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Fate of thiamethoxam from treated seeds in mesocosms and response of aquatic invertebrate communities

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Abstract

Thiamethoxam is a neonicotinoid insecticide widely applied in the Canadian Prairies. It has been detected in surface waters of agro-ecosystems, including wetlands, but the potential effects on non-target invertebrate communities in these wetlands have not been well characterized. In an effort to understand better the fate of thiamethoxam in wetlands and the response of invertebrates (zooplankton and emergent insects), model systems were used to mimic wetland flooding into planted fields. Outdoor mesocosms were treated with a single application of thiamethoxam-treated canola seeds at three treatment levels based on a recommended seeding rate (i.e., 6 kg/ha; 1×, 10×, and 100× seeding rate) and monitored over ten weeks. The mean half-life of thiamethoxam in the water column was 6.2 d. There was no ecologically meaningful impact on zooplankton abundances or community structure among treatments. Statistically significant differences were observed in aquatic insect abundance between control mesocosms and the two greatest thiamethoxam treatments (10× and 100× seeding rate). The observed results indicate exposure to thiamethoxam at environmentally relevant concentrations likely does not represent a significant ecological risk to abundance and community structure of wetland zooplankton and emergent insects.

Keywords Mesocosm · Neonicotinoids · Thiamethoxam · Wetlands · Emergent insects · Treated seeds

Introduction

In the Prairie Pothole Region (PPR) of Canada (~390,000 km^2 of southern Manitoba, Alberta, and Saskatchewan), the areas of greatest wetland density overlap with the greatest intensity of neonicotinoid insecticide use (Main et al. 2014). Approximately 80% of canola, barley, wheat, oats, and pea seeds planted in Canada are treated with a neonicotinoid insecticide (Jeschke et al. 2010). In the PPR, thiamethoxam is the most readily used neonicotinoid by mass and area in seed treatments (Main et al. 2014; Malaj et al. 2020). Consequently, it is important to understand the potential effects this neonicotinoid insecticide could have on these species-rich and productive aquatic systems.

The use of neonicotinoid insecticides has risen considerably over the last decade in part due to their specificity for the nicotinic acetylcholine receptor in insect pests, systemic nature in plants, utility on a relatively large range of insect pests, and relatively low vertebrate toxicity (Douglas and Tooker 2015). Due to their physicochemical properties, neonicotinoids have the potential to move into surface water systems via runoff and/or leaching from fields sprayed with neonicotinoids or planted with treated seeds (Anderson et al. 2015). A number of studies have reported the presence of neonicotinoids in North American surface waters (Main et al. 2014; Struger et al. 2017; Challis et al. 2018; Hladik et al. 2014), particularly in regions with

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relatively intensive agricultural activity. For example, concentrations of thiamethoxam in the surface waters of southern Ontario up to 1340 ng/L have been observed (Struger et al. 2017). Similarly, thiamethoxam in prairie wetlands at concentrations up to 1490 ng/L have been reported (Main et al. 2014).

Due to their specific mode of action, the potential effects of neonicotinoid insecticides on invertebrate communities in aquatic ecosystems is of particular concern. A number of studies have been conducted to characterize the acute and chronic toxicity of thiamethoxam to individual species of freshwater invertebrates, and been consistent in reporting that this compound is the least toxic of the neonicotinoids (Finnegan et al. 2017; Pickford et al. 2018; Maloney et al. 2018; Raby et al. 2018a; Raby et al. 2018b). Raby et al. (2018a) conducted 48- or 96-h tests with a suite of 21 laboratory-reared or field-collected freshwater invertebrates to determine thresholds for survival and immobilization. The LC50 values ranged from 5.5 to >80,000 µg/L of thiamethoxam, EC50 values ranged from 5.5 to 4775.4 µg/L (the most sensitive species being select Ephemeropterans, Trichopterans, Coleopterans, and Dipterans), and calculated HC5 values were 12.29 µg/L and 6.09 µg/L for lethality and immobilization, respectively. Consistent with previous studies, the most sensitive species were members of Insecta, while Crustacea and Oligochaeta species were less sensitive (Raby et al. 2018a).

Under chronic conditions, thiamethoxam has been reported to affect survival, growth, and emergence of aquatic invertebrates. Emergence was significantly impaired in *Chironomus dilutus* larvae exposed to thiamethoxam, with a reported 28-d EC50 of 8.91 µg/L (Maloney et al. 2018). Raby et al. (2018b) also reported reduced emergence in *C. dilutus* at an EC50 of 12.95 µg/L, as well as reduced growth and survival after 14 days of exposure to ≥21.45 µg/L of thiamethoxam. Chironomid emergence was also the most sensitive endpoint following acute and chronic GLP studies with 24 freshwater species and six marine species, including algae, macrophytes, crustaceans, molluscs, insects, and fish (Finnegan et al. 2017). The 30-d NOEC for emergence of *C. dilutus* was 10 µg/L, but mayfly (*Cloeon dipterum*) was also highly sensitive, with a 48-h EC50 of 14 µg/L (Finnegan et al. 2017).

A number of studies have investigated invertebrate responses under field conditions for thiamethoxam. The effect of a single acute exposure by direct overspray of thiamethoxam to zooplankton communities in mesocosm was investigated. No significant differences were observed in zooplankton communities between control mesocosms or mesocosms treated with up to a 500 µg/L pulse of thiamethoxam (Lobson et al. 2018). Another study observed reduced larval abundance and adult emergence in mayflies following exposure to 1.0 µg/L for 3 weeks in outdoor mesocosms (Pickford et al. 2018). Based on these results, a 35-d NOEC of 0.3 µg/L was determined for thiamethoxam for sensitive aquatic insects (Pickford et al. 2018). Collectively, previous studies have confirmed that aquatic invertebrate species can be highly sensitive to thiamethoxam.

There is the potential for these communities to be exposed to thiamethoxam due to flooding in fields planted with neonicotinoid-treated seeds. The borders of wetlands are rarely fixed and can expand significantly, with implications for macroinvertebrate communities, and the PPR specifically where this can flooding can be into planted fields (Batzer and Wissinger 1996; Gleason and Rooney 2018). The presence of treated seeds in wetlands represents a different exposure regime than the transport of thiamethoxam through mechanisms leaching, run-off, or wind erosion of dust, and has been seen in floodplain wetlands (Kuechle et al. 2019). The latter pathways of exposure likely result in acute pulsed exposures during precipitation or application events, particularly, when we consider the potential susceptibility of neonicotinoids to photodegradation in surface waters (Lu et al. 2015). The expansion of wetland borders into seeded fields presents the possibility of a slow long-term release of thiamethoxam from seeds trapped in the sediment. This situation may result in the chronic exposure of aquatic invertebrate communities to thiamethoxam in the PPR. This highlights the need for a community-level investigation of the potential effect of thiamethoxam exposure to wetland invertebrates due to release from treated seeds.

The objective of this study was to characterize the response of zooplankton and aquatic insect communities to thiamethoxam exposure via treated seeds, which may occur due to wetland expansion into seeded fields or seeding of semi-permanent wetlands that then re-fill. This study also investigated the fate of seed-associated thiamethoxam in overlying water and sediment in the model shallow wetland mesocosms. The data generated from this environmentally relevant study can be used to assess the risk of thiamethoxam to shallow (<1 m) wetland systems.

Methods

Test systems

Experiments were performed at the Prairie Wetland Research Facility (PWRF) at the University of Manitoba in 2014. The PWRF mesocosm system is composed of 18 aboveground, circular, low-density polyethylene tanks (2.7 m diameter × 0.72 m height; 5.72 m^2 surface area; 4.12 m^3 total volume), of which 12 were used in this study. In 2011, topsoil was added to each mesocosm to an

approximate depth of 23 cm to act as sediment (Anseeuw Brothers Ltd., Winnipeg, MB). The topsoil was clay-dominated with 50.9, 35.4, and 13.7% clay, silt, and sand, respectively, and organic carbon and organic matter content were 2.6 ± 0.1 and 4.5 ± 0.2% by dry weight, respectively (Cardinal et al. 2014). The mesocosms were filled with passively aged and de-chlorinated City of Winnipeg water between May 23rd and June 2nd, 2014 with an average volume of 1956L and depth of 38 cm at Day 0. They were topped up with de-chlorinated water on day 14th and day 21st (August 5th and 12th) with an average of 95 L (6% of remaining volume at the time) and 203 L (13% of remaining volume at the time), respectively to maintain water levels at around 1500 L due to evaporation.

