (Fenner and Thompson, 2005). Obranné vlastnosti semen ovlivňují jejich dormanci a s ní spjatou persistenci v půdě (Dalling et al., 2011, Shirley, 1998). V literatuře jsou popsány jednotlivé vlastnosti semen bránící semena před jejich antagonisty. Nicméně celkový systém obrany semen není detailně popsán.

Do dnešní doby nevíme, jak přesně dochází k nalézání a rozhodnutí predátora semeno napadnout a sežrat. Na základě dosavadních poznatků byly jednotlivé kroky hypotetické sekvence rozděleny do následujících podkapitol: nalezení semen, hodnocení semen po nalezení, otevírání semen a požírání semen. V jednotlivých krocích jsou popsány možnosti obrany semen.

6.5.1. Nalezení semen

Jak již bylo zmíněno, o způsobu, jakým střevlíci vyhledávají semena a vyhodnocují jejich atraktivitu jako zdroje potravy, máme zatím pouze omezené informace. Můžeme však předpokládat, že tento proces bude podobný jako u ostatních predátorů (např. Zaguri and Hawlena, 2020, Maureaud et al. 2020). Celý proces vyhledávání je pravděpodobně zahájen čichovými a zrakovými podněty (Kulkarni et al., 2017, Law and Gallagher, 2015). Při hledání kořisti predátoři nejspíše využívají semio-chemikálií pocházejících ze semen. Tyto chemické látky mohou sloužit pro jednotlivé predátory buď jako atraktanty, nebo repelenty. Orientace střevlíků za pomoci volatilních látek byla zjištěna při vyhledávání semen (Law and Gallagher, 2015, Kulkarni et al., 2017) i živočišné kořisti (např. mšic, larev a slimáků (Thomas et al., 2008, Kielty et al., 1996)). Doposud však bylo popsáno jen omezené množství látek, které by mohly být atraktivní pro střevlíkovité brouky.

Semena mají dostatečně silné a široké osemení, které nepropustí plyny ze semen, tak aby je predátoři semen nemohli jednoduše nalézt. Síla a tloušťka osemení vycházejí z jejich fylogenetické příbuznosti. Avšak znaky osemení se můžou měnit s momentálním stavem semen. Při změně stavu znaků osemení (tvrdosti a propustnosti pro plyny), ke které dochází při bobtnání semen, se může zvyšovat obsah volatilních látek, které se uvolňují ze semen. Díky vyšší míře uvolňování volatilních látek mohou být střevlíci schopni nalézt semena snadněji, než když jsou semena v suchém stavu (Davis et al., 2008, Law and Gallagher, 2015). Bylo zjištěno, že nabobtnalá semena uvolňují více oxidu uhličitého, alkoholů, aldehydů, alkanů, ketonů (Linton and Wright, 1993) nebo etylénu (Mattoo and Suttle, 1991). Ve vědecké literatuře doposud nebylo potvrzeno, že by poškozená semena mohla vypouštět ještě více semio-chemikálií, ale naznačuje tomu článek (Briese and Macauley, 1981), kde bylo zjištěno, že mravenci snadněji naleznou poškozená než nepoškozená semena. Pro zjištění změn v preferencích střevlíkovitých brouků byla vytvořena práce *Foffová et al. 2020, Acta Zoologica Academiae Scientiarum Hungaricae, 66:37 - 48.* Ta ukazuje, jak se preference střevlíkovitých liší v závislosti na momentálním stavu semen (zda jsou semena poškozená, nabobtnalá nebo suchá).

Semena se před svými predátory mohou „ukrývat“ i za pomoci své velikosti. Malá semena snadněji zapadnou do puklin v půdě a pro jejich predátory je tak mnohem obtížnější je najít (Benvenuti, 2007), přesto je predátoři mohou najít za pomoci volatilních látek, které semena produkují.

6.5.2. Hodnocení semen po nalezení

Před otevřením nalezených semen se predátor rozhoduje, zda jsou atraktivní či ne. Semena můžou na svém povrchu disponovat prvky mechanické obrany anebo látkami, které můžou predátora ještě odradit od požírání semene (např. vosky nebo mastné kyseliny) (Bewley and Black, 1982). Nicméně vliv těchto látek na predátory semen není objasněn. U celé řady semen není ani zjištěno, jaké látky mají semena na svém povrchu, a jak tyto látky mohou ovlivnit preference střevlíků na nalezená semena.

Mechanickou ochranou na povrchu semen myslíme různé výběžky, trichomy nebo drobné trny (Werker, 2000, Baskin and Baskin, 1998), popřípadě dřevnatění částí plodů (Groom and Lamont, 1997) tak, aby znepříjemnily nebo úplně zamezily požírání semen. Některá semena mají na svém povrchu mucinózní buňky, které mohou sloužit jako ochrana proti predátorům, ale i pro jejich disperzi (např. u jitrocele, *Plantago* spp.; (Grime et al., 1990). Při nabobtnání semen dochází k aktivaci těchto mucinózních buněk a dochází k přilepení celého semene k povrchu.

6.5.3. Otevření semene

Pokud predátor překoná mechanickou i chemickou obranu na povrchu semene, dojde k pokusům semena rozlousknout. Rozlousknutí semen je ovlivněno tloušťkou a tvrdostí osemení. Tvrdost semen se velmi liší mezi jednotlivými druhy semen na základě jejich fylogenetické příbuznosti a tvaru. Odolnost proti rozlousknutí však nezávisí jen na tvrdosti osemení, ale i na velikosti semen (Lundgren and Rosentrater, 2007). Malá semena je obtížnější rozlousknout nežli větší, u některých druhů je potřeba vyvinout tlak až 2000x větší než u větších semen (Fricke and Wright, 2016). Pokud jsou semena nabobtnalá, síla nutná k jejich rozlousknutí je menší nežli u suchých semen (osobní pozorování).

S tvrdostí a tloušťkou osemení také souvisí dlouhověkost semen. Čím je osemení slabší a tenčí je jeho tloušťka, tím je vyšší šance napadení semen antagonisty (Davis et al., 2008). Bohužel u většiny druhů není změřena síla potřebná k jejich rozlousknutí, přitom poznatky o síle potřebné k rozlousknutí semene by mohly odhalit, jak jsou semena náchylná k predaci. Dalším fyzikálním znakem semen, který může zabránit predaci, je jejich tvar (Thompson et al., 1993). Diskovitá semena (např. rod *Amaranthus*) jsou poměrně tvrdá a některým predátorům semen dělá problém je rozdrtit. Oproti nim plochá semena jsou jednodušší kořistí (Moles et al., 2003, Thompson et al., 1997).

6.5.4. Požírání semen

Pokud predátor rozlouskne semeno, vyhodnocuje nutriční složení semene (Crist and Macmahon, 1992, Pizo and Oliveira, 2001), množství škrobů, bílkovin, olejů (Gaba et al., 2019), sekundárních metabolitů nebo mastných kyselin (Paulsen et al., 2013, Rhoades, 1979, Hulme, 1996, Janzen, 1971). Z makroprvků se na obraně semen před antagonisty podílí vyšší koncentrace dusíku. Semena mající vyšší obsah dusíku mají větší šanci na přežití (Grubb et al., 1998), neboť jsou to semena nestravitelná a pro některé druhy dokonce toxická. Ze sloučenin mikroprvků se jedná například o silicidy akumulované v embryu (Panza et al., 2004).

Kromě jednotlivých prvků se na obraně semen před predátory podílejí sekundární metabolity. Ty rostlinám mimo jiné slouží i jako ochrana před herbivory (např. Carmona et al., 2011). Sekundární metabolity se mohou dělit z hlediska kvality na toxiny (vyskytující se v malých dávkách, mající přímou toxicitu) a na špatně stravitelné látky (vyskytující se ve vysokých koncentracích, mající vliv na vývoj nebo trávení herbivorů) (Rhoades, 1979, Janzen, 1971). Ačkoliv sekundární metabolity hrají ve vztazích mezi organismy významnou roli, o koncentraci těchto látek, nebo alespoň o zastoupení jednotlivých látek v semenech, je doposud zdokumentováno jen velmi málo informací. Tyto chemické látky obsažené v semenech mohou predátora v této fázi odradit od další konzumace, nebo jej dokonce otrávit (Pond, 2013). Zřejmě však ne všechny chemické látky semen budou odpuzovat všechny druhy predátorů. Odpověď predátorů na chemické látky v semenech bude nejspíše druhově specifická.

Pro lepší pochopení významu jednotlivých znaků semen pro preference střevlíkovitých brouků byl využit existující dataset preferencí střevlíkovitých brouků (Honek et al., 2007, Saska et al., 2019), který byl doplněn o morfologické (velikostní parametry semen a síla potřebná k rozlousknutí semen) a fytochemické znaky semen (obsah masných kyselin, obsah látek na povrchu semen a zastoupení volatilních látek). Komplexní analýzou tohoto unikátního datasetu (*Foffová et al. 2020, Insects 11:757*) bylo zjištěno, jaký vliv mají tyto znaky semen na preference střevlíkovitých brouků. Díky těmto informacím bychom mohli lépe pochopit, v čem spočívá rozdílná atraktivita semen pro predátory.

7. Výsledky disertační práce

Konkrétní výsledky této doktorské disertační práce najdete v následujících článcích:

1. Článek

   Which Seed Properties Determine the Preferences of Carabid Beetle Seed Predators?

   Autoři: Foffová, Hana; Ćavar Zeljković, Sanja; Honěk, Alois; Martinková, Zdenka; Tarkowski, Petr; Saska, Pavel

   Vydáno: INSECTS, 2020, DOI: 0.3390/insects11110757

2. Článek

   Do properties and species of weed seeds affect their consumption by carabid beetles?

   Autoři: Foffová, Hana; Bohan, A. David; Saska, Pavel

   Vydáno: ACTA ZOOLOGICA ACADEMIAE SCIENTIARUM HUNGARICAE, 2020, DOI: 10.17109/AZH.66.Suppl.37.2020

3. Článek

   Persistence and Changes in Morphological Traits of Herbaceous Seeds Due to Burial in Soil

   Autoři: Saska, Pavel; Foffova, Hana; Martinkova, Zdenka; Honek, Alois

   Vydáno: AGRONOMY, 2020, DOI: 10.3390/agronomy10030448

4. Článek

   Burial-induced changes in the seed preferences of carabid beetles (Coleoptera: Carabidae)

   Autoři: Saska, Pavel; Honek, Alois; Foffova, Hana; Martinkova, Zdenka

   Vydáno: EUROPEAN JOURNAL OF ENTOMOLOGY, 2019, DOI: 10.14411/eje.2019.015

5. Článek

Seed associated bacteria are selected from soil by seed properties and change preferences of a seed-feeding beetle

Autoři: Saska, Pavel; Kopecky, Jan; Omelka, Marek; Honek, Alois; Madrova, Pavla; Vokurková, Petra; Martinkova, Zdenka; Foffová, Hana; Sagova-Mareckova, Marketa

Under review: Plant and Soil

Which Seed Properties Determine the Preferences of Carabid Beetle Seed Predators?

Autoři: Foffová, Hana; Ćavar Zeljković, Sanja; Honěk, Alois; Martinková, Zdenka; Tarkowski, Petr; Saska, Pavel

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Which Seed Properties Determine the Preferences of Carabid Beetle Seed Predators?

Hana Foffová [1,2,*], Sanja Ćavar Zeljković [3,4], Alois Honěk [1], Zdenka Martinková [1], Petr Tarkowski [3,4] and Pavel Saska [1]

[1] Crop Research Institute, Functional Diversity in Agro-Ecosystems, Drnovská 507, Ruzyně, 161 06 Praha 6, Czech Republic; honek@vurv.cz (A.H.); martinkova@vurv.cz (Z.M.); saska@vurv.cz (P.S.)

[2] Department of Ecology, Faculty of Environmental Sciences, Czech University of Life Sciences, Kamýcká 129, Suchdol, 165 00 Praha, Czech Republic

[3] Centre of the Region Haná for Biotechnological and Agricultural Research, Department of Genetic Resources for Vegetables, Medicinal and Special Plants, Crop Research Institute, Šlechtitelů 29, 783 71 Olomouc, Czech Republic; sanjacavarsc@gmail.com (S.Ć.Z.); Tarkowski@vurv.cz (P.T.)

[4] Centre of Region Haná for Biotechnological and Agricultural Research, Department of Phytochemistry, Palacky University, Šlechtitelů 27, 783 71 Olomouc, Czech Republic

* Correspondence: foffova@vurv.cz

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Simple Summary: The carabid beetles are well known for the consumption of weed seeds in arable land, but how they choose the seeds is poorly known. In this work, we try to explain the patterns in preferences of 37 species of carabids based on eight seed properties of 28 species of seeds. Surprisingly, chemical properties of the seeds did not affect the preferences. Instead, preferences were driven mainly by seed structural properties. The importance of particular seed properties was also affected by the degree of predator specialization.

Abstract: Ground beetles are important invertebrate seed predators in temperate agro-ecosystems. However, there is a lack of information regarding which seed properties are important to carabids when they select seeds for consumption. Therefore, seed properties, such as size, shape, morphological defence, and chemical composition, were measured, and in addition to seed taxonomy and ecology, these data were used to explain carabid preferences. Carabid preferences were assessed using a multi-choice experiment with 28 species of weed seeds presented to 37 species of Carabidae. Multiple regression on distance matrices (MRM) was used to determine the importance of particular sets of seed properties for carabids. The analysis was conducted for the full set of carabids (37 species) as well as for subsets of species belonging to the tribes of Harpalini or Zabrini. For the complete set of species, seed dimensions, seed mass, taxonomy, plant strategy, and seed coat properties significantly explained carabid preferences (proportion of explained variance, $R^2 = 0.465$). The model for Harpalini fit the data comparably well ($R^2 = 0.477$), and seed dimensions, seed mass and seed coat properties were significant. In comparison to that for Harpalini, the model for Zabrini had much lower explanatory power ($R^2 = 0.248$), and the properties that significantly affected the preferences were seed dimensions, seed mass, taxonomy, plant strategy, and seed coat properties. This result suggests that the seed traits that carabids respond to may be specific to taxonomic and likely relate to the degree of specialisation for seeds. This study contributes to understanding the mechanisms that determine the preferences of carabid beetles for seeds.

Keywords: preference; ground beetles; weed seeds; seed properties

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1. Introduction

Ground beetles (Coleoptera: Carabidae) are among the most important groups of weed seed predators in temperate agro-ecosystems where they help to reduce weed seeds. These granivorous species of arable land mainly belong to the tribes Harpalini (e.g., genera *Harpalus*, *Ophonus*, *Acupalpus*, *Stenolophus*, or *Anisodactylus*) and Zabrini (genera *Amara* and *Zabrus*) [1,2], but species from other groups consume seeds as well. Recent findings suggest that granivory is more widespread within this family than previously thought [3,4]. Species that are specialised seed feeders often show distinct seed preferences [5]. Species of Zabrini prefer seeds of Asteraceae, Brassicaceae, or Caryophyllaceae, and they seem to be more selective than species of the tribe Harpalini, which prefer seeds of Violaceae or Asteraceae [6,7]. Many species of these families are considered to be problematic weeds. However, the knowledge on what drives the preferences is not fully understood. Predator identity, taxonomy, body size, size of mandibles [8,9], seed size, and other seed properties may affect carabid preferences for seeds. Understanding the driving factors of the preferences would potentially improve our ability to predict which seeds are the most vulnerable to which predators.

Seeds are usually unevenly scattered on the ground or aggregated in patches near the mother plant; therefore, insect seed predators have to locate the seeds or patches of seeds. However, the seeds try to resist predators. The defence of seeds against predators is divided into two main groups, morphological and chemical traits, which inevitably interact with each other and influence seed dormancy and persistence in soil [10,11], and in this way influence predation in the long term [12].

Although the information on the cues seed predators use is scarce and we hypothesized that the process that ultimately leads to seed predation is similar to the one described for other types of predators [13–15]. The typical process of prey location by an insect predator usually includes several steps, each having typical sets of cues involved. Visual or olfactory cues may be important when searching for seeds [16–19]. Utilising (semio-)chemicals is a common means of communication within food webs [20–22]. How important it is for seed predation is poorly understood. Few studies have shown that ground beetles detect volatiles from other animals, such as aphids, springtails, or slugs [23,24], as well as from plants [25,26] and probably seeds [16,17,24]. The chemical properties of seeds may change the behaviour of seed predators (serve as attractants or as repellents). The detection rate may be affected by the properties of seed coats because some are impermeable to gases, chemical compounds, or water [27]. This rate can also be affected by the level of imbibition [16] because the imbibed seeds release different amounts of volatile compounds, including carbon dioxide, alcohols, aldehydes, alkane, ketones, volatile acids [28], or ethylene [29], which can potentially attract or repel beetles.

Seeds vary in their morphological properties, such as mass, size and shape; as well as defensive structural traits. These properties affect seed interception and handling by a predator. Seed mass [30–32] and size [6,33–35] are major drivers in seed selection by ground beetles and there is a relationships between carabid body size and seed size or mass [6,7]. Larger seeds might be more apparent to predators [36], and they also stay on the soil surface for longer than smaller ones [37,38]. Seed shapes can also affect predation but has never been studied. The smaller, round seeds are able to escape seed predation more than flattened ones [31,39,40]. Round seeds fit better in cracks in the soil where they can escape predation [41]. In comparison to flat seeds, round ones can also be harder to handle because they pop out of mandibles (e.g., seeds of Amaranthaceae) [6].

Once seeds are found, a predator is expected to evaluate seed attractiveness. The chemical profile of the seed surface is often important in this process [17,22]. Waxes or fatty acids present on the seed surface [42] may drive a predator's decision to feed on it or not [43,44]. Other surface compounds could also affect seed predation. Other surface compounds contain mostly long-chain alkanes or their branched counterparts, which are common constituents of plant waxes [45]. These compounds protect seeds from physical, temperature-related, or water damage to ensure that the plant seed remains in a state of dormancy [46]. Once a predator attempts in feeding, crushing and opening a seed is further affected by physical traits, such as thickness [5] or the strength of the seed coat [5,47]. These are seemingly related, but this is not necessarily the case (for example, seed coats can be relatively thin but hard, e.g., *Silene latifolia alba* (Mill.) Greut. et Burdet, or thick but soft, e.g., *Fumaria officinalis* L.). This type of physical defence is potentially more effective, and in comparison to other types of defences, less costly for the plant [48]. A higher investment in a seed coat may increase post-dispersal survival [10,11,48]. That seed coat thickness can be an adaptive defensive trait is supported by the finding of Benkman [49], who documented stronger seed coats in environments with predators rather than without predators. There is also a positive relationship between seed mass and seed coat thickness [50] as well as the interaction among seed size, mass, and strength of seed coat [5]. There may also be an interaction between seed coat hardness and shape, which may explain seed preference [9].

After successfully opening, a predator further evaluates the nutritional composition of a seed (amount of starches, proteins, oils, secondary metabolites, fatty acids, etc.) [22,42,51–55], which stimulates or deters the predator from additionally feeding on conspecific seeds. Some of the chemical compounds can be distasteful or poisonous for their predator (e.g., opium and L-dopa) [56], but insects have evolved systems to detoxify these compounds. In fact, we know only very little about this hypothetical sequence of events leading to the destruction of a seed by the mandibles of insect seed predators.

In addition to seed chemical and morphological properties, plant taxonomy [6] and the life cycle strategy of plants [57] are important determinants of predator preferences. The sister taxa of plants may be more attractive for seed predators than taxa unrelated ones [6], likely because related seeds have similar properties.

The aim of this study is to explore which weed seed properties are decisive for preferences of carabid beetles. We focus on properties related to seed size, shape, mass, and morphological defence; seed chemical properties (volatile compounds, fatty acids, and other surface compounds); and seed ecology and taxonomy.

2. Materials and Methods

2.1. Seed Material

A set of 28 species of weed seeds was used (Table 1). Each year, the seeds were hand-collected de novo from the parental plant at full maturity using laboratory gloves. The seeds were cleaned from dust and admixtures of non-seed plant particles by blowing, dried at room temperature (25 °C for 30 days) and then stored in the freezer (−21 °C) until the experiments.

2.2. Preference Experiments

The preferences of 37 species of carabids were evaluated (Table 1). The preferences were determined based on a cafeteria experiment described in Honěk et al. [6] and Saska et al. [7]. The seeds of 28 species of weeds (Table 1) were presented on tin trays filled with white modelling clay (JOVI, Barcelona, Spain). The seed trays were arranged in two concentric rings in Petri dishes (20 cm in diameter) with 10 beetles for five days. The seed consumption was counted daily, after which it was summed and standardised to remove the effect of carabid body size on the total consumption and be able to compare data across the species [6]. Standardisation was performed by converting the actual consumption of seed to the proportion of the most consumed seed.

2.3. Measurement of Seed Morphological Traits

Seed mass was measured by weighing 100 seeds on a balance to a precision of 10^{-5} g (Sartorius, Göttingen, Germany). Seed dimensions were measured following Bekker et al. [38], using a digital calliper and five seeds per species: A—the longest dimension, B—the longest dimension perpendicular to A within the same plane, and C—the longest dimension perpendicular to the plane of A and B.

Table 1. List of the model organisms that were used in the preference experiment. The plant taxonomy was based on Kubát et al. [58] while that of carabids on Hůrka [2].

Plants		**Carabids**	
Species	**Family**	**Species**	**Tribe**
Amaranthus retroflexus L.	Amaranthaceae	*Acupalpus meridianus* (Linnaeus)	Harpalini
Arctium lappa L.	Asteraceae	*Amara aenea* (DeGeer)	Zabrini
Arenaria serpyllifolia agg.	Caryophyllaceae	*Amara anthobia* (A. Villa et G.B. Villa)	Zabrini
Bidens tripartita L.	Asteraceae	*Amara apricaria* (Paykull)	Zabrini
Campanula trachelium L.	Campanulaceae	*Amara aulica* (Panzer)	Zabrini
Capsella bursa-pastoris (L.) Med.	Brassicaceae	*Amara bifrons* (Gyllenhal)	Zabrini
Chenopodium album L.	Amaranthaceae	*Amara consularis* (Duftschmid)	Zabrini
Cichorium intybus L.	Asteraceae	*Amara convexior* (Stephens)	Zabrini
Cirsium arvense (L.) Scop.	Asteraceae	*Amara convexiuscula* (Marsham)	Zabrini
Consolida regalis S.F. Gray	Ranunculaceae	*Amara eurynota* (Panzer)	Zabrini
Crepis biennis L.	Asteraceae	*Amara familiaris* (Duftschmid)	Zabrini
Descurainia sophia (L.) Prantl	Brassicaceae	*Amara ingenua* (Duftschmid)	Zabrini
Fumaria officinalis L.	Papaveraceae	*Amara litorea* (C.G.Thomson)	Zabrini
Galinsoga parviflora Cav.	Asteraceae	*Amara montivaga* (Sturm)	Zabrini
Galium aparine L.	Rubiaceae	*Amara ovata* (Fabricius)	Zabrini
Lapsana communis L.	Asteraceae	*Amara sabulosa* (Audient-Serville)	Zabrini
Leonurus cardiaca L.	Lamiaceae	*Amara similata* (Gyllenhal)	Zabrini
Lepidium ruderale L.	Brassicaceae	*Amara spreta* (Dejean)	Zabrini
Melilotus albus Med.	Fabaceae	*Anisodactylus signatus* (Panzer)	Harpalini
Potentilla argentea L.	Rosaceae	*Calathus ambiguus* (Paykull)	Sphodrini
Silene latifolia alba (Mill.) Greut. et Burdet	Caryophyllaceae	*Calathus fuscipes* (Goeze)	Sphodrini
Sisymbrium loeselii L.	Brassicaceae	*Harpalus affinis* (Schrank)	Harpalini
Stellaria media (L.) Vill.	Caryophyllaceae	*Harpalus atratus* (Latreille)	Harpalini
Taraxacum officinale agg.	Asteraceae	*Harpalus distinguendus* (Duftschmid)	Harpalini
Thlaspi arvense L.	Brassicaceae	*Harpalus honestus* (Duftschmid)	Harpalini
Tripleurospermum inodorum (L.) Schultz-Bip.	Asteraceae	*Harpalus luteicornis* (Duftschmid)	Harpalini
Urtica dioica L.	Urticaceae	*Harpalus rubripes* (Duftschmid)	Harpalini
Viola arvensis Murray	Violaceae	*Harpalus signaticornis* (Duftschmid)	Harpalini
		Harpalus subcylindricus (Dejean)	Harpalini
		Ophonus azureus (Fabricius)	Harpalini
		Parophonus maculicornis (Duftschmid)	Harpalini
		Pseudoophonus griseus (Panzer)	Harpalini
		Pseudoophonus rufipes (DeGeer)	Harpalini
		Pterostichus melanarius (Illiger)	Pterostichini
		Stenolophus teutonus (Schrank)	Harpalini
		Trechus quadristriatus (Schrank)	Trechini
		Zabrus tenebrioides (Goeze)	Zabrini

These dimensions were used to calculate indices that describe seed shape, flatness, eccentricity, and volume. The shape of the seed was calculated as in Bekker et al. [38], $Vs = \sum \frac{(x - \bar{x})^2}{n}$, where x represents a division of either A, B, or C through A and $\bar{x}$ as their mean, and n is 3. vs. ranges from 0 for perfectly spherical seeds to 0.2 for seeds shaped like a thin disc or spindles. The flatness of the seeds [59] was calculated as $FI = \frac{(A+B)}{2*C}$. FI ranges from 1 for a complete sphere to higher values for plane-like or spindle-like seeds. The eccentricity of the seeds [59] was calculated as $EI = \frac{A}{B}$. EI ranges from 1 for round seeds to values > 2 for spindle-like seeds. The volume of the seeds was calculated as $V = A * B * C$ [59].

Seed coat thickness was measured using a light microscope on sections of seeds. Dry seeds were infiltrated with a 2% sucrose solution for six hours at room temperature, mounted onto cryo-gel on the alum chuck, and sectioned using a cryotome (Shandon SME, Astmoor, UK). Sections were observed using an Olympus BX51 microscope (Olympus Corp., Tokyo, Japan) and documented with an Apogee U4000 digital camera (Apogee Imaging Systems, Inc., Roseville, CA, USA). Five seeds of each species were measured 10 times. The strength of the seed coat was measured on an MTS 02 (Aviko Praha, Praha, Czech Republic), which measures the force developed by the instrument to crack the seed coat [N]. For each species, 10 seeds were measured.

2.4. Chemical Analysis of Seeds

Seeds were subjected to detailed chemical analysis, which differed in the targeted groups of compounds and methods used to detect them. The targeted groups of compounds were considered to be perceived by carabids either from a distance or during handling seeds and included surface waxes, amino acids, and volatile compounds.

Fatty acids from the ground seeds (total fatty acids) as well as from seed surfaces were isolated and derivatized into corresponding volatile methyl esters and then quantified via gas chromatography–mass spectrometry (GC–MS) [60–62]. The isolation protocol was optimised for a small-scale experiment using ~50 mg of seeds for surface fatty acids and ~25 mg of seeds for total fatty acids. After isolation with a chloroform:methanol (2:1) mixture, the fatty acids were trans-esterified with a sodium methoxide solution into corresponding methyl esters and then extracted into *n*-hexane. The solvent was then removed under reduced pressure. Dry samples were dissolved in *n*-hexane containing 0.1% *n*-undecane as an internal standard for normalization of chromatographic conditions. All samples were analysed in triplicates. Identification and quantification of fatty acid methyl esters (FAMEs) in seed samples was accomplished via an internal standard calibration curve for 35 FAMEs (Supelco, Darmstadt, Germany). The single ion monitoring (SIM) mode was used for identification and quantification of each particular analyte.

Other surface compounds (waxes, alkanes, phytosterols, etc.) were isolated by dipping 50 mg of intact seeds into chloroform for 30 s [45]. After filtration, chloroform was evaporated under reduced pressure, and isolated compounds were dissolved into *n*-hexane containing 0.1% *n*-undecane as the internal standard for normalization of chromatographic conditions. Each seed sample was analysed in triplicate, and the results are presented as the percentage content of chloroform soluble surface compounds.

The volatile compounds from plant seeds were isolated and detected by the static headspace technique. For the analysis, sets of dry and imbibed seeds were used. Imbibed seeds produce other chemical compounds because of the start of the chemical processes during germination. The dry seeds were stored in the freezer. The imbibed seeds (0.5 g of each species) were incubated for 24 h at 25 °C before measurement. Volatile compounds released by seeds were pre-concentrated during incubation into headspace vials, and, therefore, we were able to detect them via a common GC–MS platform.

2.5. Ecology and Taxonomy of Plants

Information on plant ecology (annual, biennial, annual-biennial and perennial) and taxonomic placement were determined from the literature [58,63].

