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Insecticide exposure can increase burrow network production and alter burrow network structure in soil dwelling insects (*Agriotes* spp.)

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ABSTRACT

Insecticide treated seeds are commonly used to reduce yield losses from burrowing insect damage such as wireworms. Using temporal X-ray Computed Tomography (CT) of soil-filled bioassays, we aimed to quantify changes in burrow network production and structure as a measure of wireworm behavioural change in response to three types of insecticide treated maize seed; compound X (R&D product in field trial stage of development); tefluthrin and thiamethoxam. A biopesticide alternative treatment (neem), untreated maize seed and bare soil were also investigated. Insect health outcomes were also monitored to provide toxicity/mortality data. Wireworms exposed to compound X produced greater burrow networks than untreated maize and neem treatments, similar to that in volume of those produced in bare soil. Compound X exposure also elicited the production of more complex burrow structures, a function of the number of vertices, edges and faces of a shape (V-E+F) related to the number of interconnected branches, compared to any other treatments. Compound X, tefluthrin and thiamethoxam induced mortality at greater rates than neem or untreated, suggesting all three could have potential to manage wireworm populations and reduce yield loss, but only compound X modified burrowing behaviour. With soil biopores playing an important role in soil productivity and carbon sequestration, the wider implications of this increase in burrowing activity for food security and climate change warrants further exploration.

1. Introduction

Wireworms are the soil dwelling insect larvae of click beetles, common across many parts of the globe. *Agriotes* spp. are the primary wireworm species of economic concern throughout much of Europe and North America (Benefer et al., 2012; Blackshaw and Hicks 2013) due to their feeding activity which damages roots, stems and harvestable parts of crops reducing yield and sale value (Sonnemann et al., 2012; Barsics et al., 2013; Ritter and Richter, 2013). Wireworms are semivoltine, developing underground for up to 4 years before emerging as adults (Brian, 1947; Furlan, 1996, Furlan, 1998; Furlan, 2004) undergoing an average of seven to nine developmental instars over this period (Vernon and van Herk 2013) but with great variation between and within genera. Peak feeding occurs in the spring (March–April) and autumn (September–October) in central Europe, which coincides with crop planting (Brian, 1947; Burrage, 1964; Parker and Howard 2001), with the spring feeding period particularly damaging to cereal and grain crops. In wheat, crop emergence can be reduced by over 59 % in soil with a high wireworm density (van Herk and Vernon, 2013), and in extreme cases, total crop yield losses due to wireworm damage can be as high as 100 % (van Herk et al., 2018). The threat of wireworms to maize crops is significant, with different risk levels determined by the geographical area and management strategy (Veres et al., 2020).

Controlling wireworm populations through Integrated Pest Management (IPM) programs can be challenging due simultaneous generations existing in the soil, so eradicating the above ground adults before egg laying is not immediately effective, as it may take a number of years for all existing soil larvae causing crop damage to emerge. Eradicating adults is difficult due to their ability to migrate from other areas, but pheromone traps have shown promise as an ‘attract and kill’ method to

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effectively reduce population levels and to prevent egg laying (Kabaluk, 2014; Vernon et al., 2014; Furlan et al., 2021). With increasing food security risks due to increasing populations and climate change, reducing yield losses from current levels of pest feeding activity is vital. One potential solution to reduce crop losses in cereal and grain crops from root herbivore feeding is the use of insecticide treated seeds.

Neonicotinoids, such as thiamethoxam, are broad spectrum neuroactive insecticides that act as a postsynaptic acetylcholine receptor agonist (Schroeder and Flattum, 1984) and applied as seed coatings have shown ability to protect wheat crops from stand reduction due to wireworm feeding (Vernon et al., 2009). However, the ability of thiamethoxam to cause mortality in treated wheat fields does not appear to be strong, where minimal reduction of wireworm populations has been reported (Vernon et al., 2009; Vernon et al., 2013; van Herk et al., 2018). Tefluthrin, is a broad spectrum pyrethroid compound that acts as an axonic excitotoxin, preventing the closure of the voltage-gated sodium channels in the axonal membrane (Soderlund et al., 2002). Rather than reduce population levels significantly, previous work has observed that tefluthrin tends to elicit a repulsion response with short term morbidity rather than significant mortality as a wheat seed treatment (Vernon et al., 2009; van Herk et al., 2015; van Herk et al., 2008a; van Herk and Vernon, 2007a).

Compound X is currently in trials for use in IPM programs for wireworm control. It has been supplied by Syngenta as part of a research and development program to investigate both its efficacy at population control, as well as sublethal effects on wireworm behaviour and subsequent impact on the wider soil environment. Neem oil, a plant extract from an Indian lilac (*Azadirachta indica*) native to the Indian subcontinent and Africa, has been used in traditional farming practices in India as a biopesticide for centuries (Campos et al., 2016). The oil is considered a contact insecticide, presenting systemic and translaminar activity, inhibiting feeding (Campos et al., 2016). Azadirachtin, one of the primary active ingredients in neem oil, demonstrated a repellence effect to wireworms when used as a soil treatment, but did not cause mortality, antifeeding responses, or changes in growth rate (Cherry and Nuessly, 2010).