Native invertebrate species were collected from the Oak Hammock Marsh, Stonewall, Manitoba (50.174135°N, 97.130845°W) to colonize the mesocosms. Zooplankton from the nearshore area of the marsh were retrieved using tow nets (73-µm and 35-µm). Kick nets (500-µm) were used to collect benthic invertebrates from the marsh. These two sample types were combined and equal volumes of water containing invertebrates in egg, larval, and aquatic juvenile/adult life stages from the marsh were added to each mesocosm. The mesocosms also remained uncovered to allow for natural aerial colonization by other emergent insect species. Fish and amphibians were not included in the mesocosms due to their ability to confound effects on invertebrate communities. It is also important to note that fish are not usually present in shallow wetlands due to water freezing completely to the bottom in water (Batzer and Wissinger 1996). Macrophytes included primarily *Myriophyllum sibiricum, Lemna* spp., *Stuckenia pectinata, Potamogeton* sp. and *Utricularia vulgaris*. Greater detail on the wetland mesocosms and the set-up of these test systems are provided in Cardinal et al. (2014), Lu et al. (2015), and Lobson et al. (2018).

Mesocosm treatments

The 12 mesocosms were randomly separated into four treatment groups: controls, 1× seeding rate (estimated concentration of 9.2 µg/L coated seeds), 10× seeding rate (92 µg/L), and 100× seeding rate (920 µg/L). Application rates were based on recommended rates for Cruiser 70 WS® (70% active ingredient) FarMore 100® coated canola seeds (Syngenta Seedcare™). There were no other active ingredients (e.g., fungicides) on these seeds. The total mass of canola seeds applied to each mesocosm for each treatment was based on a seeding rate of 6 kg seed/ha and 5.72 m^2 surface area of mesocosm (i.e., 1× seeding rate: 3.43 g seed/mesocosm). The resulting nominal concentration of thiamethoxam in water is based on 4.0 g of thiamethoxam in each kilogram of canola seed and approximately 1500 L of water in each mesocosm (i.e., 1× seeding rate should reach a theoretical maximum concentration of 9.2 µg of thiamethoxam per litre assuming complete dissolution of all insecticide from the seeds). In 1×, 10×, and 100× seeding rate treatments, 3.45, 34, and 344 g of seeds, respectively, were placed in each triplicate mesocosm. Seeds were allocated by mass into 20-mL scintillation vials covered with porous Teflon mesh and distributed haphazardly across four quadrants. Seeds were placed in vials to prevent them from floating at the surface of the mesocosms. The seeds were divided among 16 scintillation vials per mesocosm for the 1× and 10× treatment (mean mass of treated seeds per vial 0.21 and 2.12 g, respectively) and 52 vials were deployed in the 100x treatment (mean mass of treated seeds per vial 6.62 g). For the control mesocosms, non-coated canola seeds were deployed to rule out effects of seed presence/absence. Each control mesocosm received 344 g of untreated seeds (52 vials, 6.62 g each). Treatment day occurred on July 22, 2014. The exposure duration was 70 days with final sampling date on September 30, 2014. Water quality pre-monitoring began on June 25, 2014.

The 1×, 10×, and 100× seeding rates were selected for a number of reasons. These include capturing environmental realism (1× treatment), to try and ascertain the threshold of effect (the 10×, and 100× seeding rates), as well as induce a semi-chronic exposure scenario passively without having to repeatedly re-dose (all the treatments). They are also conservative, in that the assumption is that the entire wetland would have seed throughout, as opposed to only the margins as would be the case in an established system expanding. As well, should application or seeding rates increase, or be different amongst seed types, this experimental design can help address these scenarios.

Water and sediment sampling methods

Water samples were collected on Day −2 (prior to thiamethoxam addition), and 1 h, 8 h, and 1, 2, 4, 7, 14, 21, 28, and 42-days post-treatment. Sub-samples were collected from various locations in each mesocosm using an integrated water sampler to create a composite sample for each mesocosm with a total volume of approximately 2 L as previously performed (see Cardinal et al. 2014). Samples were stored in 250-mL amber glass bottles at 4°C for < 24 h until sample preparation. Water collected on Days −5, 14, and 28 was assessed for alkalinity, hardness, and nutrients. Alkalinity and hardness were measured using standard kits (LaMotte Company, Chestertown, MA, USA). Samples for nutrient analyses were stored in Nalgene bottles and analyzed at the Freshwater Institute in Winnipeg, Manitoba. Total dissolved nitrogen (TDN) and total dissolved phosphorus (TDP) species were determined colourimetrically (Stainton et al. 1977). The method detection limit (MDL) and limit of quantification (LOQ) values for TDN were

8 µg/L and 27 µg/L, and those for TDP were 0.58 µg/L and 1.85 µg/L, respectively.

Starting June 25, 2014, temperature, specific conductivity, pH, oxidation-reduction potential (ORP), chlorophyll-a content, and dissolved oxygen (DO) were measured 3 to 5 times per week for 27 d pre- and 70 d post-treatment with a YSI 650MDS unit, as previously (see Cardinal et al. 2014). Hobo Water Temp Pro v2 (model U22-001) data loggers were deployed 5 cm above the sediment level, and 20 cm from the western edge of each mesocosm to record water temperature at 30-minute intervals throughout the duration of the study.

Sediment was sampled to determine the fluxes of spiked thiamethoxam to sediments. This was done by deploying four 100 mL amber open glass jars (pre-ashed at 450 °C) filled with clean sediment (as described above) in each mesocosm. One jar from each mesocosm was retrieved on Days 4, 7, 14, and 42, and kept frozen until extraction and analysis.

Leaching experiments

Leaching of thiamethoxam from treated seeds was examined in the laboratory to confirm the potential for significant movement of the compound into surface water. Treated seeds were placed in 1-L clear or amber jars with 250 mL of either unbuffered Milli-Q® (pH 5.5 to 7) or mesocosm water (pH ~ 8.2). Three treatments were prepared with nominal thiamethoxam concentrations of 2, 4, and 8 µg/L (i.e., ~ 125, 250, and 500 mg of seeds/jar). The concentration of thiamethoxam in the overlying water was measured at 0, 0.5, 1, 2, 3, 8, and 24 h following the addition of treated seeds.

Analysis of thiamethoxam

Water analysis

Extraction and analysis methods for water samples followed previously published methods (Xie et al. 2011). Thiamethoxam (technical grade, purity 99.8% CDN Isotopes, Pointe-Claire QC) was dissolved in HPLC grade methanol (Fisher Scientific Fair Lawn, NJ) and used as the calibration standard. The mobile phase consisted of a 65:35 ratio of Milli-Q water and HPLC grade acetonitrile (EMD Millipore, Darmstadt, Germany).

Each 100-mL mesocosm water sample was filtered through 0.45-µm HAWP mixed cellulose ester membrane filter (Merck Millipore Ltd., Tullagreen Carrigtwohill County Cork Ireland), then loaded onto a 3cc/60 mg OASIS™ HLB SPE cartridge (Waters Corporation, Milford, MA) preconditioned with 2 mL of methanol and 2 mL of Milli-Q water. Thiamethoxam was eluted from the cartridge using 3 mL of methanol. The eluent was evaporated at 40°C under gentle nitrogen flow. The dried sample was reconstituted with 1 mL 65:35 Milli-Q® water:acetonitrile to match mobile phase conditions, and filtered through a 0.22-µm PTFE syringe filter (Restek Corporation, Bellefonte, PA).

