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Effects of experimental insecticide applications and season on soil nematode communities in a maize field

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ABSTRACT

The soil nematode abundance, species distribution, community composition and ecological indices were determined in a two-year field experiment in an agricultural field planted with *Zea mays* as well as in a complementary pot experiment to evaluate the effects of commercial insecticides that are used to control the western corn rootworm, *Diabrotica virgifera* ssp. *virgifera* LeConte. Three different insecticide variants were used in the experiment: a granular application of tefluthrin, seed treatment of clothianidin and granular application of clothianidin. In total, 39 nematode species and 35 genera were identified in the maize field. A significant impact of season was found on the relative abundance of trophic groups and all ecological and diversity indices, with the exception of nematode abundance. Non-metric multidimensional scaling (NMS) based on species diversity showed a greater difference between years than among variants. Significant differences among variants were detected for the species diversity index of trophic groups (bacterivores, fungivores and omnivores) and selected ecological indices, such as maturity and structural and basal indices. The pot experiment included the same three insecticide variants as the field experiment, as well as variants with insecticide concentrations that were five times higher than normal rate of application corresponding to control variants. Overall, 24 genera of nematodes were recorded in the pot experiment. A NMS analysis based on genera diversity separated the samples into groups by sampling date rather than by variant. A five-fold increase in the concentration of chemicals did not significantly influence the evaluated indices or trophic groups. Generally, the results showed that "season" (the year in the field experiment or the month in the pot experiment) had a greater influence on nematode communities than the chemical variants, even at a concentration five times greater than the application rate commonly used in the field.

1. Introduction

The western corn rootworm (WCR), *Diabrotica virgifera virgifera* (Coleoptera: Chrysomelidae), is one of the most devastating corn rootworm species on field maize, *Zea mays* L. It is believed that the beetle originated in Mexico and spread throughout the United States and Canada during the twentieth century as maize production increased (Krysan and Smith, 1987). In 1992, the WCR was accidentally introduced to the former Yugoslavia from North America (Bača, 1994). Its geographical expansion has continued over time, and hence, the WCR has become one of the worst maize pests in Europe. In 2000, the first occurrence of the WCR was recorded in Slovakia (Sivicek, 2000). Prior to the WCR invasion of Slovakia, there were nearly 40,000 ha of maize grown annually in monoculture. After the deleterious experiences with the WCR, the area of monoculture decreased to less than 20,000 ha in 2008, and chemical control was used to control the WCR (Cagáň, 2008).

Soil health is defined as the capacity of a soil to function within ecosystem boundaries to sustain biological productivity, maintain environmental quality, and promote plant and animal health (Doran and Zeiss, 2000). Healthy soil is stable soil that can overcome stress, is usually high in biological diversity, and is capable of maintaining nutrient cycling (Wang and Hooks, 2011). *D. v. virgifera* is a univoltine species with three larval instars that feed almost exclusively on maize roots (Moeser and Vidal, 2005; Pilz et al., 2009). To control this pest, European farmers have applied granular soil insecticides or insecticide-coated seeds to combat the larvae and foliar insecticides against the adults (Ward et al., 2004).

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Maize fields, previously almost free from insecticides, are now beginning to endanger the environment because soil insecticides and insecticide-coated seeds may cause a reduction in the diversity of soil organisms and consequently cause a reduction in soil fertility. Previous studies have shown that clothianidin variants tend to reduce the densities of the beneficial arthropod taxa Coccinellidae, Hymenoptera, Araneae, and particularly Staphylinidae and Chrysopidae, whilst tefluthrin tended to impact the Coleopteran families (Babendreier et al., 2015).

One appropriate way to assess soil health is to observe the biological structure of soil organisms. Based on numerous studies, nematode communities have the potential to be good indicators of a variety of disturbances within an ecosystem (Bongers, 1990; Brmez et al., 2008; Renčo and Čerevková, 2015). Nematodes are common, easy to sample, and well-classified into functional (feeding) groups. There are differences in feeding behaviour (Yeates et al., 1993), and omnivores and predators generally demonstrate a high sensitivity to disturbance. Nematodes can be classified based on many different criteria, but the development of the Maturity Index (MI) and the Plant Parasite Index (PPI) (Bongers, 1990; Bongers and Korthals, 1993; Bongers et al., 1997) present advanced tools for predicting ecological processes in soil ecosystems. Subsequently, Ferris et al. (2001, 2004) refined these concepts by defining the Enrichment Index (EI) and the Structure Index (SI), which provide greater resolution of the effects of enrichment, disturbance and contamination on the soil ecosystems. Ferris (2010) proposed using nematode biomass (calculated from morphometric data), cp-values and respiration for the calculation of metabolic footprints that provide metrics for the magnitude of ecosystem functions and services provided by component organisms of the soil food web.