2.6. Data Analyses

The multiple regression on distance matrices (MRM) approach (ecodist package [64] for R version 3.4.1 [65]) was used for data analysis. MRM was preferred over other methods because it allows the regression of a response matrix on multiple explanatory matrices [66]. Raw matrices were created according to the nature of the data and possible correlation between the variables, presumed mechanisms behind the expected influence on the preferences, and methods used to generate them. Most of the available data were formed as two-dimensional matrices with seed species as rows and measured quantities as columns. We considered the following matrices for the initial exploration: carabid preferences (response matrix), seed mass (mass of 100 seeds in grams), seed dimensions (dimensions of the seed on axes A, B, and C), seed shape (indices of seed shape, flatness, eccentricity and volume), seed coat (seed coat thickness and strength), plant taxonomy (family of plants), plant strategy, volatile compounds from dry seeds, volatile compounds from imbibed seeds, fatty acids from seed surface, total fatty acids, and other surface compounds. Before the MRM approach can be applied, raw data matrices must be converted into distance matrices using the vegan package [67]. Bray–Curtis dissimilarities were used to convert the seed preference matrix because consumption was standardised on a scale of 1 to 0. The Mahalanobis distance was used for matrices of seed dimensions, seed coat, surface fatty acids, total fatty acids, volatile compounds from dry seed, volatile compounds from imbibed seeds and other surface compounds because these factors contain continuous numerical variables. The data matrices of seed mass, taxonomy and plant ecology were transformed to distance matrices by using the specified distance measurement. Prior to the analysis, the correlation between the dissimilarity matrices was explored by using Mantel's permutation test for similarity of two matrices (999 permutations). The following matrices were excluded since they showed correlation with other matrices: seed shape (with seed dimensions; $p > 0.001$), volatile compounds from dry seeds (with volatile compounds from imbibed seeds; $p > 0.009$), and fatty acids from seed surface (with other surface compounds and taxonomy; $p > 0.011$). The distance matrix for carabid preferences was used as a response, and the following distance matrices were used as explanatory terms: seed dimensions, seed mass, seed coat, taxonomy, plant strategy, total fatty acids, other surface compounds and volatile compounds from imbibed seeds. Three different models were fitted that differed according to the carabid preference distance matrix: (i) one model was based on the full set of 37 carabid species, (ii) another model was calculated only for the species of Harpalini (15 species), and (iii) the final model was calculated only for the species of Zabrini (18 species). The variances with associated *p*-values from the multiple regressions were obtained using Legendre et al. (1994)'s permutation test with 9999 permutations [68]. The level of significance to reject the null hypothesis was set to $\alpha < 0.1$.

3. Results

3.1. Preferences of Carabids

Seed consumption varied among the species of carabids [7]. The highest preferences by Harpalini were on seeds of *Cirsium arvense*, *Viola arvensis*, and *Cichorium intybus*, while tribe Zabrini preferred seeds of *Taraxacum officinale*, *Tripleurospermum inodorum*, or *Crepis biennis.* The small seeds of Brassicaceae were preferred by small carabids of both tribes. The standardized consumption of all species is in Table S1.

3.2. Morphological Analysis of Seeds

The seed mass of 100 seeds ranged from 0.08 g (*Potentilla argentea* L.) to 8.72 g (*Arctium lappa* L.) (Table S2). The seed dimensions were diverse and ranged from 9.076 ± 1.264 mm (dimension A of *Bidens tripartita* L.) to 0.272 ± 0.372 mm (dimension C of *Arenaria serpyllifolia* agg.). The shape index ranged from 0.171 ± 0.002 (*B. tripartita*) to 0.005 ± 0.003 (*Fumaria officinalis* L.). The flatness index ranged from 13.496 ± 0.73 (*B. tripartita* L.) to 1.144 ± 0.052 (*F. officinalis* L.) (Table S2). Eccentricity ranged from 6.319 ± 0.322 (*Crepis biennis* L.) to 1.03 ± 0.012 (*Stellaria media* (L.) Vill.). The volume ranged from 16.932 ± 0.926 mm^3 (*A. lappa*) to 0.058 ± 0.006 mm^3 (*A. serpyllifolia*) (Table S2). The strength of the seed coat varied among the species and families as well. The seeds of *Galium aparine* L required the greatest amount of power (99.47 ± 16.818 N) to crush the seed coat, and *Urtica dioica* L. required the least amount of power to crush the seed coat (1.14 ± 0.533 N). The seed coat thickness ranged from 0.138 ± 0.043 mm (*A. serpyllifolia*) to 0.017 ± 0.006 mm (*G. aparine*) (Table S2).

3.3. Chemical Analyses of Seeds

The majority of the 35 fatty acids from the FAME standard mixture was detected in the analysed seeds (Tables S3 and S4). The greatest concentration of all fatty acids was found in the seeds of *Galinsoga parviflora* Cav. (467.75 ± 8.40 mg/g dry weight), while the lowest concentration was extracted from *G. aparine* (33.39 ± 1.26 mg/g DW). The major fatty acid in all seeds analysed was unsaturated linoleic acid, which accounted for ~50% of the total fatty acids quantified. The composition of the surface fatty acids varied between the species more than the composition of total fatty acids (Tables S3 and S4). The highest content of the sum of all surface fatty acids was found in the seeds of *Cirsium arvense* (114.12 ± 3.21 mg/g DW). Other species had a lower content of surface fatty acids. The lowest amount of all surface fatty acids (6.07 ± 0.32 mg/g DW) was found in *Amaranthus retroflexus* L. (Table S4). Some of the fatty acids were found just in one species (e.g., cis-5, 8, 11, 14, 17-Eicosapentanoic acid in *Lapsana communis* L.).

Thirteen volatile compounds were detected in seeds (Tables S5 and S6). The amounts of volatile compounds varied between dry and imbibed seeds. The highest amount of volatile compounds was found in the seeds of *Sisymbrium loeselii* L. (4.8% of determined volatiles); while no volatile compounds were detected in *A. retroflexus* (Table S5). The highest amounts of volatile compounds were found in seeds of *S. loeselii* (4.83% of determined volatiles), and the lowest amount was found in seeds of *A. retroflexus*, where 0.03% of volatiles were detected (Table S6).

Nineteen other surface compounds (Table S7) were detected in the seeds including long-chain alkanes or their branched counterparts, with significant amounts of phytosterols, such as β-sitosterol, were detected. The composition of the other surface compounds also varied between the species.

3.4. Relationships among Carabid Preferences and Seed Properties

The full model on standardized consumption included matrices on seed mass, seed dimensions, seed coat, seed taxonomy, plant strategy, other surface compounds, total fatty acids, and volatiles released from imbibed seeds. The model explained the variation in consumption across the range of carabid species ($R^2 = 0.465$, $p = 0.001$; Figure 1a), with the following matrices contributing significantly (at the level of $\alpha = 0.1$) to the explained variance: seed dimensions ($p < 0.001$), seed coat ($p < 0.001$), taxonomy ($p = 0.035$), seed mass ($p = 0.054$), and plant strategy ($p = 0.058$).

However, by re-running the analysis separately for the two major taxonomic groups of carabids, Zabrini and Harpalini, we found specific responses. The model for Harpalini fit the data comparably well to the global one ($R^2 = 0.477$, $p = 0.001$; Figure 1b), with the following matrices contributing significantly: seed dimensions ($p < 0.001$), seed coat ($p < 0.001$) and seed mass ($p = 0.062$). In no model did seed phytochemistry significantly influence the seed preferences of the carabid beetles included in this study. The model for Zabrini had much lower explanatory power ($R^2 = 0.248$, $p = 0.001$; Figure 1c), with the following matrices contributing significantly: seed coat ($p = 0.002$), seed dimensions ($p = 0.005$), taxonomy ($p = 0.005$), plant strategy ($p = 0.036$), and seed mass ($p = 0.075$).

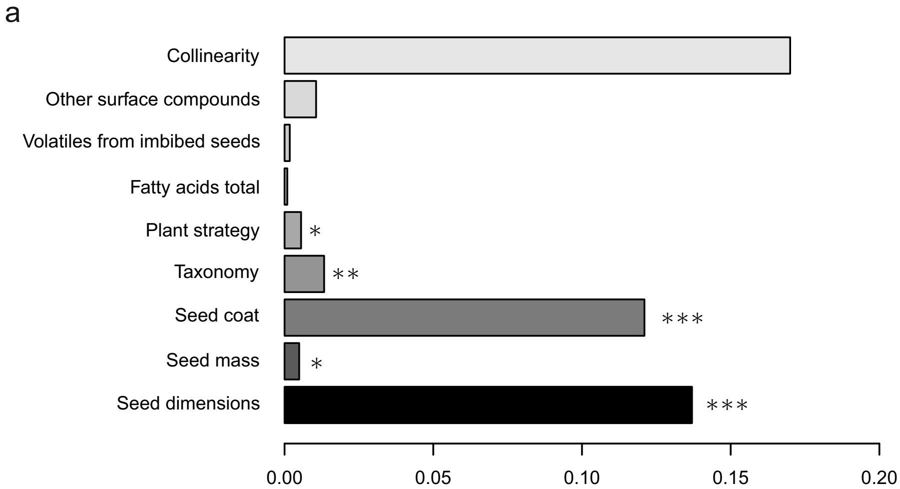

Figure 1. *Cont.*

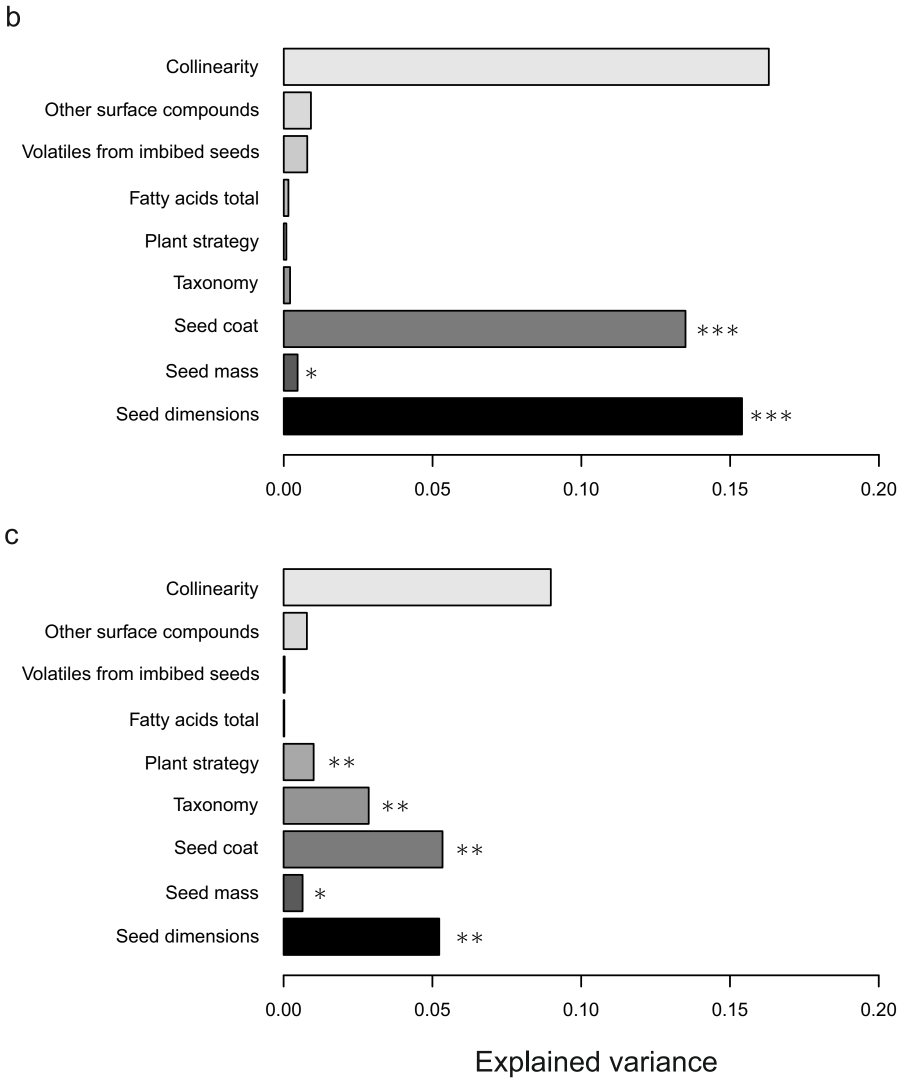

Figure 1. Contribution of the matrices of seed traits to seed preferences of carabid beetles based on a multiple regression on distance matrices (MRM) approach. The horizontal bars indicate the proportion of explained variance by a particular variable in the data. The collinearity shows part of the variation explained by the model, but which cannot be attributed solely to any of the single factors. (**a**) Full set of the 37 species of carabids (proportion of explained variance, $R^2 = 0.465$, $p = 0.01$). (**b**) Species of the tribe Harpalini ($R^2 = 0.477$, $p = 0.001$). (**c**) Species of the tribe Zabrini ($R^2 = 0.248$, $p = 0.001$); * $p < 0.1$, ** $p < 0.05$, *** $p < 0.001$.

4. Discussion

Seed properties, such as seed dimensions, mass, taxonomy, plant strategy, and physical seed coat traits were the most important properties affecting the preferences of carabid beetles in this study. The seed dimensions explaining over 13% of the preferences was the main factor affecting seed selection [6,33,34], even when other properties are considered. The interaction between seed size and the mass of carabids should not, however, be overlooked [6,7,53]. The size of the seeds also affects their chemical properties, such as the oil content or stored energy [55], which may affect seed predation.

The properties of the seed coat were also important because the seed coat protects seeds against predators. To open seeds, many species of carabids have evolved broad mandibles with large adductors [69] and bases that are generally triangular. The shape of mandibles varies among the tribes. Species of Harpalini have more asymmetrical mandibles than those of Zabrini. Quadrate mandibles with broadly rounded incisors and a basal face suggest an omnivorous diet in most Harpalini [70].

Species of the tribe Zabrini have short, square-shaped mandibles that are blunt at the tips, and are more adapted for crushing hard seeds [8]. This can explain why the seed coat properties were important properties for seed preference by Harpalini (13% of explained variance) but less so for Zabrini (5% of explained variance).

Seed preferences by species of the studied Zabrini species were related to seed taxonomy, which probably drove the significant response for the entire species set because for the tribe Harpalini, seed properties related to their taxonomy did not appear to be a significant determinant of preferences. This result is in line with the previous findings that Harpalini are less specialised than Zabrini [6]. Our results suggest that the seed traits to which the carabid seed predators respond may be species- or higher taxon-specific and perhaps dependent on the degree of carabid specialisation for seeds. Since Zabrini species are more specialised [6,71] to a narrower range of seeds, often with the same ecology or from the same family, the variables that appeared to be the most influential for preference determination were unexpected. On the other hand, Harpalini species (Figure 1b) are more generalists; therefore, it is ecologically sound that seed mass and dimensions would be the major variable explaining the variation among the matrices of traits for this tribe.

Seed chemistry did not seem to play a crucial role in seed selection by carabid beetles. Although other studies [16,24] have determined that volatiles originating from seeds can attract seed predators, our data do support these observations. There may be several reasons for this lack of support. The seeds used in the multi-choice experiment were dried and mounted on modelling clay [6], which could have limited the amount of volatiles released from the seeds [28,29] compared to those present on the soil surface. The other reason for this difference could be due the cold storage of seeds prior to seed preference assays. Although cold storage does not affect seed viability [72,73], defrosting could have potentially changed the qualitative and quantitative aspects of seed chemical ecology. This needs to be studied. The seeds could have been contaminated by fungi or bacteria [74], which also release their own suite of chemicals. In fact, ethanethiol that was found in the headspace of the tested seeds in our work suggests that some seeds were contaminated, most likely with methanogenic bacteria [75,76]. However, this occurrence should not be considered a problem because in the field, seeds are also colonised by microorganisms [77,78], so the interaction among seeds, microorganisms, and seed predators should be considered a natural component of seed predation and represents an interesting direction for future research.

5. Conclusions

Our data suggest that seed morphological properties are more important than chemical properties in determining the preferences of granivorous carabid beetles. Seed dimensions and seed coat properties were among the most important seed properties affecting carabid preferences. The preferences varied between the taxonomical groups of predators that differ in the degree of specialisation. This paper expands the knowledge on how seed defences influence seed preferences of carabid beetles.

Supplementary Materials: The following are available online at http://www.mdpi.com/2075-4450/11/11/757/s1, Table S1: Preferences of carabids, Table S2: Morphological properties of the seeds, Table S3: Surface fatty acids from seeds, Table S4: Total fatty acids from seeds, Table S5: Volatiles from imbibed seeds, Table S6: Volatiles from dry seeds, Table S7: Other surface compounds.

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Do properties and species of weed seeds affect their consumption by carabid beetles?

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DO PROPERTIES AND SPECIES OF WEED SEEDS AFFECT THEIR CONSUMPTION BY CARABID BEETLES?

Hana Foffová[1,2], David A. Bohan[3] and Pavel Saska[1]

[1]*Crop Research Institute, Functional Diversity in Agro-Ecosystems*
Drnovská 507, 161 06 Praha 6 – Ruzyně, Czech Republic
E-mail: foffova@vurv.cz, https://orcid.org/0000-0001-5528-7211
saska@vurv.cz; https://orcid.org/0000-0003-1397-2987
[2]*Czech University of Life Sciences, Faculty of Environmental Sciences, Department of Ecology*
Kamýcká 129, 165 00 Praha 6 – Suchdol, Czech Republic
[3]*Agroécologie, AgroSup Dijon, INRAe, Université de Bourgogne Franche-Comté*
F-21000 Dijon, France; E-mail: David.Bohan@inrae.fr; https://orcid.org/0000-0001-5656-775X

Seed predators are an integral part of agroecosystems, where they can reduce the populations of weeds. The preference of predators for seeds and the observed predation rate may be affected by the properties of seeds (e.g. taxonomy, chemical composition, physical defence). In this work, we focused on seed consumption of *Taraxacum officinale* Web. and *Stellaria media* (L.) Vill., from France and the Czech Republic, by three species of ground beetle that are seed predators (Coleoptera: Carabidae): *Poecilus cupreus* (L.), *Pterostichus melanarius* (Illiger) and *Anchomenus dorsalis* (Pontoppidan). The seed species were offered in arenas, simultaneously, under three different experimental manipulations of moisture and seed coat conditions: dry and intact, water-imbibed and intact, and water-imbibed with a damaged seed coat. Seed consumption was checked after 0.5, 1, 2, 24, and 48 hrs of exposure. *Anchomenus dorsalis* largely refused to feed on seeds. *Taraxacum officinale* seeds with damaged coats were most preferred by the remaining two species of carabids. The consumption by *P. cupreus* of *T. officinale* seeds with damaged coats increased from 0.18% after 0.5 hrs to 83.83% after 48 hrs, and by *P. melanarius* from 13.76% after 0.5 hrs to 76.77% after 48 hrs. Seeds of *S. media* were consumed less. There was a significant difference in consumption rates due to the country of origin of the seeds, but there were no differences between the carabid sexes. That carabids preferred water-imbibed and damaged seeds may suggest an involvement of olfactory clues in the seed selection process, and/or shorter seed-handling times.

Keywords: carabid beetle, weed seeds, preference, imbibed seeds, seed predator, granivory.

INTRODUCTION

Ground beetles are one of the most important seed predator groups in agroecosystems (Tooley & Brust 2002, Gallandt *et al.* 2005, Honek *et al.* 2005,). Seeds constitute a vital part of the diet for both adults and larvae (Saska 2005, 2008, Klimes & Saska 2010). Seed predators can remove 18 to 70% of seeds produced annually (Westerman *et al.* 2003) thus they can reduce the input of seeds into the soil bank in arable fields (Bohan *et al.* 2011). Numerous factors affect seed predation and selection by carabid beetles, such as seed size

(Forsythe 1983, Acorn & Ball 1991), seed mass (Honek *et al.* 2007), seed coat thickness (Lundgren & Rosentrater 2007), chemical composition (Bewley & Black 1982, Janzen 1982, Hulme 1996), time after ripening of the seeds (Saska *et al.* 2019*a*), the hardness of seeds (van der Meij & Bout 2000) and state of seed imbibition (Law & Gallagher 2015), amongst others. One understudied effect is the geographical origin of the seed. Both physical and chemical properties of seeds may vary among locations (Widmer *et al.* 2007, He *et al.* 2020), which may influence the seed choice by granivores (Honek *et al.* 2011).

The attributes of the ground beetle predator may also affect seed consumption. There is a positive correlation between the mass of the carabid and the mass of the most preferred seeds (Honek *et al.* 2006, Saska *et al.* 2019*b*). The sex of the carabid also influences seed consumption, with females consuming more seeds than males (Marshall & Moonen 2002, Sasakawa 2010, Saska *et al.* 2010, Kulkarni *et al.* 2015), probably because they require more energy for reproduction. Dietary preferences are also influenced by the evolutionary history of carabids: species of the tribe Zabrini prefer seeds of Asteraceae, while those of the tribe Harpalini may feed upon seeds of the Violaceae and other Asteraceae usually not selected by the Zabrini (Honek *et al.* 2007, Saska *et al.* 2019*b*).

Carabids seem to use olfactory (Law & Gallagher 2015) and tactile cues (Kielty *et al.* 1996) when searching for seeds. The type or amount of chemical cues released from seeds might be affected by conditions of the seeds, e.g. the state of the seed coat or the levels of imbibition. Many seed predators are not able to detect seeds with an impermeable coat, most likely because chemical cues are not released from the seed (Vander Wall 1998). Beetles appear to prefer water-imbibed seeds over the dry seeds (Kulkarni *et al.* 2016, 2017) because the olfactory cues of the previous are stronger and the handling time of these seeds is also shorter (personal observation).

There is a lack of information about the preferences of carabids for seed species in interaction with water-imbibed seeds or seeds with a damaged seed coat. In this study, we conducted a multiple-choice test to determine the preference of the three carabid species: *Pterostichus melanarius*, *Poecilus cupreus* and *Anchomenus dorsalis*. Although the representatives of the genera *Pterostichus*, *Poecilus* and *Anchomenus* are commonly regarded as predominantly carnivorous species, they have been frequently observed to consume seeds (Goldschmidt & Toft 1997, Honek *et al.* 2003, Hurst & Doberski 2003, Deroulers & Bretagnolle 2019). The presence of plant DNA in carabid regurgitates of *P. melanarius* and *P. rufipes* was found to be substantial (>70% of individuals) and independent of carabid species, sex, region and the time of sampling (Frei *et al.* 2019). In particular, *P. melanarius* has been described as a generalist predator (Bohan *et al.* 2000, Langan *et al.* 2004, Bargmann *et al.* 2016), but this species also consumes weed seeds (Tooley *et al.* 1999, Honek *et al.* 2003). *P. melanarius* has been found to readily accept fresh, dry and imbibed seeds of *Brassica napus* (Koprdova *et al.* 2012). On average, *P. melanarius* consumed three seeds of *S. media* seeds in 48 hrs in a cafeteria test (Tooley *et al.* 1999). *P. cupreus* is known as an omnivorous species (Lövei & Sunderland 1996, Honek *et al.* 2003, Bargmann *et al.* 2016, Saska *et al.* 2019*b*). Very little is known about seed consumption of *A. dorsalis*. This species consumed 1.7±1.4 seeds of *Capsella bursa-pastoris* during a three days experiment (Honek *et al.* 2003). Because it is one of the dominant species in European agroecosystems, the capacity of this species to eat seeds is worth exploring.

The preferences of the three different conditions of seeds (dry/intact, imbibed/intact and imbibed/damaged) were tested on three different species of carabids, separately for each sex. We expected: (i) carabid species-specific responses to particular species of seed; (ii) specific responses to the different seed conditions; (iii) sex-specific consumption; and (iv) that geographical origin of the seeds would affect consumption.

MATERIAL AND METHODS

Ground beetles

Three species of carabids were used in this study: *Pterostichus melanarius*, *Poecilus cupreus* and *Anchomenus dorsalis*. These species were selected because they are amongst the most abundant carabids in arable fields in France. The ground beetles were collected using pitfall traps from two field edges on the INRAe experimental farm near Dijon, France (47.233°N, 5.097°E) between the end of September and October 2018. We used 160 trapped individuals of each species for the experiments. Beetle identification was made according to Hůrka (1996). Carabids were starved for 3 days before the trial. The beetles were individually kept in plastic containers (7.5 cm in diameter) covered with a lid at room temperature to standardise their hunger level (Lundgren & Rosentrater 2007) and water was provided by placing a wet cotton roll on the bottom.

Seeds

We used seeds of *Taraxacum officinale* (Asteraceae) and *Stellaria media* (Caryophyllaceae); species that have previously been used for experimentation with adults as well as larvae of carabid beetles (Honek *et al.* 2005, Saska 2008, Petit *et al.* 2014). The seeds of *T. officinale* are obconic achenes 2.5 x 0.9 mm long on average (Bojnanský & Fargašová 2007), and are rich in proteins (25.65%), lipids (27.86%) and carbohydrates (23.05%) (Bretagnolle *et al.* 2016). The seeds of *S. media* are round, ca. 1 mm diameter (Bojnanský & Fargašová 2007), and have lower protein (16.44%) and lipid (5.19%) content than *T. officinale*, but are higher in carbohydrate (65.22%) (Bretagnolle *et al.* 2016). Seeds from the Czech Republic (collected near to CRI, Prague, 50.086°N, 14.302°E) and France (collected near to INRAe Dijon, 47.316°N, 5.068°E) were used. All seeds were dried at room temperature (22 °C) for 4 weeks. Plant identification was made according to Kubát *et al.* (2002).

Experiment design

The effects of seed species, conditions, location of origin and the sex of the beetles, and all interactions, on seed consumption by ground beetles, were tested in a multi-choice experiment. The experimental arenas consisted of Petri dishes (14 cm in diameter) lined with moist filter paper on the bottom. Both species of seeds, *T. officinale* and *S. media*, were placed simultaneously in each arena. Three seed conditions were used: dry/intact, imbibed/intact and imbibed/damaged. To assure that the dry treatment seeds remain dry at the time of exposure, they were placed into the arena immediately before the start of the trial. The water-imbibed seeds were left to soak on wet filter paper for 24 hrs before the start of the trial. The imbibed/damaged seeds were first imbibed on filter paper for 24 hrs, after which a small hole was made through the seed coat using an entomological pin number 0 (0.35 mm in diameter), so the seeds remained viable (confirmed by conducting a parallel germination test on a subset of 20 seeds per species and country; data not shown). Each condition was replicated five times per dish, so 30 seeds were presented at a time (Fig. 1). The French and Czech seeds were examined separately. The photoperiod was set for 10 hrs light/14 hrs dark and constant temperature of 17 °C, in order to prevent any temperature effect on seed consumption (Saska *et al.* 2010). The experiment was conducted from the end of October to the beginning of December 2018. Individual beetles were released into arenas and the remaining seeds were counted after 0.5, 1, 2, 24, and 48 hrs. Any seed that was more than half-eaten was considered eaten and removed, as were the empty seed coats.

Statistical analysis

The statistical analysis was performed by generalised mixed effects models in R version 3.5.3 (R Core Team) using the package *glmmTMB* (Brooks *et al.* 2017). Since *A. dorsalis* ate very few seeds (a total of 89 out of the 4800 seeds offered), the data for this species were not analysed. The preferences of *P. melanarius* and *P. cupreus* were tested separately. Since the data contained a high proportion of zeros, we fitted models using the binomial and beta-binomial distributions, with or without terms that accounted for zero-inflation, and with or without arena as a random factor (because the consumptions of different seed conditions within the same arena were not independent from each other). Models based on beta-binomial distribution without the zero-inflation term and with random effect were superior (justified by the change in Akaike Information Criterion). As the preferred models did not show any signs of overdispersion and zero-inflation (checked using the DHARMa

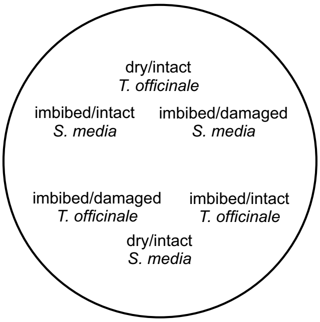

Fig. 1. Design of the testing arena

package; Hartig 2016), beta-binomial models were used in the subsequent steps of model simplification. Significance of individual terms in the models was assessed using the Anova function from the car package (Fox & Weisberg 2019).

RESULTS

Seed consumption was affected by seeds species and conditions, and it varied between the carabid species (Table 1). For *P. melanarius*, seed preference was affected by seed conditions, species and geographical origin. There were also significant two- and three-way interactions between seed conditions and seed origin, and seed species, seed conditions and geographical origin (Table 2). Seeds of *T. officinale* were preferred more than *S. media*. Dry/intact seeds were consumed significantly less than imbibed/intact and imbibed/damaged seeds. The seeds from France were consumed significantly more than the Czech seeds. There was no effect of the sex of the beetle (Table 2).

Poecilus cupreus also preferred seeds of *T. officinale*. Dry/intact seeds were the least consumed seed conditions followed by imbibed/intact seeds. There was a significant interaction between seed species and seed conditions, as well between seed conditions and seed origin (Table 3). There was also no significant difference in consumption between the sexes of the beetles.

Table 1. The mean proportions (95% confidence intervals) of consumed seeds by two species of carabids in 48 hrs.

Species of carabids/ Condition of seeds	*Stellaria media*		*Taraxacum officinale*	
	France	Czech Republic	France	Czech Republic
Pterostichus melanarius				
Imbibed/damaged	0.401	0.208	0.712	0.761
	(0.304, 0.507)	(0.142, 0.294)	(0.571, 0.821)	(0.629, 0.856)
Dry/intact	0.312	0.096	0.803	0.187
	(0.226, 0.412)	(0.059, 0.151)	(0.683, 0.886)	(0.107, 0.304)
Imbibed/intact	0.217	0.166	0.67	0.224
	(0.149, 0.304)	(0.110, 0.241)	(0.522, 0.790)	(0.132, 0.353)
Poecilus cupreus				
Imbibed/damaged	0.196	0.102	0.853	0.702
	(0.113, 0.317)	(0.054, 0.187)	(0.656, 0.946)	(0.456, 0.868)
Dry/intact	0.144	0.061	0.843	0.361
	(0.080, 0.245)	(0.030, 0.119)	(0.642, 0.942)	(0.168, 0.612)
Imbibed/intact	0.135	0.078	0.751	0.241
	(0.075, 0.232)	(0.039, 0.149)	(0.506, 0.899)	(0.101, 0.474)

Table 2. The minimum model for the seed preference of *Pterostichus melanarius* determined after 48 hrs. Generalised mixed-effects model with beta-binomial distribution was used.