Quantification of thiamethoxam in samples was performed by Agilent 1200 (Agilent Technologies, Mississauga, ON) high performance liquid chromatography (HPLC) coupled with a UV diode array detector (DAD) and ultra-high performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) with an electrospray ionization (ESI) source in positive mode coupled to a 6410 triple quadrupole MS/MS. If the concentration of thiamethoxam was below the method detect limit (MDL) for DAD, then MS/MS was used. The liquid chromatography mobile phases were prepared with 0.05% formic acid in Milli-Q® water and HPLC-grade acetonitrile and a Waters (Milford, MA) Symmetry C_{18}, 4.6 mm × 150 mm, 3.5 µm analytical column and a Phenomenex (Torrance, CA) SecurityGuard C_{18} Guard Cartridge (4 mm × 3.0 mm ID). Thiamethoxam was resolved isocratically with a 65:35 Milli-Q® water:acetonitrile eluent at 1.0 mL/min with an injection volume of 15 µL and column temperature of 40 °C. Quantification was by isotope dilution using thiamethoxam-d_3 (CDN Isotopes). Thiamethoxam and thiamethoxam-d_3 were quantitatively determined by monitoring the *m/z* transitions of 292.0 (precursor ion) to 211.0 (quantifier product ion) and 295.0 to 214.0, respectively. The detection limits of thiamethoxam and thiamethoxam-d_3 were confirmed by the qualifier product ion *m/z* 181 and 184, respectively. Fragmentation voltage and collision energy were set as 111 V. Instrumental source parameters were as follows: gas temperature at 300 °C, nebulizer gas flow 15 psi at 11.0 $L \cdot min^{-1}$ and capillary voltage 4000 V. A total of ten thiamethoxam standard solutions were prepared in methanol over a concentration range of 0.1–200 mg/L. All solutions were kept in amber vials. The MDL and LOQ for HPLC-DAD were 0.8 and 2.6 µg/L, respectively, and the MDL and LOQ for UHPLC-MS/MS were 0.02 and 0.06 µg/L, respectively. Instrumental source parameters are outlined in Table S1 in the SI.

Sediment analysis

Collected samples were left at room temperature in the dark to thaw for one day. A 5-g sub-sample of wet sediment was weighed from each sample and transferred to a beaker covered by aluminum foil. An internal standard of 50 ng/mL thiamethoxam-d_3 was added to each sample. The samples were then extracted with 10 mL of acetonitrile, sonicated for 30 min at 25°C, and centrifuged for 15 min at 10,000 *g*. The first supernatant was transferred to a clear glass tube, and the remaining sediment was extracted and centrifuged again by the same method with an additional 10 mL of

acetonitrile. The combined supernatants were evaporated on the nitrogen-evaporator until dry. The samples were then reconstituted with 1 mL of the mobile phase (65 Milli-Q®: 35 Acetonitrile) and filtered with a 0.22-µm filter. Thiamethoxam in the extracts of sediments was also detected by UHPLC-MS/MS with an ESI source in positive mode, using an Agilent 1200 UHPLC (Agilent Technologies) coupled to a 6410 triple quadrupole MS/MS, as described above. The MDL and LOQ for thiamethoxam in sediment were 1.58 and 5.26 ng/g dw, respectively.

Zooplankton

Zooplankton sampling occurred on Days −14 and −5 pre-treatment and Days 0, 1, 4, 7, 14, 28, 42, and 71 post-treatment. The zooplankton community was sampled from two locations in each mesocosm using activity traps, which consisted of 900-mL glass jars with a 243-mL Nalgene polypropylene powder funnel attached by two s-hooks and an elastic band (adapted from Murkin et al. 1983 and Sibley et al. 2001). The traps were first filled with water filtered through 53-µm mesh from the mesocosm being sampled. After a 24-h deployment, the traps were retrieved and their contents filtered through 53-µm mesh. Organisms were narcotized by adding soda water (5 mL) to each sample jar. In the laboratory, 5 mL of sucrose formalin solution was added to preserve the samples until identification and enumeration (Haney and Hall 1973; USEPA 2016). Zooplankton samples were adjusted to a consistent volume of 50 mL prior to enumeration. Zooplankton samples were mixed to ensure an even distribution of organisms, then a 5-mL sub-sample (i.e., 10% of sample volume) was transferred into a Bogorov zooplankton counting chamber using a glass pipette. The sub-sample was then analyzed using a dissecting microscope (4 to ×5 magnification) and taxa were counted in the following order: rotifers, nauplius larvae, cladocerans, copepods, and ostracods (USEPA 2016). If the number of organisms counted was less than 50 individuals in the initial aliquot of sample, an additional 5-mL aliquot was enumerated (USEPA 2016). Cladocerans were identified to genus, copepods to order, ostracods to class, and rotifers to phylum. Dichotomous keys developed by (Eddy and Hobson 1950 and Balcer et al. 1984) were used to identify zooplankton. Total abundance of zooplankton, abundance of individual zooplankton species, and diversity metrics (species richness, species evenness, Shannon's diversity, and Simpson's diversity) were calculated for each mesocosm. Diversity indices were calculated using the *vegan* package with RStudio (Version 1.1.383; R Version 3.4.0) (RStudio Team 2015; Oksanen et al. 2017; R Core Team 2017).

Aquatic insects

To collect emergent insects, one emergence trap was placed on each of the study mesocosms. Emergence trap parameters: base = 0.012 m^2, top = 0.0041 m^2, height = 0.023 m^2. The trap was composed of PVC loft piping (¾ inch), "T" and "L" shaped PVC Joints, and mesh (glued directly to the PVC frame via all-weather caulking). The trap was anchored in placed to the mesocosm-water interface via three wooden stakes, and fastened by rope (shown in Fig. S1). A container near the top of each emergence trap was filled with 100 mL of 70% ethanol (renewed after each collection) to attract insects and preserve them for collection and sorting (Thomson 1973). Aquatic insects were collected every Tuesday and Friday over the course of the study duration. Traps were first deployed on Friday, July 18, 2014 (i.e., four days pre-treatment).

Aquatic insects captured in emergence traps were identified to family and preserved in 70% ethanol in 20-mL scintillation vials. Identification was accomplished using a Stemi 2000-C stereomicroscope and an insect identification guide (Merritt et al. 2008).

For quality assurance and quality check (QA/QC) purposes, one randomly selected scintillation vial was selected for each sample day. Three members of the research team counted and identified the insects within the randomly selected scintillation vial. The average and standard deviation per sample day were determined, as well as the identity of the specimen(s) within each scintillation vial, for comparison. Any discrepancies (i.e., counts by an individual that did not fall within the average plus or minus the standard deviation) were then immediately corrected (re-enumeration or identification). Out of the 23 randomly selected samples, three samples contained discrepancies between the counters, and none were misidentified. In only one of the 23 randomly selected samples was the primary enumerator for the emergent insects found to be causing the count discrepancy. However, following a recount, the primary enumerator's count total was found to be within a standard deviation of the mean.

Total abundance of aquatic insects, abundance of individual aquatic insect species, and diversity metrics (species richness, species evenness, Shannon's and Simpson's diversity) were calculated for each mesocosm. Diversity indices were calculated using the *vegan* package with RStudio (Version 1.1.383; R Version 3.4.0) (RStudio Team 2015; Oksanen et al. 2017; R Core Team 2017).

Statistical analysis

A Shapiro-Wilk test for normality and Brown-Forsythe test for equal variance were performed before analysis of variance (ANOVA). A two-way ANOVA was used to

determine if significant differences in water quality parameters (temperature, specific conductivity, pH, ORP, chlorophyll-a, and DO) occurred among treatments over the course of the study, both pre-, as well as post-exposure to determine if there were any differences between treatments. If a significant difference was found, a post-hoc Tukey's test was used. One-way ANOVA with a Dunnett's post-hoc test was used to test for difference in zooplankton and aquatic insect abundance and diversity metrics between the control and the treatment concentrations at each sampling time point. Repeated measures one-way ANOVA with a Dunnett's post-hoc test was used to test for differences in the cumulative abundance of Diptera (the most abundant emergent insect taxa) over the course of the study among the treatments. If response variables did not satisfy assumptions of normality and homoscedasticity, a Kruskal-Wallis test was performed with a post-hoc Tukey's test. Analyses were performed in Sigma Plot and Sigma Stat (Systat Software Inc., 2006 and 2008, San Jose, CA, USA) with an alpha value of 0.05.

