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The soil-dwelling earthworm *Allolobophora chlorotica* modifies its burrowing behaviour in response to carbendazim applications

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ABSTRACT

Carbendazim-amended soil was placed above or below unamended soil. Control tests comprised two layers of unamended soil. *Allolobophora chlorotica* earthworms were added to either the upper or the unamended soil. After 72 h vertical distributions of earthworms were compared between control and carbendazim-amended experiments. Earthworm distributions in the carbendazim-amended test containers differed significantly from the 'normal' distribution observed in the control tests. In the majority of the experiments, earthworms significantly altered their burrowing behaviour to avoid carbendazim. However, when earthworms were added to an upper layer of carbendazim-amended soil they remained in this layer. This non-avoidance is attributed to (1) the earthworms' inability to sense the lower layer of unamended soil and (2) the toxic effect of carbendazim inhibiting burrowing. Earthworms modified their burrowing behaviour in response to carbendazim in the soil. This may explain anomalous results observed in pesticide field trials when carbendazim is used as a control substance.

1. Introduction

The fungicide carbendazim is known to be highly toxic to earthworms and is recommended for use as the reference substance in standardised guidelines for testing the effects of pesticides on earthworms in field situations (ISO, 1999). However, results using carbendazim in field trials have been highly variable (Römbke et al., 2004; Ellis, 2008). This paper reports a study into the behavioural response of *Allolobophora chlorotica* to carbendazim as part of a wider investigation into this variability.

Carbendazim has limited movement in the soil profile and studies have recorded up to 97% of the applied total to remain in the upper 5 cm of the soil profile (Ellis, 2008; Jones et al., 2004; Holmstrup, 2000). The exposure of earthworms to carbendazim in the field will therefore, in part, be determined by their vertical distribution and their ability to detect the chemical and modify their vertical burrowing behaviour as a consequence of this. A field study (Römbke et al., 2004) showed the vulnerability of earthworms to the toxic effects of carbendazim to differ between species. This difference was attributed to the different feeding preferences of the species and their distribution in the soil profile. Species, which typically feed on vegetation at the surface of the soil where carbendazim concentration was highest, including *Lumbricus terrestris* and *L. rubellus*, had higher mortality than geophageous species including *Apporectodea caliginosa*, which were not dependant on the surface for food and subsequently had lower exposure to the chemical (Römbke et al., 2004). While certain species may be more vulnerable due to their feeding behaviour, earthworms can occupy a range of depths in the soil profile and can adjust their burrowing depth behaviour based on soil conditions (Edwards and Bohlen, 1996). The geophageous species *A. chlorotica*, for example, is typically found above a depth of 8 cm when soil conditions are favourable but will burrow to below 8 cm to avoid extremes of temperature or dry soil at the surface (Gerard, 1967). In earthworm avoidance studies, in which earthworms are given a choice between horizontally adjacent soils (usually a control, contaminant-free soil and a contaminant-bearing soil, e.g. Yeardley et al., 1996; da Luz et al., 2004; Environment Canada, 2007; ISO, 2008) the earthworm species *Eisenia andrei* (Loureiro et al., 2005) and *E. fetida* (Garcia et al., 2008) have been shown to significantly avoid carbendazim and benomyl at concentrations ≥ 1 mg kg^{-1}. However, for chemicals such as carbendazim, which have limited mobility through the soil profile, the most significant concentration gradient encountered in the field will be in the vertical plane and a key question is whether or not earthworms are able to modify their behaviour to avoid such chemicals. Horizontal avoidance studies provide useful information on the ability of earthworms to detect and respond to

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adverse concentrations of chemicals, but they do not provide information on whether this avoidance driver is sufficient for earthworms to modify their normal behaviour to avoid such chemicals.

The aim of this study was therefore to determine whether the presence of carbendazim led to a modification of the burrowing behaviour of the earthworm *A. chlorotica*.

2. Method

2.1. Earthworm species

A. chlorotica is a widely abundant species in the UK. It was selected as a suitable species for the study as it occupies a range of depths in the soil profile, is geophageous (is not dependant on the soil surface for feeding) (Edwards and Bohlen, 1996) and is known to adjust its burrowing depth in response to unfavourable conditions (Gerard, 1967). Earthworms were collected by manual digging and hand sorting of soil from a pasture field at the University of Reading farm at Sonning, Berkshire, UK, and kept in a 3:1 mix of sandy loam soil and sphagnum peat moss at a temperature of 15 °C until the test.

2.2. Test substance

Delsene 50 Flo, obtained from Nufarm UK Ltd. Belvedere, Kent, UK, was selected as a suitable test substance for the study as it is a commercially available water-based suspension concentrate containing carbendazim at a concentration of 500 g l^{-1}. The Delsene 50 Flo was diluted using deionised water to a concentration of 46 mg l^{-1}.

2.3. Test soil

Kettering loam, a commercially available sandy loam soil obtained from Broughton Loam, Kettering, UK (Table 1 for soil properties), was used in the avoidance studies. The soil was air dried and sieved to < 2 mm prior to use. A carbendazim concentration of 8 mg kg^{-1} was used, as significant avoidance behaviour was observed in previous studies using similar concentrations (Loureiro et al., 2005; Garcia et al., 2008). Using the relationship of Jänsch et al. (2006), which assumes a soil density of 1500 kg m^{-3}; this concentration is approximately twice that in soil after the typical application rate of 4 kg ha^{-1} used in field trials (ISO, 1999). The diluted carbendazim suspension was mixed thoroughly with the soil using a house-hold mixer (Kenwood A907D), to give a soil moisture content of 60% of the soil water holding capacity. As the aim of the study was to determine whether carbendazim led to a modification of earthworm burrowing behaviour, and as all experiments were established at the same time from the same carbendazim-amended batch of soil, the decision was taken to not analyse the soil for carbendazim. For the control soil, Kettering loam was mixed with deionised water only. The moisture contents of the carbendazim-treated and the control soil were the same.

