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Major effects of glucosinolates and minor effects of erucic acid on predation of *Brassica* seeds by mice

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Abstract

Seeds of cultivars of the crop oil seed rape (*Brassica napus*) differ widely in their content of glucosinolates (GS) and erucic acid (EA), while seeds of the wild relative *B. rapa* have a high content of both. If mice were to distinguish between the seed types this could affect persistence of plant populations.

In a choice experiment in two habitats seven seed types were offered to seed predators, mainly mice, and to captured individuals of the wood mouse *Apodemus sylvaticus*.

Seed predators clearly distinguished between seeds of the two species and between different cultivars of *B. napus*. In three experiments the amount of seeds eaten had a high, negative correlation with GS content (rank correlation coefficient −0.81 to −0.91). Correlations with EA were lower (−0.33 to −0.52) and not statistically significant. When offered a choice between two seed types with similar GS content, mice chose those with the lower EA content.

Double-zero (low GS and low EA) cultivars of *B. napus* suffer heavily from post-dispersal seed predation while cultivars with high GS and/or EA suffer less, making the latter potentially more persistent. We suggest that the consistently high GS and EA content in seeds collected in wild populations of *B. rapa* is an adaptation that reduces seed predation.

Zusammenfassung

Samen von modernen und alten Kulturrassen der Feldfrucht *Brassica napus* unterschieden sich in ihrem Gehalt an Glukosinolaten (GS) und Erucasäure (EA). Samen des Unkrauts *B. rapa* haben demgegenüber einen hohen Gehalt von beiden Substanzen. Wenn Mäuse zwischen Samen verschiedener Rassen differenzieren, wird das die Persistenz von Pflanzenpopulationen beeinflussen.

Hier wurden in Wahlexperimenten im Freiland (in zwei Habitaten) Samen von sechs Kulturrassen an frei lebenden Prädatoren, vor allem Mäusen, angeboten. Daneben wurden Laborexperimente mit gefangenen Individuen der Waldmaus *Apodemus sylvaticus* durchgeführt.

Samenprädatoren unterscheiden zwischen Samen von *B. napus* und *B. rapa* und zwischen Samen von Kulturrassen von *B. napus*. In allen Experimenten war der Anteil der Samen, die gefressen wurden, stark negativ korreliert mit dem GS-Gehalt (Rang-Korrelationskoeffizienten −0.81 bis −0.91). Rang-Korrelationen zwischen dem Anteil gefressener Samen mit EA waren niedriger (−0.33 bis −0.52) und nicht signifikant.

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In den Freilandexperimenten selektierten die Mäuse auf Basis des GS- und EA-Gehalts. Doppel-Null-Kulturrassen (mit geringen GS- und EA-Gehalten) erlitten hohe Samenverluste. Kulturrassen für die Biodieselproduktion wiesen geringe Prädation auf, und das erhöht potentiell die Persistenz dieser Rassen.

Keywords: *Apodemus sylvaticus*; Cafeteria experiment; Invasive species; Plant defence; Plant–herbivore interactions; Weed

Introduction

Plants produce a number of metabolites as a defence strategy against a suite of herbivores and pathogens, both above and below ground (Burow, Halkier, & Kliebenstein 2010; van Dam & Heil 2011). The type and quantity of these metabolites in seeds may be quite different from that in the rest of the plant, depending on the challenging animals. If a seed predator spoils some seeds, aiding their dispersal, its effect on fitness could be positive. In this case, plants may be selected to produce entirely palatable seeds (Janzen 1971) that require a long handling time and are therefore more likely to be dispersed and stored, rather than eaten on the spot (Vander Wall 2010). However, when seed predation reduces fitness, plants are expected to develop morphological (Reader 1993; Paulsen, Högstedt, Thompson, Vandvik, & Eliassen 2014) or chemical defences against their predators.