Variables	χ^2	Df	P
Seed species	181.872	1	<0.001
Seed conditions	45.223	2	<0.001
Seed origin	14.207	1	<0.001
Seed species × Seed conditions	2.799	2	0.246
Seed species × Seed origin	3.421	1	0.064
Seed conditions × Seed origin	36.934	2	<0.001
Seed species × Seed conditions × Seed origin	26.784	2	<0.001

The cumulative proportion of seeds consumed increased over time (Fig. 2). The most preferred seeds were those of *T. officinale* with imbibed/damaged seed coat. The consumption of these seeds differed between carabid species: *P. cupreus* consumed 0.18% of the seeds after 0.5 hrs, while *P. melanarius* consumed 13.76% of the seeds over the same time period. The consumption of these seeds, regardless of seed origin, gradually increased and *P. cupreus* and *P. melanarius* consumed 83.83% and 76.77% of seeds after 48 hrs, respectively.

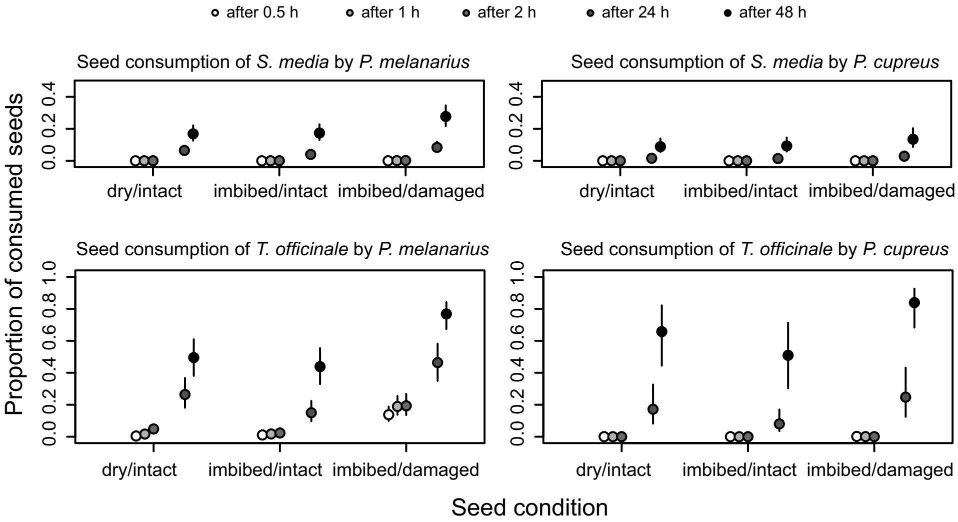

Fig. 2. Average cumulative consumption rate by carabids during the time of 0.5, 1, 2, 24, and 48 hrs. Each point represents the mean consumption, and the vertical bars represent 95% confidence interval. Points within particular seed condition are spaced based on log(time of exposure). Note that the ranges of y-axis differ between the top and bottom panels.

Table 3. The minimum model for the seed preference of *Poecilus cupreus* determined after 48 hrs. Generalised mixed-effects model with beta-binomial distribution was used.

Variables	χ^2	Df	P
Seed species	293.469	1	<0.001
Seed conditions	26.124	2	<0.001
Seed origin	4.347	1	0.037
Seed species × Seed conditions	7.039	2	0.029
Seed conditions × Seed origin	6.462	2	0.039

The seed consumption of *S. media* was lower than the consumption of *T. officinale*. The most preferred seeds of the *S. media* sets were the imbibed/damaged conditions. Consumption after 48 hrs, irrespective of the origin of the seeds, was 13.46% of seeds for *P. cupreus,* and 27.69% of seeds for *P. melanarius*.

DISCUSSION

In this study, the seed preferences of two omnivorous carabid species were found to depend on the seed species, conditions and the geographical origin of the seeds. *Pterostichus melanarius* and *Poecilus cupreus* consumed a rather high number of seeds of *Taraxacum officinale* in our study, and both consumed more of the water-imbibed than dry seeds. In other studies, carabids also made significant preference choices for imbibed seeds (Hurst & Doberski 2003, Kulkarni *et al.* 2016).

Several mechanisms could explain why the dry and unimbibed seeds were less consumed by carabids (Fig. 2). These seeds release less olfactory volatiles than those that are imbibed (Paulsen *et al.* 2013) and more attractive to predators. Dry and unimbibed seeds may also deter seed predators (Cardina *et al.* 1996), because of the chemical compounds in the seed coat. Alternatively, it may be that the effect of imbibition on the hardness or thickness of the seed coat (Lundgren & Rosentrater 2007), is to soften the seed coat making penetration by the carabids relatively easier. The seeds of *S. media* have stronger and harder seed coats than *T. officinale*, which may partly explain the higher predation of seeds of *T. officinale* observed. Another mechanism may be related to the chemical properties of seeds which are not changed during imbibition, such as the volatile compounds (Paulsen *et al.* 2013), waxes and other secondary metabolites that are present (Janzen 1982). Both mechanisms may also explain the increase in consumption in the case of imbibed seeds with damaged coats. It is possible that the handling time of these seeds by the carabids was shorter (Mikheev & Wanzenböck 2010), although this was not measured in our study.

On average, after one day of seed exposure, consumption by *P. melanarius* was 3.44 seeds of *T. officinale* and 1.9 seeds of *S. media*, and that by *P. cupreus* was 2.46 seeds of *T. officinale* and 0.3 seeds of *S. media*. The daily consumption of *T. officinale* is lower than observed by (Petit *et al.* 2014). The consumption rates observed here could be negatively affected by the relatively late time of the year that the experiment was conducted in October. Although the experiment was carried out in the laboratory, the beetles were collected in the field near the end of their period of seasonal activity. The predation rate has been found both to be low in the winter months (Cardina *et al.* 1996) and to decline from late summer (Honek *et al.* 2003). The season of the experiment could also affect the consumption by the different beetle sexes (Honek *et al.* 2006) because in the autumn females no longer need energy for reproduction.

Current information about carabid selection for seeds with different geographical origin is limited. Honek *et al.* (2011) demonstrated that the locale of seed origin played an important role in preferences of carabids. In our study, the origin of the seed significantly affected seed consumption and preferences by both species of carabids (Tables 1 & 2). The French beetles used in our study preferred French seeds; unfortunately, we could not make the same comparison with Czech beetles since these were not available at the time of the experiment. Differential consumption by origin might be explained by micro-species of plants (Mogie & Ford 1988). Perhaps seeds from particular geographical regions differ e.g. in starch (Widmer *et al.* 2007) or oil content (He *et al.* 2020) to which local carabids respond, and this aspect of seed-predator interaction deserves further attention.

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Article

Persistence and Changes in Morphological Traits of Herbaceous Seeds Due to Burial in Soil

Pavel Saska [1,*], Hana Foffová [1,2], Zdenka Martinková [1] and Alois Honěk [1]

[1] Crop Research Institute, Group Functional Diversity in Agro-Ecosystems, Drnovská 507, 161 06 Praha 6—Ruzyně, Czech Republic; hana.foffova@gmail.com (H.F.); martinkova@vurv.cz (Z.M.); honek@vurv.cz (A.H.)

[2] Department of Ecology, Faculty of Environmental Sciences, Czech University of Life Sciences, Kamýcká 129, 165 00 Praha—Suchdol, Czech Republic

* Correspondence: saska@vurv.cz; Tel.: +420-2-330-22-416

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Abstract: Seeds in soil banks can survive for many years before conditions become more suitable for germination. Meanwhile, seeds undergo changes in morphology and viability. In this study, we launched an artificial seed bank experiment that included 26 species of seeds. We excavated cohorts for 6–8 consecutive years after burial (*YAB*) in order to determine changes in the morphology (mass, volume, density, seed form) and proportion of fresh (thus persistent) seeds using a crush test as a measure of persistence. The change in seed morphology was fitted by linear and logistic regression, and the proportion of persistent seeds was fitted by logistic regression (effectively by the binomial GLM), which enabled estimation of 50 and 5% persistence times (PT_{50} and PT_{05}). We found that in most species, seed mass, volume and proportion of persistent seeds declined with *YAB*, while other morphological traits were less variable, and the decline in these traits with *YAB* was best fitted with logistic regression. The decline in the proportion of persistent seeds was better fitted by the change in mass than by *YAB* in some species. Among the species included in this study, PT_{50} ranged from 1.2 to 10.5 years, and PT_{05} ranged from 2.1 to 24.3 years. These results can contribute to better understanding of the ecology of weed seed bank persistence in soil. Describing the morphological changes that the seeds undergo in the soil bank may improve our understanding of the biology of seed persistence and facilitate the identification of seeds from the soil bank.

Keywords: seed survival; seed decay; seed persistence in soil; crush test; seed morphology; germination

1. Introduction

The soil seed bank represents a natural storage of plant seeds in soil, in which seeds may persist and remain viable for many years [1]. Depending on the above-ground vegetation dynamics, soil seed banks contain different quantities of seeds. These quantities are largest in arable land, where numbers may exceed 10^5 seeds m^{-2}, and in grasslands (up to 10^4 seeds m^{-2}) [2,3]. Soil seed banks thus provide a "back-up" for future situations when environmental conditions become more suitable for germination (e.g., soil and canopy disturbance) and may prevent local extinction.

Plant species largely differ in the persistence of seed banks. Some species form only a transient seed bank, as seeds that do not germinate quickly do not usually survive more than a year [2,4]. Seeds of other species remain alive for a long time, even hundreds of years [5], and form so-called persistent seed banks [2]. Longevity correlates with seed morphology [6–9], taxonomic affiliation [10,11] and plant ecology [12]; for example, arable species tend to have more persistent seeds compared to forest species [4]. However, within evolutionary constraints of a species, seed persistence is not a fixed trait, and may vary with soil disturbance, type and pH, nutrient content in soil and (micro-) climatic conditions such as soil moisture and temperature and depth at which the seeds are buried [13–22]. Thus, the realized seed persistence is a combination of seed characteristics and the immediate environment [12].

A variety of approaches have been applied to determine seed persistence in soil [2]. Studies of changes in seed bank composition over time often rely on analyses of series of samples from sites with known time from the last disturbance [5], which is used as a proxy of the minimum seed age. The limitations of these studies for understanding the dynamics of seed persistence in soil lie in the fact that the age determination of the retrieved seeds is very rough and that the initial seed cohort size is unknown. Another common approach includes the creation of artificial seed banks by either sowing the seeds in soil followed by repeatedly taking soil cores and viability testing the retrieved seeds [23,24] or by burying seed bags followed by periodic exhumation and viability testing [2,14,20,25]. With these approaches, the deterioration of the seed population can be followed, parametrized and used for making predictions. Such studies do not usually cover more than 5–6 years [14,21], and those that would span over longer periods of time usually do not collect data on yearly basis [22,26].

In addition to changes in viability, seeds in soil also undergo changes in external morphology [27] caused by soil chemistry and activity of soil microorganisms. There is a lack of literature records that would provide a qualitative description of these changes, which may include changes in colour and loss of trichomes and other extremities or of the seed coat entirely. The little attention paid to these changes may result from the fact that the research has focused mainly on seed persistence per se and that morphological changes have been perceived as too descriptive and thus unimportant.

However, there are several ecological as well as practical reasons to have these changes described for an array of seed species. In the first place, many seeds undergo physical dormancy, i.e., they can germinate only if the seed coat is scarified, i.e., damaged, such as by soil microorganisms or abrasion during movement through soil. Knowing over the course of morphological changes then may become useful for predicting the germinability of the seeds. In the second place, knowledge of the morphological changes can be used in seed identification from soil samples, particularly in species in which these changes are substantial. Knowledge on changes in seed morphology may become relevant in connection with seed predation of exhumed seeds [27–29] because predators are known to select seeds based on seed morphology [30,31]. The aim of this paper is therefore to describe and analyse (i) seed persistence and (ii) changes in morphological seed traits in 26 species of herb seeds in the course of 6–8 years of burial in artificial soil seed banks. Seed persistence was determined over up to 8 years spent in soil, 50 and 5% persistence times were predicted for each seed species based on logistic regression modelling of the proportion of persistent seeds, and changes in morphological traits that can be perceived by seed predators, such as mass, volume, density and form, were examined.

2. Materials and Methods

Seed material. Seeds of 26 species of herbs, mostly weeds, were introduced into artificial seed banks in 2005 and 2006 (Table 1). The species of seeds were selected so they differed in morphology, taxonomic affiliation and presumed persistence in soil, and based on availability. Seed materials were sampled in Prague-Ruzyně (western Czech Republic) in a c. 10 × 10 km area surrounding Crop Research Institute (CRI) and centred at 50.09 N and 14.30 E. The seeds were harvested from mother plants by hand at full ripeness in July-October 2005 and October 2006 and stored dry at 5 °C until the burial. Furthermore, a subset of the seeds was stored at −20 °C as a control cohort (0 years). Seeds of 9 species were buried on 8 November 2005, and the remaining 17 species were buried on 24 October 2006 (Table 1). Seed batches destined for burial for the time period of a particular length (seed cohorts) were prepared in a standard way. Approximately 10^4 seeds were mixed with soil dug from a 0.6 m depth and sieved through 0.05 mm mesh, placed in bags of nylon fabric and buried at a 20 cm depth under grassland on a ground of CRI. Mixing seeds with soil is important to prevent excessive degradation [32], and fine sieving of the soil facilitates separation of the seeds after exhumation. Seed material was divided into 8 (2005) or 6 (2006) batches per species. Each seed batch packed in a bag with soil was buried separately and connected by a nylon cord with a label on the ground surface. Every year, one batch per seed species was exhumed, and recognizable seeds were separated from soil, dried at 25 °C and 40% r.h., and then stored at −20 °C for experimental use (a cohort). The last batches of seeds buried in 2005 were thus excavated in 2013 after 8 years of burial, and those buried in 2006 were excavated in 2012 after 6 years of burial, so there were 8 or 6 burial cohorts per species, respectively. For *Crepis biennis* 2011 excavation and for *Plantago lanceolata* 2012 excavation, no seeds were available due to complete deterioration of the respective batches.

Table 1. Species of seeds buried in 2005 and 2006 with longevity index (LI) and seed persistence index (SPI) calculated based on the burial data taken from [2]. Number of records indicates how many individual data entries from the database of [3] were used for calculation of the indexes. The nomenclature was based on Kubát et al. [33].

#	Species	Family	Year of Burial	Number of Records	LI	SPI
1	*Amaranthus powellii* S. Watson	*Amaranthaceae*	2006			
2	*Amaranthus retroflexus* L.	*Amaranthaceae*	2006	7	1	3
3	*Atriplex sagittata* Borkh.	*Amaranthaceae*	2006			
4	*Campanula trachelium* L.	*Campanulaceae*	2006			
5	*Capsella bursa-pastoris* (L.) Med.	*Brassicaceae*	2005	20	1	2.55
10	*Chenopodium album* agg.	*Amaranthaceae*	2006	15	1	2.87
11	*Chenopodium glaucum* L.	*Amaranthaceae*	2006			
12	*Chenopodium polyspermum* L.	*Amaranthaceae*	2006			
6	*Crepis biennis* L.	*Asteraceae*	2005			
7	*Geum urbanum* L.	*Rosaceae*	2005			
8	*Hyoscyamus niger* L.	*Solanaceae*	2006			
9	*Hypericum perforatum* L.	*Hypericaceae*	2006	1	1	3
13	*Lavandula angustifolia* Mill.	*Lamiaceae*	2006			
14	*Leonurus cardiaca* L.	*Lamiaceae*	2005			
15	*Lycopus europaeus* L.	*Lamiaceae*	2005			
19	*Persicaria lapathifolia* (L.) Delarbre	*Polygonaceae*	2006	3	1	3
16	*Plantago lanceolata* L.	*Plantaginaceae*	2006	14	0.93	2.43
17	*Plantago major* L.	*Plantaginaceae*	2006	7	0.86	2.57
18	*Plantago media* L.	*Plantaginaceae*	2006	1	1	2
20	*Portulaca oleracea* L.	*Portulacaceae*	2006	6	1	3
21	*Silene noctiflora* L.	*Caryophyllaceae*	2006			
22	*Silene vulgaris* (Moench) Garcke	*Caryophyllaceae*	2006	6	1	3
23	*Thlaspi arvense* L.	*Brassicaceae*	2005	14	1	2.86
24	*Tripleurospermum inodorum* (L.) Schultz-Bip.	*Asteraceae*	2005	18	0.94	2.5
25	*Urtica dioica* L.	*Urticaceae*	2005	1	1	2
26	*Urtica urens* L.	*Urticaceae*	2006	3	1	3

Changes in seed morphology with duration of burial. Prior to measuring changes in seed morphology with duration of burial, subsamples of seeds from each cohort were cleaned from fine soil particles in an ultrasound cleaner (Sonorex RK 31, Bandelin electronic, Berlin, Germany), submerged in water for 2 min, and dried in an oven for 24 hr at 75 °C. The following measurements were made on seeds from each cohort:

Seed mass (M)—For each species and cohort, the average seed mass was determined based on five batches of 20 seeds using analytical balances (CP225D-0CE, Sartorius AG, Göttingen, Germany) with a precision of 0.00001 g.

Seed dimensions—following [19], five seeds per cohort were measured by digital scales NTD12P-15CX (Mitutoyo Corp., Kawasaki, Japan) with a precision of 0.01 mm. The following dimensions were measured: length (L; the longest dimension of the seed), width (W; the longest dimension perpendicular to L within the same plane), and height (H; the longest dimension perpendicular to the plane of L and W). Based on these measurements, the following metrics of the seed form were calculated for each of the five seeds per combination of species and cohort, except for density, which could only be estimated as a mean value:

Volume—calculated as $V = L * W * H$ [34]

Density—calculated as $D = \frac{M}{V}$ [34]; only mean values of mass and volume were used, as these were measured on different seeds.

Shape—according to [19], the seed shape can be expressed as a dimensionless measure: $Vs = \sum \frac{(x-\overline{x})^2}{n}$, where x represents a division of either L, W, and H through L and $\overline{x}$ as their mean, and n is 3. Vs ranges from 0 for perfectly spherical seeds to 0.2 shaped as a thin disc or spindles.

Flatness Index—calculated as $FI = \frac{(L+W)}{2 * H}$ [34]. It ranges from 1 for a complete sphere to greater values for plane or spindle like shaped seeds.

Eccentricity Index—calculated as $EI = \frac{L}{W}$ [34]. It ranges from 1 for round seeds to values greater than 2 for spindle like seeds.

Seed persistence in soil. Seed persistence was measured on another subsample of seeds. We used the so-called imbibed seed crush test (ICT), which was found to be reliable for estimation of the true viability of weed seeds [35–37]. This test was performed in order to determine the proportion of fresh [38], i.e., persistent and viable seeds. In these seeds the seed coat does not collapse when crushed with the tips of a pair of forceps, or have apparent and intact cotyledons or embryos when the seed coat is broken. In other cases, the seeds were considered dead. In this test, 20 seeds per cohort tested, and were left imbibe for 3 days in laboratory conditions (20 °C, 12 h light: 12 h dark) prior testing.

Statistical analysis. Prior to analysis, the duration of burial was expressed as a continuous numerical vector, year after burial (*YAB*), and used as the explanatory variable in most of the analyses. Frozen (control) seeds were given age 0, those exhumed after one year were given age 1, etc., so the maximum age was 8 for seeds buried in 2005 and exhumed in 2013 and age 6 for those buried in 2006 and exhumed in 2012. *YAB* 1 then denotes a cohort of seeds that were buried for 1 year and so on. The analyses were performed in R version 3.3.1 [39]. The variation in morphological seed traits with *YAB* was tested within seed species by regression methods [40]. For comparative reasons across species of seeds, the values of M, V and D were converted to relative scale against the mean values of the respective trait for control seeds and denoted rM, rV and rD (i.e., relative mass, relative volume and relative density). The three seed form indexes were not relativized because they are inherently dimensionless. For each species of seeds, the rM, rV and rD were regressed against *YAB* by fitting three different models were used to fit:

(a) Linear: $y = a + b * YAB$, where y is the respective trait on relative scale, a is the intercept, and b is the slope of the linear regression line;

(b) 3-parameter logistic: $y = \frac{A}{1+e^{(D-YAB)/C}}$, where y is the respective trait on relative scale, A is the upper asymptote, C is the scale parameter on the x-axis, and D is an inflexion point of the curve;

(c) 4-parameter logistic: $y = A + \frac{B-A}{1+e^{(D-YAB)/C}}$, where y is the respective trait on relative scale, A is the upper asymptote, B is the lower asymptote, C is the scale parameter on the x-axis, and D is an inflexion point of the curve.

The non-linear curves were fitted as the self-starting functions SSlogis and SSfpl for 3- and 4-parameter logistic functions (nls package of R). The explanatory power of the three models was compared based on the Akaike Information Criterion (AIC) [40], and the one with the lowest AIC value (ΔAIC = 2 as threshold) was chosen as best and presented. Only the linear model was fitted to *Shape*, *FI* and *EI* based on a priori visual data inspection. In case the diagnostic graphs [40] suggested that outlying *YAB* values could influence the parameter estimates, these were removed from model fitting. To better interpret the general pattern in seed form variation with *YAB*, we performed principal component analysis by implementing the *prcomp* function from stats package of R and including *rM*, *rV*, *rD*, *Shape*, *FI* and *EI* as variates, and the resulting principal scores for the first and second axes (*PC1* and *PC2*) were used for interpretation of changes in the proportion of persistent seeds [9].

The variation in the proportion of persistent seeds was assessed based on the logistic regression: $y = \frac{1}{1+e^{-(a+b * x)}}$ [41], and linearized by implementing generalized linear modelling, with a being the intercept and b the slope of the regression, using binomial distribution of errors and logit link function (GLM-b) [17]. This approach takes the nature of the data (proportions of viable seeds in case of germination test and binary response variable: 0—dead, 1—live into account without a need for transformation [40] and allows for making predictions [17]. The parameter x is either *YAB*, *PC1*, *PC2* and $\overline{rM}$ (i.e., mean relative seed mass per *YAB* cohorts) and the models were compared based on the AIC as above. The seed persistence in the soil was estimated for each species of seed based on the parameters of the model with *YAB*, predicting the 50 and 5% persistence time in *YAB* (PT_{50} and PT_{05}, respectively), i.e., the time after burial when 50 and 5% of the initial seed population were still viable. To identify which morphological traits might have affected the predicted seed persistence across species, we regressed PT_{50} and PT_{05} separately against all traits measured on control seeds against *rM*, *rV* and *rD* for each separate *YAB* cohort and against slopes of change in *Shape*, *FI* and *EI* across *YAB*.

Seed longevity literature survey. Seed longevity data for some of the study species can be excerpted from the literature. The monograph [2] was used as a starting point for the survey. We used only seed persistence data originating from burial experiments (method coded 1–3 after work [2]), which were available for only 14 out of 26 species of seeds included in this study. From the literature data, we calculated two indexes, the longevity index (*LI*) and seed persistence index (*SPI*). *LI* was calculated as: $LI = \frac{R_{sp}+R_{lp}}{R_t+R_{sp}+R_{lp}}$, where R_t, R_{sp} and R_{lp} are the proportion of records classifying the species as transient (persisting less than 1 year), the proportion of records classifying the species as short persistent (persistent for more than 1 year but less than 5 years), and the proportion of records classifying the species as long persistent (persistent for more than 5 years), respectively [4]. *SPI* was calculated as: $SPI = \frac{T+2*SP+3*LP}{total\ number\ of\ records}$, where T, SP and LP represent the number of records reporting transient, short persistence and long persistence, respectively [8]. The values of *LI* and *SPI* with the number of literature records used for calculations are listed in Table 1.

3. Results

Seed mass (as *rM*) decreased with *YAB* in 25 out of 26 species of seeds available. The only exception was *H. niger*, the seeds of which did not change their *rM* with *YAB* (Figure 1a). Logistic curves appeared to fit the decline in *rM* better than the simple linear fit in 17 cases, suggesting that in the majority of species, the rate of mass reduction is slow during the first years after burial, then accelerates and finally slows down again. The 4-parameter logistic curve that included a lower asymptote described the data best in 10 cases, and the 3-parameter curve without the lower asymptote curve did so in seven cases. Examples are in Supplementary Materials. Seed volume (as *rV*) declined with *YAB* in 14 species, of which the linear decrease was found in 10 and logistic in four species, respectively (Figure 1b). The surprising observation that relative seed mass or volume increased after burial in some species of seeds (Figure 1a,b) has to be viewed as an artefact of natural variability among the seeds. Seed density (as *rD*) was even less variable with *YAB* in terms of the number of significant changes (in 11 species only), but the changes were both positive (1 case) and negative (10 cases), suggesting that the mechanisms that lie behind the variation in morphological traits over time spent in soil are diverse across species. Three species of seeds showed a significant linear change, and eight species showed a significant logistic course of change in *rD* with *YAB* (Figure 1c).

The interspecific interrelationship of the three morphological traits and temporal dynamics of their change across *YAB* is exemplified in Figure 2 using fitted values from the above models for *YAB 1*, *5* and *8*. After one year in soil, there was little variation in *rM*, *rV* and hence *rD* (Figure 2a). With the course of time spent in soil, it becomes evident that the rate of change in *rM* and *rV* and hence in *rD* becomes more variable across species (Figure 2b,c)—species distributed along the line representing slope = 1 through the origin are those in which both mass and volume decreased in similar rate, species below the line are those in which the volume decreased more rapidly than mass and species above the line are those in which seed mass declined more rapidly than volume.

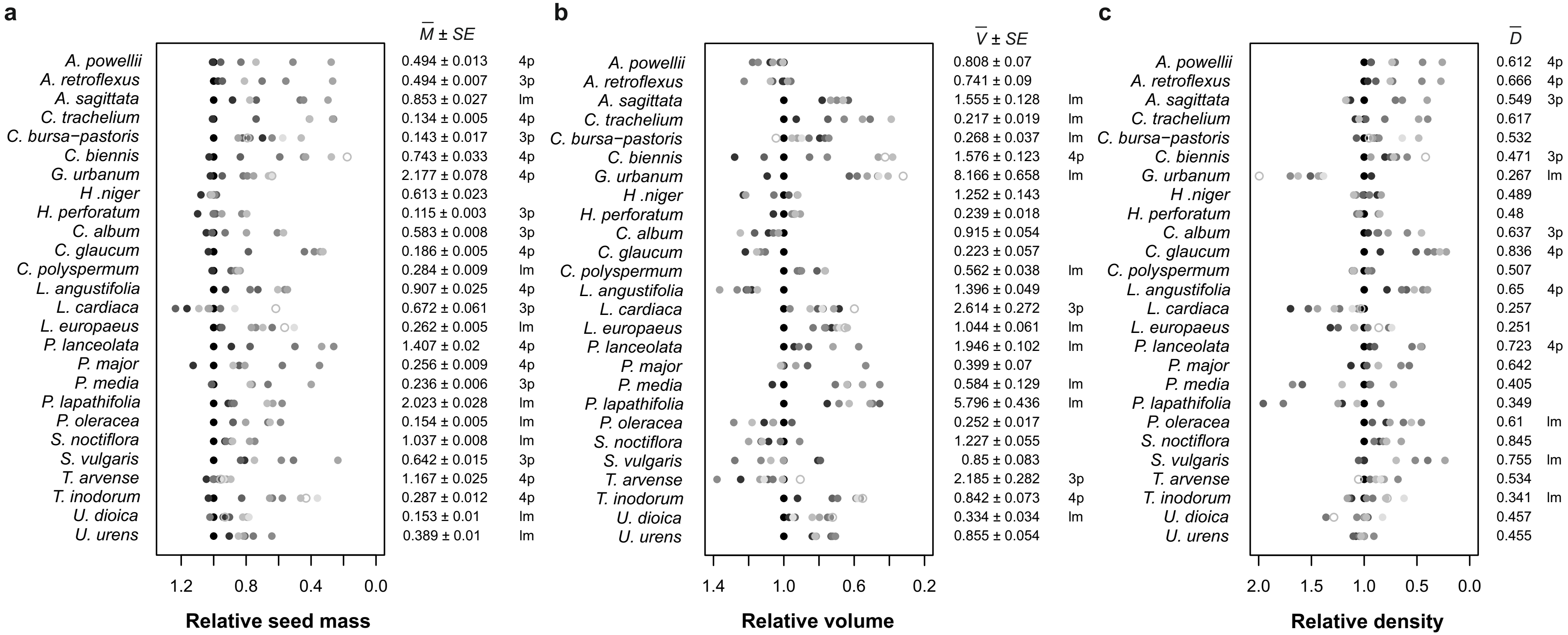

Figure 1. Variation in seed morphology with year after burial (*YAB*) for 26 species of seeds: (**a**) relative seed mass (*rM*); (**b**) seed volume (*rV*); (**c**) relative seed density (*rD*). The decrease in the intensity of grey colour refers to the increase in *YAB*: black = control, white with grey lining = *YAB* 8 are the two extremes). $\overline{M}$ ± SE and $\overline{V}$ ± SE denote the mean dry mass of control seeds ± standard error. $\overline{D}$ denotes the mean dry mass of control seeds. Lm (linear), 3p (3-parameter logistic) and 4p (4-parameter logistic) indicate the model that fitted the change in the seed trait over time based on the Akaike Information Criterion, if any of the three models were significant. See Table 1 for generic names of each species.