Few studies have sought to investigate the burrowing behaviour of wireworms, owing to the difficult nature of studying the opaque soil habitat. Previous work has primarily focused on the location of wireworms in the soil profile (Fisher et al., 1975) and utilisation of existing pore structures (van Herk and Vernon. 2007b) rather than quantifying the extent of burrow structures. Recently Booth et al. (2020) demonstrated the capability of using X-ray CT to measure wireworm activity in response to different crop species, and Booth et al. (2022) further demonstrated the methods ability to quantify differences in burrow structure and attributed this to potential differences in foraging strategy. X-ray Computed Tomography (CT) is a non-destructive image-based method that has previously been used to track insect movements in soil (Harrison et al., 1993; Johnson et al., 2004), as well as burrow networks produced by other invertebrate species (Jégou et al., 2002; Jégou et al., 1998; Jégou et al., 1999; Joschko et al., 1993; Joschko et al., 1991; Bastardie et al., 2003). By applying this method to soil columns planted with insecticide treated seeds, we aimed to determine if insecticide exposure influences wireworm behaviour as measured through changes in the production and structure of wireworm burrows, and discuss possible implications for crop protection and the wider soil ecosystem. In addition, by monitoring the health outcomes of the wireworms following exposure we also aim to evaluate the efficacy of compounds to protect crops through population management.

2. Materials and methods

2.1. Soil core preparation and seedling growth

A loamy sand soil from the Newport series (sand 83.2 %, silt 4.7 %, and clay 12.1 %; pH 6.35; organic matter 2.93 %) collected from the University of Nottingham farm at Bunny, Nottinghamshire, UK (52.9°N, −1.1°E) was air dried and sieved to 2 mm. Soil columns (68 mm internal diameter × 120 mm height) were prepared using the method of Mairhofer et al. (2017). Columns were filled with the sieved soil to a depth of 100 mm at a bulk density of 1.2 g^{-1} cm^3, and saturated overnight in H_2O. Columns were then allowed to drain freely to a moisture content of 10 % by weight (an approximated field capacity state), which was maintained for the duration of the experiment.

Untreated *Zea mays* (maize) seeds and treated maize seeds (compound X coated- 0.5 mg^{-1} seed, thiamethoxam coated- 0.25 mg^{-1} seed and tefluthrin coated- 1.0 mg^{-1}seed) were obtained from and prepared by Syngenta Crop Protection (Stein, Switzerland) and one seed was planted in each of the prepared columns (n=10 for controls and each treatment). For the neem oil treatment, two solutions of neem oil were used as a soil drench treatment prepared using cold pressed organic neem oil concentrate (Pink Sun Organic, UK) with a biodegradable horticultural soap carrier (Pink Sun Organic, UK) to a concentration of 0.5 mg^{-1} seed of neem for maize. Neem treatments are commonly applied as a foliar spray for above ground pests, and as a soil drench treatment for below ground pests (e.g. Acharya et al., 2023). This solution was applied surrounding an untreated maize seed (n=10) at the point of planting via a syringe. Control columns, containing soil only with no seedlings (n =10) were also prepared. Columns were incubated in a Conviron A1000 growth chamber (Controlled Environments Ltd, Winnipeg, MB, Canada) at 25 °C day/23 °C night, 50–60 % relative humidity and a 14:10 h light/dark photoperiod for 8 days before the first X-ray scanning.

2.2. Wireworm maintenance

Wireworms (*Agriotes* spp.) were obtained from Syngenta Crop Protection (Stein, Switzerland), and were stored at 10 °C in moist (15 % moisture by weight) soil containing potato as a food source. Wireworms were collected via bait trapping and digging in fields with known infestations in the upper Rhine plain between Offenburg and Raststatt in the southwest of Germany. Approximately 70 % of the wireworms were collected from maize fields on either young emerging maize or on old but still green root stocks of maize. The other 30 % of wireworms were collected from fields with different wild herbs such as camomile, millet and thistle wild rye. *Agriotes lineatus* is the dominating species of wireworm in that region. A sample of 50 individual larvae was taken from the population and identified to a species level using the method of Klausnitzer (1994), and it consisted of 32 *Agriotes lineatus*, 17 *Agriotes obscurus* and 1 *Agriotes ustulatus*. As previous work demonstrated, there are no significant differences in burrow characteristics between the two major species of this population (Booth et al., 2022). In addition, pest management products target not just a single species of wireworm, but rather protect broadly against all species of wireworm. Therefore, wireworm species were not separated for this work and treated as a single mixed population. Only actively feeding wireworms were selected for addition to the soil columns by using the potato food source as bait, observing those that were attracted to the tuber and burrowed into it to feed. The selected insects were not fed for 48 h before the start of the experiment as preconditioning for the trial and then moved to the growth chamber to acclimatise to the temperature and humidity conditions. Only insects with a head width of 1 mm or over were selected (7th instar stage or greater) for trials. The health of the insects harvested from the soil columns following the final X-ray CT scan was monitored for 32 days after trials to evaluate the toxicity and mortality effects of compound exposure using modified methods of Vernon et al. (2008), where leg and mouth movement and mouth movement only are combined into a singular category; appendage movement. Observations were conducted in a 15 cm diameter dish containing a moistened 12.5 cm diameter filter paper with an 8 cm diameter circle drawn in the centre. Wireworms placed in the dish were observed closely for 2 minutes, and their body movements and coordination recorded.

Wireworms that did not leave the 8 cm circle within the 2 min were considered affected (writhing), and those that were not visibly moving were further examined under a magnifying glass for leg and mouthpart movement (appendage movement). With no movement at all, larvae were considered dead, but this was only confirmed following continued monitoring for signs of visible decay. A total of 94 individual insects (2 per column) were assessed in this study.