There are several examples of the role of secondary metabolites in the defence of seeds. High concentrations of monoterpenes in seeds of the conifer *Abies alba* discourage squirrels from eating them (Lobo 2014). Low concentrations of alkaloids were observed to deter agoutis from feeding on seeds (Guimaraes, Jose, Galetti, & Trigo 2003). Tannins in artificial seeds reduced seed predation by mice (Wang & Yang 2015). The toxic protein ricin in *Ricinus communis* (Barnes, Baldwin, & Braasch 2009) occurs only in the seeds and protects against seed predation by mammals and beetles (do Nascimento et al. 2011). Glucosinolates (GS) play a role in seed defence against mice (Samuni-Blank et al. 2012). Other substances such as selenium, cyanogenic glycosides, saponins, phenolics and endopeptidase inhibitors have also been suggested to play a major role in defence mechanisms of seeds (Janzen 1971).

Seeds are also rich in fats. Fatty acids are primarily used in the cotyledons to support fast growth of the emerging seedling. Given the variation in the seed fatty acid profile between species (Wolff et al. 2001) it is possible that fatty acids could play a role in plant defence. This idea is supported by observations on the preference of many vertebrates for diets that contain relatively short fatty acids with a low degree of saturation (Karasov & Martínez del Rio 2007) or on passerine birds that preferred diets with unsaturated fatty acids to those with saturated fatty acids (Ríos, Barceló, Narváez, Maldonado, & Sabat 2014). Erucic acid (EA) is a relatively long fatty acid with a single double-bond (22:1) and a relatively high melting point of 33.5 °C. EA is a major fatty acid in seeds of the Brassicaceae (Tonguç & Erbaş 2012; Sun et al. 2013). EA is unpalatable to some animals and unhealthy for rats and mice, affecting heart condition and survival in laboratory feeding tests (Badawy, Atta, & Ahmed 1994; Bozcali, Suzer, Gursoy, Atukeren, & Gumustas 2009). A study by Sanyal and Linder (2013) showed that EA content of *Arabidopsis thaliana* seeds increased towards the south of Europe and these authors suggested that increased EA content could be an adaptation to reduce seed predation. While certain fatty acids (for instance EA) may be harmful, the total fat content of seeds still had a positive effect on rodent seed predation (Wang & Chen 2012).

Oilseed rape (*Brassica napus*) is a good model species for studying the chemical aspects of seed predation because there is so much variation in its chemistry. The cultivars that were grown before 1980 typically have a high content of both GS and EA. In the 1980’s double-zero or canola cultivars with reduced GS, especially progoitrin, and reduced EA content in seeds were introduced. The canola cultivars are considered healthier for humans allowing a revival of *B. napus* as an oilseed crop. Recently cultivars with high EA contents, known as high erucic acid rape (HEAR), have been developed and these cultivars are now grown specifically for producing biofuel. Extensive studies were done on herbivores of modern canola low in EA and GS and varieties high in both EA and GS (Giamoustaris & Mithen 1995; Bruce 2015). Birds and slugs showed clear preferences for modern canola, while many insects feeding on leaves or in fruits showed no such preference. Moshgani, Kolvoort, and de Jong (2014) reported a strong negative correlation between aliphatic GS and herbivory on seedlings by slugs. Post-dispersal seed predation was not a part of the comparative studies by Giamoustaris and Mithen (1995). Rodents are generalists and it is not known whether they discriminate between seeds from different *Brassica* cultivars and whether chemical defence plays any role in this preference. GS occur in seeds, roots and leaves and clearly play a role in defence against many different herbivores. EA occurs only in the cotyledons because fatty acids are not transported to the first leaves. EA will not effect herbivory on the root or the shoot, but could play a role in seed predation. EA could also deter herbivores, such as slugs, that feed on the cotyledons immediately after germination. We performed preliminary experiments with the slug *Arion lusitanicus* (T.J. de Jong and C.C. Molenaar, unpublished results, 2015). In these experiments slugs fed as much on seedlings of high-EA cultivars as they did on low-EA cultivars. We thus have no indications that EA defends seedlings against slug herbivory.