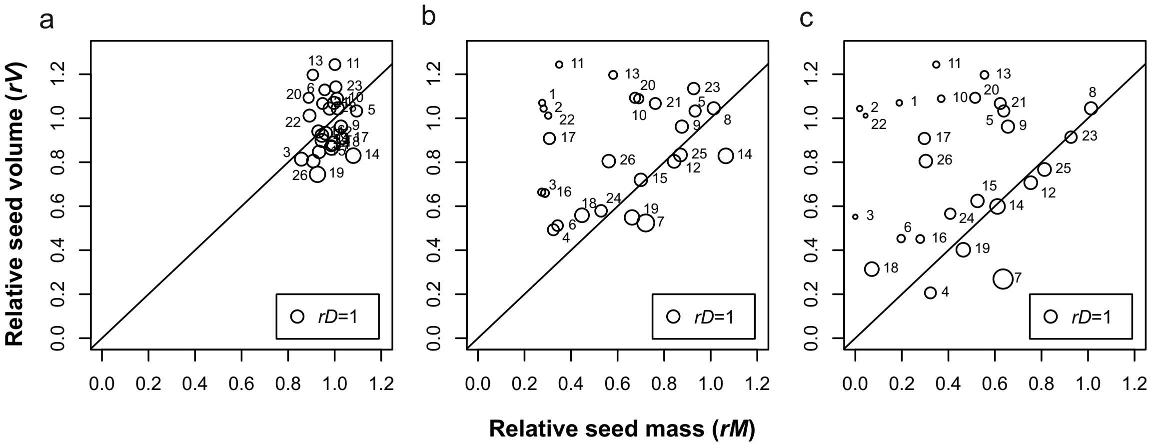

Figure 2. Interrelationship between relative mass (*rM*), volume (*rV*) and density (*rD*) of 26 species of seeds (for species codes, see Table 1). Fitted values from the best models shown in Figures a, b, and c were used, the line of slope = 1 indicates the situation when relative mass and volume declined at the same rate so *rD* did not change. Sizes of the symbols are scaled according to the size of change in *rD*, the symbol size shown on the bottom right equals *rD* = 1, smaller or larger symbols refer to proportional decrease or increase in *rD*, respectively. (**a**) *YAB 1*; (**b**) *YAB 5*; (**c**) *YAB 8.*

The shape of seeds remained similar overall, as the three indexes of the seed form varied with *YAB* in only a few species (Figure 3a–c). Principal component analysis, however, revealed patterns in seed form changes across seed species and *YAB* (Figure 4). The PC1 axis explained 48.4% of the variance and presented the change in external seed form as *Shape*, *FI* and *EI* correlated positively and *rV* negatively with this axis. This suggests that seeds that change their shape also lose their volume as they either shrink and become flatter or lose their extremities. The PC2 axis explained 27.3% of the variance and was well correlated positively with *rM* and *rD*, i.e., represented by seeds that did not change their volume but changed their mass and hence density. The individual variation in all morphological traits with *YAB* for each of the seed species can be found in the Appendix S1: panels A–F.

Alternative models for the proportion of persistent seeds provided mixed results. While the two models based on *PC1* and *PC2* together improved the explanatory power of the variation compared to the ICT model in two cases only (*PC1*: *L. cardiaca*, AIC = 185.28; *PC2*: *C. album*, AIC = 63.86), models based on *rM* explained the change in the proportion of persistent seeds better than did the initial models in 10 cases (Table 2) suggesting that the decline in the proportion of persistent seeds may be associated with the decline in seed mass more tightly than solely to *YAB*, especially when large or inconsistent scatter in *rM* across *YAB* appeared. The change in the proportion of persistent seeds with *YAB* for each of the individual species can be found in the Appendix S1: panels G, and with relative seed mass in the Appendix S1: panels H.

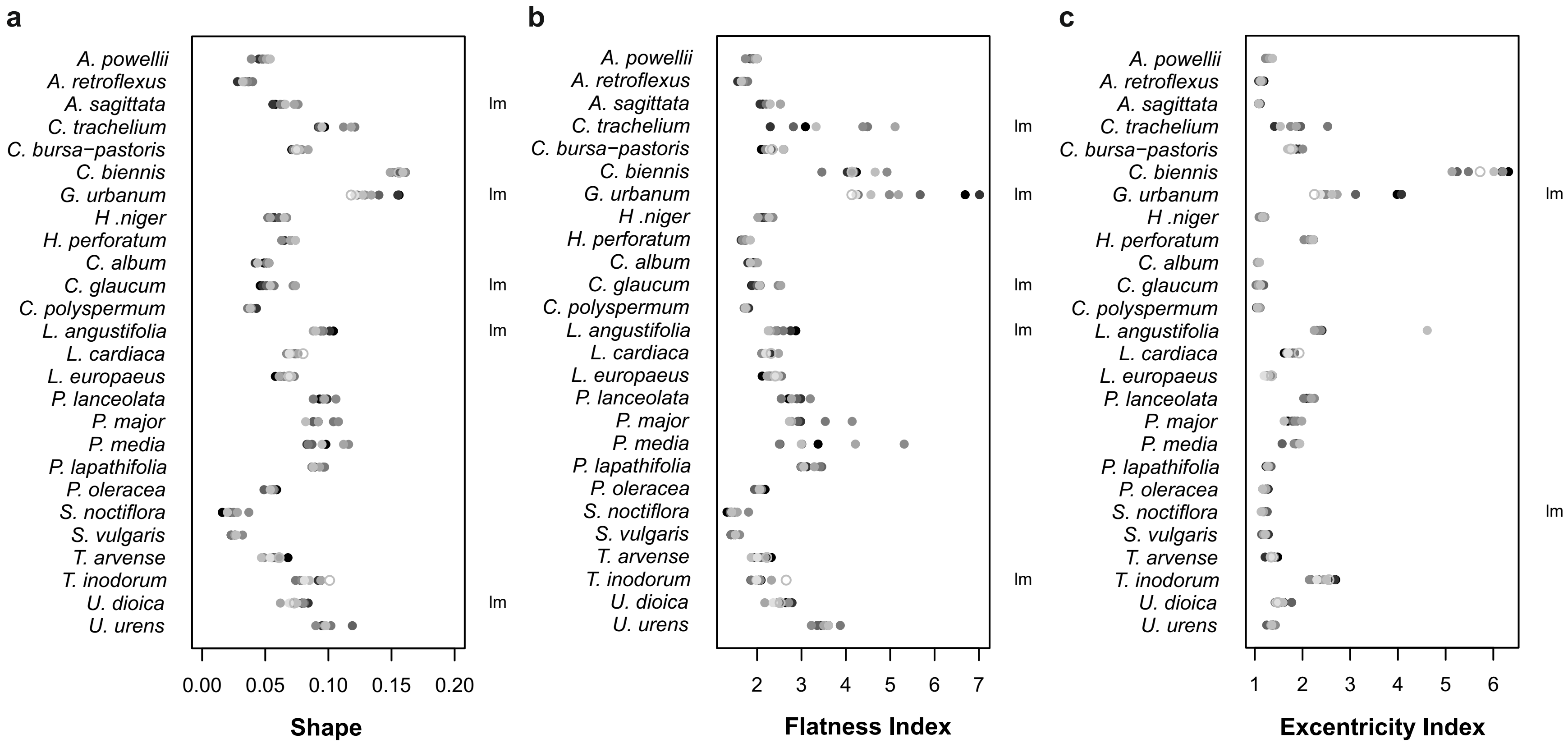

Figure 3. Variation in seed form with year after burial (*YAB*) for 26 species of seeds: (**a**) seed shape; (**b**) flatness index; (**c**) eccentricity index. The value of 0 indicates perfectly spherical seeds, while the value of 0.2 indicates flat or elongated seeds (Figure 3a). The larger the number the flatter (Figure 3b) or eccentric (Figure 3c) the seeds were. The decrease in the intensity of grey colour refers to the increase in *YAB* (i.e., a decrease in the intensity of grey colour): black = control, white with grey lining = *YAB 8* are the two extremes). Lm (linear) indicates the cases when the change in the trait over time was significant according to the linear model. See Table 1 for generic names of each species.

Table 2. Persistence of 26 species of seeds in soil as estimated by logistic regression based on the imbibed crush test (ICT). See Table 1 for the genus of plants. Year after burial (*YAB*) or seed mass relative to control seeds were used as explanatory variable based on 5–8 *YAB* cohorts (N *YAB*). Intercept and slope are estimated parameters a and b, respectively, of the model: $y = \frac{1}{1+e^{-(a+b*x)}}$, accompanied with 95% confidence intervals (CI), where y is the proportion of viable seeds and x is *YAB*. AIC—Akaike Information Criterion for the respective model. Significantly better models (ΔAIC>2) are in bold. Persistence times PT_{50} or PT_{05}, accompanied with standard errors (s.e.), show the time in *YAB* when 50 or 5% of the initial cohort of seeds, respectively, were predicted to be still viable.

Species	**N *YAB***	***YAB***						**Relative Mass**	
		Intercept (95% CI)	**Slope (95% CI)**	**P-Value**	**AIC**	**Persistence Time ± s.e. [*YAB*]**		***P*-Value**	**AIC**
						PT_{50}	PT_{05}		
A. powellii	6	6.578 (4.229–8.926)	−1.963 (−2.641–−1.285)	< 0.001	**70.617**	3.4 ± 0.16	4.9 ± 0.31	< 0.001	102.66
A. retroflexus	6	9.884 (5.372–14.396)	−3.785 (−5.445–−2.125)	< 0.001	**37.37**	2.6 ± 0.12	3.4 ± 0.2	< 0.001	62.65
A. sagittata	6	1.833 (0.891–2.775)	−1.519 (−2.070–−0.968)	< 0.001	**82.18**	1.2 ± 0.19	3.2 ± 0.38	< 0.001	92.14
C. trachelium	6	5.051 (2.838–7.265)	−2.727 (−3.846–−1.609)	< 0.001	**52.30**	1.9 ± 0.14	2.9 ± 0.26	< 0.001	54.77
C. bursa-pastoris	8	4.051 (2.900–5.202)	−0.809 (−1.033–−0.585)	< 0.001	**149.39**	5.0 ± 0.26	8.6 ± 0.60	0.215	243.91
C. album	6	4.938 (3.320–6.557)	−1.130 (−1.505–−0.756)	< 0.001	106.17	4.4 ± 0.22	7.0 ± 0.53	< 0.001	**64.09**
C. glaucum	6	41.940 (-)	−21.663 (-)	< 0.001	24.02	1.9 ± 9.8	2.1 ± 11.05	< 0.001	24.02
C. polyspermum	6	4.537 (2.915–6.159)	−0.791 (−1.133–−0.449)	< 0.001	**105.66**	5.7 ± 0.41	9.5 ± 1.13	< 0.001	115.02
C. biennis	7	2.785 (1.637–3.933)	−1.428 (−1.94–−0.909)	< 0.001	87.49	2.0 ± 0.19	4.0 ± 0.43	< 0.001	86.55
G. urbanum	8	2.448 (1.601–3.295)	−0.238 (−0.393–−0.083)	0.002	174.23	10.3 ± 1.96	22.7 ± 5.96	0.001	172.40
H. niger	6	4.769(2.539–6.998)	−0.513 (−0.976–−0.049)	0.014	**59.25**	9.3 ± 2.32	15.1 ± 4.91	0.537	64.95
H. perforatum	6	2.821 (1.819–3.823)	−0.495 (−0.730–−0.260)	< 0.001	140.85	5.7 ± 0.61	11.6 ± 1 93	< 0.001	**136.22**
L. angustifolia	6	1.595 (0.799–2.392)	−1.000 (−1.334–−0.666)	< 0.001	114.67	2.1 ± 0.16	5.3 ± 0.34	< 0.001	**101.73**
L. cardiaca	8	2.387 (1.600–3.174)	−0.353 (−0.499–−0.207)	< 0.001	198.99	6.8 ± 0.64	15.1 ± 2.23	< 0.001	**189.88**
L. europaeus	8	2.182 (1.360–3.004)	−0.916 (−1.181–−0.652)	< 0.001	132.60	2.4 ± 0.25	5.6 ± 0.50	< 0.001	131.99
P. lanceolata	6	5.099 (2.859–7.340)	−2.192 (−3.158–−1.225)	< 0.001	58.17	2.2 ± 0.18	5.2 ± 0.6	< 0.001	57.60
P. major	5	0.959 (0.312–1.606)	−0.161 (−0.335–−0.013)	0.067	187.35	6.0 ± 1.91	24.3 ± 11.74	< 0.001	**125.59**
P. media	6	2.382 (1.495–3.270)	−0.909 (−1.193–−0.625)	< 0.001	129.86	2.6 ± 0.25	5.9 ± 0.56	< 0.001	**90.92**
P. lapathifolia	6	5.449 (3.595–7.303)	−1.650 (−2.187–−1.114)	< 0.001	82.56	3.3 ± 0.18	5.1 ± 0.35	< 0.001	**73.21**
P. oleracea	6	3.064 (1.988–4.140)	−1.184 (−1.548–−0.820)	< 0.001	**108.33**	2.6 ± 0.21	5.1 ± 0.44	< 0.001	128.00
S. noctiflora	6	3.545 (2.153–4.937)	−0.415 (−0.724–−0.106)	0.004	95.29	8.5 ± 1.79	15.6 ± 4.41	< 0.001	**88.63**
S. vulgaris	6	3.126 (1.790–4.461)	−2.025 (−2.770–−1.279)	< 0.001	67.18	1.5 ± 0.16	3.0 ± 0.31	< 0.001	**62.9**
T. arvense	8	26.566 (-)	0.000 (-)	1	4	inf	inf	1	4
T. inodorum	8	2.357 (1.528–3.185)	−0.860 (−1.101–−0.619)	< 0.001	141.61	2.7 ± 0.25	6.2 ± 0.52	< 0.001	**127.15**
U. dioica	8	2.777 (1.835–3.719)	−0.265 (−0.432–−0.097)	0.001	**158.69**	10.5 ± 1.91	21.6 ± 5.40	0.015	163.42
U. urens	6	2.284 (1.380–3.188)	−0.781 (−1.073–−0.489)	< 0.001	**130.70**	2.9 ± 0.28	6.7 ± 0.81	< 0.001	135.24

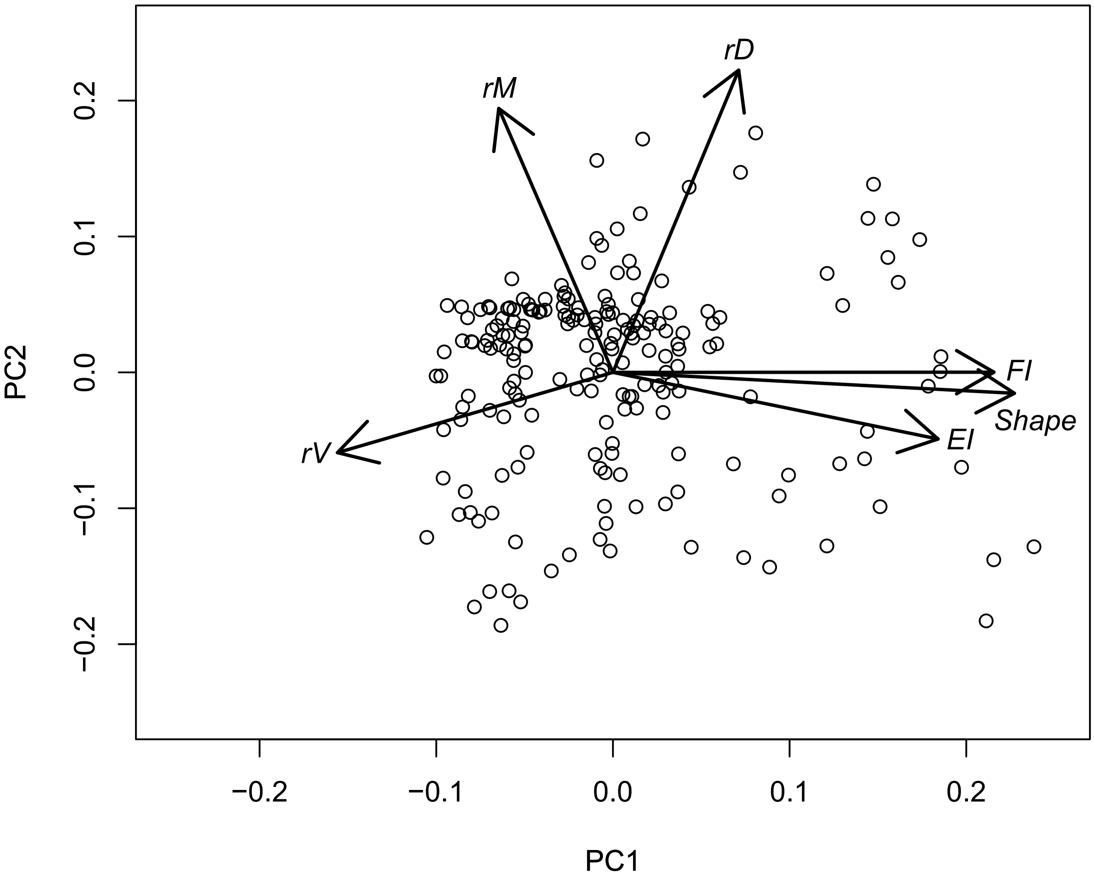

Figure 4. Principal component analysis in variation in seed form across species of seed and year after burial (*YAB*). PC1 and PC2 together explain 75.7% of the variance. Symbols indicate combination of seed species and *YAB*.

The persistence times of particular seed species in soil predicted based on the ICT are shown in Table 2. In some species, such as *A. sagittata*, the PT_{50} was predicted to be as low as 1 year, while in *G. urbanum*, the PT_{50} reached 10 years. The PT_{05} ranged from 2 (*C. glaucum*) to more than 20 years (*U. dioica* and *P. major*). As all seeds were found to be viable in *T. arvense*, the persistence in soil could not be estimated for this seed based on our data.

Across the species, predicted persistence was not related to *M*, *V*, *D* nor any of the three indexes for seed forms, using data for control seeds (results not shown). On the other hand, the PT_{50} was negatively related to the *rM* of buried seeds (Figure 5a). The relationship of the *rM* with PT_{50} for *YAB 1* and *YAB 2* cohorts was much steeper compared to the remaining *YAB* cohorts, suggesting that the seed species losing their mass in the first two years of burial survive in soil relatively shorter. The results were also similar for PT_{05}, with the exception of the insignificant relationship with *YAB 1* (not shown). The relative volume of neither cohort significantly explained the PT_{50} nor PT_{05} (not shown), and neither did the relative density of *YAB 1* and *YAB 2*. In contrast, seed persistence was negatively affected by the decrease in relative density in cohorts of *YAB 3–8* (Figure 5b), suggesting that seeds that lose relatively more mass than volume suffer from shorter lifespan in soil. Change in seed form indexes seemed to affect the predicted persistence (Figure 5c), but the effect was due to the outlying *G. urbanum* seed, which greatly changed its shape as a result losing seed coat and its appendages, so the trends were no longer significant when this species was removed from the analyses.

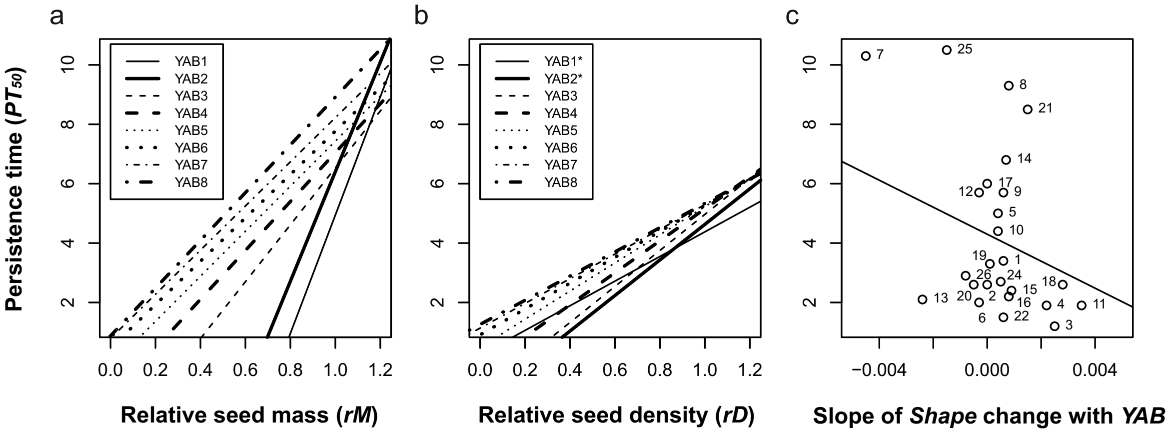

Figure 5. Effects of changes in selected morphological seed traits in particular cohorts on PT_{50}. (**a**) Relative seed mass (only regression slopes are shown); (**b**) Relative seed density (only regression slopes are shown; * slopes not significantly different from 0); (**c**) Slope of change in seed shape over years after burial (*YAB*). Symbols indicate seed species, for species codes, see Table 1.

4. Discussion

The seed characteristics investigated in this study involve traits that directly affect the fitness of plant species as well as traits that are most likely relevant only with respect to the perception and attractiveness of seeds to seed predators. The seeds were generally losing viability gradually over time when buried in the soil, and this loss of viability was accompanied by more or less noticeable morphological changes. Of these changes, the loss of mass and volume were the most prominent, although changes in other traits (seed density and shape) appeared to be significant in some species of seeds as well.

Seed persistence. Seed viability is known to non-linearly decline with time spent in soil as seeds decay or age [14,17,25,42], and this was true for the vast majority of species included in this study. Exponential decay rates were previously used for fitting decline in seed viability and for estimating the annual decay rate [14,43]. However, the visual inspection of the plots in the abovementioned studies clearly shows that the exponential curve was not capable of catching the uneven decline rate over time (see, e.g., Figure 1 in Conn et al. [43], plots for *C. bursa-pastoris* or *T. inodorum*, among others), resulting in the prediction of unrealistic annual decay rates. In this paper, we took advantage of using GLM with a binomial distribution of errors, which is a linearized form of a logistic regression, for describing the course of decline in seed viability, approximated by using the change in the proportion of persistent seeds in artificial seed banks over time. The method for analysing and predicting the persistence of seeds from soil seed banks at the population level was previously used, e.g., in work of Pakeman et al. [17], and we encourage others to do so as well.

We found this method to be robust, well grounded in mathematical theory, and easy to perform and interpret, and more importantly, it can be used for modelling seed persistence in soil. The greatest advantage of this approach is that one can describe the dynamics of seed persistence in time (or with any other variable that may have influenced the persistence, such as mass also used in this study) in a realistic way and estimate the persistence times. Using this method more frequently, the estimation of seed persistence in soil would approximate the reality closer, and would be more comparable among studies. The prerequisite for using this method is that a proportion of a seed population at each time point must be known, as estimated by a subset of the retrieved seeds, as in this study. Another advantage is that by using the appropriate distribution and link function, no data transformation is needed. Other researchers used various logistic regression models for analysing the decay/survival of seeds in soil [42,44] or complicated probit-based models [25]. Depending on the nature of their data (either Poisson or binomial distribution of errors), various data transformations were applied to approach counts (Poisson) or proportions (binomial) to Gaussian distribution, allowing for fitting the chosen model. Although fitting some of these models may provide seed persistence curves and hence help with making predictions, we argue that GLM with binomial error structure shall be the preferred method in such studies due to its simplicity and accuracy, and this should be taken into consideration already when seed persistence data are collected.

We consider that wider application of logistic regression models in seed bank persistence studies would greatly increase our understanding of the dynamics of seed banks and that this approach represents a major step forward from classifying species into groups according to the detected persistence of their seeds, e.g., transient (< 1 year), short persistent (1–5 years) and long persistent (> 5 years) [2]. Such classification might erroneously suggest that seed persistence in soil is a discrete process, although the logistic approach has demonstrated that losing viability is continuous process and that a minor proportion of seed population may persist for a long time. For example, it can be predicted for *C. trachelium*, one of the least persistent species in our study, that 2.1 seeds out of a hypothetical 10^5 initial seeds will survive for five years in the soil, using the parameters describing the course of change in the proportion of persistent seeds estimated here, and their detection (and classification in the respective persistence class) depends on the sampling method, sample sorting precision and, of course, coincidence of finding viable seeds in soil samples. Therefore, literature data on seed persistence are extremely variable for each species (see, e.g., database of Thompson et al. [2]), and it is not rare that a species is classified as transient in one study and long persistent in another (e.g., *A. retroflexus* used also in this study according to the database of Thompson et al. [2]). There are obvious ecological reasons that may have caused such differences, including interpopulation or geographical variation, soil conditions and microflora [9,12,15,17,45], but such variation can also be largely attributed to the methods used for data collection (burial vs. emergence, soil depth) and evaluation. It is a well-established fact that seeds tend to germinate earlier and thus remain in a seed bank for less time when buried at shallow depths compared to deeper burial [25]. It also seems that seed emergence data are less reliable than seed burial experiments basically because species with larger seed production are more likely to be detected in soil for a longer period of time [46]. To address the variability among studies, several authors developed indexes [4,8,19] that consider the relative frequency of studies reporting a class of seed persistence and unite the known persistence data in one number. However, these indexes are inherently biased when different methods of seed persistence evaluation are combined for calculation [46] and therefore ecologically meaningless, despite their frequent use in ecological papers. For the sake of demonstration, we calculated the longevity index [4,19] and seed persistence index [8] for the 14 species for which data were available using only the burial data from the database of Thompson et al. [2]. The variation in the indexes values were in fact minor among species (Table 1), and all of them could be classified as long persistent if one would wish to do so, despite the variation in persistence times found in our study. In fact, papers that would provide reliably calculated predictions of seed persistence times are scarce, making difficult any comparison with persistence times PT_{50} and PT_{05} predicted in this study. For the species included in this study, we found only one reference [17], and interestingly, by using binomial GLM, the estimated PT_{50} for *P. lanceolata* (2.35 ± 0.43) was nearly identical to that from our study (2.2 ± 0.18). Others provided persistence times based on an exponential decay formula, but the resulting values are not comparable due to lack of fit (see above). We attempted to find patterns in the variation in persistence times and ecological explanation of these patterns by using morphological data of the control seeds as well as data describing the morphology change of seeds over time spent in soil. We found that predicted persistence times were unrelated to any of the morphological traits of the control seeds available, which contradicts the previously published literature data (e.g. [6,7,19,45,47]). Nevertheless, we found that a relative change in seed mass affected the predicted persistence. Seed species that lose mass rapidly already in the first two years of burial survive in soil relatively shorter. Seeds that lose relatively more mass than volume, i.e., seeds that have a rather strong seed coat, would suffer from a shorter life span in the soil as the embryo deteriorates. Additionally, relative seed mass explained the decline in the proportion of persistent seeds better than *YAB* in a significant portion of the species studied, mainly in those in which an increase in the proportion of persistent seeds occurred after a period of decline. Such reversal periodically appears in the literature [14,21,22,26]) and is sometimes explained as a result of microsite variation in soil conditions favourable or disfavouring seed persistence. Additionally, the persistent times estimated in this study might be overestimating those that would occur in the field since these were based on identifiable seeds only and neglecting those which deteriorated completely. As seed mass generally declines with *YAB* non-linearly, using seed mass as an explanatory variable for the decline in the proportion of persistent seeds partly removed this scatter from the data. Congeneric species included in this study were expected to have similar persistence times because of common evolution and thus presumed taxonomic constraints on seed biology and similar life histories [12]. Contrary to this expectation, the variation among congeners was quite large in most cases; only the species of *Amaranthus* had rather similarly short persistence times. It is difficult to provide an explanation for such a scatter based on a limited number of species per genus (or family), and more likely, functional traits of plants and their habitat requirements might help to disentangle the ecological significance, if it exists, of such a striking scatter.

Seed morphology. The limited availability of descriptions of changes in external morphology and seed mass through time spent in soil is evident because these traits have not seemed to be important for species' ecology or economic importance. They may, however, have vital importance in determining the consumption rate of seed predators [48] and for identifying seeds from seed banks. We provide evidence that over the study period, the seed mass, volume and density largely become altered. There are several possible causes that result in changes in seed mass, volume, density and form through seed aging and decay. These relate to deterioration of the external structures, decay of the embryo and shrinking of the seed. As changes in specific descriptors of seed morphology were often correlated for a particular species of seed, the nature of the correlations provides insight into the manner of morphological changes the seeds underwent during soil burial. Significant change in seed shape indicators with *YAB* was caused by the reduction in volume. This can be a consequence of shrinking soft seeds due to death and deterioration of the embryo (e.g., *C. trachelium*), so the density remained unchanged. In a special case of *G. urbanum,* the seed coat of the control seeds is soft and equipped with a hook. Both structures deteriorate over time, which changes the overall seed shape and even increases seed density. Our results suggest that the seed coat does not have protective function in this species since *G. urbanum* was also one of the longest persistent species in our study. Many seeds did not change their volume over time in soil but mass (e.g., *A. powellii*), which indicates that these seeds are hard-coated with embryos dying and deteriorating. The fact that in some species of seeds the relative mass and volume was observed to "increase" after burial has to be viewed as a result of natural variability in seed size; alternatively it may partly be an artefact that the smallest seeds deteriorated first, so these were not available for taking morphological measurements.

The morphological changes and changes in seed mass over time spent in soil can also be well described by logistic regression in many species, and it seems plausible that this approach will find wide application in seed ecology studies. In addition to seed persistence [17,42,49] and morphology (this study), logistic models were recently applied for describing germination response to environmental conditions [50]. Thus, we propose using these models in situations in which the response of this type of seed to a focal variable can be expected.

5. Conclusions

In this study, we show on 26 species of seeds, how selected seed traits change with the course of burial in soil. Using this unique dataset, we described the patterns in the change of the proportion of persistent seeds and in morphology by using modern statistical methods such as logistic regression, which is a robust tool and provides comparable results across studies. Describing these changes using robust tools is important for obtaining better insight into seed bank biology and ecology and for making more accurate predictions of seed persistence of, e.g., arable weeds, and better plan their management.

Supplementary Materials: Appendix S1—Variability in seed traits over time spent in seed bank for 26 species of seeds. Supplementary materials can be found at http://www.mdpi.com/2073-4395/10/3/448/s1.

