



Efficacy of *Brassica juncea* granulated seed meal against *Melolontha* grubs



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ABSTRACT

The grubs of *Melolontha melolontha* and *Melolontha hippocastani* damage the roots of many plants and are therefore considered serious pests in agriculture, horticulture and forestry in central and eastern Europe. Since the implementation of legislative regulations banning the use of synthetic insecticides against these pests in soil, alternative methods have gained high priority. The plants of the family Brassicaceae have recently received much attention due to the high content of glucosinolates (GSLs) in their tissues. The GSL breakdown products are highly biocidal in relation to many soil-borne pests. We tested *Brassica juncea* granulated seed meal against *Melolontha* grubs under semi-natural (the concentration-response experiments) and field conditions. The efficacy and the indirect lethal concentrations of GSLs that were necessary to obtain 50% and 95% grub mortality (ILC50 and ILC95, respectively) were estimated.

The concentration-response experiments revealed that the ILC50 of GSLs dominated by sinigrin (97%) for the L1, L2 and L3 grubs was 118.4, 167.1 and 173.5 $\mu\text{mol/L}$, respectively, and the ILC95 was 293.3, 312.7 and 401.7 $\mu\text{mol/L}$, respectively. Under field conditions, the mortality of the L3 grubs in the plots that were treated with granulated *B. juncea* seed meal at ILC99.8 was almost twice as high (82.2%) as that of the grubs in the untreated plots (45.4%), and the changes in the grub density with time in these plots were significantly different.

The results of our experiments demonstrate the high potential of soil biofumigation using *B. juncea* granulated seed meal to control *Melolontha* grubs.

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1. Introduction

The two common cockchafer species, the May cockchafer *Melolontha melolontha* (L.) and the forest cockchafer *Melolontha hippocastani* F., are considered serious pests in agriculture, horticulture and forestry in many European countries (e.g., Keller and Zimmermann, 2005; Malinowski, 2007; Švestka, 2010; Wagenhoff et al., 2014). After overwintering in the soil, the beetles emerge in late April through May and begin maturation feeding in the crowns of several tree species, primarily the silver birch *Betula pendula* Roth, pedunculate oak *Quercus robur* L., European beech *Fagus sylvatica* L. and European larch *Larix decidua* Mill. (Sierpiński, 1975; Śliwa, 1993). However, the damage is mainly caused by the larvae (grubs), which develop through three instars in the soil and feed on the roots of many plant species during four consecutive years.

The first attempts to control these pests were primarily based on mechanical methods, i.e., collecting the adult bee-

gles during the swarming period or killing the grubs when ploughing the soil (Satkowski, 1899; Zimmermann, 2010). Since their introduction in the 1950s, synthetic pesticides were successfully used to reduce the cockchafer population densities. These insecticides were either aerially sprayed to control the adult beetles feeding on the foliage or, most often, applied to the soil to kill the grubs (Głowacka and Sierpińska, 2012; Zimmermann, 2010). Due to the high toxicity of pesticides, their ability to accumulate in living organisms and their overall negative environmental impact, a number of legislative regulations were implemented to limit their use (e.g., Regulation (EC) No 1107/2009 <http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:32009R1107> and Directive 2009/128/EC <http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:32009L0128>). Consequently, alternative methods to reduce the number of cockchafer have gained high priority in recent years.

The use of defence mechanisms of various plant species is of particular interest. The plants of the family Brassicaceae have received much of attention due to the relatively high quantities of the glucosinolates (GSLs) in their tissues (Fahey et al., 2001; Kirkegaard and Sarwar, 1998; Oleszek, 1995). When plant tissue is damaged,

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GSLs are hydrolysed by the enzyme myrosinase, which is also present in plant tissues, to a range of hydrolysis products, such as nitriles, thiocyanates and isothiocyanates (ITCs) (Brown et al., 1991). This glucosinolate-myrosinase system is a defence mechanism that was developed by certain plants to prevent herbivore attack (Ahuja et al., 2011; Textor and Gershenzon, 2009). The products of GSL conversion have extensive biocidal activity (reviewed in Brown and Morra, 2005; Piekarska et al., 2010; Vig et al., 2009), including bactericidal (Lin et al., 2000), fungicidal (Angus et al., 1994; Sarwar et al., 1998), nematocidal (Avato et al., 2013; Lazzeri et al., 2009; Mojtahedi et al., 1991, 1993; Ploeg, 2008) and insecticidal (Borek et al., 1995, 1997; Furlan et al., 2010; Shaaya and Kostyukovsky, 2009; Tsao et al., 2002) effects. Thus, mustards, such as Indian mustard *Brassica juncea* (L.) Czern. or rapeseed *Brassica napus* L. can be used as green manures or seed meal amendments to suppress harmful organisms in the soil, via a procedure known as “biofumigation”, and there is an increasing interest in this approach as an alternative to synthetic pesticides and soil fumigants (Barker et al., 2014; Kirkegaard et al., 1999; Noble and Sams, 1999; Piekarska et al., 2010; Shaaya and Kostyukovsky, 2009). In addition to the biofumigation effect, the Brassicaceae plants and their seed products also have a fertilizing effect by enriching the soil with nitrogen and other macroelements (Snyder et al., 2010).