The minimum detectable difference (MDD) was calculated for the abundance of each zooplankton taxon and total zooplankton abundance at each sampling point in the study (Brock et al. 2015). The MDD is a measure of the difference between the means of a treatment and the control required to detect a statistically significant effect. The MDD was calculated using the equation below, where $t_{1\text{-}\alpha,df,k}$ is the quantile of the t-distribution, df is the degrees of freedom, k is number the number of treatments, s is the residual standard error from a one-way ANOVA, and n_0 and n are the sample sizes (Brock et al. 2015). The MDD% was calculated by dividing the MDD by the mean of the control treatment and multiplying the quotient by 100.

$$MDD = t_{1-\alpha,df,k} \cdot s\sqrt{\frac{1}{n_0} + \frac{1}{n}}$$

If the MDD% is <100% at a specific sampling point, a treatment-related difference in abundance can be demonstrated. If the MDD% is >100% at a specific sampling point, the power of the experiment is considered too low to identify a treatment-related significant change in abundance (Brock et al. 2015).

Canonical correspondence analysis

Environmental variables (temperature, conductivity, pH, ORP, chlorophyll, concentration of thiamethoxam, and day) and zooplankton and aquatic insect community data collected from the mesocosms over the course of the study were used to perform a canonical correspondence analysis (CCA). The *vegan* package in RStudio (Version 1.1.383) was used to conduct the CCA (RStudio Team 2015; Oksanen et al. 2017; R Core Team 2017). A model to describing the variability in the zooplankton and aquatic insect community was constructed using permutation tests to perform a step-wise addition of significant environmental variables. The R code used to perform the CCA is presented in the SI (Table S2).

Principle response c

Principle response curve (PRC) analysis was performed to compare the response of the zooplankton and aquatic insect community across thiamethoxam treated mesocosms relative to the untreated mesocosms (Van den Brink and ter Braak 1998; 1999). The *vegan* package in RStudio was also used to perform the principle response curve analyses (Version 1.1.383) (RStudio Team 2015; Oksanen et al. 2017; R Core Team 2017). The effect of treatment at each sampling point was examined using a Monte Carlo permutation test. A Dunnett-Contrasts test was used to determine when treatments were significantly different from the control treatment at each sampling time point. The R code used to perform the PRC analysis is presented in the SI (Table S3). The R code used for PRC analysis was modeled after the code presented by Szocs (2012).

Results and discussion

Water quality parameters

Overall, there were no significant differences among treatments prior to the introduction of the canola seed for any of the water quality parameters. Chlorophyll-a increased significantly following the addition of seeds; however, there was no significant difference between treatments relative to the pre-exposure period, as well as no interaction ($p > 0.05$, Table 1). Conductivity increased significantly following the addition of treated seeds, and conductivity in treatments 10× and 100× was significantly greater than in the control ($p < 0.05$, Table S2). DO and temperature decreased significantly after addition of seeds; no significant difference between treatments was observed (Table 1). There was also no significant difference in the density of filamentous algae among treatments. Alkalinity, hardness, TDN, and TDP were not significantly different among treatments at any time point, and these parameters were not significantly different before and after addition of thiamethoxam-treated seeds (Tables 1 and S4). The pre-exposure DO, pH, conductivity, and ORP measurements recorded for the current study were consistent with those measured pre-exposure by Lobson et al. (2018) at the same facility. While Lobson et al. (2018) did not observe a consistent increase in

Table 1 Mean (±SE) water quality parameters measured in mesocosms in 2014

	DO (mg/L)		pH		Conductivity (µS/cm)		ORP (mV)		Temp. (°C)		Chlorophyll-a (mg/L)		Alkalinity (mg/L $CaCO_3$)		Hardness (mg/L $CaCO_3$)	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
0x	9.1 (0.4)	7.7[a] (0.6)	9.2 (0.1)	9.3 (0.1)	510 (17)	647[a] (23)	457.8 (4.7)	594.6 (46.1)	19.1 (0.4)	17.7[a] (0.5)	5.6 (0.2)	9.0[a] (0.3)	177 (5)	255 (13)	127 (15)	165 (7)
1x	8.5 (0.4)	7.7[a] (0.5)	9.2 (0.1)	9.5 (0.03)	520 (8)	598[a] (8)	461.9 (4.8)	529.7 (18.6)	19.1 (0.4)	17.7[a] (0.5)	7.4 (0.4)	11.3[a] (2.2)	185 (8)	234 (9)	153 (6)	140 (10)
10x	8.9 (0.4)	7.8[a] (0.5)	9.2 (0.1)	9.2 (0.1)	546 (17)	699[a] (25)	458.8 (6.5)	497.8 (14.6)	18.9 (0.4)	17.7[a] (0.5)	6.6 (0.4)	10.7[a] (2.0)	183 (12)	271 (34)	150 (0)	173 (25)
100x	7.8 (0.4)	6.5[a] (0.4)	8.9 (0.1)	9.0 (0.1)	620 (21)	749[a,b] (9)	464.1 (5.1)	518.4 (15.5)	19.0 (0.4)	17.7[a] (0.5)	7.6 (0.3)	11.4[a] (0.6)	213 (45)	284 (19)	167 (21)	190 (10)

DO dissolved oxygen, *ORP* oxidation-reduction potential

[a]Significantly different from pre-exposure ($p < 0.05$, ANOVA)

[b]Significantly different from control ($p < 0.05$, ANOVA)

conductivity with the addition of TMX to the tanks, Cardinal et al. (2014) did note an increase in conductivity (and chlorophyll-a) in the systems following spiking with wastewater.

Fate of thiamethoxam

Leaching experiments

Laboratory-based leaching experiments showed 43 to 100% of thiamethoxam leached from the treated seeds within 30 min of being placed in water (Table S5). This finding is consistent with the high water solubility of thiamethoxam in water (4.1 g/L; Tomlin 2006) and with previous leaching studies performed with thiamethoxam-treated seeds. For example, rapid leaching of thiamethoxam from treated soybean and corn seeds was reported in a 24-h laboratory study (Smalling et al. 2017). An equilibrium concentration between ~68 and 72% of the initial thiamethoxam was reached within the first 5 h in deionized buffered water (pH 7.0) (Smalling et al. 2017). The percentage of thiamethoxam leaching from the seeds increased with the number of seeds in the treatment. For example, the percent leached in the 2 µg/L treatment after 30 min was 43%, while 86% leached in the 8 µg/L treatment (Table S5). The leaching of thiamethoxam followed the same trend in Milli-Q® or mesocosm water and amber or clear jars (Table S5). There was less than a 10% difference between leached percentages for the two jar types in all but three samples (Table S5). Leaching in Milli-Q tended to be slightly less than in mesocosm water, but this trend also concentration-dependent, with ~20 to 30% greater leaching in mesocosm water at a concentration of 2 µg/L, and <1% difference between waters for all but the initial measurement (Table S5). This experiment illustrates the rapid rate with which thiamethoxam on treated seeds will move into the water phase, validating the concern of exposure to wetlands from treated seeds.

Concentration in mesocosm water

The concentration of thiamethoxam in the water of mesocoms increased over the first 24 h in the 1× and 10× treatments and over the first 48 h in the 100× treatment (Fig. 1). The mass of thiamethoxam in overlying water of mesocosms increased in the initial day(s) of the test, likely due to the diffusion of thiamethoxam from treated seeds at an initial rate greater than the rate of degradation. The concentration of thiamethoxam then dissipated relatively rapidly until Day 7, followed by slower dissipation from Days 7 to 70 (Fig. 1). Thiamethoxam was not detected in mesocosms from the 1× treatment after Day 20, but was detected and quantified in mesocosms from the 10× and

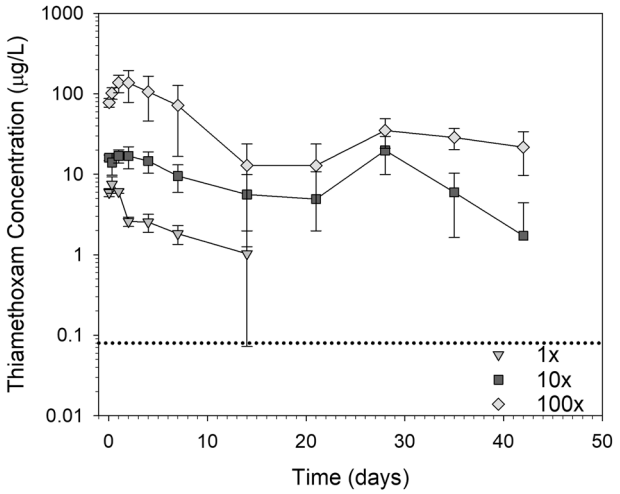

Fig. 1 Mean (±SD, $n = 3$) concentration of thiamethoxam measured from July 22 to September 2, 2014, in mesocosms treated at different seeding rates. On days 21, 28, 35, and 42, the concentrations of thiamethoxam in samples taken from the 1× treatment were less than the method detection limit (MDL = 0.08 µg/L; dotted line). The concentration of thiamethoxam was below the MDL in all water samples taken from control mesocosms