2.3. X-ray CT scanning and analysis

After 7 days growth, plants showing good shoot emergence (n=8 of each treatment except n=6 for neem-treated maize due to lower emergence rates) were selected for wireworm trials. Two wireworm larvae were added to each column, and then returned to the growth chamber. Twenty-four hours after wireworm inoculation, a 'fast mode' scan (Continuous rotation with no image averaging), consisting of 1800 projection images using a detector timing of 250 ms, an X-ray source energy setting of 180 kV and 156 μA current was collected for each core using a v|tome|x M 240 kV X-ray CT system (Waygate Technologies, Wunstorf, Germany) based at the Hounsfield Facility, University of Nottingham. The distance of the sample (FOD) and detector (FDD) from the X-ray source were 327.5 mm and 818.7 mm, respectively, resulting in a spatial resolution of 80 μm. The total scan time was 8 min. A rapid scan protocol was utilised to minimise any image blurring associated with movement of the wireworms during the scanning process. This same scan method was also repeated at 48 hours, 72 hours and 96 hours after wireworm inoculation. Between scans, the columns were returned to the growth chamber, and the insects manually harvested from the column for health assessments after the final 96-hour scan.

2.4. Scan reconstruction and analysis

All projection images were reconstructed into volumetric data sets using Datos|x REC software (GE Sensing and Inspection Technologies). Segmentation of wireworms and burrow morphology was made using the 3-D region growing tool in VGStudioMAX version 2.2 (Volume Graphics GmbH, Heidelberg, Germany) using methods outlined in Helliwell et al. (2017) and Booth et al. (2020). Furthermore, burrow quantification in the form of Euler characteristic was collected using the connectivity function of the Bone-J plugin (Doube et al., 2010) for Image-J (Rasband, W.S., ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA, https://imagej.nih.gov/ij/, 1997–2022.). The Euler characteristic describes a topological shape regardless its orientation, giving the complexity of the shape (Euler, 1758), and is calculated using the number of vertices, edges and faces of a shape (V-E+F), becoming more negative in value as a shape becomes more complex. the overall connectivity of the structure contributes to Euler characteristic. This has previously been used as a measure of wireworm burrow network complexity Booth et al. (2022). More complex burrow networks would generally have a greater number of interconnected branches than less complex burrow networks of similar overall volume. This was only measured at the final time point (96 hours) in order to determine the cumulative effect of varied treatment exposure on burrow network structure, and its subsequent impact on soil porosity.

2.5. Statistical analysis

All data were tested for normality and analysis of variance (repeated measures) with Greenhouse–Geisser correction (to adjust degrees of freedom) and post-hoc Tukey tests were performed to determine any significant differences in burrow volume (factors: crop treatment, time and interaction). Burrow complexity was analysed using a regular ANOVA and post-hoc Tukey test (factor: crop treatment). Kaplan–Meier curves and pairwise Cox regressions were used to analyse wireworm mortality data. All burrow data analysis was conducted using GenStat statistical software (19th edition; VSNi, Hemel Hempstead, UK) and mortality data was analysed using SPSS statistical software (IBM SPSS Statistics for Windows, Version 28.0. Armonk, NY: IBM Corp).

3. Results

3.1. Visualisation of wireworm burrow networks

In columns planted with maize (Fig. 1), different burrowing activity was observed between treatments, with pore networks in compound X treated columns greater in volume and more complex in structure, with more loops and branches compared to columns planted with untreated maize or neem treated maize. An increase in the burrow network volume was also observed over time. (Fig. 1).

3.2. Burrowing activity between treatments

The volume of wireworm-produced burrows increased over time in all columns ($F_{(3,\ 111)} = 48.83$, $P < 0.001$, $\varepsilon = 0.57$) (Fig. 2) suggesting burrowing activity continued from initial inoculation. Significant differences were observed in burrow volume between treatments ($F_{(5,\ 37)} = 6.26$, $P < 0.001$, $\varepsilon = 0.57$). Following post hoc comparisons, the mean burrow volume in columns of Compound X treated maize (4306 mm^3, a) and bare soil (4799 mm^3, a) were significantly different from columns containing seeds of either untreated maize (1892 mm^3, b) or neem treated maize (1454 mm^3, b) (Fig. 2). No significant difference was observed in the interaction between treatment and time ($F_{(15,\ 111)} = 1.79$, $P = 0.091$, $\varepsilon = 0.57$).

3.3. Burrow network structure between treatments: complexity

Average burrow complexity differed significantly between the treatment groups (Fig. 3), but following post hoc comparisons, only the burrows in columns planted with compound X-treated maize (-96.5, a) were significantly different from columns of bare soil (-43.5, b), untreated maize (-8.3, b), tefluthrin treated maize (-29.25, b), thiamethoxam treated maize (-32.5, b) and neem treated maize (-10, b) ($F_{(5,42)} = 7.91$, $P < 0.001$), which were similar in their structural complexity.

3.4. Effects of compound exposure on wireworm health and mortality

Exposure to seeds treated with tefluthrin (Fig. 4d) caused the greatest levels of mortality of the total population of wireworms compared to the other treatments and control after 32 days. Similarly, wireworms exposed to compound X-treated seeds demonstrated the highest levels of toxicity symptoms upon removal from the columns (Fig. 4e). Thiamethoxam caused similar levels of mortality to compound X after 32 days (Fig. 4c). Exposure to neem treated seeds caused no symptoms of toxicity or mortality (Fig. 4b).