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ORIGINAL ARTICLE

Burial-induced changes in the seed preferences of carabid beetles (Coleoptera: Carabidae)

Pavel SASKA[1], Alois HONĚK[1], Hana FOFFOVÁ[1,2] and Zdenka MARTINKOVÁ[1]

[1] Crop Research Institute, Functional Diversity in Agro-Ecosystems, Drnovská 507, 161 06 Praha 6 – Ruzyně, Czech Republic; e-mails: saska@vurv.cz, honek@vurv.cz, foffova@vurv.cz, martinkova@vurv.cz

[2] Czech University of Life Sciences, Faculty of Environmental Sciences, Department of Ecology, Kamýcká 129, 165 00 Praha 6 – Suchdol, Czech Republic

Key words. Coleoptera, Carabidae, food preferences, granivory, seed consumption, seed burial, weeds

Abstract. Seeds of many species of plants may survive for a long time in the soil and germinate when brought to the surface, but whether they are subsequently eaten by seed predators is unknown. We examined the preferences of three species of carabids (Coleoptera: Carabidae) for 25 species of seeds and determined the difference in palatability between freshly dispersed and those buried for six years. The stability of their preferences was tested using a collection of seeds of different species, each of which was offered fresh or after being buried. Carabid beetles readily accepted previously buried seeds as food. In total, *Pseudoophonus rufipes* and *Amara littorea* ate more fresh seeds than previously buried seeds, while the opposite was true for *Harpalus affinis*. The seeds of some species were even more attractive to carabids after burial than in the fresh state. For all the species of carabids tested, the diet breadth was similar when the beetles were fed fresh or buried seeds, but the preferences for fresh and buried seed of particular species were correlated only in *P. rufipes* and *A. littorea*. We measured the seed characteristics (mass and viability) likely to be associated with the loss of attractiveness to carabids during burial. The change in carabid consumption was not related to changes in any of these characteristics. This finding indicates that factors responsible for variation in seed acceptability are complex. This study provides the first conclusive evidence that invertebrate seed predators will feed on seeds from seed banks, although they prefer fresh seeds.

INTRODUCTION

Seed mortality is an important factor in the population biology of plants (Harper, 1977; Larios et al., 2017), and an important component of seed mortality is post-dispersal seed predation, which typically occurs on the surface of soil where seed released from mother plants are exposed before germinating or entering the soil seed bank. While predation immediately following seed dispersal has been intensively studied (Kulkarni et al., 2017), little attention has been paid to predation of seeds that previously were buried in the soil for a period of time. Seeds may persist in the soil and remain viable for many years (Baskin & Baskin, 1998). Some species form only a transient seed bank, while seeds of other species remain alive in soil for many years (Thompson et al., 1997; Fenner & Thompson, 2005; Long et al., 2015). Plant species that form a persistent seed bank are typically characterised by a strong and impermeable seed testa (Gardarin & Colbach, 2015), which helps them to survive the soil conditions until they become favourable for germination. In soil, seed mortality is caused mainly by soil microbial pathogens or decomposers (Blaney & Kotanen, 2001; Schafer & Kotanen, 2004; Davis et al., 2006; Wagner & Mitschunas, 2008) but is also due to predation by earthworms (Eisenhauer et al., 2010), carabids (Kulkarni et al., 2015) and rodents (Hulme, 1998). Buried seeds are less likely to be found by surface active seed predators compared with seeds that remain exposed and uncovered (Hulme, 1988; White et al., 2007; Kulkarni et al., 2015; but see Ruzi et al., 2017).

A proportion of the seeds buried in soil eventually come to the surface as a result of soil mixing activities, tillage, freeze-thaw cycles and bioturbation. This provides an opportunity for the seed to germinate but also exposes it to surface predators for the second time. Predation may occur during this period, but very little is known about the predation of previously buried seeds. To date, this topic is considered in only two studies. Martinkova et al. (2006) studied the fate of the seeds of six species of weeds exposed to two species of ground beetle. Following burial for half a year, the consumption of the seed of four species did not change, one species (*Tripleurospermum inodorum* (L.) Schultz-Bip.) was accepted at a higher rate than when fresh and one was not eaten by the predators (*Taraxacum* agg., section Ruderalia). Koprdova et al. (2012) report different responses of invertebrate predators to previously buried, fresh, imbibed and germinating seed of *Brassica napus* ssp. *napus* L.

Predators use scent to locate (Kulkarni et al., 2017) and physical traits to evaluate the quality (Honek et al., 2007; Lundgren & Rosentratter, 2007) of seed prior to accepting it. Therefore, any change in the properties and traits of seed that occurs during burial may affect the likelihood of it being eaten by predators (Martinkova et al., 2006). A study that includes the seed of more species of plants and predators of seeds and compares the consumption of fresh seed with that seed buried for some time is warranted to elucidate the extent of predator pressure on seed released from the seed bank.

In this paper, we investigate the response of three carabid species in terms of seed consumption of the seed of 25 species of herbaceous plants provided fresh and after being buried in soil for six years. In this study, we address three questions: (1) Is the consumption by carabid predators higher or lower when offered previously buried seed compared to fresh seed? As a previous study (Martinkova et al., 2006) shows that the patterns in the change in consumption of six species of seed is variable, we do not predict the rate and direction of carabid response to previously buried seed. Instead, using a wider range of species of seed, we explore what proportion of the different species of seed becomes less or more preferred after burial. (2) How does the diet breadth change when carabids are offered fresh or previously buried seed? In theory, an animal may adjust its diet breadth in response to a change in the available range and quality of food (Sexton et al., 2017). It is likely that the size of such an adjustment depends on the degree of food specialization of particular species. The guild of carabid granivores is quite diverse and includes unspecialized omnivores that accept a wide range of different seeds (have a broad seed diet breadth), while other species show marked preferences (have narrow seed diet breadth) (e.g., Talarico et al., 2016). We hypothesize that the diet breadth will remain the same or become broader in generalists because seed that loses its morphological or chemical defence will become more acceptable to predators. Generalists enlarge the range of acceptable food or "replace" species of seed that loses its attractiveness or decays. In contrast, we expect that the diet breadth of a specialist would tend to become narrower as preferred seed is more likely lose its attractiveness during burial in contrast to non-preferred seed, which becomes more attractive. (3) Is the change in consumption after burial related to a change in seed properties? Seed transformation during burial may include changes in biological, chemical and physical properties. At the population level, seed mass varies with time spent in soil (authors' unpubl. data) because the proportion of dead seeds increases or their seed coats are eroded. These changes in seed characteristics may have consequences for their attractiveness to predators. As it is known that changes occur in the seed material used in this study (authors' unpubl. data), we test whether the change in consumption is related to change in seed viability or mass due to burial. We hypothesize that the change in consumption will be greater for the seed of plants that produce a high proportion of dead seed. The response in terms of a decrease in consumption due to seed mass can be more variable and more difficult to predict, depending on the cause of the reduction in seed mass, as mentioned above.

MATERIALS AND METHODS

Seed material

We used seed of 25 common herbaceous plants that differ in size, morphology, taxonomic position and persistence in soil and are likely to be available to carabid seed predators (Table 1) (Thompson et al., 1997; Grime et al., 2007). Shortly after dispersal from mother plants, these seeds differ in attractiveness for carabid seed predators (Honek et al., 2007). Seed was collected from stands of wild herbaceous plants in 10 × 10-km area centred at 50.088N and 14.274E (surroundings of Prague – Ruzyně, western Czech Republic). The seed was collected from mother plants at full ripeness in July–October 2005 and October 2006, dried at room temperature for 20 days and then stored at 5°C until buried. The seed of each species were each placed in a separate bag. The nylon fabric bags were made to contain approximately 1000 seeds mixed with sieved soil. Mixing with soil is important as it prevents the seed amassing into large clumps and its subsequent degradation (Van Mourik et al., 2005). Finely sieved soil facilitates the separation of the seed when the bags are dug up. For the entire experiment (including seed burial and preference trials), soil was obtained from one place at one time at a depth of 0.6 m and sieved through 0.05-mm mesh. This prevented the soil being contaminated with seed from the natural soil seed bank.

At the beginning of November in the years when the seed was collected (2005 and 2006, Table 1), the bags were buried at a depth of 20-cm in grassland in the grounds of the Crop Research Institute at Prague – Ruzyně (50.08581N, 14.29727E). To facilitate recovery, the bags were connected by a nylon cord to a label on the surface of the ground. Six years after burial, the bags were dug up in late April. The seeds were separated from the soil, dried at 25°C and 40% r.h., and then stored at –20°C until used in the experiments. Samples of fresh seeds used in this experiment (a control) were stored at –20°C from the beginning of the experiment. Hereafter, we refer to freshly frozen control seeds, as "fresh" seeds, and seeds dug up after six years, as "buried" seeds.

Seed quality

In this study, we measured two seed properties/traits presumed to change during burial: seed viability and seed mass. Viability of seeds was estimated by means of the imbibed seed crush test, the results of which are highly correlated with those of the classical tetrazolium chloride test (Borza et al., 2007). Twenty seeds per cohort were left to absorb water for 3 days and then crushed with the tips of a pair of forceps. If cotyledons or embryos appeared or the seed did not collapse, the seed was considered to be viable; if not, the seeds were considered to be dead. Seed mass was measured using seeds cleaned of dust and fine soil particles in an ultrasound cleaner (Sonorex RK 31, Bandelin Electronic, Berlin, Germany), submerged in water for 2 min and dried in an oven for 24 h at 75°C. The average seed mass was determined based on five batches of 20 seeds per species and cohort using an analytical balance (CP225D-0CE, Sartorius AG, Göttingen, Germany) with a precision of 0.00001 g. The fresh seeds and buried seeds are henceforth referred to as "cohorts".

Table 1. Species of seeds used in this study, their dry mass (control seeds) and use in the experiments with three species carabids. The plant nomenclature follows Kubát et al. (2002). Carabid species: PR – *Pseudoophonus rufipes*; HA – *Harpalus affinis*; AL – *Amara littorea*.

Species	Family	Year of burial	Dry mass [mg ± SE]	PR	HA	AL
Amaranthus powellii S. Watson	Amaranthaceae	2006	0.494 ± 0.013	x	x	x
Amaranthus retroflexus L.	Amaranthaceae	2006	0.494 ± 0.007	x	x	x
Campanula trachelium L.	Campanulaceae	2006	0.134 ± 0.005	x	x	
Capsella bursa-pastoris (L.) Med.	Brassicaceae	2005	0.143 ± 0.017	x	x	x
Chenopodium album agg.	Amaranthaceae	2006	0.583 ± 0.008	x	x	x
Chenopodium glaucum L.	Amaranthaceae	2006	0.186 ± 0.005	x	x	x
Chenopodium polyspermum L.	Amaranthaceae	2006	0.285 ± 0.009	x	x	x
Crepis biennis L.	Asteraceae	2005	0.743 ± 0.034	x		
Geum urbanum L.	Rosaceae	2005	2.177 ± 0.073	x		
Hyoscyamus niger L.	Solanaceae	2006	0.613 ± 0.023	x	x	x
Hypericum perforatum L.	Hypericaceae	2006	0.115 ± 0.003	x	x	x
Lavandula angustifolia Mill.	Lamiaceae	2006	0.907 ± 0.025	x	x	x
Leonurus cardiaca L.	Lamiaceae	2005	0.672 ± 0.061	x	x	x
Lycopus europaeus L.	Lamiaceae	2005	0.262 ± 0.005	x	x	x
Persicaria lapathifolia (L.) Delarbre	Polygonaceae	2006	2.023 ± 0.028	x	x	x
Plantago lanceolata L.	Plantaginaceae	2006	0.256 ± 0.009	x	x	x
Plantago major L.	Plantaginaceae	2006	0.236 ± 0.006	x	x	x
Plantago media L.	Plantaginaceae	2006	1.407 ± 0.020	x		
Portulaca oleracea L.	Portulacaceae	2006	0.154 ± 0.005	x	x	x
Silene noctiflora L.	Caryophyllaceae	2006	1.037 ± 0.008	x	x	x
Silene vulgaris (Moench) Garcke	Caryophyllaceae	2006	0.642 ± 0.015	x	x	x
Thlaspi arvense L.	Brassicaceae	2005	1.167 ± 0.025	x	x	x
Tripleurospermum inodorum (L.) Schultz-Bip.	Asteraceae	2005	0.287 ± 0.012	x	x	x
Urtica dioica L.	Urticaceae	2005	0.153 ± 0.010	x	x	x
Urtica urens L.	Urticaceae	2006	0.389 ± 0.010	x	x	x

Predators

In this study, we used three species of ground beetles (Coleoptera: Carabidae) abundant in arable fields: *Pseudoophonus rufipes* (DeGeer) (body length = 13.8 mm, dry body mass = 31.3 mg), *Harpalus affinis* (Schrank) (body length = 10.2 mm, dry body mass = 14.1 mg) and *Amara littorea* C.G. Thomas (body length = 7.7 mm, dry body mass = 6.7 mg). Body length was taken from Hůrka (1996), and dry body mass was calculated from the body length using the formula of Jarošík (1989). The differences in carabid body size enabled the testing of the effects of (i) the carabid seed size ratio that appears to be important in determining the seed preferences of carabid beetles (Honek et al., 2007) and (ii) the diversity of body sizes of carabid seed predators in natural communities in arable fields. The carabids were collected by means of pitfall traps in the same area as the seeds in June and July 2014 (*P. rufipes*) and 2015 (*H. affinis* and *A. littorea*). To standardize hunger, the beetles were starved for 24 h prior to the experiments. According to previous experiments (Honek et al., 2003, 2007) the carabid species selected accept a range of the different species of seeds used in preference experiments.

Preference experiments

The preferences of the carabid beetles were examined by means of multi-choice cafeteria experiments. Petri dishes measuring 25 cm in diameter with a 0.8-cm deep layer of sieved soil at the bottom served as experimental arenas. The seeds were mounted on a tin tray filled with white modelling clay (Plasticina JOVI®, Barcelona) and pressed into the layer of soil so that the clay surface was flush with the soil surface (Honek et al., 2003; Saska et al., 2014). Trays filled each with thirty fresh or thirty buried seeds of a particular species were placed in a Petri dish and arranged in two concentric circles. Each Petri dish was then considered an experimental replicate and contained the full available range of different species of seed for a particular cohort presented simultaneously (Table 1). Five replicates (dishes) with fresh seed and five replicates with buried seed, each with three beetles per dish, were used for *P. rufipes*, four replicates with fresh seed and four replicates with buried seed, each with four beetles for *H. affinis* and four replicates with fresh seed and four replicates with buried seed, each with seven beetles for *A. littorea*. The difference in number of replicates was because the availability (abundance in the open) of the carabids differed. The number of beetles per tray differed because their body mass differed. The objective of this was to standardize the expected overall seed exploitation. The beetles removed and consumed the seed in the tin trays and the remaining seeds were counted daily. Trays were replaced if more than 15 seeds per tray were consumed. The experiments each lasted for four days.

Data analysis

The difference in overall seed consumption by individual carabid species was tested using GLM with Poisson distribution of errors (GLM-p) with counts of consumed seeds as the response variable and seed cohort (fresh vs. buried) as the factor. To investigate whether the diet breadth changed depending on provision of fresh or buried seeds, a modification of the standardized Levins' niche breadth index B_A was computed (Krebs, 2009) for each carabid species and seed cohort as follows:

$$B_A = \frac{\dfrac{1}{\sum p_i^2} - 1}{n-1}$$

where p_i is the proportion of total seed consumption made up of species of seed i, and n is the number of species of seeds. Diet breadth is narrow (low values of B_A) if a species accepts a low number of different species of seeds. Broad diet breadth (high values of B_A) means that a carabid accepts high numbers of different species of seeds. To classify the seed in terms of its attractiveness to carabids, consumption of each species of seed was standardized relative to the consumption of the most preferred species (the consumption of which was set at 1). Species were arbitrarily (Krebs, 2009) classified as preferred if the standardized consumption was > 0.2, consumed if > 0.05 but < 0.2, and rejected if < 0.05.

The change or stability in overall preferences was initially tested using Pearson correlation test for the complete dataset (species combined) and for each species of carabid separately (Crawley, 2007), assuming that a significant correlation indicates that the overall carabid preferences remains stable for both cohorts. Overlap of the diet breadth O_{jk} was computed according to Pianka (1973) as follows:

$$O_{jk} = \frac{\sum p_{ij}\, p_{ik}}{\sqrt{\sum p_{ij}^2\, p_{ik}^2}}$$

where p is the proportion of total seed consumption made up of seed species i, and j and k are the seed cohorts of fresh or buried seeds, respectively. The effect of cohort (fresh or buried) on consumption of individual species of seeds was further tested using the mixed effect model (R package lme4; Bates et al., 2015). Poisson distribution of errors was used as seeds of the most consumed species were replenished. The models included different species of seeds, burial (fresh vs. buried) and their interaction as fixed terms and replicate (Petri dish) as a random term. The analysis was repeated for each species of carabid separately. The significance of differences in the consumption of particular species of seeds between cohorts was assessed based on the confidence intervals of the differences. A difference was considered significant if the confidence interval of the difference excluded zero (Zar, 1999).

To test the hypothesis that particular seed traits affected the change in seed consumption, we calculated the differences in seed mass and seed viability of fresh and buried cohorts. As the decrease in seed mass and viability may be correlated and thus describe the same thing, we first estimated the strength of their relationship. Indeed, the two traits were correlated (Fig. S1), and an R^2 value that was not very high (0.42) and scattered along the line indicated that some species of seeds that lost mass were still viable. Using the change in both traits for explaining the change in seed consumption of the different cohorts is justified. All analyses were performed in R 3.3.1 (R Core Team, 2017).

RESULTS

Preferences for fresh seeds

The different species of carabid differed in their preferred species of fresh seeds (Fig. 1) and diet breadth. *Pseudoophonus rufipes* was the least choosy and preferred (standardized consumption of 0.2 or higher) 21 out of 25 species of seeds, resulting in a diet breadth of $B_A = 0.687$. The most preferred species of seeds in the fresh state were *U. urens*, *L. angustifolia* and *H. niger* (Fig. 1). *Harpalus affinis* preferred 15 out of 22 species of seeds ($B_A = 0.658$), and the most preferred fresh seeds were *U. urens*, *L. angustifolia* and *C. album* (Fig. 1). *Amara littorea* was the species with most restricted choice and preferred 4 out of 21 species of seeds ($B_A = 0.248$). The most preferred seeds for this carabid were *C. bursa-pastoris*, *T. arvense* and *T. inodorum* (Fig. 1).

Consumption and preferences for seeds after burial

Summed across seed species, *P. rufipes* and *A. littorea* consumed fewer buried seeds than fresh seeds (GLM-p, *P. rufipes*: $\chi^2_8 = 127$, $P << 0.001$; *A. littorea*: $\chi^2_6 = 8.66$, $P = 0.003$), while *H. affinis* consumed more buried than fresh seeds (GLM-p, $\chi^2_6 = 17.9$, $P < 0.001$). In *P. rufipes* and *H. affinis*, respectively, the diet breadths were similar ($B_A = 0.698$ for both species) and slightly broader in *A. littorea* ($B_A = 0.364$). Diet breadths of all the species of carabids largely overlapped between groups when fed fresh and buried seeds (O = 0.863 for *P. rufipes*, 0.801 for *H. affinis*, and 0.727 for *A. littorea*).

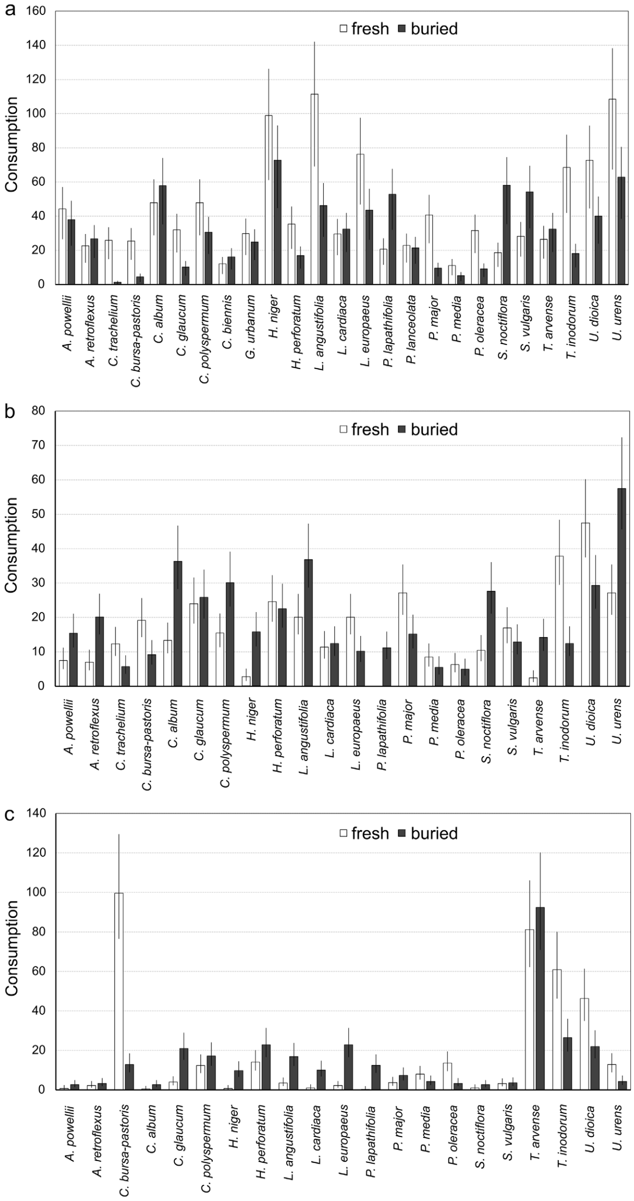

Fig. 1. Preferences for seeds of three species of carabids based on consumption in multi-choice experiments. Error bars indicate 95% confidence intervals. a – *P. rufipes*; b – *H. affinis*; c – *A. littorea*.

The overall preferences for seeds were correlated ($P < 0.05$) for fresh and buried seeds when data for all three species of carabids were combined, and similar results were obtained for *P. rufipes* and *A. littorea* (Table 2). In contrast, the preferences were not correlated (at $P < 0.05$) in *H. affinis* (Table 2). Low values of the correlation coefficients (close to 0.5) were significant only for *P. rufipes* and *A. littorea*, indicating that the consumption of fresh and buried seeds does vary. The presence of this variation was con-

Table 2. Correlation of carabid preferences for fresh and buried seeds.

Model	r	*t*	df	P-value
Species combined	0.707	8.109	66	<< 0.001
P. rufipes	0.524	2.948	23	0.007
H. affinis	0.356	1.701	22	0.104
A. littorea	0.596	3.232	19	0.004

firmed by the significant interaction of species of seeds and cohort in all mixed effect models (Table 3). Plots of the confidence intervals for differences in mean consumption between cohorts (Fig. 2) also reveal that the response of carabids to buried seeds largely depended on the species of seeds.

Regressing the changes in consumption on the changes in seed properties (viability and mass) did not reveal any significant trends (Fig. 3). The absence of a relationship between change in seed characteristics and carabid consumption indicates that the decline in viability or seed mass does not influence carabid preferences. In fact, each of the carabid species preferred the seed of at least one species of buried seed of which all were dead, over fresh seed. Obvious examples were *S. vulgaris* in the case of *P. rufipes*, *A. retroflexus* and *A. powellii* in the case of *H. affinis*, and *C. glaucum* in the case of *A. littorea* (Fig. 2).

DISCUSSION

This paper presents information on the consumption of seeds from the soil seed bank by carabids. Because of interspecific variation in the response of carabids to fresh and buried seed, it is difficult to generalize the results. Carabids prefer similar species of seeds in both fresh and buried states but consume smaller amounts of buried seed. The exception was *H. affinis*, which consumed more buried than fresh seed. The diet breadth remained the same or very similar when fresh of buried seeds were offered, regardless of the degree of food specialization of the carabid. This study thus revealed that carabid predation of previously buried seed is substantial and potentially an important component of the population dynamics of plants.

Using a modification of Levins' index (Krebs, 1999), we quantified the diet breadths of the three species of carabids. We expected that if it changed, there would be opposite changes in the diet breadth of generalists and specialists. However, a large change was not found, and all the carabids preferred similar number of species of the array provided. This finding may be related to the fact that for all the species, regardless of the similarity in their diet breadths and existing correlations between their preferences for fresh and buried seed, the actual number of fresh and buried seeds of a particular species consumed was significantly different, but the direction of change was both negative and positive. *A. littorea* is known to prefer seeds of *Brassicaceae* (Honěk et al., 2007), which was also confirmed in this study, as it consumed more seed of *C. bursa-pastoris* and *T. arvense* followed by *T. inodorum* of the family *Asteraceae*. The consumption of seed of *C. bursa pastoris* dramatically decreased after burial. In fact, the decrease in overall

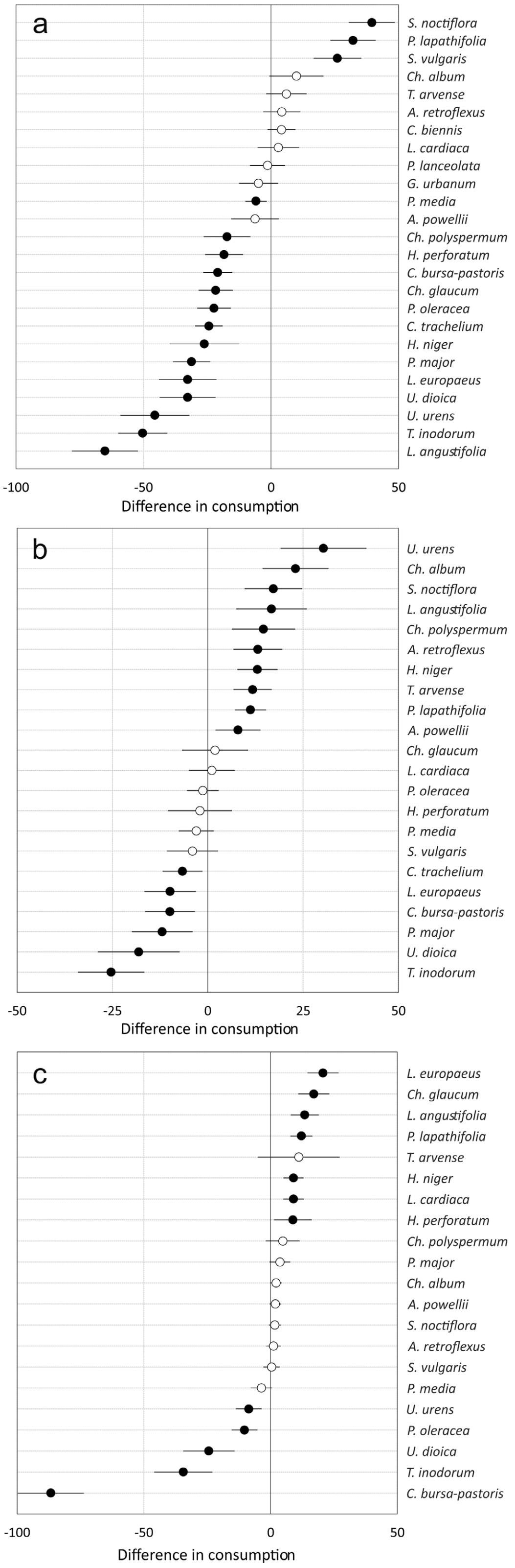

Fig. 2. Mean difference in consumption of fresh and buried seeds by three carabid beetles in multi-choice experiments. Points located on the right of the y-axis indicate seed species that were consumed more after 6 years in soil compared with the control; points located on the left of the y-axis indicate seed species in which burial for 6 years resulted in a reduction in consumption. Solid circles indicate species for which the mean difference was significant; open circles indicate species for which the mean difference was not significant. Horizontal lines indicate 95 % confidence intervals of the mean difference. a – *P. rufipes*; b – *H. affinis*; c – *A. littorea*.

Table 3. Effect of seed species and burial on consumption (GLMM) and standardized consumption (LME) by three carabid beetles.

Term	*P. rufipes*		*H. affinis*		*A. littorea*	
	χ^2 (df)	P-value	χ^2 (df)	P-value	χ^2 (df)	P-value
Seed * *Burial* [1]	1028.4 (24)	<< 0.001	450.1 (21)	<< 0.001	785.3 (20)	<< 0.001
Burial [2]	1.9 (1)	0.168	1.4 (1)	0.236	0.7 (1)	0.420
Seed [3]	3020.7 (24)	<< 0.001	932.2 (21)	<< 0.001	3357.7 (20)	<< 0.001

[1] Deletion test: *Seed* * *Burial* + (1|*Dish*) vs. *Seed* + *Burial* + (1|*Dish*); [2] Deletion test: *Seed* + *Burial* + (1|*Dish*) vs. *Seed* + (1|*Dish*); [3] Deletion test: *Seed* + *Burial* + (1|*Dish*) vs. *BURIED* + (1|*Dish*).

consumption of buried seed is mainly driven by this species. Conversely, seven species of seeds were significantly more consumed after burial, but their consumption was still notably low. In contrast, *P. rufipes* consumed the seed of different families before and after burial. Before burial, *L. angustifolia* (Lamiaceae), *U. dioica* (Urticaceae) and *H. niger* (Solanaceae) were the most preferred. After burial, *H. niger*, *U. urens* (Urticaceae) and *S. noctiflora* (Caryophyllaceae) were the most eaten. Overall, more species of buried seeds were consumed less than fresh seeds, and the total consumption after burial decreased. *Harpalus affinis* was exceptional in consuming more species of seeds after burial than in a fresh state. Seeds of *U. dioica*, *T. inodorum* and *P. major* (Plantaginaceae) were the most preferred in a fresh state, while *U. urens*, *L. angustifolia* and *C. album* were the most consumed after burial. Why the response of this species is different from that of the other two species of carabids is difficult to explain.

One interesting finding is that the seed of *P. lapathifolia* (Polygonaceae) was preferred by all species of carabids more after burial than fresh. In contrast, the seeds of *T. inodorum* and *C. bursa-pastoris* were consistently consumed less after burial than fresh. Many other species of seeds showed the same direction of change in consumption for two species of carabids. Seed qualities responsible for changes in preference are likely to be perceived and evaluated accordingly.