100× treatments until day 42. First-order non-linear regression was used to determine half-lives (with 95% confidence interval) of 2.8 (2.1 to 4.0), 9.3 (5.9 to 21.9), and 6.4 (3.5 to 44.9) days for 1×, 10×, and 100× treatments, respectively. The half-lives of thiamethoxam observed in 2014 were similar to those reported by Lobson et al. (2018). In that mesocosm study, half-lives ranged from 0.8 to 17.8 days, but mesocosms were dosed by direct addition of thiamethoxam to the water column. In another mesocosm study using thiamethoxam concentrations between 1 and 100 µg/L, the dissipation half-life from the water column ranged from 1.6 to 5.2 d (Finnegan et al. 2017), which is fairly consistent with the current study. Thiamethoxam remained detectable in the water column for a longer period of time in the current study compared to the previous mesocosm study at the same location (Lobson et al. 2018). In study, thiamethoxam could not be detected in the water of the mesocosm treated at 25 µg/L and 100 µg/L after 10 h and 5 days post treatment, respectively. In the current study, thiamethoxam was detected for up to 14 days post treatment and longer (Fig. 1). Thiamethoxam is susceptible to degradation via natural sunlight, as was demonstrated at the same mecososm facility in unplanted tanks, with a mean reported half-life of 0.98 d at the surface (Lu et al. 2015). However, photodegradation was substantially reduced (≥89%) at depths of 8 and 18 cm in clear tanks in that study, which is consistent with the current study (e.g., less light at depths, greater half-lives). These data indicate that release from treated seeds may represent a mechanism of chronic exposure for non-target receptors.

Concentration in mesocosm sediment

The concentration of thiamethoxam in sediment sampled from the mesocosms was below the MDL (i.e., <1.58 ng/g dw) in the control and 1× treatment mesocosms (Table S6). Thiamethoxam was only detected on Day 4 post-treatment in one 10× treatment mesocosm and was not detected in any 10× treatment mesocosms on Days 7, 14, or 42 (Table S6). The concentration of thiamethoxam was above the LOQ (i.e., 5.26 ng/g dw) on post-treatment Days 4 and 7 in the 100× mesocosms, with a mean concentration of 10.74 and 9.19 ng/g dw, respectively. The mean concentration was below the LOQ at 14 and 42 d post-treatment (Table S6). After 42 d post-treatment, the concentration in sediment had declined 66% from the concentration on Day 4. The relatively low concentration of thiamethoxam in sediment, even at 100× seeding rate, corresponds with the relatively low *Koc* of thiamethoxam (i.e., 70 mL/g). This experiment confirms thiamethoxam's low affinity for organic carbon in sediment in wetland ponds, and suggests it has a half-life in sediment between 7 to 14 d post treatment (Table S6). There are few studies that have reported thiamethoxam in sediments in order to contrast. Most recently, sediments in flood plains that had been inundated in Missouri, USA that had been actively planted with crops that contained NNI seed-treatments rarely detected thiamethoxam (4% detects; n = 157) with a max concentration of 1.09 µg/Kg sediment (Kuechle et al. 2019), which in the range of those observed in this study. That said, our experiment did not actually introduce the seeds into the sediments (as they would float away) and thiamethoxam would have need to transfer from the water column to the sediment phase. It is possible that our study underestimated sediment exposures, and overestimated waterborne exposures.

Zooplankton

A total of thirteen zooplankton taxa were identified in mesocosms (*Simocephalus* sp., *Scapholeberis* sp., rotifers, *Polyphemus* sp., ostracods, nauplius larvae, *Diaphanosoma* sp., cyclopoid copepods, chydorids, *Ceriodaphnia* sp., calanoids, bryozoans, and amphipods). No significant differences in zooplankton total abundance (Fig. S8) or metrics of diversity (Simpson's and Shannon's diversity, species richness, and species evenness) were observed among treatments over the course of the study. The abundances of *Simocephalus* sp. and *Polyphemus* sp. were significantly greater in the 100× and 1× treatment relative to the controls on Day 14. The abundance of cyclopoid species was significantly greater in the 100× treatment relative to control on Day 71. The abundance of *Diaphanosoma* sp. was significantly lower in all three treatments relative to the control on Day 0. There were no other significant differences in the

Table 2 Percent minimum detectable difference (MDD%) for total zooplankton abundance, individual zooplankton taxa, and diversity metrics at each sampling point from Day 1 to 71 of the study

Sampling day									
Taxa / Diversity metric	1	4	7	14	28	42	71	Mean	Standard deviation
Total abundance	36.6	43.0	38.2	52.8	37.6	68.8	36.9	44.8	12.0
Diaphanosoma sp.	67.0	119.4	89.1	61.3	43.4	60.6	67.0	73.5	26.9
Ceriodaphnia sp.	108.0	133.5	133.5	288.4	176.1	180.7	119.5	162.0	61.8
Chydorids	79.1	92.2	109.8	88.7	n/a	63.1	50.2	80.5	21.4
Simocephalus sp.	253.2	104.8	n/a	1417.7	41.0	73.0	98.5	331.4	537.2
Scatholebris sp.	n/a	n/a	n/a	58.8	n/a	48.0	98.5	68.5	26.6
Polyphemus sp.	125.8	n/a	n/a	n/a	119.0	208.8	n/a	151.2	50.0
Cyclopoids	55.9	31.5	26.6	29.8	62.5	48.4	67.5	46.0	16.8
Calanoid	39.2	n/a	n/a	83.2	32.9	73.3	117.7	69.3	34.6
Nauplii	86.1	45.8	167.4	297.5	187.7	78.0	396.9	179.0	128.0
Rotifers	57.3	63.6	68.9	73.2	68.0	679.9	312.2	189.0	235.1
Ostracods	93.6	115.8	69.3	50.0	119.0	n/a	38.7	81.1	33.7
Amphipods	343.7	n/a	n/a	10786.2	1532.6	1977.7	111.0	2950.2	4450.1
Bryozoans	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Shannon's diversity	10.0	17.8	23.0	12.1	11.9	11.4	23.9	15.7	5.8
Simpson's diversity	7.5	17.9	18.7	11.2	11.9	19.1	22.9	15.6	5.5
Evenness	9.5	16.4	18.9	11.0	11.4	9.6	25.5	14.6	6.0
Richness	6.1	11.0	19.6	14.7	17.2	13.6	16.1	14.0	4.4

n/a: MDD% could not be calculated because the mean of the control treatment was 0

abundance of individual taxa among the treatments relative to control during the study.

The mean MDD% for total abundance of zooplankton was 45% and the MDD% for the diversity metrics ranged from 14 to 16% (Table 2). According to the European Food Safety Authority's Panel on Plant Protection Products and their Residues (MDD classes: Class 0, >100%; Class I, 90–100%; Class II, 70–90%; Class III, 50–70%; Class IV, < 50%), this would indicate that small effects on total abundance of zooplankton and the measured diversity metrics for the zooplankton community due to exposure thiamethoxam-treated seeds could have been detected in this study (EFSA 2013; Brock et al. 2015). However, in terms of individual taxa, small effects could only have been observed in cyclopoid copepods, medium effects in *Scatholebris* sp., calanoids and *Diaphanosoma* sp., and large effects in chydorids and ostracods (Table 2). No effects could be determined statistically for the remaining individual taxa observed due to their MDD% being >100% (Table 2) (EFSA 2013; Brock et al. 2015).

The only environmental variable identified as a significant factor for developing a model to explain changes in the zooplankton community using CCA was the application rate of thiamethoxam-treated seeds (Table S2). The PRC analysis indicated that application of rate of seeds and time explained 28.5 and 10.7% of the variance, respectively, in the zooplankton community among treatments (Fig. 2; Table S3). The Monte Carlo permutation tests indicated that a significant effect of treatment was present on Day 4, but the effect was not present on Day 7 through 71; p-values for Day 1, 4, 7, 14, 28, 42, and 71 were 0.24, 0.02, 0.20, 0.73, 0.73, 0.75, and 0.22 (Table S2). The Dunnett-Contrasts tests showed that there was no significant difference in the zooplankton community among the treatments at any sampling days (Fig. 2; Table S3). The PRC analysis indicated that *Ceriodaphnia* sp. and *Simocephalus* sp. experienced the greatest increase with increasing seeding rate while rotifers and nauplius larvae saw the greatest decrease (Fig. 2).