Comparing wireworm mortality over time (Fig. 5), thiamethoxam, tefluthrin and compound X treatments all caused significantly greater mortality compared to the untreated control (thiamethoxam: $X^2 = 6.623$, $P = 0.010$, tefluthrin: $X^2 = 9.316$, $P = 0.002$, compound X: $X^2 = 5.440$, $P = 0.020$) and neem treatment (thiamethoxam: $X^2 = 6.623$, $P = 0.010$, tefluthrin: $X^2 = 9.316$, $P = 0.002$, compound X: $X^2 = 5.440$, $P = 0.020$), but not significantly different mortality compared to each other (thiamethoxam-tefluthrin: $X^2 = 0.541$, $P = 0.462$, thiamethoxam-compound X: $X^2 = 0.080$, $P = 0.777$, tefluthrin-compound X $X^2 = 1.199$, $P = 0.274$,).

4. Discussion

Significantly different levels of activity of larvae were found when exposed to different treatments or the absence of roots (Fig. 1). The similarity of burrow network volume between compound X-treated maize and bare soil columns compared to untreated maize and neem treated maize suggests compound X causes a similar activity response in wireworms as that of bare soil (Fig. 2). Previous work has reported

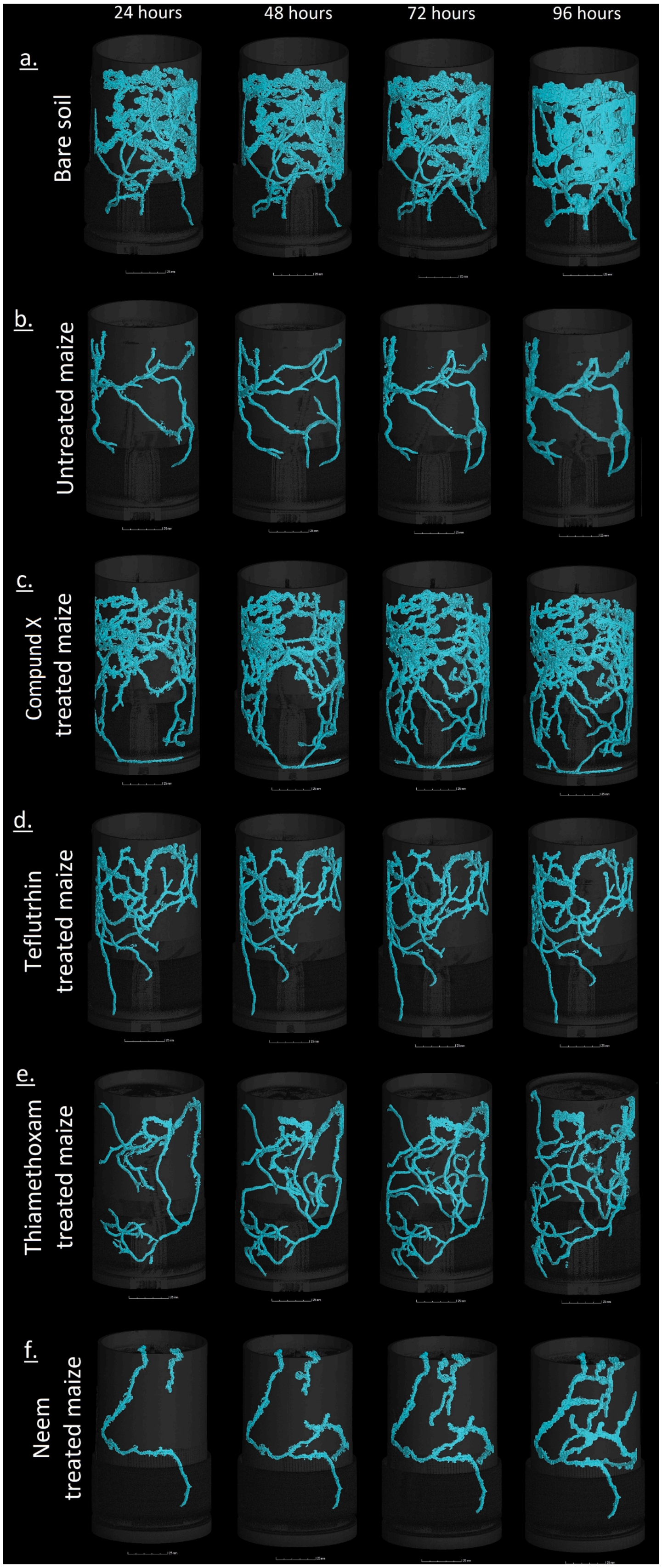

Fig. 1. Example images from temporal X-ray Computed Tomography scans showing burrow network development 24 h, 48 h 72 h and 96 h after wireworm inoculation in columns of a) bare soil and columns planted with b) untreated maize, c) compound X treated maize, d) tefluthrin treated maize, e) thiamethoxam treated maize and f) neem treated maize. Scanned with a resolution of 80 µm.