Few studies have focused on the effectiveness of biofumigation against soil-living insects. A high larval mortality of the click beetle *Limonium californicum* (Mann.) (Elateridae), a pest of potatoes and sugar beet in North America, was observed when *B. napus* meal was added to the soil (Elberson et al., 1996). Similar results were obtained when larvae of the black vine weevil, *Otiorhynchus sulcatus* (F.) (Curculionidae), a common root pest in Europe and North America, were exposed to soil that was amended with rapeseed meal (Borek et al., 1997). The toxic effect of *B. juncea* on the larvae of the Masked chafers, *Cyclocephala* sp. (Scarabaeidae), has also been demonstrated (Noble et al., 2002). The successful control of *Agriotes* sp. (Elateridae) wireworms using the same plant was also confirmed in both laboratory and field studies (Furlan et al., 2010).

The aims of our research were: (1) to test the efficacy of *B. juncea* granulated seed meal against *Melolontha* spp. grubs at the three larval instars, (2) to assess the concentration of GSLs (μmol per litre of soil) that is necessary to indirectly, through their breakdown products, cause a 50% and 95% mortality of grubs (called an indirect lethal concentration and hereinafter described as ILC50 and ILC95, respectively), and (3) to evaluate the efficacy of soil biofumigation under field conditions.

2. Materials and methods

2.1. Experimental insects

The *Melolontha* spp. grubs of the three instars, L1, L2 and L3, were used in our experiments. There is no reliable diagnostic key that can be used to differentiate these grubs to the species level (Krell, 2004). However, it was possible to determine the instar of the grubs by measuring the width of the head capsule (L1: approx. 2.6–2.7 mm, L2: approx. 4.2–4.5 mm, L3: approx. 6.5–6.9 mm) (Śliwa, 1993). The grubs were collected in the soil of young forest plantations, clear-cut areas and post-agricultural lands in different regions of Poland; however, in each experiment we usually used grubs that were sampled from the same population.

2.2. Materials used in concentration-response experiments

Black plastic pots with an upper external diameter of 13.3 cm, a lower diameter of 9.8 cm, and a height of 10.3 cm were used. The bottom parts of the pots were covered with a fabric to prevent the

grubs from escaping from the pots through the drainage holes. The soil that was used in the experiments was a mixture of sand and garden soil in a 3:1 ratio. The garden soil consisted of peat that was de-acidified to pH (H_2O) = 5.5–6.5 with chalk (CaCO_3) and macro- and microelements.

Each pot was filled with 0.7 L of soil that was previously mixed in a larger container with the required amount of the *B. juncea* granulated seed meal (hereinafter named granulate), which was supplied by Kosmalki Herbs & Spices (Inowrocław, Poland). The required amount of the granulate (g/0.7 L of soil) was calculated by dividing the desired concentration of GSLs by their content in the granulate (see below) and multiplying by 0.7 L. The soil in the untreated pots was not mixed with the granulate.

Each grub of the selected instar for a particular experiment was placed in a hole in the soil to a half depth of the pot and covered with a thin layer of soil. Then, a small piece of carrot was added as food for the grub.

2.3. Estimation of the GSL amount in the granulate

The concentration of GSLs in the granulate was determined by the Institute of Animal Reproduction and Food Research of Polish Academy of Sciences (Olsztyn, Poland). The GSLs were extracted according to the method in the Commission Regulation (EEC) No. 1864/90. Briefly, 200 mg of the granulate was extracted thrice with boiling 70% methanol. A pre-defined amount of glucotropaeolin was added to the sample immediately prior to the first extraction as an internal standard for the HPLC analysis. The isolation, desulphation and HPLC of GSL were carried out according to the method that was modified by Heaney et al. (1986). Desulpho-GSL were separated in the HPLC system with an auto injector (20 μl loop), Spherisorb ODS-2 3 Micron column (150 \times 4.6 mm) and 1.2 ml/min flow rate at 32 °C by eluting with a gradient of water (A) and 20% acetonitrile (B) as follows: isocratically 1% B for 1 min, gradient to 99% B for 30 min (curve – 3), isocratically 99% B for 6 min, linear gradient to 1% B for 5 min, and 1% B for 8 min. The GSL were detected at $\lambda = 229$ nm. The sample content of GSL was quantified based on the internal standard and relevant relative response factors. The total amount of GSLs in the granulate was 104.14 $\mu\text{mol/g}$, including 101.2 $\mu\text{mol/g}$ of 2-propenyl GSL (sinigrin), which is 97% of the total GSLs.

2.4. Concentration-response experiments

The granulate was preliminary tested against the L1 grubs at three concentrations under laboratory conditions. The minimal concentration was 160 $\mu\text{mol/L}$, which was a concentration of the total GSLs that was effective against wireworms (Furlan et al., 2010). The two other tested concentrations were 320 and 640 $\mu\text{mol/L}$. The mortality rates of the L1 grubs after 7 days were 25, 50 and 100%, respectively. Using these results, we tested three other GSL concentrations (640, 1280 and 2560 $\mu\text{mol/L}$) against the L2 grubs. The minimal concentration was that which was 100% effective in the first experiment. All of the tested concentrations led to 100% mortality estimated after 7 days, whereas all grubs survived in the untreated pots.

Based on the results of the two preliminary tests, the experiments to estimate the ILC50 and ILC95 for the grubs at three different instars were conducted from the end of May through mid-June 2012. These tests were performed outdoors in a tent with a wooden frame covered with a net providing 30% shade. The granulate quantities corresponding to five GSL concentrations (80, 160, 320, 640 and 1280 $\mu\text{mol/L}$) were tested against the L1, L2 and L3 grubs. Each variant was tested in 20 replicates. The pots were prepared as described above. Water (50–70 ml) was applied on the first day, after which the pots were watered every day for 5 min

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