In this study, we a priori hypothesized that decreases in seed mass and viability are traits that explain the patterns in the change in seed attractiveness. However, our data do not support this hypothesis as the change in consumption was not correlated with decreases in seed mass or viabil-

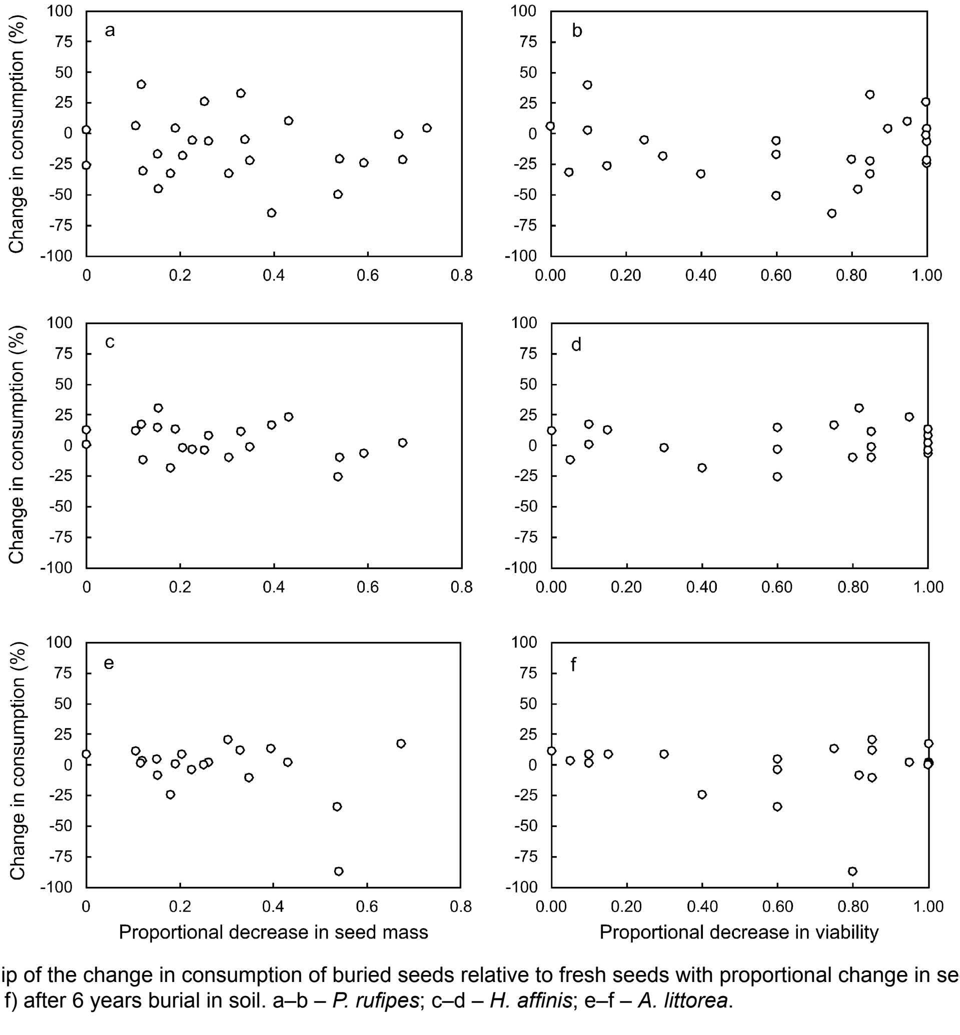

Fig. 3. Relationship of the change in consumption of buried seeds relative to fresh seeds with proportional change in seed mass (a, c, e) and viability (b, d, f) after 6 years burial in soil. a–b – *P. rufipes*; c–d – *H. affinis*; e–f – *A. littorea*.

ity. Changes in qualities not evaluated in this study were potentially involved in the changes in carabid preferences. Seeds in the soil are under continuous attack by soil-living microorganisms (Blaney & Kotanen, 2001, 2002; Dalling et al., 2011) and under the influence of soil chemistry (Long et al., 2009) which, in combination, may alter the chemical, biochemical and mechanical properties of the seed coat, including coat hardness and the semiochemicals present on the surface (Davis et al., 2008, 2016; Tiansawat et al., 2014). The cues that carabids use for selecting seeds are not fully understood. Carabid beetles use visual, tactile and olfactory cues when searching for food (Bauer & Kredler, 1993) and the cue that predominates can be deduced from carabid morphology, with those that have large eyes orientating visually and those with small eyes and few ommatidia using mainly olfactory cues (Bauer & Kredler, 1993). The morphology of the species used in this study indicate they may respond primarily to olfactory cues. Indeed, carabids locate seeds using the smells they produce when germinating (Kulkarni et al., 2017), which explains why more granivorous carabid beetles are caught by pitfall traps surrounded by seed (Honek & Martinkova, 2001). In this study, the change in preference is most likely due to changes in the (bio)chemical composition of the seed surface, which is faster in species in which the seed persists for only a short period than those that persist for a long period. This is attributed to the fact that short lived seeds rely more on chemical than mechanical defence (Davis et al., 2008). However, the hardness of the testa of seeds also decreases with time spent in soil (Tieu & Egerton-Warburton, 2000; Davis et al., 2016; but see Zalamea et al., 2015). Decreasing the strength of this mechanical protection facilitates the crushing and eating of seeds by carabids. We did not measure the toughness of the testa in this study, but noted a difference in a number of seed species during the viability assessment. In the crush test, the testa of buried seed was markedly thinner, softer or more fragile than that of fresh seed. The best example was *P. lapathifolia*, which was preferred more after being buried for a prolonged period due to the weakening of the (originally) thick testa. In some cases, carabids preferred even dead exhumed seeds to fresh seeds. Carabids thus may scavenge dead plant material and have no effect on plant population dynamics. The changes that seeds undergo in soil are very complex, and selecting only one seed characteristic as the explanatory variable for change in carabid preference might be misleading.

In conclusion, this study showed the effect of carabids on the mortality of previously buried seeds. Compared with fresh seeds, the consumption of buried seeds increased or decreased depending on both the species of seed and carabid. Determining to what extent the predation of buried seeds may have on the population biology of herbaceous species of plants requires further study.

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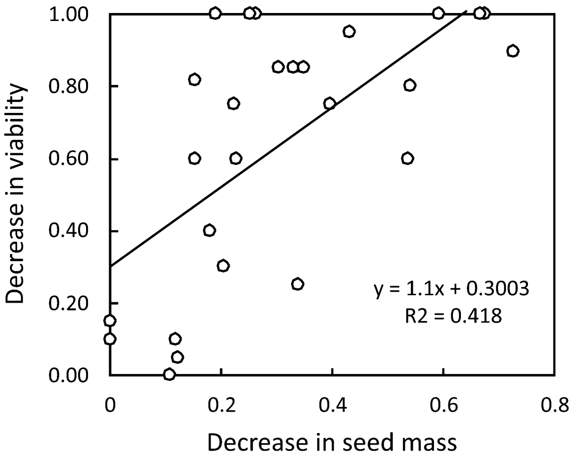

Fig. S1. Correlation between the changes in seed mass and viability after burial in soil for six years.

Seed associated bacteria are selected from soil by seed properties and changepreferences of a seed-feeding beetle

Autoři: Pavel Saska, Jan Kopecky, Marek Omelka, Alois Honek, Pavla Madrova, Petra Vokurková, Zdenka Martinkova, Hana Foffová, Marketa Sagova-Mareckova

Under review: Plant and Soil

Seed associated bacteria are selected from soil by seed properties and change preferences of a seed-feeding beetle.

Pavel Saska[1], Jan Kopecky[2], Marek Omelka[3], Alois Honek[1], Pavla Madrova[2], Petra Vokurková[4], Zdenka Martinkova[1], Hana Foffová[1,5], Marketa Sagova-Mareckova[6]

[1] Crop Research Institute, Group Functional Ecology, Drnovska 507, Prague 6 – Ruzyne, 161 06, Czech Republic

[2] Crop Research Institute, Group Epidemiology and Ecology of Microorganisms, Drnovska 507, Prague 6 – Ruzyne, 161 06, Czech Republic

[3] Charles University, Faculty of Mathematics and Physics, Dept. of Probability and Mathematical Statistics, Sokolovska 83, Prague 8, 186 75, Czech Republic

[4] Czech University of Life Sciences, Faculty of Agrobiology, Food and Natural Resources, Dept. of Soil Science and Soil Protection, Kamycka 129, 165 00, Prague – Suchdol

[5] Czech University of Life Sciences, Faculty of Environmental Sciences, Dept. of Ecology, Kamycka 129, 165 00, Prague – Suchdol

[6] Czech University of Life Sciences, Faculty of Agrobiology, Food and Natural Resources, Dept. of Microbiology, Nutrition and Dietetics, Kamycka 129, 165 00, Prague – Suchdol

* Corresponding author**:**

+420 702 087 624; mareckova@af.czu.cz

Abstract

Bacterial communities inhabiting seeds may interact with plant host but also with seed predators. The study aimed in showing bacterial communities associated with fresh seeds of seven weed species to compare them with seed communities after burial in soil for two years, and to demonstrate how these changes relate to seed mass, viability loss and seed attractiveness for a seed predator, *Pseudoophonus rufipes* (DeGeer) (Coleoptera: Carabidae). Bacterial diversity, assessed by Illumina MiSeq sequencing of 16S rRNA gene amplicon, generally increased, while seed viability and seed mass decreased with burial time. Mostly, the seed species differed in their microbiomes as well as in changes of seed properties together with their influence of seed attractiveness to the beetle. Seed microbiomes remained specific even after burial and contained taxa characteristic for both plant endophytes but also insect guts. In all seeds, 5 ZOTUs were common after one year of burial, after second year, only one common ZOTU remained. Seeds of *T. officinale* and *T. inodorum* lost their attractivness for the beetle, while seeds *T. arvense* improved their atractiveness after soil exposure, which was partially explained by dominance of endophytic and gut bacterial taxa in the seeds, respectively. We concluded that species specific relationships between seeds, soil-born bacterial community and beetle predation exist and may be relevant to ecosystem functioning including population dynamics of plants and weed management.

Key words: bacterial diversity; endophytes; seed bank; seed predation; three-level interactions; Carabidae

Introduction

Seeds of plants that do not germinate immediately after release from mother plant become part of a soil seed bank. The time, for which the seeds persist in soil is highly variable. Besides evolutionary constraints (some species inherently form only transient seeds while others tend to survive for many years), seed persistence correlates with seed size, shape, coat thickness (Thompson et al. 1993; Hodkinson et al. 1998; Gardarin et al. 2010; Schutte et al. 2014), plant ecology (Thompson et al. 1998; Long et al. 2009), but also with soil type and pH, nutrient content, disturbances, burial depth and (micro-)climatic conditions such as soil moisture and temperature (e.g. Bekker et al. 1998; Davis et al. 2005; Long et al. 2009; Pakeman et al. 2012). The seed persistence is, therefore, determined by both seed characteristics and their immediate environment (Long et al. 2015).

During their deposition in soil, seeds are colonised by soil microorganisms, which participate in seed decomposition and directly affect seed persistence in soil through altering seed morphology and physiology (Dalling et al. 2011; Long et al. 2015; Saska et al. 2020). Yet, some groups of microorganisms also have beneficial effects such as protecting seeds from decomposers and pathogens by chemical defence, and thus slowing down decay of seed enclosing structures or serving as plant growth promoting organisms after seed germination (Dalling et al. 2011; Long et al. 2015). Seed associated microorganisms may also produce signal compounds, which enable complex communication among bacteria themselves but also between bacteria and plants and consequently influence the entire soil food web (D'Alessandro et al. 2014). Most agricultural weeds are early successional plant species and, as such, are particularly apt to thrive in disturbed habitats like those created on agricultural land. Evidence is accumulating that the endophytic and associated microbiota support weeds' ability to strive in suboptimal environments, so their associations with microorganisms might be particularly strong (Trognitz et al. 2016).

Currently, plant core microbiome is sought to identify microorganisms closely associated with the host. The core microbiome may be defined at various scales from individual plants, populations, and species to ecosystems (Vandenkoornhuyse et al. 2015). Additionally, the interactions between the plant host and associated microorganisms may be extend by interactions with environment and environmental microbes, and defined as eco-holobionts to stress the transitory nature of many microorganisms associated with plants. The observed heterogeneity in the plant microbiome is probably the consequence of adaptation processes to given environmental conditions of the holobiont and allows rapid buffering of environmental changes (Singh et al. 2020). Yet until recently, many studies have focused on fungal endophytes in seeds, while the presence and role of bacterial seed endophytes are less known (Truyens et al. 2015).

Carabid beetles are major invertebrate seed predators in arable fields (Kulkarni et al. 2015) and their preferences for seeds are related to seed morphology, seed size ratio, seed coat thickness (Lundgren and Rosentrater 2007; Honek et al. 2007; Saska et al. 2019b) and both, seed and carabid taxonomy (Honek et al. 2007; Saska 2008; Saska et al. 2019b). Most results on predation were obtained on fresh seeds but burial of six seed species in soil for 6 months revealed differences in seed consumption for two seed species but not for the other four after release from the seed bank (Martinková et al. 2006). Further to that, the study of 26 seed species (Saska et al. 2019a) indicated that mostly decrease in viability and seed mass with years after burial affected seed preference and consumption by carabid beetles, and it is likely that these changes in seed quality resulted from the activity of soil microorganisms (Davis et al. 2016). Bacterial diversity may be particularly important because many interactions between seeds and predatory insects are mediated by specific bacterial taxa (Lundgren and Lehman 2010; Schmid et al. 2015). The selection pressure of predators might be further transfered to the core microbiome because bacterial symbionts hosted by insect herbivores have been identified as important components of ecosystems that can mediate trophic interactions with diverse ecological and evolutionary effects on their hosts (Berendsen et al. 2012; Zytynska and Meyer 2019). Seed-associated microorganisms might be particularly important for carabid beetles because of their symbiotic digestive activities. Although the seeds represent a highly nutritious food source, they are distinct from the ancestral diet of Carabidae consisting of small soil organisms (Evans and Forsythe 1985) and thus, their digestion requires cellulolytic and ligninolytic enzymes provided by microbes (Lundgren and Lehman 2010). Recent studies have discovered that the gut microbiota participate in insect physiology, including nutritional metabolism, development, morphogenesis, immunity, and behavior. Moreover, some gut microbes actively degrade toxic compounds such as phytotoxins and pesticides (Jang and Kikuchi 2020). Thus, the microbial interactions connecting weed seeds and seed predators might be relatively strong because of the weed life strategies on one side, and the seeds recognized as the non-inherent food for the beetles on the other.

In this study, we aimed in determining how seed bacterial communities change during deposition in soil. Firstly, we wanted to identify, which bacterial taxa remain in seeds and which are exchanged by taxa from soil after one and two years of soil exposure. Secondly, we also seeked to see how seed microbiomes modified by exposure to soil change preferences for seed eating beetles. Those questions come from the previous observation that even in one plant host different microorganisms are subject to various selection pressures, which leads to transient species within holobionts (Singh et al. 2020) and that these changes may have long-term consequences in interactions within soil environment (Chen et al. 2021).

Thus, we compared microbiomes of seven seed species and related the composition of bacterial communities to seed mass loss, viability and consumption by carabid beetle *Pseudoophonus rufipes*. According to previous studies, the selected seed species differ in size, morphology, chemical properties and also in palatability to carabid beetles (Honek et al. 2003; Saska et al. 2019b). The development of bacterial communities and changes of seed properties in soil were studied in a field experiment by burial at one site for up to two years, the consumption by *P. rufipes* was studied by laboratory experiment, in which fresh seeds and the excavated seeds after one and two years were offered to beetles. The study suggested the persistence of particular bacterial taxa in seeds deposited in soil and changes in predation by beetles in connection to bacterial communities. Consequently, the study also showed the effectiveness of the individual seed species elimination in the soil bank by means of both decomposition and consumption. This knowledge may improve our understanding of seed endophytes functions, reveal the effects of microorganisms on plant demography (Larios et al. 2017) and provide a step in weed management targeted on seeds (Chee-Sanford and Fu 2010; Müller-Stöver et al. 2016).

Material and Methods

Seed species and collection

Seeds of seven herbaceous species were used: *Crepis biennis* L., *Taraxacum officinale* agg., *Tripleurospermum inodorum* (L.) Schultz-Bip. (all *Asteraceae*), *Plantago lanceolata* L. (*Plantaginaceae*), *Thlaspi arvense* L. (*Brassicaceae*), *Silene latifolia* ssp. *alba* (Mill.) Greuter et Burdet (*Caryophyllaceae*) and *Leonurus cardiaca* L. (*Lamiaceae*). The species were selected because they represent common weeds in central European fields and adjacent habitats, are abundantly available, differ in size and shape, and also in palatability to carabid beetles (Honek et al. 2003; Saska et al. 2019b). The seed morphological traits are shown in Supplementary Table S1, chemical composition in Supplementary Table S2, and photographs in Supplementray Fig. S1. Seeds of three *Asteraceae* are non-dormant, while the remaining four species undergo physiological dormancy (Baskin and Baskin 1998). Seeds of the *Asteraceae* and *P. lanceolata* are relatively short-living in soil, those of *T. arvense* and *L. cardiaca* are more persistent (Thompson et al. 1997; Saska et al. 2020). Seeds were collected from standing plants during July-August 2014 in Prague area and air dried at room temperature on sterile Petri dishes.

Seed burial experiment

The experiment was set up in Prague – Ruzyně (50.08° N, 14.29° E) Czechia. The site was selected for convenience but also with respect to the knowledge of higher seed decomposition occurring in unmanaged fields (Nikolić et al. 2020). The field is a fallow for about 10 years, is described as a loam haplic Luvisol according to WRB classification (WRB 2015) and its basic physico-chemical characteristics are in Supplementary Table S3. The experiment started on Aug 28, 2014. Air-dried seeds of each species were filled to bags made of synthetic cloth with a mesh size of ca. 0.15 mm. Each bag contained 2 grams of seeds and 20 g of fine-grain autoclaved sand. Mixing seeds with a substrate is essential to prevent high seed deterioration rates in soil (Van Mourik et al. 2005). Bags with seeds were connected by synthetic ropes to a pole so they could be easily found. Prior to burial seed bags were stored in a freezer at -20 °C. Seed bags were buried at a field margin to the depth of 25 cm in a block design, so each of 14 (2 years x 7 replicates of each seed species) blocks contained one bag per seed species. The seeds from the first seven blocks were excavated after one year on Sept 10, 2015 (YAB 1), the remaining seeds after two years on Sept 23, 2016 (YAB 2). Excavated seed bags were manipulated with sterile gloves and kept separately in order to minimize the risk of contamination. They were frozen immediately at -80 °C until further processing. Samples of fresh seeds were kept frozen at -80 °C as controls (YAB 0).

Soil characteristics

The total content of C and N was determined by an Elementary Analyzer. Particle size distribution was determined by the hydrometer method (Gee and Bauder 1986). Water extraction were made according to (Hubová et al. 2018). Briefly, fresh samples were subjected to deionised water (conductivity $< 0.055\ \mu S \cdot cm^{-1}$, Crystal Adrona and simultaneously $< 2\ ng \cdot L^{-1}$ TOC) extraction (ratio soil/water 1:10 w/v, 60 min extraction on a reciprocal shaker at a stable laboratory temperature). The suspension was then centrifuged at 4000 rpm for 10 min; finally, extracts were filtrated through a 0.45 μm nylon membrane filter (Cronus Membrane Filter Nylon, GB). In aqueous extracts the following chemical parameters were analysed: pH (pH meter inoLab pH Level 1 WTW, Germany) and content of selected elements using inductively coupled plasma optical emission spectrometer (ICP-OES). Dissolved organic carbon (DOC) content was determined by a modified wet dichromate oxidation method according to (Tejnecký et al. 2014).

Determination of seed properties

Seeds were cleaned from dust and fine soil/sand particles in an ultrasound cleaner (Sonorex RK 31, Bandelin electronic, Berlin, Germany) for 3 minutes. The first subsample of 5 times 10 seeds were weighed using Sartorius balances (CP225D-0CE, Sartorius AG, Göttingen, Germany) (precision of 10^{-5} g), and mean seed mass was calculated for each seed bag. The second subsample of 5 times 10 seeds was used in a germination test. The seeds were placed on top of a moist (1 ml of tap water) filter paper in a Petri dish (diameter 9 cm, height 1.5 cm) and incubated for three weeks at continuous darkness while checked twice a week. Germinated seeds were removed and recorded. The seeds that did not germinate were subjected to an imbibed seed crush test (Borza et al. 2007). In this test, the seeds were gently pressed by tips of a pair of forceps and seeds that crushed or collapsed without revealing intact cotyledons or embryo were considered dead, and the number of viable/dead seeds per dish was recorded. The results of this test proved to be highly correlated with the tetrazolium chloride test, while being less demanding for labour (Borza et al. 2007).

Microbial communities

In the third subsample, 100 mg of seeds were were homogenized using a MiniBeadBeater-16 (Biospec Products, Bartlesville, USA) in steel vials with one 4 mm bead for 3000 rpm for 1 – 6 minutes depending on the seed species. DNA was extracted by method described in Sagova-Mareckova *et al*. (2008). Bacterial 16S rRNA gene including the variable region V4 was amplified by PCR using universal primers with overhang adapters CS1-515F (5’-ACACTGACGACATGGTTCTACAGTGCCAGCMGCCGCGGTAA-3’) and CS2-806R 5’-TACGGTAGCAGAGACTTGGTCTGGACTACHVGGGTWTCTAAT-3’) (Caporaso et al. 2011). PCR was performed in 50 uL mixture using AccuPrime buffer II and AccuPrime Taq DNA polymerase (ThermoFisher Scientific, Waltham, USA). Construction of amplicon libraries and sequencing using MiSeq sequencer (Illumina, San Diego, USA) were done at the DNA Services Facility, Research Resources Center, University of Illinois, Chicago, USA. The resulting paired sequence reads were merged, filtered, aligned using reference alignment from the Silva database (Quast et al. 2013), and chimera checked using integrated Vsearch tool (Rognes et al. 2016) according to the MiSeq standard operation procedure (Miseq SOP, September 2021) (Kozich et al. 2013) in Mothur v. 1.44.1 software (Schloss et al. 2009). A taxonomical assignment of sequence libraries was performed in Mothur using the Silva Small Subunit rRNA Database, release 138 (Yilmaz et al. 2014) adapted for use in Mothur (https://mothur.s3.us-east-2.amazonaws.com/wiki/silva.nr_v138.tgz) as the reference database. Sequences of plastids, mitochondria, and those not classified in the domain *Bacteria* were discarded. The sequence library was clustered into ZOTUs using the Unoise algorithm in Usearch v. 11.0.667 software (Edgar 2016). The ZOTU table was used to calculate the rarefaction curves, Bray-Curtis distance matrices describing the differences in community composition between the samples, and composition of core microbiomes for groups of seed samples with tools implemented in the Mothur software. The core microbiome was defined as a subset of ZOTUs present in at least 80% of samples in the group in proportion (relative abundance) over 0.01% of the whole community. The non-metric mutidimensional scaling plots with environmental variables were constructed in vegan package v. 2.5-7, core microbiomes were compared using made4 package v. 1.66.0, and areas under the rarefaction curves were calculated using trapezoidal rule, all in the R v. 4.1.0 software (R Core Team 2021). The Illumina MiSeq 16S rRNA gene amplicon sequences have been deposited in the NCBI Sequence Read Archive under accession number SRP150525 (https://www.ncbi.nlm.nih.gov/sra/?term=SRP150525).

Seed consumption experiment

The fourth subsample of fresh seeds and seeds from both years of the burial experiment were presented to *Pseudoophonus rufipes* (DeGeer) (Coleoptera: Carabidae), the model species of a generalist invertebrate seed predator, on small tin trays filled with white modelling clay (Plastilina JOVI, Barcelona, Spain) following the protocol of Honek *et al.* (2003) and Saska *et al*. (2014). Each tray contained 30 seeds from one bag (or fresh seeds). Seeds were stuck by ca. half their diameter so they could be easily picked up by the beetles. Trays from all bags were presented simultaneously in an arena, which consisted of a glass Petri dish (25 cm in diameter and 5 cm in height) with a 1 cm layer of sieved soil that did not contain any seeds on the bottom. Seed trays (21 in total) were randomly arranged in two concentric rings (15 + 6 trays) by pressing them in the soil so they were flush with soil surface. This arena set-up was replicated seven times; in each replication were the seeds of the same YAB (buried for one or two years, respectively) were from the same block. Three beetles were released in each arena and their consumption was determined for four consecutive days on a daily basis. If more than 50 % of seeds on a tray was removed, the respective tray was replaced to assure that the preferred seeds were always present in excess. After the experiment, the consumption of particular seed bag was summed over the entire period and expressed as consumption in number of seeds (N) and consumption in seed mass (mg).

Statistical analysis

Seed properties (seed mass and viability) and the diversity of bacterial communities (area under the rarefaction curve) were compared between seed species using Friedman, Kruskal-Wallis, and pairwise Wilcoxon rank sum tests. The effect of YAB was tested for seed mass, seed viability, diversity of the bacterial community (rf) and consumption of each species of seeds. Consumption in number of seeds was analysed using generalized linear model with quasi-poisson family, and consumption in mass was analysed by linear model. Each model included seed species, one of the variables and their interaction, and a covariable indicating the replication (arena) was also included in order to account for dependency of consumption within each dish. To improve the fit of the linear models the logarithmic transformation was used for consumption in mass and for seed mass, when appropriate (McArdle and Anderson 2001). All analyses were conducted in R version 4.1.0 (R Core Team 2021).

Results

Seed properties

Seeds of *Plantago lanceolata* had the highest and *Tripleurospermum inodorum* had the lowest mass (Supplementary Table S1). Seeds differed in all measured properties: seed mass, seed mass loss, viability and bacterial diversity (Supplementary Table S4A). Seed mass loss after one year of soil exposure differed between 21 pairs of seeds and after the second year of exposure between 15 pairs of seeds (Fig. 1A, Supplementary Table S4B). The highest seed viability was in *T. arvense* and the lowest in *L. cardiaca*, *P. lanceolata* and *T. inodorum.* Significant differences occurred between one pair after the first year and nine pairs after the second year of soil exposure (Fig. 1B, Supplementary Table S4B). Bacterial diversity (expressed as the area under the rarefaction curve - rf) was highest in *L. cardiaca*, *S. latifolia* and *T. officinale* and lowest in *P. lanceolata* and *T. arvense* in fresh seeds. Significant differences in bacterial diversity occurred between two pairs in fresh seeds, three pairs after the first year and nine after the second year of soil exposure (Fig. 1C, Supplementary Table S4B).

Changes in seed properties with burial in soil

Both, seed mass and viability declined significantly with time (year after burial, YAB) in all seeds but the rate differed significantly among species and individual years ($P < 0.001$; Fig. 1A,B; Supplementary Table S5A,B). The fastest decline in seed mass was observed in *C. biennis* and lowest in *T. arvense* in both years. The fastest decline in viability was in *L. cardiaca* in the first year and in *P. lanceolata* in the second year (Supplementary Table S5B). The changes in bacterial diversity varied significantly between seed species ($P < 0.001$; Fig. 1C). The decline in viability and seed mass was correlated in all seven seed species (Pearson's r ranging from 0.72 to 0.97). Bacterial diversity increased mostly between the fresh seeds and the first year soil exposure, while the difference between the first and second year was not significant (Supplementary Table S5A).

Seed bacterial communities

The proportions of dominant bacterial phyla *Proteobacteria*, *Actinobacteriota*, *Chloroflexi*, *Verrucomicrobiota, Firmicutes* and *Spirochaetes* varied between both seed species and burial time (Fig. 2). Bacterial communities differed in the overall test (Amova, $P < 0.05$) between all seed species except between *T. officinale* and *C. biennis*, and *L. cardiaca* and *T. inodorum* (Supplementary Table S6).

In a more detail taxonomic analysis within *Proteobacteria,* the proportion of *Gammaproteobacteria* (predominantly *Enterobacteriales* and *Pseudomonadales*) and *Betaproteobacteria* (*Burkholderiales*) decreased, while *Alphaproteobacteria* (*Rhizobiales*) increased (Supplementary Fig. S2). In fresh seeds, *Gammaproteobacteria* formed a large proportion in *T. officinale*, *C. biennis*, *S. latifolia* ssp. *alba* and *T. inodorum*, *Enterobacteraceae* reached up to 40%, *Pantoea* (*Enterobacterales*; *Erwiniaceae*) was found in high proportions in the fresh seed communities of *T. officinale*, *C. biennis*, *S. latifolia* ssp. *alba* and *T. inodorum* (20-30%). *Hafnia* (*Enterobacterales*; *Hafniaceae*) occurred also mostly in the fresh seeds. *Pseudomonadaceae* occurred in all species (up to 30%) and were always highest in the fresh seeds. Within *Xanthomonadales*, *Stenotrophomonas* occurred in *T. officinale* and *C. biennis* reaching the maximum after one year, but also in the fresh seeds of *T. inodorum* and *S. latifolia* ssp. *alba*. *Xanthomonas* occurred particularly in the fresh seeds of *S. latifolia* ssp. *alba* and decreased with burial. *Betaproteobacteria*, namely *Burkholderiales* occurred more in *T. officinale* and *C. biennis* in the fresh seeds, then decreased (average 7 %). *Burkholderia* was present in most samples but in low proportions. *Massilia* was present only sporadically in all seeds and times, and in higher percentage in the fresh seeds of *P. lanceolata*.

In *Actinobacteriota* (Supplementary Fig. S3), *Micrococcales* were typical for fresh seeds, *Micromonosporales* and *Streptomycetales* slightly increased with burial time, and

Solirubrobacterales and *Pseudonocardiales* appeared only with soil exposure. More specifically, *Micrococcales* were found in high proportion in the fresh seeds in *T. officinalis*, *L. cardiaca* and *S. latifolia* ssp. *alba, Streptomycetales* increased after one year in *T. officinalis* and *P. lanceolata*, while *Propionibacteriales* after two years in *T. arvense. Corynebacteriales* appeared in *P. lanceolata* and *C. biennis*, and *Cellulomonas* after burial particularly in *P. lanceolata*, *L. cardiaca* and *S. latifolia* ssp. *alba*.