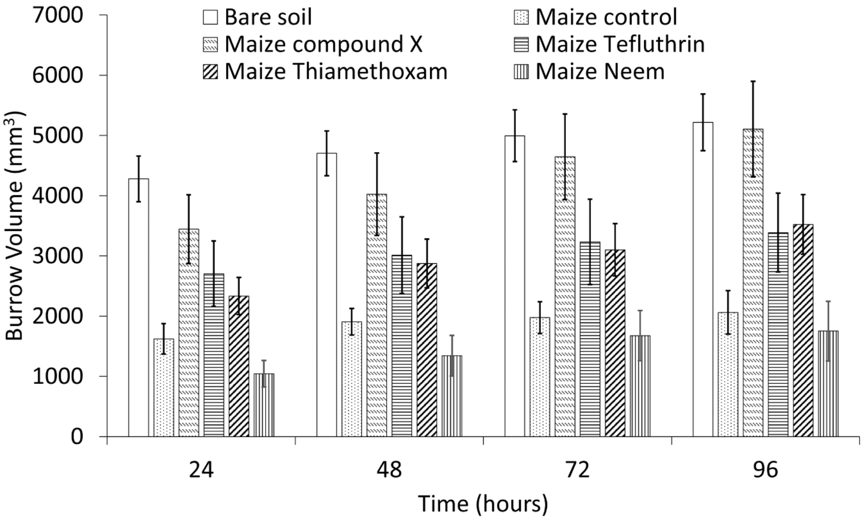

Fig. 2. Mean wireworm produced burrow volume at 24, 48, 72 and 96 h after wireworm inoculation for columns of bare soil and columns planted with untreated maize, compound X treated maize, tefluthrin treated maize, thiamethoxam treated maize and neem treated maize ± 1 SE.

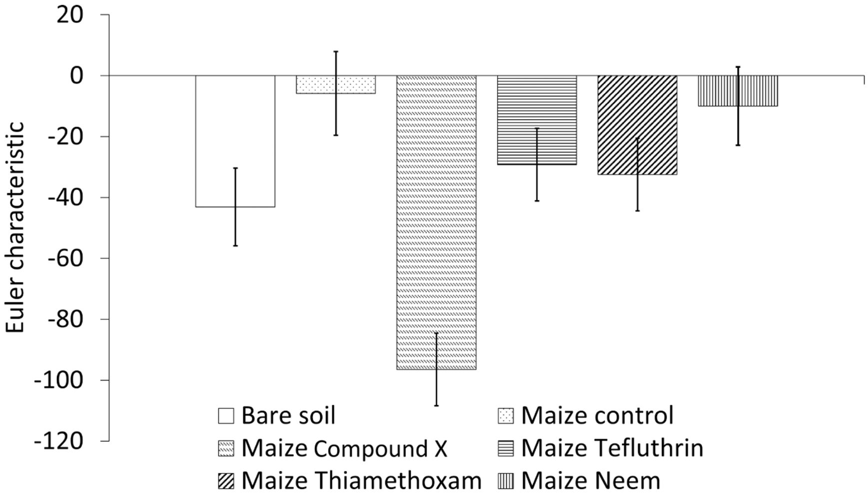

Fig. 3. Mean wireworm produced burrow complexity 96 h after wireworm inoculation for columns of bare soil and columns planted with untreated maize, compound X treated maize, tefluthrin treated maize, thiamethoxam treated maize and neem treated maize ± 1 SE.

higher burrowing activity in bare soil compared to soil planted with maize crops which was attributed to wireworm searching behaviour due to a lack of availability of root material for feeding (Booth et al., 2022). Like many soil dwelling herbivores, wireworms orientate towards root structures through CO_2 (Sonnemann et al., 2014) and volatile organic compounds (VOCs) gradients (Gfeller et al., 2013; Barsics et al., 2017) produced through cell respiration and in root exudates.

In unplanted soil, without the presence of living root structures, wireworms are unable to orientate towards suitable food sources, affecting foraging strategies, and so demonstrate increased searching behaviour, which results in the formation of larger burrow networks (Booth et al., 2022).

Wireworms exposed to compound X-treated maize produced significantly greater burrow networks than untreated maize, similar to bare soil, suggesting compound X may be eliciting a similar searching response in larvae to that of unplanted soil. This could be due to the presence of compound X making the roots unpalatable to the larvae, and so either due to contact with the insecticide, or after ingestion through feeding, the insects determine the compound X-treated maize as an unpalatable food source and increases searching. This could also be attributed to an escape response following exposure to compound X, where following exposure, larvae seek to move away from the insecticide source which can lead to an increase in burrow network volume. This response is well documented in wireworms exposed to insecticides (Lange et al., 1949; Vernon et al., 2008; Langdon et al., 2018), and so could be the mechanism leading to increased burrow volume, but may not explain the greater complexity of burrow networks in response to compound X compared to tefluthrin and thiamethoxam exposure.

In maize (Fig. 3) columns planted with compound X had significantly more complex burrow networks than bare soil, untreated maize, and treatments of tefluthrin, thiamethoxam or neem. This was to be expected due to the high activity observed through burrow volume in compound X columns compared to other treatments (Fig. 2), with higher volume structures generally expected to be more complex. However, compound X-treated maize columns and bare soil columns would be expected to have burrow networks of similar complexity due to their similarity in volume. Booth et al. (2022) observed significantly more complex burrow networks in columns of bare soil and barley compared to those planted with maize crop. They attributed this to increased searching behaviour to the lack of or lower volume of root material to orientate towards. With significant differences between compound X treated and unplanted maize columns, the more complex burrow structures