2.4. Experimental procedure

2.4.1. Arrangement of soils

The test containers comprised two sections, one section containing the carbendazim-amended soil and the other the clean unamended soil. The two sections were stacked vertically and earthworms were able to move freely between the two soils. The behavioural response of *A. chlorotica* to carbendazim was tested with the soils in two arrangements (Fig. 1). The first arrangement (*Field arrangement*) reflected carbendazim application in the field with the carbendazim-amended soil at the top and the unamended soil below. In the second arrangement (*Alternative arrangement*) the carbendazim-amended soil formed the bottom section. Control tests (with unamended soil in both sections) were used to determine the natural distribution of earthworms without the influence of carbendazim. The test containers were designed to account for the typical burrowing behaviour of *A. chlorotica*. *A. chlorotica* usually forms temporary horizontal burrows in the upper 8 cm of the soil profile (Edwards and Bohlen, 1996). The test containers comprised of two open-ended, translucent PVC cylinders wrapped in black adhesive tape to exclude light, 8 cm high and with a diameter of 7.5 cm. Four hundred grams (dry weight equivalent) of soil were added to each container, which were placed on top of each other. The top of the upper container was covered with mesh (1 mm size) to prevent individuals escaping and to allow light onto the surface of the soil. The bottom of the lower container was closed to prevent earthworm escape. The test containers were kept in a temperature controlled room at 18 °C with a photo period of 12:12 h (light:dark).

Table 1
Selected mean chemical and physical properties of the Kettering loam test soil ($n=3\pm$standard error).

Soil property	
pH	6.2 ± 0.2
Organic matter content/%	7.06 ± 0.09
Texture	11.8 ± 1.3% clay
	21.7 ± 0.3% silt
	66.9 ± 1.0% sand
Water holding capacity/%	29 ± 4

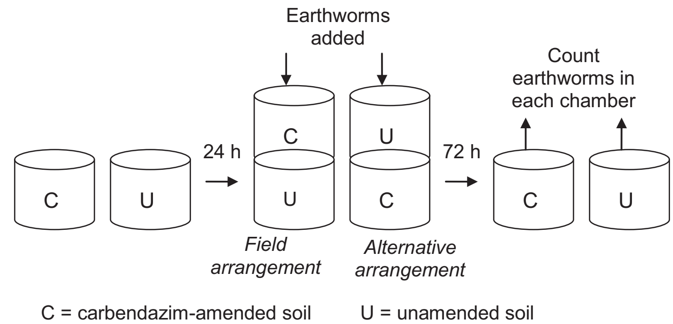

Fig. 1. Diagrammatic representation of Method 1 for assessing vertical avoidance behaviour of earthworms in which earthworms are added to the upper surface of the upper soil.

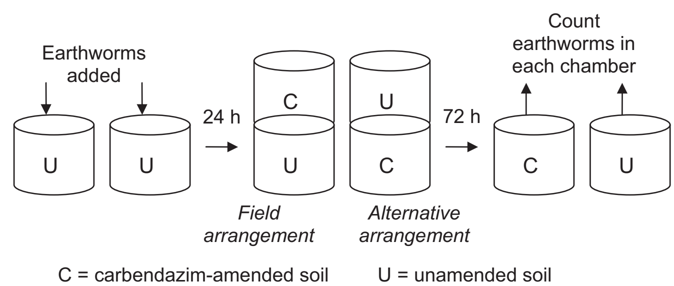

Fig. 2. Diagrammatic representation of Method 2 for assessing vertical avoidance behaviour of earthworms in which earthworms are added to the upper surface of the unamended soil.

2.4.2. Earthworm addition

Earthworms were added to the containers in one of two ways. In both methods the earthworms were added 24 h after the soil had been mixed and added to the containers. Five replicates were used per soil arrangement with ten individuals used per replicate. Five replicates were also used for each control. The tests were run for 72 h to ensure that earthworms had sufficient time to burrow into the soil. After 72 h the sections were separated using a card divider and the number of individuals in each section determined by hand sorting.

Method 1 (Fig. 1): Earthworms were added to the soil surface at the top of the test container. Thus when the carbendazim-amended soil was in the upper container earthworms were added to the upper surface of the 8 cm thick carbendazim-amended soil. This method allowed us to assess the response of the earthworms when they experienced direct dermal contact with carbendazim-amended soil.

Method 2 (Fig. 2): This was intended to be more representative of a field scenario where carbendazim would be sprayed onto the soil surface. Earthworms were initially added to unamended soil and allowed to acclimatise for 24 h before the carbendazim-amended soil was added, either above or below the unamended soil. This method allowed us to assess whether *A. chlorotica* would modify its burrowing behaviour in response to either an over-lying or under-lying layer of carbendazim-amended soil. In this method *A. chlorotica* began the test in two different positions in the test container (either the top or bottom section), dual controls were used for both arrangements. For each arrangement, 5 replicates plus 5 dual controls were used.