This study determined the influence of insecticides applied for the control of the WCR on nematode communities. Similar complex studies have not been previously conducted because the application of soil insecticides to maize fields has increased in recent years in response to the introduction of the WCR to Europe. Preliminary partial results of this work have been presented in Čerevková and Cagáň (2013). The current study provides a detailed survey of nematode communities during a two-year field study, supplemented by a pot experiment. In the field experiment, the influence of chemical applications under different climatic conditions was studied during the growing season. In the pot experiment, the influence of chemicals under a constant level of soil moisture (not less than 90%) was studied. The aim of this study was to explain: 1) the importance of the seasonal effect on nematode communities; 2) the impact of selected insecticides commonly used against the WCR on nematode communities; 3) the interaction between season and insecticides on nematode communities; 4) differences in the development of nematodes under field and pot conditions; and 5) the influence of a five-fold increase in the concentration of chemicals on nematode communities under pot experimental conditions.

2. Materials and methods

2.1. Field experiment and soil characteristics

Field trials were conducted at Komoča (47°58′N; 18°02′E) in southwest Slovakia during the years 2011 and 2013. The site was situated in a warm region with an average of 50 or more summer days annually and a warm, dry sub-region with a mild winter. The average daily temperature in January is above −3 °C, and the average daily temperature in July reaches 20–21 °C. The mean annual precipitation is 500–550 mm. The location is characterised by calcaric fluvisols associated with gleyic and arenic calcaric fluvisols from carbonate alluvial segments. The soil has a clay texture (Miklós, 2002).

The ratio of humus in the soil was up to 3%, the pH of the soil was 6.9, and soil analysis by the Central Control and Testing Institute (ÚKSUP) showed 7200 mg of calcium, 45 mg of phosphorus, 143 mg of potassium and 741 mg of magnesium per kg of soil.

The fields were ploughed in October in both years and were dragged 25 days before sowing. Urea (324 kg per hectare) was applied on the soil surface and combined immediately. Sowing was performed at a deep of 5–6 cm.

The maize was sown on April 27, 2011 and on April 25, 2013. Granular insecticides were simultaneously applied directly to the rows. Seed-coating with a liquid form of the insecticide was performed one hour prior to sowing. The maize hybrid Realli (Caussade Semescens) was used in the experiments. The field trials included four variants randomly arranged in 5 replicates. The plot size was 100 m^2. Table 1 provided detailed information of field experimental design.

In 2011, the average daily temperature reached 13–15 °C during last week of April; increased from the beginning of May to the end of May from 7 °C to 23 °C; and was typically above 20 °C during June, July and August. The total precipitation was less than half of normal values in April (26 mm) and May (16 mm), normal in June (62 mm), very high in July (84 mm) and low in August (22 mm).

In 2013, the last week of April was very warm with average daily temperatures of 16–21 °C; in May, temperatures were below average (15 °C), and during June, July and August temperatures were typically above 20 °C with average daily temperatures often above 25 °C. In April, the total precipitation was 22 mm, but at the beginning of May, the rainfall totalled more than 20 mm. The precipitation was 70 mm in May, 64 mm in June, 31 mm in July and 57 mm in August.

Sampling was conducted over five months in both years (2011 and 2013) during the first week of May (one week after sowing), and subsequently during the first week of June, July, August and September.

As a result, the first sampling was taken 7 days after the application of the chemicals and the subsequent samples were taken after 38, 68, 99 and 130 days.

For each research variant, five bulk samples of soil (one per plot) were used for investigating nematode communities. A bulk sample consisted of five sub-samples collected at a depth of 15 cm on each sampling date.

2.2. Pot experiment

In 2013, the pot experiment was organized outside at Vráble, 50 km from the village of Komoča. Soil samples for the pot experiment were collected from the site of the field experiment in Komoča. In total, 20 samples of soil were collected from the upper soil layer (15 cm). All samples were carefully mixed to create an average soil sample. Each pot was filled with 2 kg of soil from the mixed sample. The sowing of maize in the pot experiment was completed at the same time as the field trial.

In one half of the pot experiment, the variants in the field trial were duplicated. In the second half of the pot experiment, the concentration of chemicals was 5 times higher than the normal rate of application. The pot experiment consisted of eight variants randomly arranged in 5 replicates. Granular insecticides were applied to the pots in granular form. Five time higher dose of granules represented five time higher concentration of insecticide in one pot. Seed-coating insecticides were applied to the seeds. Five treated seeds in one pot delivered the equivalent quantity of chemicals as applied in the maize field. To achieve the higher dose of insecticide in one pot, it was necessary to plant 25 treated seeds