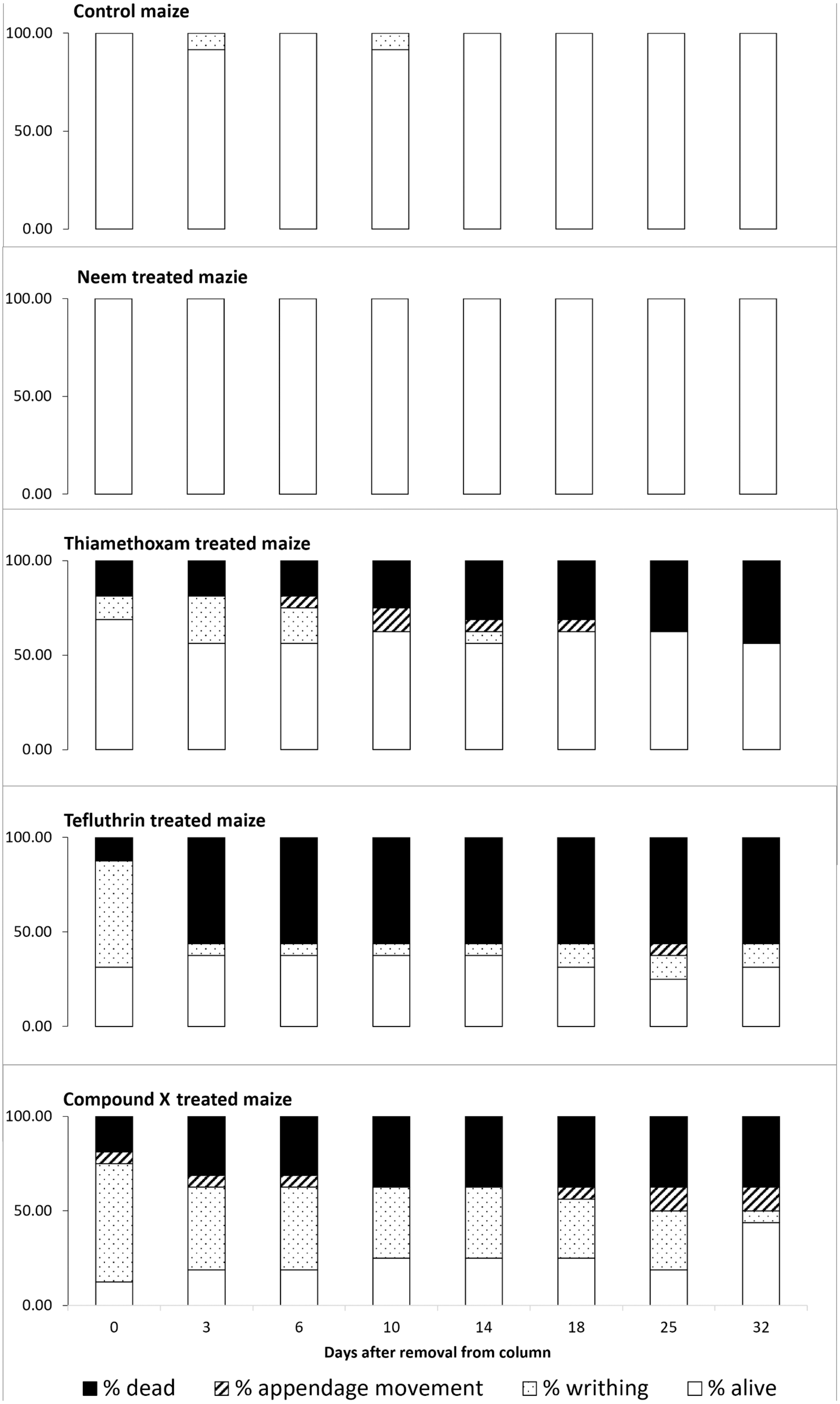

Fig. 4. Proportional health status of wireworm populations exposed to a treatment as determined by the modified methods of Vernon et al. (2008) for insects exposed to a) untreated maize b) neem treated maize c) thiamethoxam treated maize d) tefluthrin treated maize e) compound X treated maize. Conducted at 0, 3, 6, 10, 14, 18, 25 and 32 days after removal from columns.

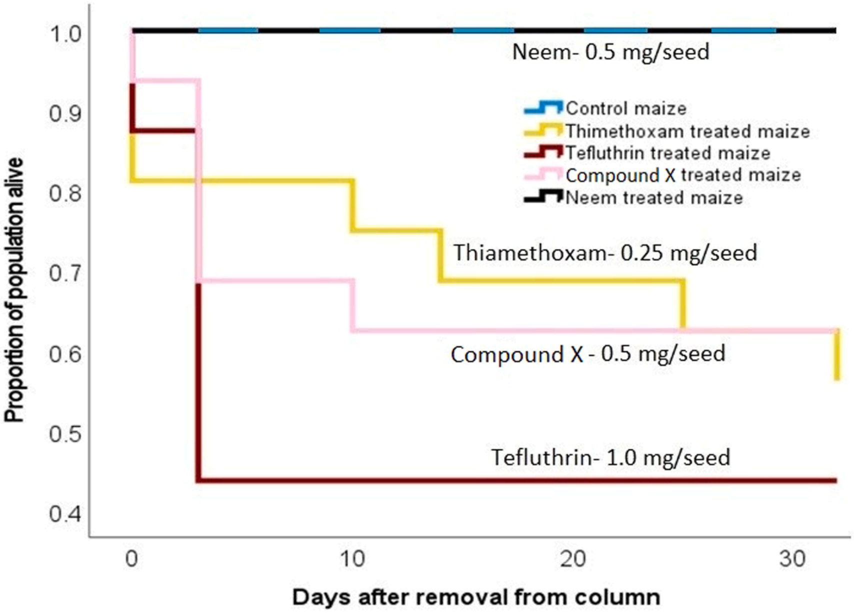

Fig. 5. Kaplan-Meier plots graphically visualising the survival functions of wireworm populations exposed to either untreated maize, neem treated maize, thiamethoxam treated maize, compound X treated maize or tefluthrin treated maize, with rates of compound attributed to each (no rate for untreated (blue) and where proportion of population alive remained at 1.0 throughout).

observed in compound X treated maize columns could be attributed to repulsion rather than a searching or escape response.