Some families of *Bacteroidota* (Supplementary Fig. S4) were seed specific (*Flammeovirgaceae*), while some increased with time (*Chitofagaceae*) or decreased (*Sphingobacteriaceae*) after the first year, particularly in *T. officinalis, T. arvense, P. lanceolata* and *S. latifolia* ssp. *alba*.

Firmicutes (Supplementary Fig. S5) seemed to be seed specific in both fresh seeds and after burial, were represented mostly by the genera *Paenibacillus* and *Bacillus*, which were highest after the first year of burial particularly in *T. arvense*, less in *P. lanceolata* and *L. cardiaca*; also *Planococcaceae* were present specifically in *T. arvense*.

Core microbiomes of fresh seeds and seeds after one year soil exposure

Comparison of core microbiomes between fresh seeds and seeds buried for one year showed, which bacterial taxa survived in the seed and which were lost or enriched after burial. In all seeds 5 ZOTUs survived in seeds after the first year. These were represented by genera of *Pantoea* and *Burkholderia-Caballeronia*, *Methylobacterium-Methylorubrum* (*Proteobacteria*), *Kineosporia* (*Actinobacteriota*), and *Candidatus* Cardinium (*Bacteroidota*). Only one ZOTU, *Kineosporia* (*Actinobacteriota*), survived in core microbiome to the second year. Mostly *Alphaproteobacteria* and *Actinobacteriota* enriched the seed microbiomes after the first year in soil.

The proportions of the dominant taxa changed in microbiomes of individual seed species. Mostly, the core microbiomes differed in proportions of *Proteobacteria*, *Actinobacteriota* and *Bacteroidota*, the highest proportion of *Proteobacteria* was in *C. biennis* and *T. officinale*, highest proportion of *Actinobacteriota* in *L. cardiaca* and highest proportion of *Bacteroidota* in *P. lanceolata*. Additionally, *S. latifolia* comprised higher numbers of *Chloroflexi* ZOTUs. The unique part of fresh seed microbiomes, which did not survive in soil differed even more in the proportion of *Proteobacteria*, *Actinobacteriota* and *Bacteroidota*. The characteristic microbiomes of the individual seed species also encompased *Firmicutes*, highest in *T. inodorum* and *Chloroflexi* occurring in *T. arvense*. The most noticeable differences between seed species occurred in microbiomes after the first YAB. Highest proportion of *Proteobacteria* was in *T. officinale*, *Actinobacteriota*, *Chloroflexi* in *T. inodorum*, *Firmicutes* in *T. arvense*, *Bacteroidota* in *C. biennis*, and there were also significant proportions of *Planctomycetota* in *T. officinale* and *T. inodorum*, and *Verrucomicrobiota* in *T. arvense*. Mostly *Alphaproteobacteria* and *Actinobacteriota* enriched the seed microbiome after the first year in soil (Fig. 3).

Microbiomes of individual seed species

C. biennis was a seed typical by high mass loss but low viability loss (Fig. 1, Supplementary Table S5B). In the species, besides ZOTUs common to all species, also *Pseudomonas*, *Stenotrphomonas*, *Massilia* (*Proteobacteria*) and *Microbacteriaceae* (*Actinobacteriota*) survived, mostly *Raoultella*, *Pseudomonas*, and also *Rathayibacter* (*Actinobacteriota*) disappeared, and many ZOTUs from *Alphaproteobacteria*, *Gammaproteobacteria*, *Actinobacteriota* and *Bacteroidota* were enriched after the first year (Supplementary Fig. S6).

L. cardiaca and *P. lanceolata* seeds were typical by high viability loss and relatively low bacterial diversity (Supplementary Table S5B). *L. cardiaca* had relatively high number of ZOTUs surviving the first year, mostly *Pseudomonas*, *Alphaproteobacteria* and *Micrococcales* (*Actinobacteriota*) (Supplementary Fig. S7). *P. lanceolata* besides the typical taxa shared *Massilia, Bartonella* and many other *Gammaproteobacteria* and *Alphaproteobacteria*, *Candidatus* Cardinium (*Bacteroidota*), some *Actinobacteriota* and *Firmicutes*. In both seeds, unique ZOTUs in the fresh seeds were dominated by *Massilia,* interesting genera were also *Bartonella* and *Wohlbachia, Sphingomonas* and other *Alphaproteobacteria*, and relatively similar percentage of *Actinobacteriota*, *Bacteroidota* and *Firmicutes*. After one year, both seeds were enriched by similar percetage of *Alphaproteobacteria* and *Gammaproteobacteria*, but different ZOTUs of mostly *Rhizobium*, *Bradyrhizobium*, *Sphingomonas* and others. Both seeds were enriched also with similar percetage but various ZOTUs of *Actinobacteriota*, *Firmicutes* and *Bacteroidota*. Additionally, in *P. lanceolata* occurred high proportions of *Bartonella*, *Candidatus* Cardinium and *Wolbachia* (*Proteobacteria*) (Supplementary Fig. S8).

S. latifolia ssp. *alba* was typical by low mass loss (Supplementary Table S5B). After the first year, mostly *Pantoea* and *Massilia* (*Gammaproteobacteria*) survived in the microbiome but also *Pseudoarthrobacter* (*Actinobacteriota*), *Nitrososphearaceae* (*Thaumarchaeota*) and *Firmicutes*. Relatively high number of ZOTUs was unique in fresh seeds and surprsingly, those were dominated by *Actinobacteriota*, mostly *Curtobacterium* and *Frondihabitans*, large proportions also belonged to *Gammaproteobacteria* and *Alphaproteobacteria*, namely *Pseudomonas* and *Stenotrophomonas*, but also *Bacteroidota*, mostly *Chryseobacterium*, *Pedobacter*, *Sphingobacterium* and *Firmicutes*, mostly *Paenibacillus*. The seed was enriched mostly by *Alphaprotebacteria* but also

Gammaproteobacteria, *Actinobacteriota*, *Chloroflexi* and *Firmicutes* after one year of soil exposure (Supplementary Fig. S9).

T. oficinale was typical by high seed mass loss, high bacterial diversity and strong decline of consumption rate after soil exposure (Supplementary Table S5B). The shared microbiome between fresh seeds and one YAB was strongly dominated by *Pantoea*, *Klebsiella*, *Pseudomonas* and *Stenotrophomonas* (*Gammaproteobacteria*), other ZOTUs included *Micrococcales* (*Actinobacteriota*) and *Pedobacter* (*Bacteroidota*). The fresh seeds were unique in ZOTUs of *Pseudomonas*, *Enterobacteriales* (*Gammaproteobacteria*), *Sphingomonas* (*Alphaproteobacteria*), *Microbacteriaceae* and *Nocardiaceae* (*Actinobacteriota*) and *Pedobacter* (*Bacteroidota*). Similarly to other seeds after soil exposure, the microbiome was enriched mostly in *Alpha* and *Gammaproteobacteria*, *Actinobacteriota* but also all other typical soil phyla (Supplementary Fig. S10).

T. arvense was typical by low mass and viability loss, low bacterial diversity but increased consumption rate after soil exposure (Supplementary Table S5B). The shared microbiome of fresh seeds and seeds after one YAB was dominated by *Pseudomonas*, *Burkholderia-Caballeronia-Paraburkholderia*, *Pantoea*, *Rikettsiella* (*Gammaproteobacteria*), *Rhizobiaceae*, *Bartonella*, *Wohlbachia*, *Methylobacterium*, *Sphingomonas* (*Alphaproteobacteria*), high proportion of *Candidatus* Cardinium (*Bacteroidota*) and typical taxa of *Actinobacteriota*, and additionally also *Bacillus* (*Firmicutes*). The ZOTUs unique in fresh seeds were dominated by *Caulobacter*, *Sphingomonas* (Alphaproteobacteria), *Pseudomonas*, *Paucibacter* (*Gammaproteobacteria*) but also interestingly *Staphylococcus* and *Romboutsia* (*Firmicutes*) and *Catenullispora* (*Actinobacteriota*). The enrichment from soil was dominated by *Bacilli* (*Firmicutes*), *Alpha* and *Gammaproteobacteria*, *Actinobacteriota* dominated by *Actinoplanes*, *Bacteroidota*, *Chloroflexi* and others (Supplementary Fig. S11).

T. inodorum was typical by high bacterial diversity and strong decline of consumption rate after soil exposure. ZOTUs surviving the first year in soil belong mostly to *Pantoea*, *Pseudomonas*, *Massilia*, *Xanthomonas*, *Sphingomonas* (*Gammaproteobacteria*), and *Frondihabitans* and *Curtobacterium* (*Actinobacteriota*). The fresh seeds were unique by dominance of *Paenibacillus* (*Firmicutes*) and then *Pseudomonas*, *Sphingomonas* (*Proteobacteria*), *Ratayibacter*, *Clavibacter*, *Leifsonia* (*Actinobacteriota*) and *Chryseobacterium* and *Pedobacter* (*Bacteroidota*). The unique ZOTUs from soil exposure were dominated by *Micromonosporaceae*, *Kineosporia* and other *Actinobacteriota*, *Alpha* and *Gammaproteobacteria*, *Chloroflexi*, *Bacteroidota* and others (Supplementary Fig. S12).

Seed consumption

Seed consumption in total numbers (N) or by seed weight (mg) provided similar results. The highest consumption of fresh seeds occurred in *T. officinale* the lowest in *T. arvense* but after two years the highest consumption was in *T. arvense* and lowest in *P. lanceolata*. Significant differences in consumption between seed species were revealed by an overall test for the fresh seeds and the both burial times (Fig. 4; Supplementary Table S7A), while in post-hoc pairwise test the differences were significant for three pairs of fresh seeds only. The effect of burial time (YAB) differed between seed species. In consumption of seed numbers the effect of YAB was significant for *C. biennis*, *S. latifolia*, *T. officinale* and *T. arvense*, in consumption by weight it was for *C. biennis*, *T. officinale*, *T. arvense* and *T. inodorum* (Supplementary Table S7B).

Some seeds (*T. inodorum* and *T. officinale*) were losing their initial attractiveness with YAB, but *T. arvense* seeds were becoming more attractive. The pattern typical for the latter species was that the consumption increased after one year of burial and decreased again after the second year in soil, which however, still exceeded the original consumption (Fig. 4).

Therefore, consumption differed between seed species over time, while in *C. biennis*, *T. officinale*, *T. arvense* and *T. inodorum* the burial time had significant effect on consumption, in *L. cardiaca*, *P. lanceolata* and *S. latifolia* the consumption did not change over burial time (Supplementary Table S7B).

Changes of interactions between seed properties, consumption and bacterial communities

The non-metric multidimensional scaling plots with instrumental variables show that the relationship between bacterial communities and seed properties changed over time (Fig.5). In the beginning, all properties i.e. seed mass, viability, bacterial diversity and consumption were correlated with bacterial communities. Seed mass and bacterial diversity were directed towards *P. lanceolata* seeds, viability towards *T. arvense* seeds and consumption to *C. biennis* and *T. officinale*. Bacterial diversity opposed consumption. After one year of seed exposure to soil, only seed mass and bacterial diversity were correlated with bacterial communities, seed mass pointing towards *S. latifolia* and bacterial diversity towards *L. cardiaca*, *T. arvense* and *T. inodorum.* After two years of seed soil exposure, seed mass together with viability and consumption that were weakly correlated with bacterial communities and pointing towards relatively heavy and viable seeds of *T. arvense* and *S. latifolia*, while bacterial diversity pointed towards *L. cardiaca*, *T. inodorum* and *T. officinale*. Overall, all seed properties were dependent on bacterial community composition, viability and seed mass were mostly negatively correlated with diversity. Seed mass and diversity were always correlated with bacterial communities, while viability and consumption rate were correlated in fresh seeds but weakly correlated or uncorrelated after burial (Supplementary Table S8). The shared microbiome of seeds of *T. officinale*, *T. inodorum* and *T. arvense*, which were typical by significant changes of consumption rate represented 44% of bacterial communities. It was dominated by *Actinobacteriota* (*Micromonosporales*, *Micrococcales*, *Streptosporangiales* and

Corynebacteriales). Unique sequences of *T. arvense* that was characterized by increased consumption after burial represented 21 % of bacterial communities and were typical by high percentage of *Firmicutes*, *Paenibacillus* and *Bacillales*. Seeds of *T. officinale* and *T. inodorum* typical by high decrease of consumption after burial were characterized by high percetage of *Actinobacteriota* (Supplementary Fig. S13). Thus, changes in bacterial communities due to burial affected seed properties as well as consumption by *P. rufipes*.

Discussion

Microorganisms mediate a range of ecological interactions among phylogenetically distant taxa. In this work, we propose that changes in bacterial communities of seeds buried in soil are linked to seed survival and also to their consumption by a carabid beetle.

The "basic" seed endophytes

The predominant bacterial phyla of *Proteobacteria*, *Actinobacteriota* and *Bacteroidota* that occured in studied seeds are commonly present in seed endophytic communities (Truyens et al. 2015; Nelson et al. 2018; Escobar Rodríguez et al. 2020). The shared microbiome of all seeds, fresh and after one year burial, consisted of 5 ZOTUs belonging to genera *Pantoea*, *Burkholderia-Caballeronia-Paraburkholderia*, *Methylobacterium-Methylorubrum* (*Proteobacteria*), *Kineosporia* (*Actinobacteriota*) and *Candidatus* Cardinium (*Bacteroidota*), which come from these phyla. Additionally, the only ZOTU of the genus *Kineosporia* was shared in all seed species after the second year of soil exposure. Thus, only a very restricted part of the otherwise diverse bacterial community was common to all seeds and withstood the soil exposure. Since the seeds of weeds stay in the soil for several years until conditions are favorable for germination (Trognitz et al. 2016), the genera conserved in the microbiome, may be particularly important for germination in the season following their maturation or other interactions in soil (Singh et al. 2020; Bredon et al. 2021).

The multilevel interactions of these genera are also probable because they were linked with functions not only in plants but also in insects (Zytynska and Meyer 2019). For example, *Pantoea* comprises versatile lifestyles, including plant pathogens, plant growth promoters, strains used for biocontrol of phytopathogens (Truyens et al. 2015) and simultaneously *Pantoea* is an important member of the carabid beetle gut community (Lundgren and Lehman 2010). The genus *Burkholderia* was recently divided to two major new genera *Paraburkholderia* and *Caballeronia*, so *Burkholderia* in now connected to pathogenicity, although mutualistic partnerships with insects and contributions to plant health were also identified (Kaltenpoth and Flórez 2020). *Paraburkholderia* primarily contains nonpathogenic strains with beneficial effects on plants such as nitrogen fixation in legume root nodules, degradation of aromatic compounds, phosphate solubilization, or induction of stress resistance or antifungal compounds production (Suárez-Moreno et al. 2012) and the genus *Caballeronia* harbors mostly environmental species common in soil or water (Kaltenpoth and Flórez 2020). *Methylobacterium-Methylorubrum* was identified as plant growth promoting bacterium suppressing growth of some pathogenic fungi and regulating salt stress (Grossi et al. 2020). *Kineosporia*, the most persistent genus, was together with *Variovorax* and *Acidovorax* identified as a keystone taxon driving fungal-bacterial balance (Durán et al. 2018). The candidatus genus *Cardinium* is rather connected to the beetle predators since it is a common intracellular symbiont of many insects and similarly to *Wolbachia* can be horizontally transmitted between different insect species through plants, where it resides as an endophyte (Frank et al. 2017).

Changes of seed microbiomes in soil

Many various taxa enriched the seeds in soil. Although they came from several phyla, the dominant part belonged among the common endophytic phyla of *Proteobacteria*, *Actinobacteriota*, *Bacteroidota* and *Firmicutes*. After one year in soil, the fresh seeds were mostly depleted of *Pseudomonas*, *Sphingomonas*, *Bartonella* (*Proteobacteria*), which represent taxa typical for symbiotic and/or pathogenic interactions with both plants and insects (e.g. Trognitz et al. 2016; Segers et al. 2017; Kong et al. 2019; Vacheron et al. 2019). Interestingly, after the second year in soil the seeds were again mostly enriched by strains belonging to typical endophytic phyla, *Proteobacteria* and *Actinobacteriota*, but after the first year, when most commonly the germination occurs, the seeds were enriched with strains from less common phyla *Firmicutes* (*Paenibacillus*), *Chloroflexi* (uncultured) or *Bacteroidota* (*Chryseobacterium*, *Flavobacterium* and *Pedobacter*). However, some of those genera, namely *Flavonobacterium* and *Pedobacter,* were also previously linked with germination but disapeared from seed microbiomes after one year of growth in sterile conditions (Escobar Rodríguez et al. 2020). Also *Paenibacillus* belongs among typical seed endophytes with potential antifungal activities (Truyens et al. 2015; Rybakova et al. 2016).

Although many strains belonged to endophytic phyla, in the individual seed species they appeared in various proportions and together with many other species-specific taxa. We also observed that the direction of changes in bacterial communities after burial was mostly similar for the studied seed species (Fig. 5) but the specific selection of endophytes occurring in fresh seeds seemed to continue after burial. Thus, the individual seeds were differently connected with the surrounding soil bacterial communities. That can be partially explained by their different surface structures and chemical composition because those may influence selection of bacteria from soil (Chen et al. 2021). Also microorganisms already present on the seed surface might later act as gatekeepers that prevent either pathogenic attack or slow decay of hard seed enclosing structures (Dalling et al. 2011; Links et al. 2014). Therefore, this study showed that bacteria associated with seeds kept their species-specific communities during soil deposition, which further supports close plant-soil feedbacks (Miller et al. 2019). Also the results support the suggestion that although soil microbiome serves as a primary source of plant microbiomes it is strongly and sequentially filtered by the plant rhizosphere, rhizoplane and growth stages (Singh et al. 2020), and in case of our study including the seed stage.

Seed traits and survival of individual seed species

The seeds deposited in soil were losing mass and viability but gaining bacterial diversity during their two year exposure in soil. Mass loss and viability were inter-correlated probably due to bacterial decomposing activities of seeds. Bacterial communities seemed to explain particularly seed mass loss because it was correlated with bacterial communities in fresh seeds as well as after one or two years of burial in soil, while viability and consumption rate were correlated with bacterial communities only in fresh seeds and later only weakly after two years of burial.

The most common loss of seeds deposited in soil is due to decomposing microorganisms, which influence seedbank longevity (Nikolić et al. 2020). The proportion of typical decomposers such as *Actinobacteriota* and *Firmicutes* increased in all seed species after burial and therefore, suggest their role in decomposition (Bastian et al. 2009). However, some *Actinobacteriota* (*Micrococcales*) were rather typical for fresh seeds of *S. latifolia*, *T. officinale* and *T. inodorum* and decreased with burial time, so they probably represent seed endophytes (Truyens et al. 2015). Similarly, *Proteobacteria* although present in all seed species, they dominated in fresh seeds. Particularly, the decreasing proportion of *Enterobacteriaceae* (*Gammaproteobacteria, Enterobacteriales*) may be related to changes in seed attractiveness (Truyens et al. 2015). Finally, the seeds of *T. officinale* and *T. inodorum* (both *Asteraceae*) had high bacterial diversity, which negatively correlated with viability and seed mass, suggesting a relatively fast seed decay (Thompson et al. 1997; Baskin and Baskin 1998).

As it has been already explained the disappearance of the individual seeds from seed bank followed different patterns. *C. biennis* was typical by high seed mass loss but low viability loss, average diversity and average and stable consumption rate. Similarly, *L. cardiaca* and *P. lanceolata* had average consumption rates, average mass loss and diversity but relatively high viability loss. In *L. cardiaca*, the latter may be supported by production of alkaloids, e.g. stachydrine (Kuchta et al. 2013), which possibly interfere with carabid consumption. Seeds of *T. officinale*, *T. inodorum* and *T. arvense* were removed significantly by consumption, fresh or after burial, respectively.

Generally, the seed traits such as strengths of seed coat and the proportion of lignocellulose compounds in it (i.e., cellulose, hemicellulose, and lignin) could also have consequences on microbiome structure (Colman et al. 2012; Jang and Kikuchi 2020). The strongest coating was in *P. lanceolata* and *S. latifolia*. Several taxa such as *Proteobacteria* of *Moraxellaceae*, *Enterobacteriaceae*, and *Pseudomonadaceae* but also *Klebsiella*, *Serratia* and *Enterobacter* commonly present in these seeds were idenfied with capabilities to digest plant-based compounds (Blankenchip et al. 2018).

Microbiomes affect seed consumption

The rates of seed consumption were similar for fresh seeds and seeds after one or two year's burial for *C. biennis*, *L. cardiaca*, *P. lanceolata* and *S. latifolia*. Yet, the consumption differed between the fresh and buried seeds in *T. arvense*, *T. officinale* and *T. inodorum.* In the previous studies, these three seeds belonged to the most preferred species by many carabids (Saska et al. 2019b) and their consumption rate by *P. rufipes* changed after burial (Martinková et al. 2006; Saska et al. 2019a). However, the observed changes were not always in agreement. The consumption of *T. inodorum* seeds decreased after 1, 2 (this study) and 6 years of burial (Saska et al. 2019a), while increased after burial for only 6 months (Martinková et al. 2006). Similarly, in *T. arvense* an increase in consumption by *P. rufipes* occurred after 1 and 2 years of burial (this study), while no effect was found after 6 months (Martinková et al. 2006) or 6 years (Saska et al. 2019a). Among the seed species used in this study, a variable response was observed also when different carabid species were used, in *L. cardiaca* (increase in consumption by *Amara littorea* but stable consumption by *P. rufipes* and *Harpalus affinis*) and *T. arvense* (increase in consumption by *H. affinis* but not in *P. rufipes* and *A. littorea*). The different consumption trends might be explained by various seed – microbe interactions due to a different burial site, seed origin and duration of burial (Martinková et al. 2006; Johnston-Monje et al. 2016; Escobar Rodríguez et al. 2020).

Pseudoophonus rufipes generally seemed to prefer fresh to buried seeds in previous studies (Saska et al. 2019a). That was true for seeds of *T. officinale* and *T. inodorum* in this study. Interestingly, the microbiomes of *T. officinale* and *T. inodorum* seeds showed considerable similarities, possibly because they belong to the same family *Asteraceae*. In both, there was a great increase in diversity between fresh seeds and seeds after burial. Since the loss of viability was not high in these seeds it seems that the diversity enrichment was not connected to a fast decay (Nikolić et al. 2020). Their shared microbiomes of fresh seeds and after one year of burial were also quite similarly dominated by *Proteobacteria* (namely *Pantoea*, *Pseudomonas*), *Actinobacteriota* (*Frondihabitans*, *Curtobacterium*) and *Bacteroidota* (*Pedobacter*). These taxa were associated with both plant seeds and insect guts and they are also known for antagonistic activities (e.g. Frank et al. 2017; Durán et al. 2018; Hou et al. 2018). The microbiomes unique in fresh seeds also had noticable similartities, namely the presence of *Clavibacter* and *Rathayibacter* (*Actinobacteriota*), and again *Pedobacter* (*Bacteroidota*), the genera known for various functions in both plants and insects (e.g. Sharma et al. 2021). The specific microbiomes of the two seed species after one year were quite different, but after the second year in soil they were both dominated by *Actinobacteriota*, which differentiated them from the other seeds that were mostly dominated by *Proteobacteria*. In seed eaters particularly, the microbiomes were strongly dominated by *Proteobacteria* (Jang and Kikuchi 2020) so *Actinobacteriota* dominance may represent another, yet unknown interaction with potencial multilevel influence. That may be relatively important because it was observed that most insect guts contain rather few microbial species, so only small diversions in their food source may lead to changes in gut microbiome (Engel and Moran 2013).

The consumption after burial increased for seeds of *T. arvense*, which was further connected to its small loss of mass and viability and to a small increase in bacterial diversity suggesting that the seeds were still mostly alive over burial time. Possibly, low diversity of seed microbiomes is advantageous because high diversity may result in negative interactions with intestine bacteria (Schmid et al. 2015). The microbiome of *T. arvense*, for which the consuption increased after soil exposure, was relatively different from the other microbiomes. In fresh seeds, the unique ZOTUs belonged for example to *Catenullispora* (*Actinobacteriota*), and the soil exposed seeds were enriched by a relatively high proportion of *Firmicutes* (*Paenibacillus*, *Bacillus*) compared to the other seed species, which were mostly enriched by *Proteobacteria* and *Actinobacteriota*. *Paenibacillus* and *Bacillus* are typical endophytic taxa with plant growth promoting and antagonistic activities against fungi (Truyens et al. 2015; Torres-Cortés et al. 2018). *Bacillus* is also a very typical endosymbiont of insects (Lundgren and Rosentrater 2007). Yet, *Bacillus* may also indicate decaying processes (Yilma and Bekele 2021) or rather poor growth conditions of previous seed generations (Tannenbaum et al. 2020). So, various factors might participate in this seed microbiome and lead to feeding by the beetle.

Interestingly, the unique sequences of bacterial communities in seeds with low consumption rate either fresh as *P. lanceolata*, or after soil exposure as *T. officinale* and *T. inodorum*, were inhabited by many taxa with known antibiotic activities such as *Streptomyces*, *Thermomonospora*, *Micromonospora* or *Catenullispora*, while bacterial communities in seeds with overall relatively high consumption *L. cardiaca*, *S. latifolia* and *T. arvense* (Supplementary Fig. S13) were inhabited by taxa known for association with insects such as *Nitrosomonadaceae* (*Betaproteobacteriales*) and archaea *Nitrososphaeraceae* providing nitrification or *Methylophilaceae* feeding on gut released methane and *Chitinophagaceae*, which might be connected to feeding on fungi (Ebert et al. 2021). Additionally, seed microbiomes of *P. lanceolata* and *T. arvense* shared relatively high proportions of typical insect and invertebrate symbionts such as *Bartonella*, *Wohlbachia* and *Candidatus* Cardinium that might influence the seed consumption (Jones et al. 2013).

Conclusions

The study showed that bacterial communities inhabiting seeds change during soil exposure, are connected to seed properties and modulation of attractiveness for carabid beetle predators. Due to seed deposition in soil, it was possible to show how permanently certain bacterial taxa reside inside seeds and that may have implications for the stability and functions of bacterial endophytes. The generalization of results is limited because the study was conducted on one site. However we propose that the ZOTUs common to all seeds even after burial and the high specificity of individual seed-microbe interactions will occur at various sites. Finally, we found that species specific relationships between seeds, bacterial community and beetle predation change after seed burial and thus, may represent novel three-level trophic interactions, which have evolutionary consequences for plant demography but also practical importance for weed management.

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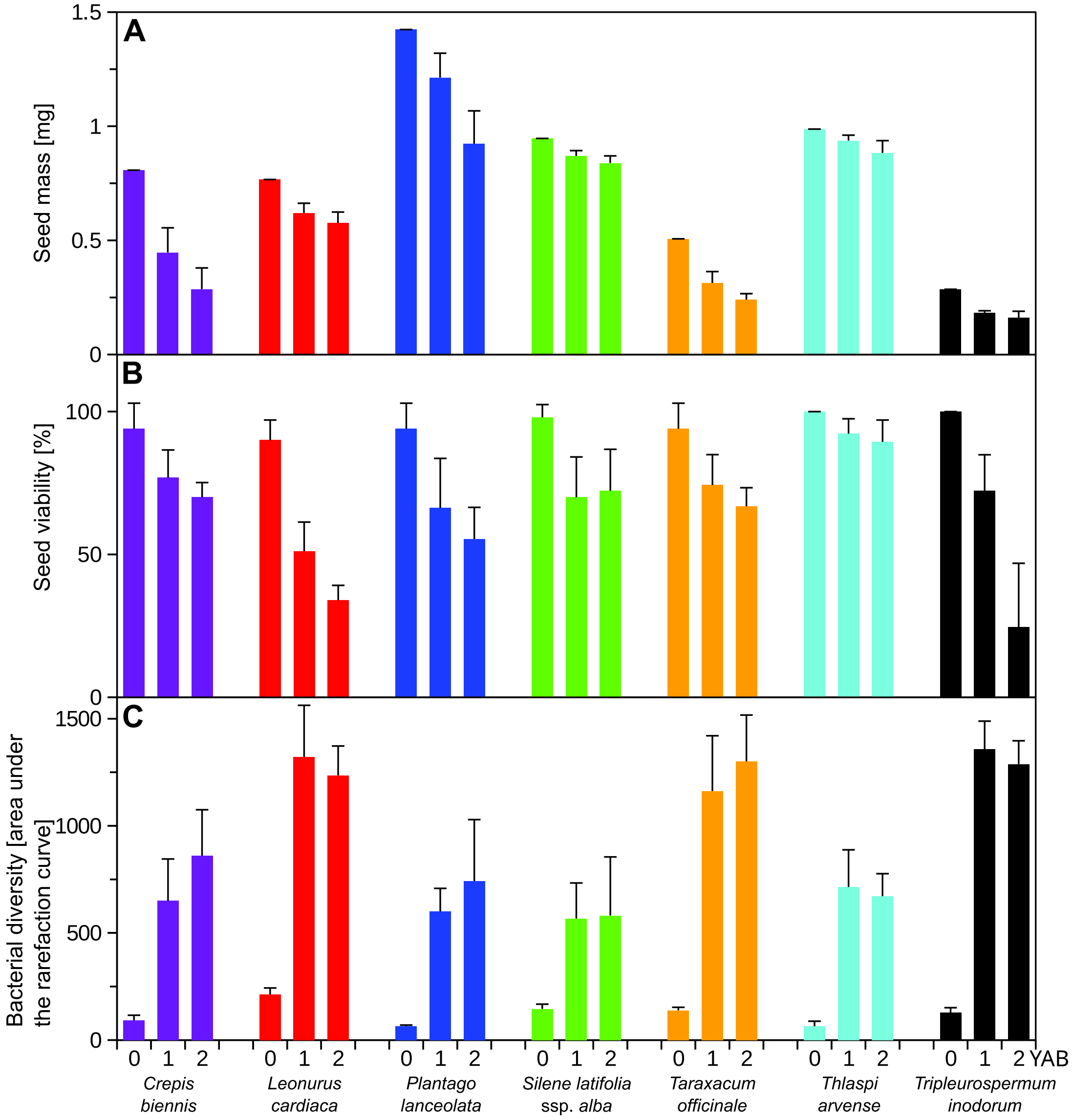

Fig. 1. Seed mass (A), viability (B), and associated bacterial diversity (C) in the fresh seeds and 1 or 2 years after burial (YAB). Averages with respective SD, n=7.