Other mesocosm studies have observed that exposure to insecticides (e.g., chlopyrifos, ivermectin) can result in a significant decline in zooplankton populations (Van den Brink et al. 1996; Sanderson et al. 2007). A previous study exposed a zooplankton community, representative of communities found in the PPR, to thiamethoxam via a single direct overspray application in a mesocosm, which resulted in nominal concentrations in overlying water ranging from 0 to 500 µg/L (Lobson et al. 2015). They observed that exposure to a single pulse of thiamethoxam had little effect on zooplankton communities. PRC analysis did not show a significant concentration-dependent difference in the zooplankton community across the treatments over a 56-d period (Lobson et al. 2018). The community-level effects of thiamethoxam exposure appeared to be consistent with effects in individual taxa, as Lobson et al. (2018) also reported the greatest species weight for

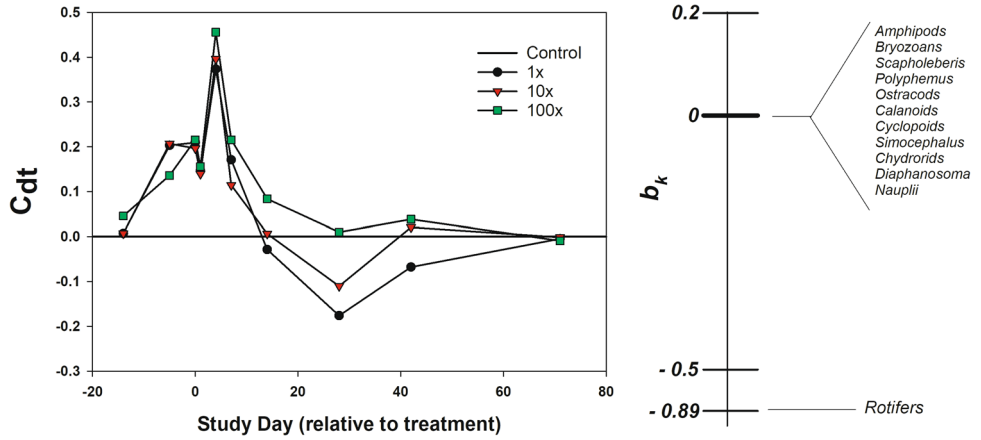

Fig. 2 Principle response curves diagram showing the response of zooplankton community in mesocosms exposed to different application rates of thiamethoxam-treated seeds (0, 1×, 10×, and 100×) over time. Species weights are provided on the right axis

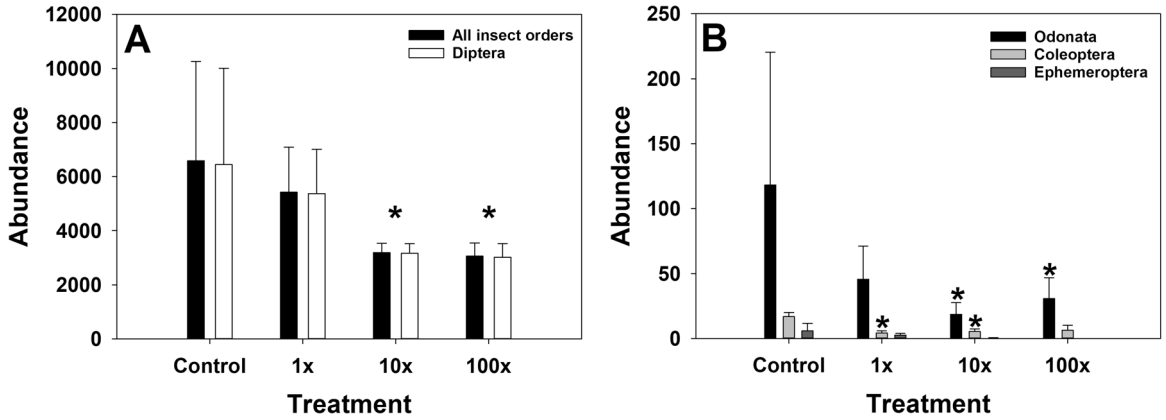

Fig. 3 Mean (±SE, $n = 3$) cumulative abundance of (**A**) all aquatic insects and Diptera, (**B**) Odonata, Coleoptera, and Ephemeroptera among control and thiamethoxam-treated mesocosms. Samples were collected from July 22 to September 30, 2014 (sample days = 22). Significant difference from the control is indicated by an asterisk

Ceriodaphnia sp. and lowest species weights for rotifers and nauplius larvae. The zooplankton communities in the current study were exposed to greater concentrations of thiamethoxam for a longer period of time than in Lobson et al. (2018) due to the release of thiamethoxam from treated seeds over the course of the study (Fig. 1). Mesocosms were also used by Finnegan et al. (2017) to investigate effects of thiamethoxam on multiple trophic levels, including zooplankton. Concentrations of thiamethoxam up to 100 µg/L did not result in significant effects to zooplankton at the community-level over a 92-d study, which is consistent with the community-level trends observed in the current study. There were changes in individual species/classes (e.g., Cyclopoida, *Simoncephalus vetulus*), but with the exception of a single-day NOEC of 30 µg/L for *Eudiaptomus* sp., all NOEC values below 100 µg/L indicated an increase in the number of individuals present relative to controls (Finnegan et al. 2017). The mesocosms used by Finnegan et al. (2017) were dominated by the rotifer *Keratella quadrata*, copepod nauplii, and the rotifer *Synchaeta* sp., but in contrast to the current study, rotifers and copepod nauplii were not affected by thiamethoxam exposure.

Aquatic insects

In total, 62,819 emerged aquatic insects were enumerated; 16,918 before treatment of mesocosms (day −4 and 0) and 45,901 following treatment Days 1 to 70. A total of four insect Orders and 24 Families were identified (Table S7). The abundance of Diptera and Odonata were greatest among the four Orders (Fig. 3). The total abundance of insects and the abundance of the four observed Orders among the treatments over the duration of the study are presented in Figs. S2–S5. The abundance of individual taxa and Orders among the treatments were not significantly different from the control at any time point in the study with the exception of the 100× treatment on Day 14 (Figs. S2–S6). The emergence of Diptera was greatest for the 100× treatment on Day 0, but quickly declined relative to other

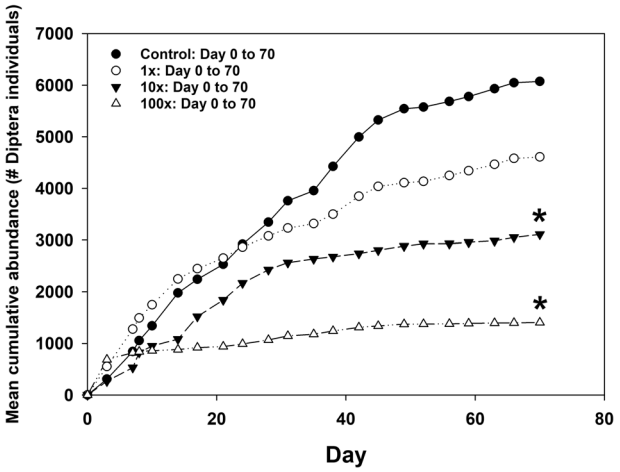

Fig. 4 Mean cumulative abundance of Diptera taxa on each sampling day between July 25 and September 30 (21 days) in control and thiamethoxam-treated mesocosms. Significant difference from the control is indicated by an asterisk