Repulsion effects of insecticides are well documented in wireworms, with studies demonstrating both short and long-range repulsion of larvae to insecticides (van Herk and Vernon. 2007b; van Herk and Vernon, 2018). Additionally, aversion learning, where a repulsion response to a non-toxic compound is observed after pre-conditioning with a toxic compound, has been reported in wireworms (van Herk et al., 2010). With limited data available on impacts of compound X, it is feasible that the changes in burrow size and structure could be due to this repulsion effect, with the insects actively migrating from areas of a higher concentration to lower concentration. Within the confines of a closed system like that used in this study, repulsion behaviour could result in the type of burrow structures observed here.

However, previous work has suggested tefluthrin is primarily effective at protecting crops through a repellence reaction without causing mortality (Vernon et al., 2009; van Herk et al., 2015) and so is likely to cause a repulsion response. If tefluthrin had a repellence effect in this study, then it was not demonstrated through changes in burrow production or structure, as the volume and complexity of these structures was not significantly different to columns containing untreated maize. This suggests that it is not a repulsion response leading to modification of burrowing behaviour.

When exposed to toxic compounds, wireworms display a variety of symptoms, including compound specific ones (Vernon and van Herk 2013). Generally, when exposed to insecticides, wireworms display symptoms of toxicity through full body writhing, where movement is possible but without directionality, leg and mouthpart movement, where leg and mouthparts move but not the whole body, and mouthpart movement only (Vernon and van Herk 2013). In this study, leg and mouth movement and mouth movement were combined into one category, 'appendage movement'. We hypothesise that intoxication from compound X treated maize may lead to high complexity of burrow networks observed in the columns (Fig. 3), with the non-directional movement from full body writhing as a state of hyperexcitation creating the high volume-high complexity burrow networks observed. When considering the health effects of larvae after compound exposure (Fig. 4), compound X has the highest incidence of toxicity symptoms upon removal from the column compared to other treatments and untreated control. The high incidence of toxicity occurring in wireworms exposed to compound X-treated maize, alongside these wireworms producing the largest and most complex burrow networks suggests that compound X intoxication may be linked to changes in burrow production and structure.

However, of the wireworms exposed to tefluthrin treated maize, over 50 % demonstrated toxicity symptoms after removal from soil, but not with a corresponding increase in burrow volume or complexity. Previous studies have demonstrated mixed results with the use of thiamethoxam, with most agreeing it protects crops through intoxication (Vernon et al., 2009; van Herk et al., 2018; Onsacer and Rusk, 1969; van Herk et al., 2008b). Although toxicity was low for thiamethoxam in this study, if intoxication was the mechanism causing alteration of burrow production and structure, more similarities between compound X, tefluthrin and thiamethoxam treated maize columns may have been observed. It is worth noting however that symptoms of intoxication can vary between compounds, with compounds such as fipronil causing specific symptoms not observed when exposed to other compounds (Vernon and van Herk 2013), so presentation of a hyperexcitation intoxication symptoms that significantly alter burrow structure may be specific to compound X only. Level of intoxication must be considered also, with higher levels of intoxication, leading to appendage movement only are too intoxicated to be motile and therefore produce complex burrow structures.

A study utilising constant recording methods of insect behaviour in soil filled bioassays, akin to soil window methods, has the potential to test the hypothesis of compound X exposure leading to presenting of specific symptoms of intoxication: a state of hyperexcitation leading to increased burrowing activity. This would include measures of insect location, state and soil displacement from movement. Infrared recordings of thin (<5 mm soil thickness) soil filled bioassays offer a possible avenue for study, as an adapted version of van Herk and Vernon (2007b) and van Herk et al., (2008a).

Considering just mortality effects in isolation, the high toxicity symptoms observed in wireworms exposed to compound X-treated maize (Fig. 4e,) at 0 days after removal from soil demonstrates the compounds' ability to negatively impact wireworm health. Whilst some individuals went on to die over the 32-day observation period, many recovered following removal from treated columns. This may be due to the removal of the insects from the soil after the 4-day period and no subsequent exposure, with more prolonged exposure potentially leading to higher mortality rates, as mortality rates to insecticides are a function of insecticide concentration and exposure time (Morales-Rodriguez and Wanner, 2014). Regardless of recovery by some individuals, compound X exposure caused significantly greater wireworm population mortality compared to untreated maize and neem (Fig. 5), but not compared to tefluthrin or thiamethoxam treatments.

Conversely to literature (Vernon et al., 2009; van Herk et al., 2015), tefluthrin also clearly demonstrated its ability to cause toxicity followed by mortality in wireworms after exposure (Fig. 5) compared to untreated control. Despite wireworms previously documented ability to recover from insecticide intoxication even after displaying symptoms for 150+ days following tefluthrin exposure (Vernon and van Herk 2013), the rapid mortality of insects displaying tefluthrin intoxication in this study contradicts many previous studies. They suggest that tefluthrin is effective at protecting crops through repellence effects without causing mortality, and if it does cause toxicity then this rarely leads to mortality (Vernon et al., 2009; van Herk et al., 2015).