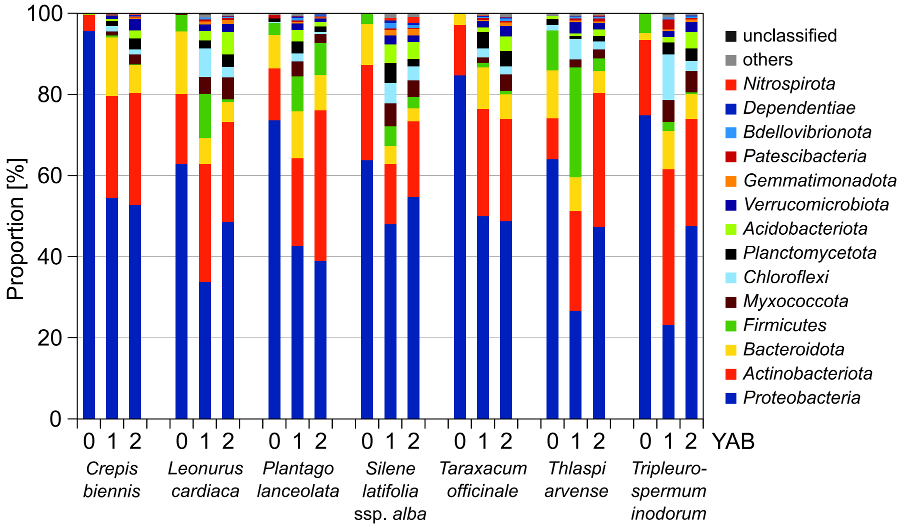

Fig. 2. Average proportions of bacterial phyla in 16S rRNA gene amplicon sequence libraries (n=7) from seeds of seven plant species before (0) and after one and two years of burial (1 and 2 YAB).

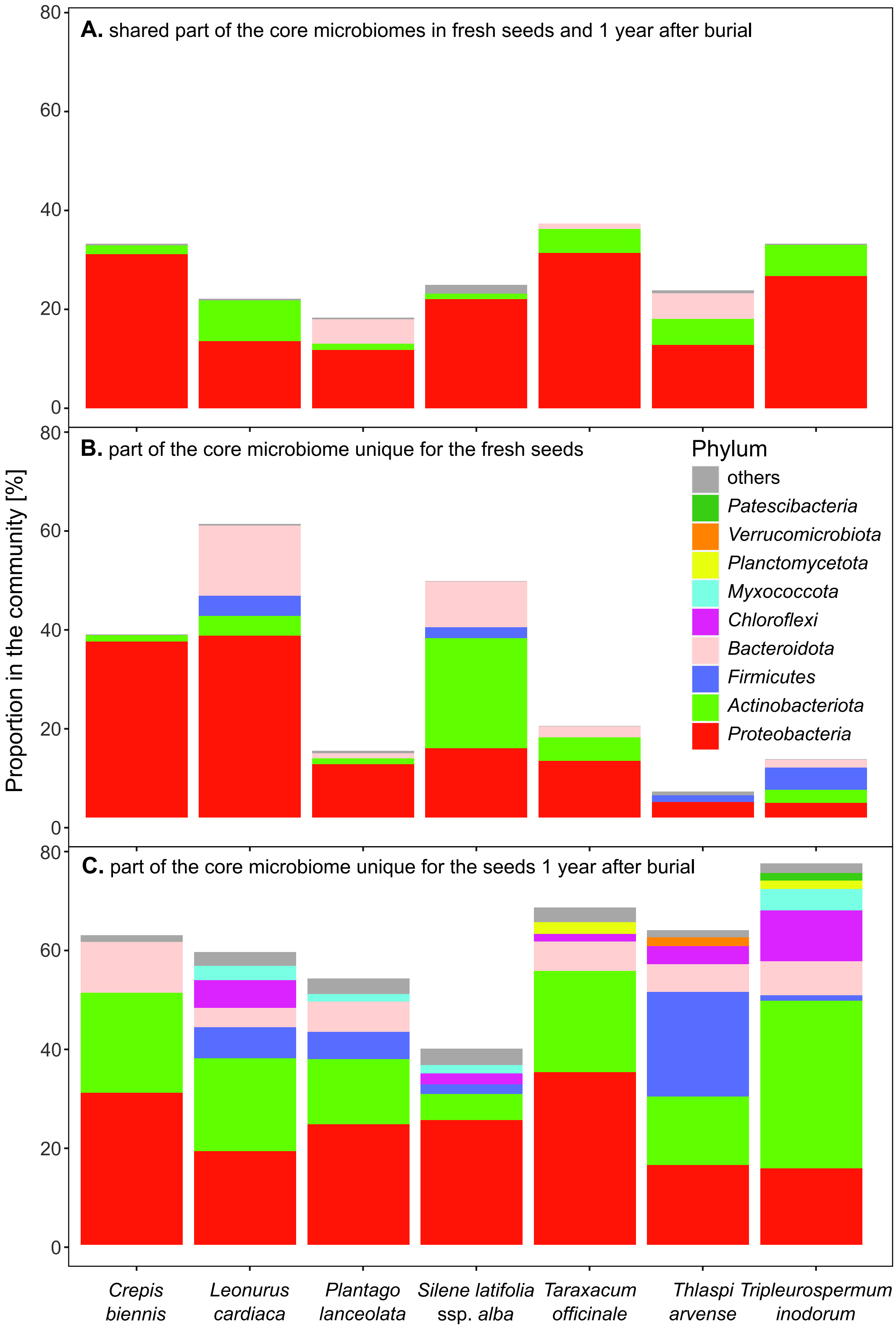

Fig. 3. Comparison of core microbiomes of the seed species between freshly harvested seeds and seeds one year after burial. The columns show the proportions of ZOTUs, grouped by taxonomic classification at the phylum level, shared by core microbiomes of the both fresh and buried seeds (A), and those unique for the fresh seeds (B), and the seeds sampled one year after burial (C).

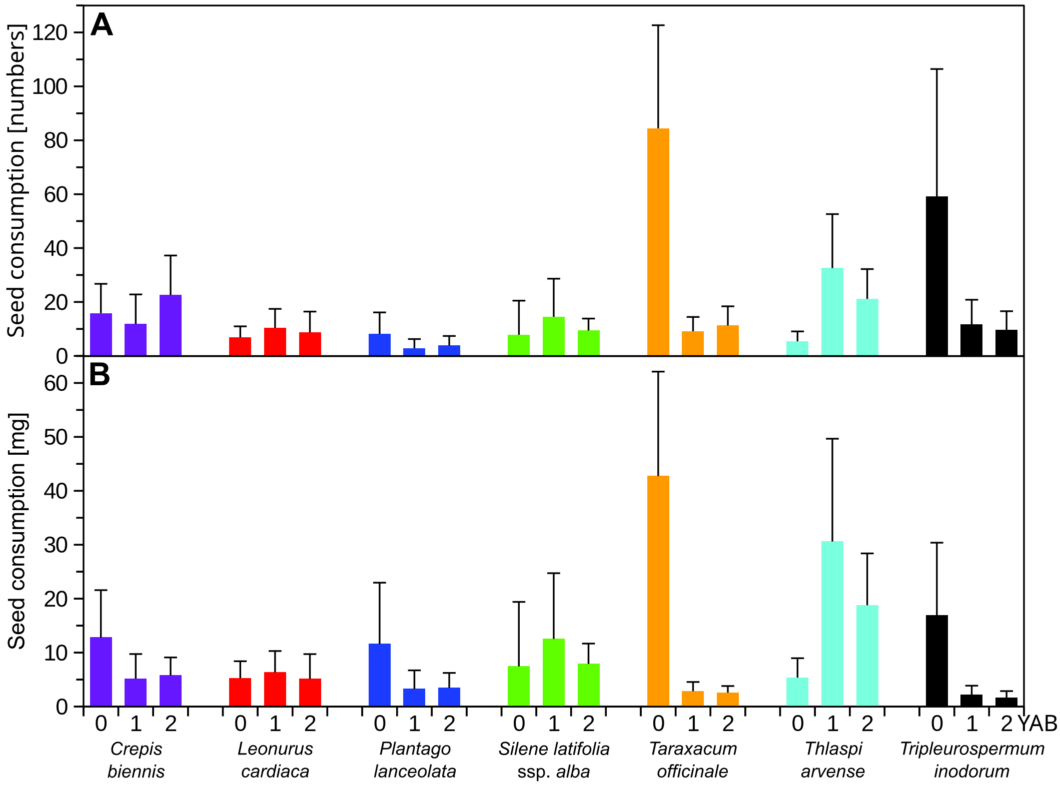

Fig. 4. Seed consumption by *P. rufipes* in numbers (A) and weight (B) of seven species of seeds and three times of burial.

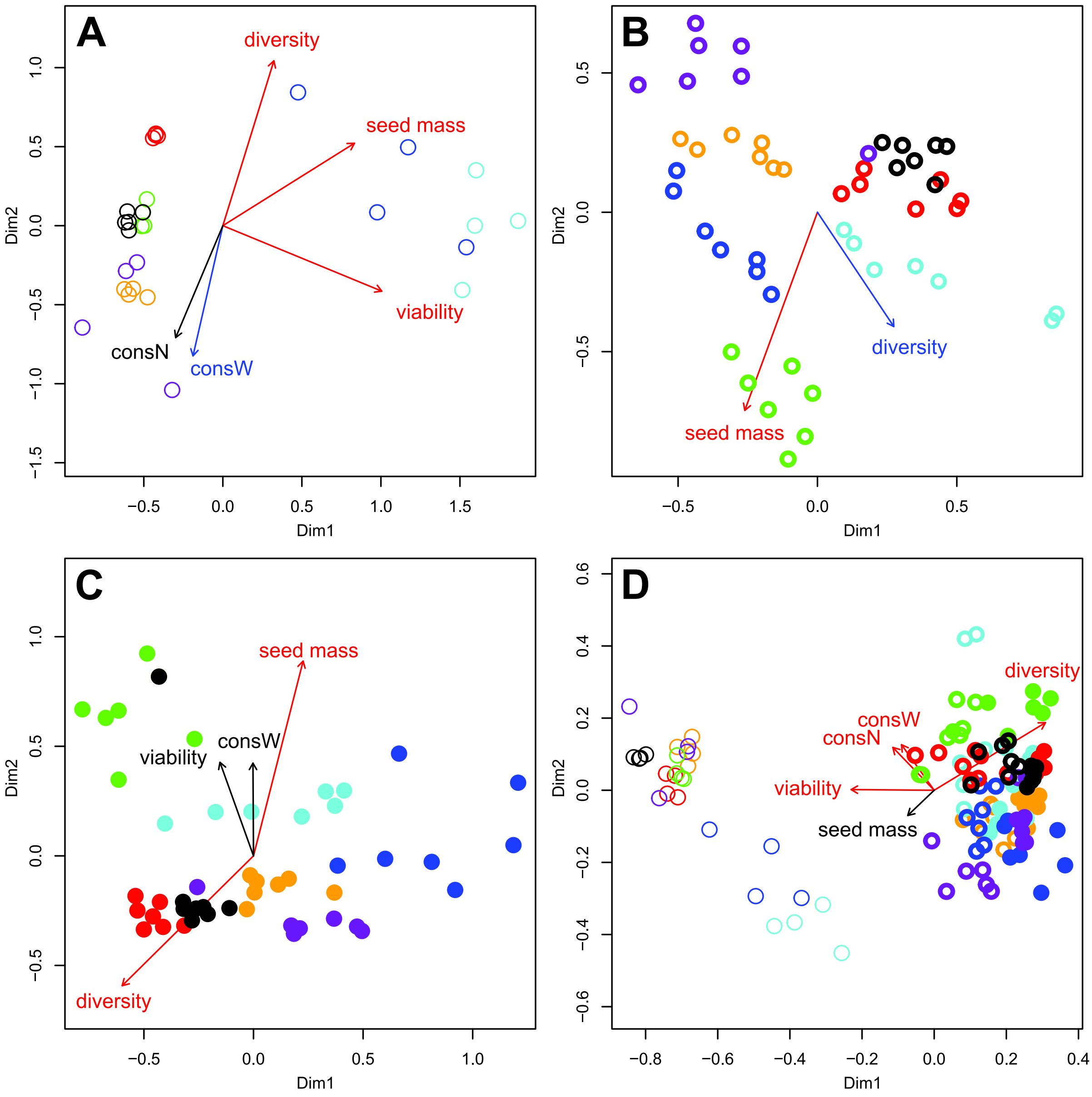

Fig. 5. Differences in composition of seed-associated bacterial communities of the fresh seeds (A, open thin symbols), after one (B, open thick symbols) and two years (C, closed symbols) of burial, and of all samples analyzed together (D). The seeds were of seven species: *C. biennis* (purple), *L. cardiaca* (red), *P. lanceolata* (blue), *S. latifolia* ssp. *alba* (green), *T. officinale* (yellow), *T. arvense* (cyan), and *T. inodorum* (black). The vectors show linear variables correlated with the community composition: seed mass and viability, diversity of the bacterial community, and rate of seed consumption by *P. rufipes* expresed in seed numbers (consN), and weight (consW). Vector colors show statistical significance of the correlation (p<0.05, black; p<0.01, blue; p<0.001, red)Non-metric multidimensional scaling of distance matrices based on Bray-Curtis index.

8. Závěrečná diskuze

Předmětem této disertační práce bylo studium predace semen v souvislosti s preferenčním chováním střevlíků k vybraným druhům plevelů. Jako nejvýznamnější znak semen ovlivňující preference střevlíkovitých brouků se v literatuře uvádí velikost (např. Honek et al., 2003, Honek et al., 2011, Kotze et al., 2011). Dalšími znaky pak bývá uváděna hmotnost (např. Moles et al., 2003, Saska et al., 2019, Law and Gallagher, 2015), tvrdost osemení (Lundgren and Rosentrater, 2007) nebo fylogenetická příbuznost mezi druhy semen (Moles et al., 2003, Saska et al., 2019, Kulkarni et al., 2016).

Výsledky této dizertační práce naznačují, že preference střevlíků jsou ovlivněny znaky semen, které jsou dány jejich fylogenetickou příbuzností. Tyto znaky semen se mění v čase i v prostředí. Zároveň tyto změny významně ovlivňují preference střevlíků. Mezi hlavní znak semen ovlivňující preference střevlíků řadíme velikost, tvar, hmotnost, vlastnosti osemení (tloušťka osemení a síla potřebná k rozlousknutí jednotlivých druhů semen), jejich taxonomickým zařazením a životní strategií. Tyto výsledky se shodují s pracemi, kde jsou jednotlivé znaky semen popsány jako významné pro preference semen. Překvapivě v našich experimentech nebyl potvrzen vliv chemických látek v semenech na preference střevlíkovitých brouků (*Foffová et al. 2020, Insects 11:757).* Důvodů k tomu může být více. Jedním z nich je použití plastelíny jako „nosiče“ semen (Honek et al., 2007), která by mohla potenciálně přispět k pozměnění chemických znaků semen nebo produkovat volatilní látky. Mohlo například dojít k omezení vylučování volatilních látek a semena tak predátor nemohl najít (Linton and Wright, 1993). Dalším důvodem může být zamražení semen před analýzou. Zamražení semen nemění jejich životnost (Jordan and Jordan, 1982), ale mohlo dojít ke změnám jejich chemického složení. Bohužel v práci *Foffová et al. 2020, Insects 11:757* nebyly měřeny chemické látky, jako je např. obsah olejů v semenech. Olejnatost semen se zdá být také jedním z významných znaků semen určují jejich predaci (Gaba et al., 2019).

V práci *Foffová et al. 2020, Insects 11:757* bylo zjištěno, že fylogenetická příbuznost druhů střevlíků určuje, jaké znaky semen se podílejí na výsledných preferencích střevlíků. V této práci byli studováni zástupci dvou tribů (Zabrini a Harpalini). Bylo prokázáno, že znaky semen určující preference mezi triby se liší. Preference zástupců tribu Zabrini jsou ovlivněny strategií rostlin, taxonomií, vlastnostmi osemení, hmotností semen a rozměry semen. Oproti tomu preference zástupců tribu Harpalini byly ovlivněny pouze znaky osemení, hmotností a rozměry semen. Jelikož vysvětlená variabilita dat byla v případě tribu Zabrini menší nežli u tribu Harpalini, lze toto interpretovat tak, že zástupci tribu Harpalini jsou méně specializovaní při výběru semen oproti zástupcům tribu Zabrini (Honek et al., 2007). Díky vyšší míře specializace u zástupců tribu Zabrini může každý druh reagovat na jiné, specifické podněty při výběru semen. V dalších dostupných pracích bylo také dokázáno, že preference predátorů semen se liší mezi jednotlivými fylogenetickými skupinami (např. Deroulers and Bretagnolle, 2019, Saska et al., 2019). Tyto preference mohou být ovlivněny stavbou mandibul predátora (Deroulers and Bretagnolle, 2019, Lundgren and Rosentrater, 2007, Evans and Forsythe, 1985). Pro další fylogenetické skupiny střevlíků, které se podílejí na predaci semen, je potřebné provést další experimenty, neboť jejich preference nejsou příliš známy.

Dále bylo zjištěno, že preference střevlíků jsou ovlivněny momentálním stavem semen. Střevlíci preferují nabobtnalá nebo poškozená nabobtnalá semena více, nežli suchá semena (*Foffová et al. 2020, Acta Zoologica Academiae Scientiarum Hungaricae, 66:37 - 48*). Toto tvrzení se shoduje s pracemi (Cardina et al., 1996, Koprdova et al., 2008, Hurst and Doberski, 2003). Nabobtnalá semena také více požíral *Harpalus pensylvanicus* (Degeer, 1774) v práci Law & Gallagher (2015). V této konkrétní práci autoři uvádějí, že vyšší počet sežraných semen je možné vysvětlit vyšší šancí predátorů najít semena díky olfaktorickým vjemům střevlíků. Stejný výsledek pak přináší práce Kulkarni et al. (2017). Únik atraktivních látek z nabobtnalých semen by mohl také vysvětlit nárůst predace semen v práci Lundgren et al. (2006), kdy střevlíci v deštivém týdnu žrali více semen nežli v suchém týdnu. V literatuře zcela schází informace o predaci semen, která by byla nějak poškozená. Tato práce jako první poukazuje na to, že střevlíci nejvíce žerou poškozená životaschopná semena.

V dalších experimentech je potřebné změřit, jak se mění síla potřebná k rozlousknutí semen při bobtnání. Lze očekávat, že s nabobtnáním semen se bude snižovat síla potřebná k otevření semen. Dále je potřebné se zaměřit na tzv. handling time, tj. čas potřebný pro zpracování kořisti, konkrétně jestli po nabobtnání semen nebo při poškození osemení se handling time zkracuje. Zkracující se handling time by potenciálně souvisel s možností predátora rychleji a efektivněji sežrat semena. V neposlední řadě je potřebné ověřit, jak se mění spektrum a koncentrace volatilních látek uvolňovaných z nabobtnalých oproti suchým semenům. V našich experimentech bylo zjištěno, že ze suchých semen se uvolňuje menší spektrum volatilních látek s nižší koncentrací než ze suchých semen. Je možné, že detekce takto malých koncentrací není zatím možná. Dále práce *Foffová et al. 2020, Acta Zoologica Academiae Scientiarum Hungaricae, 66:37 - 48* ukazuje, že vyjma momentálního stavu semen mají na preference vliv i znaky semen vycházející z jejich fylogenetické příbuznosti. Stejně jako práce Kulkarni et al. (2017), která zjistila, že kromě nabobtnání semen mají na preference semen střevlíky vliv i znaky semen, které vycházejí z jejich fylogenetické příbuznosti. Střevlíkovití brouci v experimentu *Foffová et al. 2020, Acta Zoologica Academiae Scientiarum Hungaricae, 66:37 - 48* preferovali semena ze shodné geografické lokality, kde se vyskytovali i střevlíci. Stejně tak tomu bylo v experimentu Honek et al. (2011), kde střevlíci preferovali semena ze stejné oblasti (česká semena *Taraxacum* agg. před italskými). Tato zjištění je možné vysvětlit adaptací střevlíků na změny znaků semen mezi jednotlivými populacemi rostlin.

Již zmiňované znaky semen vycházejí z jejich fylogenetické příbuznosti. Tyto znaky společně s životností semen se mění s dobou, po kterou setrvávají v půdě (*Saska et al. 2020, Agronomy, 10:448*). V této práci bylo potvrzeno, že životnost semen je nelineární a postupem doby setrvání v půdě se snižuje počet životaschopných semen. Toto tvrzení bylo také zjištěno kolektivy autorů Lutman et al. (2002) nebo Masin et al. (2006). V naší práci však bylo použito modelování změn v podílech životných semen pomocí logistické křivky. Tento přístup považujeme za vhodnější pro pochopení dynamiky změn a ekologii půdní zásoby semen. Tento postup by mohl nahradit klasifikaci dlouhověkosti semen do umělých kategorií, která v literatuře v současnosti převládá (např. Thompson et al. 1997). Novost této práce je také ve změření znaků semen a jejich změny v průběhu setrvání v půdě, neboť jejich změny s dobou setrvání v půdě nebyly doposud zdokumentovány. Přitom právě změny znaků semen nám mohou napomoci určit životnost jednotlivých semen, které najdeme v půdě. Práce *Saska* *et al. 2020, Agronomy, 10:448* také naznačuje, že ne u všech druhů semen má stárnutí a poškození osemení vliv na životnost semen. S průběhem doby setrvání v půdě se zvyšuje poškození osemení a semena jsou různě deformovaná (např. semena *Geum urbanum* L.). Přes změny osemení a deformaci semen byla životnost semen tohoto druhu vysoká po celou dobu experimentu. Oproti tomu semena *Amaranthus powellii* S. Watson nevykazovala žádné výrazně viditelné změny v osemení, tvaru či hmotnosti, ačkoliv životnost semen tohoto druhu byla jedna z nejkratších ze zkoumaných druhů. Nicméně veškeré znaky semen i jejich životnost může být ovlivněna geografickým původem semen, půdními podmínkami nebo antagonisty semen v přirozeném prostředí (např. Cerabolini et al., 2003, Pakeman et al., 2012, Davis et al., 2005).

Na preference střevlíků má vliv i stáří semen (*Saska et al. 2019, EJE, 116:133 - 140*). V tomto článku bylo zjištěno, že druhy střevlíků *Pseudoophonus rufipes* (DeGeer, 1774) a *Amara littorea* C. G. Thomson, 1857 preferují čerstvá semena před zakopanými. Oproti tomu druh *Harpalus affinis* (Schrank, 1781) preferuje zakopaná semena před čerstvými. Schopnost predátorů semen žrát i semena, která byla součástí půdní banky, byla potvrzena i dalšími autory. Například Hulme (1998) potvrdil, že hlodavci jsou schopni sežrat semena, která vstoupila do uměle vytvořené půdní banky. Martinkova et al. (2006) potvrdili, že střevlíci žerou semena, která byla v půdní zásobě po dobu 6 měsíců. Změny preferencí střevlíků k zakopaným nebo čerstvým semenům nejde vysvětlit zkoumanými morfologickými změnami v semenech. Možným vysvětlením může být, že v semenech došlo ke změně koncentrací nebo přítomnosti některých chemických látek (Davis et al., 2008), došlo k napadení semen mikrobiálními antagonisty (Blaney and Kotanen, 2001, Dalling et al., 2011) nebo došlo ke změně v osemení (Tiansawat et al., 2014). Nicméně předpoklad, že došlo ke zmiňovaným změnám, nemůžeme v tuto chvíli podpořit žádnou analýzou, avšak v budoucnu plánujeme takové analýzy udělat.

Preference semen střevlíky jsou ovlivněny i diverzitou a početností jednotlivých druhů bakterií vyskytujících se na semenech nebo uvnitř semen. Znaky semen, která setrvala v půdě, se mění i díky bakteriím. V práci *Saska et al., PLSO – under review*, bylo zjištěno, že diverzita společenstev bakterií zakopaných semen roste, zatímco životnost a hmotnost semen se snižuje. Mezi druhy bakterií na zkoumaných druzích semen je vysoká variabilita. Jednotlivé druhy semen mohly být vystaveny různým selekčním tlakům mikroorganismů (Singh et al., 2020). Variabilita v přítomných druzích bakterií může ovlivnit preference jednotlivých predátorů semen. Příkladem mohou být semena druhů *Taraxacum officinale* L. nebo *Tripleurospermum inodorum* (L.) Sch. Bip., jejichž preference střevlíky se snížily, zatímco počet druhů diagnostikovaných bakterií se navýšil. Naopak semena druhu *Thlaspi arvense* L. začala být po zakopání preferovanější nežli čerstvá semena. Poznatky o druhové biodiverzitě bakterií na semenech jsou nezbytné pro pochopení interakcí v půdním prostředí (Chen et al., 2021). Tyto znalosti mohou zlepšit i naše chápání funkcí endofytů semen, odhalit účinky mikroorganismů na populace semen v půdní zásobě (Larios et al., 2017) nebo změny v mikrobiomu trávicího traktu střevlíků (Engel and Moran, 2013), které mohou ovlivnit individuální preference.

Tato disertační práce přináší nové poznatky týkající se fenoménu predace semen a s ní spojeným výběrem semen jejich predátory. V této práci jsou zahrnuty poznatky o obranných znacích semen před jejich predátory a o jejich změnách v průběhu doby setrvání v půdě, při nabobtnání, poškození nebo při kolonizaci bakteriemi. Tyto znaky a jejich změny společně s poznatky o perzistenci semen v půdní zásobě semen nám pomáhají pochopit populační dynamiku plevelů a ekologii jejich semen v půdní zásobě semen. Tyto výsledky by mohly přispět k lepšímu pochopení vztahů mezi predátory semen a jejich kořistí, tj. semeny. Dále by výsledky zařazené do této práce mohly v budoucnu napomoci k přesnějšímu modelování dynamiky predace semen a následné kvantifikaci účinků ekosystémové služby predace semen, neboť bylo zjištěno, že střevlíci požírají i semena, která setrvala v půdní zásobě. Dále bylo u některých druhů stanoveno, kolik průměrně sežerou semen za určitý časový úsek. Také bylo zjištěno, že střevlíci preferují nabobtnalá semena, takže k ekosystémové službě predace semen plevelů by mohlo docházet častěji za vlhčích podmínek nežli za sucha. Zjištěné poznatky zjištěné v této disertační práci mohou být dále použity v pracích, které modelují změny v půdní zásobě semen (např. Carbonne et al. 2021; Bohan et al. 2011) nebo kvantifikují potravní sítě predátorů semen (např. Pocock et al., 2021).

9. Kompletní seznam výsledků

9.1. Odborné články v databázi Web of Science

Carbonne, B., Bohan, D. A., Foffová, H., Daouti, E., Frei, B., Neidel, V., Saska, P., Skuhrovec, J. and Petit, S. 2022. Direct and indirect effects of landscape and field management intensity on carabids through trophic resources and weeds. J Appl Ecol. 59:176–187 DOI: 10.1111/1365-2664.14043

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9.2. Ostatní publikace

Saska P., Skuhrovec J., Foffová H., Řezáč M. 2020. Ekosystémové služby poskytované bezobratlými v zemědělství: opylování a regulace škůdců a plevelů. Vydáno VÚRV ve spolupráci s Českou technologickou platformou pro zemědělství. ISBN: 978-80-7427-344-5.

Foffová H.; Saska P. 2019. Predace semen - ekosystémová služba přispívající k regulaci půdní zásoby semen. Selská revue,7,82-84.

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Foffová H.; Skuhrovec J., Saska P. 2021. Minisérie: Ekosystémové služby v zemědělství (2): Regulace plevelů ekosystémovými službami. Agromanuál 9-10:30-32.

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9.3. Aktivní účast na konferencích a seminářích

2021: Konference: Dolní Dunajovice: Pohled přes hranice na téma Rostlinolékařství není jen chemie (trendy ochrany rostlin v duchu evropských strategií „Grean Deal“, „Farm to Fork“) – přednáška na téma Ekosystémové služby v zemědělství

2020: Webinář: Agro-environmentální opatření v zemědělství - přednáška na téma: Agro-enviromentální opatření (AEKO) se zaměřením na ekosystémové služby v zemědělství

2019: Seminář pro odbornou veřejnost: Vliv systémů pěstování plodin na změny spektra škodlivých organismů, Praha - přednáška na téma: Ekosystémové služby se zaměřením na regulaci plevelů

2019: Konference: Proceedings 7th meeting of the EWRS working group "Weeds and biodiversity", Německo – přednáška na téma: Do the properties of seeds affect their predation?

2019: Konference: 19th European Carabidologist meeting, Italie – poster na téma: Some seed properties affecting seed choice by *Poecilus cupreus*

2018: Konference - 18th EWRS Symposium - Lublaň, Slovinsko - prezentace na téma: Seed persistence and changes in seed coat colouration with time spent in soil

2018: Konference: Kostelecké inspirování, Česká Republika – přednáška na téma: Změny životnosti semen a jejich obalů v závislosti na délce setrvání v půdě

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