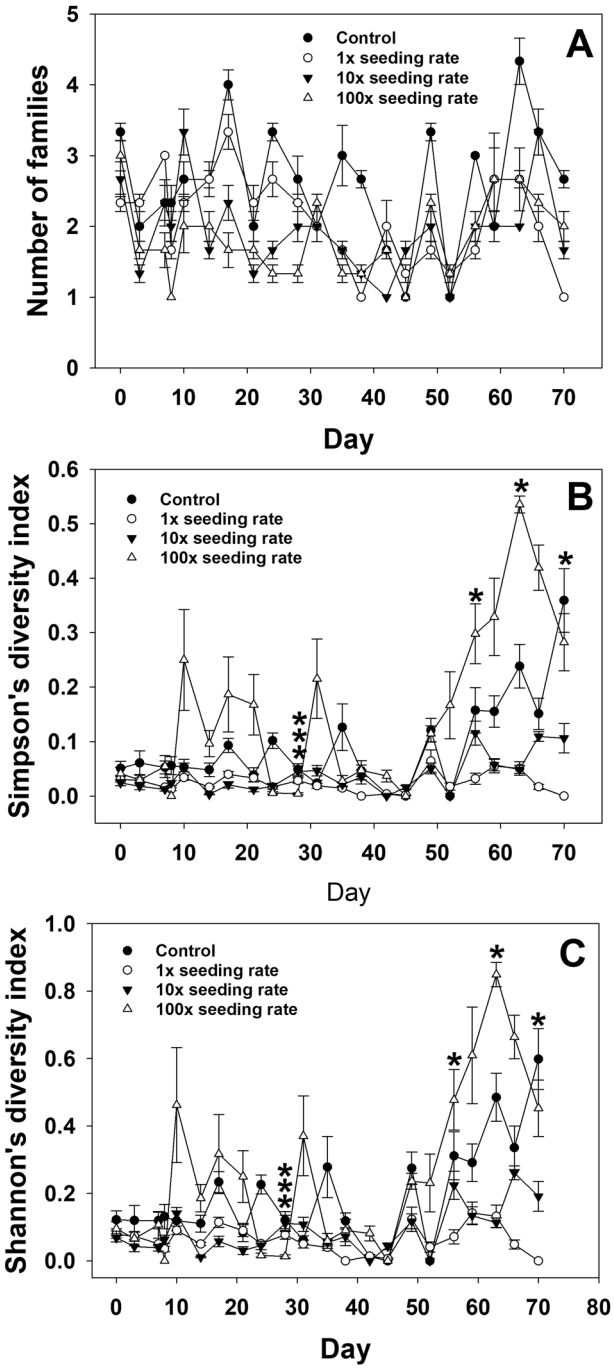

Fig. 5 Mean (±SE, $n = 3$) (**A**) Family richness, (**B**) Simpson's and (**C**) Shannon's diversity indices for aquatic insect community among treatments over the course of the study. Duration displayed from July 22 to September 30, 2014 (sample days = 22). Significant difference from the control is indicated by an asterisk

treatments (Figs. S2, S3). The abundance of Chironomidae was significantly lower in the 100× treatment relative to control on Day 14. Consequently, due to the dominance of Chironomidae in the insect community, the abundance of Diptera and total abundance of insects were also significantly lower in the 100× treatment on Day 14 (Figs. S2, S3). Odonata emergence in the 10× and 100× treatments declined relatively rapidly compared to 1× and control treatments (Fig. S4). The abundance of Coleoptera and Ephemeroptera were relatively low compared to the other two insect orders, and Coleoptera abundance increased over the course of the study (Figs. S5, S6). Ephemeroptera were not observed in the 100× treatment (Fig. S6).

The cumulative total abundance of emerged insects at the conclusion of the test declined as the concentration of thiamethoxam increased (Fig. 3). Cumulative total abundance of insects and abundance of Diptera, Odonata, and Chironomidae were significantly lower in 10× and 100× treatments relative to the controls (Fig. 2). The cumulative abundance of Coleoptera was significantly lower in 1× and 10× treatments but not 100× treatment compared to controls. The cumulative abundance of Ephemeroptera was not significantly different among treatments. The cumulative abundance of Diptera over the course of the study was significantly lower in the 10× and 100× treatment relative to the control (Fig. 4).

Species richness was not significantly different among the treatments over the course of the study (Fig. 5A). Shannon's diversity was significantly lower in the 10× and 100× treatment relative to control on Day 28 and Simpson's diversity was significantly lower in the 1×, 10×, and 100× treatment on this sampling day (Fig. 5B, C). The two diversity indices, along with evenness, was significantly greater in 100× treatment relative to the control on Days 56, 63, and 70 (Fig. 5B, C).

The MDD% for Chironomidae and species richness were <50% (Table 3), indicating that a small effect due to exposure to thiamethoxam-treated seeds could have been

Table 3 Percent minimum detectable difference (MDD%) for total aquatic insect abundance, individual aquatic insect taxa, and diversity metrics at each sampling point from Day 1 to 71 of the study

Taxa / Diversity metric[a]	Sampling day										Mean	Standard deviation
	1	14	21	28	35	42	49	56	63	70		
Total abundance	47.2	31.3	58.4	70.2	74.2	71.0	69.5	72.2	69.1	84.1	64.7	15.2
Diptera	47.2	31.4	59.2	70.8	74.4	71.0	69.4	72.2	70.9	85.7	65.2	15.6
Coleoptera	93.0	49.9	41.2	36.0	52.0	117.9	144.2	n/a	44.0	62.9	71.2	38.3
Odonata	763.2	70.4	76.5	55.8	84.6	83.2	n/a	83.3	101.8	n/a	164.9	242.1
Shannon's diversity	51.0	103.6	124.3	28.6	132.7	122.3	61.3	78.1	47.3	49.9	79.9	37.8
Simpson's diversity	30.8	113.5	81.6	29.1	59.4	156.0	74.8	78.1	43.1	56.2	72.3	39.0
Evenness	121.0	88.2	50.7	41.4	42.2	96.0	90.2	72.0	62.2	45.6	71.0	27.1
Richness	44.1	32.1	83.2	83.2	35.5	33.3	27.7	29.2	43.7	35.5	44.8	21.0
Chironomidae	47.2	31.4	43.7	53.5	56.1	49.9	59.0	30.2	51.1	50.8	47.3	9.7
Ceratopoginidae	n/a	85.2	n/a	148.8	n/a	n/a	381.6	110.9	938.4	204.0	311.5	324.8
Ephydridae	n/a	88.3	50.0	78.5	117.9	144.2	117.9	83.1	n/a	151.9	104.0	35.0
Culicidae	n/a	n/a	n/a	n/a	n/a	n/a	58.9	n/a	n/a	93.0	75.9	24.1
Dolichopodidae	n/a	n/a	72.0	93.0	n/a	n/a	93.0	n/a	n/a	n/a	86.0	12.1
Nymphomyiidae	83.3	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	83.3	n/a
Hydraenidae	n/a	87.7	36.7	83.3	83.2	n/a	n/a	n/a	n/a	n/a	72.7	24.1
Noteridae	n/a	n/a	n/a	117.9	n/a	83.3	n/a	n/a	47.5	36.7	71.3	36.9
Hydrophilidae	83.3	83.3	n/a	117.9	62.4	n/a	n/a	n/a	83.3	n/a	86.0	20.0
Elmidae	n/a	n/a	n/a	n/a	n/a	n/a	83.3	n/a	101.8	117.9	101.0	17.3
Staphylinidae	n/a	n/a	n/a	83.3	n/a	n/a	n/a	n/a	n/a	n/a	83.3	n/a
Melyridae	n/a	83.3	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	83.3	n/a
Baetidae	n/a	n/a	n/a	83.3	60.0	n/a	83.3	93.0	84.3	n/a	80.8	12.3

n/a: MDD% could not be calculated because the mean abundance of the control treatment was zero

[a]MDD% not reported for Ephemeroptera, Sciomyzidae, Dryomyzidae, Tipulidae, Psychodidae, Chrysomelidae, Cuclionidae, Salpingidae, and Caenidae because the mean abundance in the control treatment was zero at all sampling points

detected (EFSA 2013; Brock et al. 2015). Medium effects could have been observed in total abundance of aquatic insects, Diptera, Coleoptera, Shannon's and Simpson's diversity, species evenness, Culicidae, Hydraenidae, and Noteridae (Table 3). A significant treatment effect in the remaining taxa would have required a large effect or would not have been possible to detect statistically (Table 3). A MDD% could not be calculated for a number of taxa and/or sampling points due to the taxa not being observed in the control mesocosms (Table 3).