Thiamethoxam-treated maize also caused significantly greater mortality than untreated maize and neem treated maize (Fig. 5). Previous studies have demonstrated mixed results with the use of thiamethoxam, with most agreeing that it is able to protect crops through intoxication but does not lead to significant population reduction through mortality (Vernon et al., 2009; van Herk et al., 2018; Onsacer and Rusk, 1969). Others have reported the capability of thiamethoxam to reduce wireworm populations by up to a third (Badawi et al., 2013). These studies focus on the protection of wheat crops, for which the rates of compound used within the seed treatments are generally lower compared to maize, and rate of compound has significant impact on mortality rates (Morales-Rodriguez and Wanner, 2014). Therefore, more work on the efficacy of thiamethoxam seed treatment at causing mortality when protecting maize crops is required for more robust comparisons of the mortality data presented here.

Neem caused no symptoms of toxicity (Fig. 4b), nor any significant mortality compared to untreated maize (Fig. 5). This matches previous work that observed neem caused repellence effects without significant toxicity or mortality (Campos et al., 2016; Cherry and Nuessly, 2010). With neem having no apparent effect on behaviour or wireworm health, it appears from the result of this study that neem does not suitably protect crops form wireworm feeding through behavioural change, intoxication or mortality effects.

Although all three (compound X, tefluthrin and thiamethoxam) traditional style pesticides caused significant mortality in wireworms in this study populations following exposure, it is difficult to translate this to a large population in the field. The experimental system used for this study are individual closed mesocosms that contain a single plant, and due to this, insects are in constant proximity to insecticide treatments. In a field setting, individual insects may utilise alternative food sources, and are similarly not constrained and free to move in the environment away from stimuli such as insecticides. These factors may have led to an increased mortality of larvae exposed to the tefluthrin and thiamethoxam treatments in this study compared to many previous studies (Vernon et al., 2009; van Herk et al., 2018; van Herk et al., 2015; Onsacer and Rusk, 1969). Further work is needed in field trials to suitably compare treatment efficacy to protect crops and manage wireworm populations compared to control and each other.

Only the compound X treatment had any observed significant effect on burrow production and structure compared to untreated maize. Further work into the mechanism and wider effect of this behavioural change is required. Regardless of the mechanism, this modification in behaviour itself could have implications for the wider soil ecosystem. A large number of studies have investigated the effects of soil characteristics on insecticides and likewise the effects of these compounds on the biology and chemistry of soil (Spyrou et al., 2009; Monard et al., 2011; Zaller et al., 2014; Farenhorst et al., 2000). The effects of macrofauna-produced biopores (burrows) on the transport of agrochemicals is well established, with the transport of compounds occurring much faster in soil with higher volume of hydraulically connected biopores (Worrall et al., 1998; Ramesh et al., 2019). Additionally, biopores can directly influence the mineralisation of agrochemicals by the microbial community contained within the pore, with hydraulically active biopores acting as hot spots of this mineralisation compared to the surrounding soil (Spyrou et al., 2009).

What has been observed in this work is that insecticide use can significantly modify the physical properties of soil via secondary action: altering the behaviour of soil dwelling insects leading to increased burrow production and therefore increased porosity of soil. Soil porosity is a key factor that dictates hydraulic conductivity, facilitating the availability and movement of air or water, particularly when connected to the surface (Ramesh et al., 2019). In addition, soil porosity (and biopore, length, shape, size distribution and connectivity over a range of scales) are also important for soil biodiversity, by providing habitats for soil flora and fauna including the diverse microbial communities (Six et al., 2004; Kinyangi et al., 2006). Existing work has also observed positive correlations between soil porosity and SOC (Fukumasu et al., 2022)

Although from this work we can conclude that insecticide exposure has the potential to modify the burrowing behaviour of wireworms, further investigation is required to determine the mechanism of this behaviour, why it was only seen in response to a singular compound, and the implications for both crop protection and the wider soil ecosystem. Future experiments might focus on different soil conditions (texture, moisture, management systems etc.), crop species, insecticide compounds or other target organisms. From a crop protection perspective, all three traditional style insecticides (compound X, tefluthrin and thiamethoxam) were able to cause significant mortality in wireworms compared to an untreated control. However, as the system used in this study is a closed, single pot experiment, the health effects following exposure to these compounds may not be comparable to a field context, so conclusions on the ability of these compounds manage populations through mortality would require further study utilising field trials.

CRediT authorship contribution statement

Samuel W. Booth: Conceptualisation, Data curation, Formal analysis, Investigation, Methodology, Project administration, Validation, Visualisation, Writing – original draft, Writing – review & editing. **Craig J. Sturrock:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Project administration, Methodology, Investigation, Funding acquisition. **Sacha J. Mooney:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization. **Benedikt J. Kurtz:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization. **Martine I de Heer:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Samuel Booth reports financial support was provided by Syngenta International AG. Samuel Booth reports a relationship with Syngenta International AG that includes: funding grants. Sacha Mooney reports a relationship with Syngenta International AG that includes: funding grants. Craig Sturrock reports a relationship with Syngenta International AG that includes: funding grants. Martine de Heer reports a relationship with Syngenta International AG that includes: employment. Benedikt Kurtz reports a relationship with Syngenta International AG that includes: employment. N/A If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data will be made available on request.

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