Time, chlorophyll-a concentration, DO, and seeding rate of thiamethoxam-treated canola seeds were identified using CCA as significant factors explaining changes in the aquatic insect community (Table S2). The PRC analysis indicated that time explained 29.3% of the variance in the aquatic insect community among the treatments relative to the control, and that seeding rate, plus the interaction with time, explained 32.2% of the variance (Fig. 6; Table S3). The Dunnett-Contrasts tests indicated that the aquatic insect community in the 100× treatment was significantly different from the control on Day 14 only (Fig. 6; Table S3). The PRC analysis indicated that *Lestidae* sp. and *Dolichopodidae* sp. experienced the greatest increase with increasing seeding rate, while Chironomidae and *Libellulidae* sp. saw the greatest decrease (Fig. 2).

The type of wetland system used in the current study likely explains the prominence of certain taxa. Other studies have shown that in highly ephemeral wetland habitats in general, Coleoptera and Diptera (Cuclicidae) are found to predominate, whereas areas submersed for extended periods are found to be dominated by Diptera (Chironomidae) and Odonata (Schalles and Shure 1989; Batzer and Resh 1992; Jefferies 1994), as was observed in this study. Ephemeroptera primarily inhabit free-flowing water bodies, which likely explains their low abundance in PWRF mesocosms and other model ecosystems (Beketov et al. 2008; Pestana et al. 2009; Mohr et al. 2012; Van Dijk et al. 2013). However, some genera of Ephemeroptera, such as Baetidae, prefer lentic systems (Merritt et al. 2008), and these were observed in the current study. In the PPR specifically, Gleason and Rooney (2018) found that for macroinvertebrates, community composition was distinct among the pond-permanence classes. Despite the differences amongst wetlands, their most common and abundant

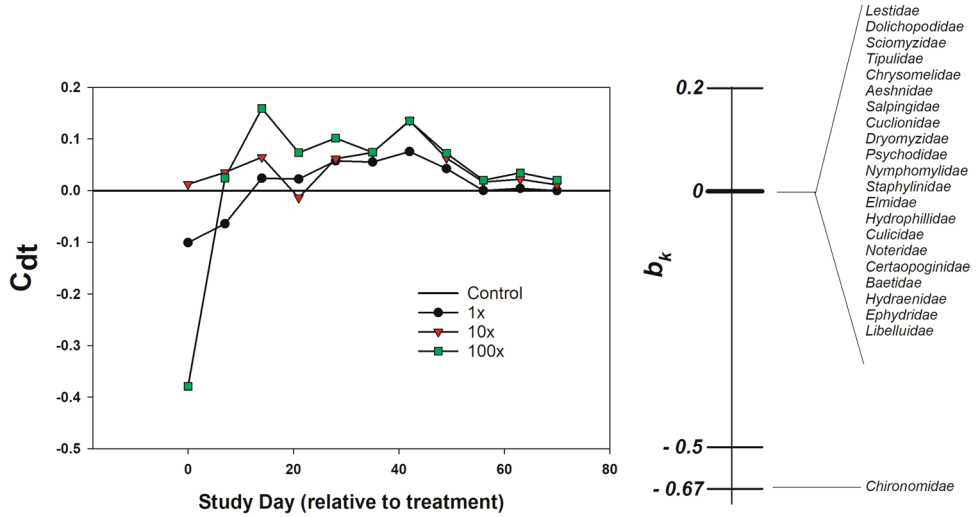

Fig. 6 Principle response curves diagram showing the response of aquatic insect community in mesocosms exposed to different application rates of thiamethoxam-treated seeds (0, 1x, 10x, and 100x) over time. Species weights are provided on the right axis

species were chironomids of 87 sampled sites (600 samples in total), similar to the current study.

The diversity of emergent insects in the mesocosms of this study was less than has been observed in wetland ponds. It has previously shown that mesocosms contain lower insect diversity than wetland ponds, particularly in relation to Coleoptera, Trichoptera, and Heteroptera taxa (Williams et al. 2002); as was observed in the current study. The physical structure of mesocosms (i.e., verticals walls, homogenous depth) has shown to have a greater influence on diversity of the emergent insect community compared to water quality and colonization constraints (Williams et al. 2002). A gradient in depth has been associated with more diverse benthic insect communities in wetland ponds due to greater niche diversity (Williams et al. 2002). Consequently, it is important to consider that the mesocosms used in this study are most representative of small shallow ponds.

It was observed that even in the control mesocosms, insect abundances declined over time throughout the duration of the experiment (Figs. S2–S7). This was likely caused by the relationship between seasonal growth rate and temperature. Tronstad et al. (2007) observed that as temperature declines, so too did density of the insect orders Diptera, Odonate, Coleoptera and Ephemeroptera. In this study, average water temperature significantly declined following the addition of seeds as summer progressed to fall (July 22 to September 30) (Table 1). The family Chironomidae dominated the emergent insect community across treatments. The dominance of Chironomidae was likely due to their capability to rapidly colonize large areas and ability able to deposit eggs days after inundation (Davies 1974; Walton et al. 1990; Williams et al. 1993).

Over the duration of the study the number of Families were consistently greater in the controls relative to the other treatments (Fig. 4A). The dominance of Chironomidae taxa declined over the course of the study in the control and 100× treatments (Fig. S7). The decline in dominance of Chironomidae over the course of the study explains the increase in Simpson's and Shannon's diversity index of emergent insects in the 100× treatment (Fig. 4B & C). Chironomidae have shown to be relatively sensitive to neonicotinoids compared to other emergent insect taxa, e.g., Odonata, Coleoptera (Morrissey et al. 2015). Consequently, in the 100× treatment, thiamethoxam may have exhibited a greater toxicity to Chironomidae relative to other emergent taxa. The greater diversity in control mesocosms may have been due to the continued presence of the taxa most sensitive to TMX.

Other mesocosm studies investigating the effect of thiamethoxam on emergent insect communities have been conducted. Over the course of a 92-d monitoring period, Finnegan et al. (2017) observed no significant effects of exposure to thiamethoxam at concentrations up to 100 µg/L on macroinvertebrate communities. As in the current study, Chironomidae dominated the emergent insects (69%) collected in emergence traps. They observed transient reductions in abundance of Chironomidae at 30 and 100 µg/L and in emergence at 100 µg/L, but recovery was observed before the end of the study. This result is consistent with reported lab-based EC50 values for emergence of *Chironomus riparius* of 35 to 71 µg/L (Finnegan et al. 2017). Emergence of damselflies (member of the Odonata order) was not affected by thiamethoxam exposure up to 100 µg/L (Finnegan et al. 2017), while in the current study, exposure to ~92 µg/L did result in a significant reduction in emergence in Odonata species (Fig. S4).

The absence of a significant difference between the control and 1× treatments for abundance of Diptera,

Odonata, and Chironomidae indicates that recommended planting rates of thiamethoxam-treated seeds may not have an adverse effect on the abundance of these taxa in shallow prairie wetlands (Figs. 3, 4). The relatively low abundance of Coleoptera and Ephemeroptera among treatments in this study limits the ability to conclude whether current rates of seeding would affect the abundance of these two taxa.

Conclusions

The application of thiamethoxam-coated seeds at the recommended seeding rate did not have a significant effect on the structure and abundance of the zooplankton and aquatic insect communities in shallow wetland mesocosms. Based on the maximum concentration of thiamethoxam measured in wetlands of the Canadian Prairies (i.e., 1.49 µg/L; Main et al. 2014), thiamethoxam exposure from inundation of crop land planted with treated seed appears to present a negligible risk to zooplankton and emergent insects under environmentally relevant planting conditions. However, deleterious effects are evident at heavier seeding rates. The overall consensus in the literature appears to be thimamethoxam at environmentally relevant concentrations, acutely or chronically, does not appear to impair invertebrates during the open water seasons, of which our study is in agreement. Should seeding or application rates to seeds increase, there appears to be the potential for effects under specific circumstances (e.g., wetland expansion). We believe future work with thiamethoxam should focus on better characterizing sediment exposures and the potential impacts on benthic insects, as this has yet to be examined effectively.

Data availability

Much of the data are available in SI, or will be available upon request of the authors.

Code availability

R code used in the statistical analysis can be found in the SI.

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Compliance with ethical standards

Conflict of interest MLH has published previously with the registrant of thiamethoxam (Syngenta Crop Protection LLC) on this compound.

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